Effect of Tolvaptan on the Hepatobiliary Disposition of Bile Acids in Human B-CLEAR® Hepatocytes

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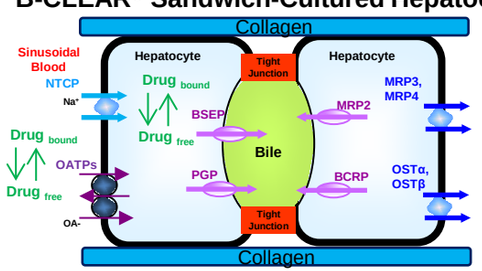
INTRODUCTION

Bile acid homeostasis in the liver is tightly controlled through various regulatory 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. Thus, an *in vitro* model that has all transport proteins expressed, localized, and functioning as *in vivo* is critical to predict accumulation and the net effect of uptake and efflux inhibition, as it occurs *in vivo*. In these studies, the effect varying concentrations of tolvaptan (0.15 µM to 50 µM) on the hepatobiliary disposition of a model bile acid, taurocholate (TCA), were evaluated in sandwich-cultured hepatocytes using B-CLEAR® technology. To mimic physiologic conditions and account for nonlinear protein effects, experiments were performed in the presence of a physiologic concentration of protein (4% BSA).

B-CLEAR® Sandwich-Cultured Hepatocytes



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

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METHODS

Human Hepatocyte Isolation and Culture. Transporter Certified™, cryopreserved human hepatocytes (Triangle Research Labs - HUM4059) were cultured in 24-well BioCoat™ plates and overlaid with Geltrex™ 24 hours post-seeding. Qual-Gro™ culture medium was changed daily; studies were performed on Day 5.

Experimental. Using B-CLEAR® technology, the effects of increasing concentrations of tolvaptan on the hepatobiliary disposition of TCA were determined. Briefly, cell culture medium was removed from the hepatocytes and hepatocytes were washed twice with Plus (+) Buffer or Minus (-) Buffer followed by incubation 10 minutes at 37°C with conditioning solutions of Plus (+) Buffer or Minus (-) Buffer prepared with and without tolvaptan or Positive Control Inhibitor. After conditioning, the Plus (+) and Minus (-) Buffers were removed and replaced with the incubation solutions containing 2.5 uM of d_8-TCA in the absence or presence of tolvaptan (0.15, 1.5, 15, 20, and 50 µM) or erythromycin estolate (100 µM) in the presence of a physiological concentration of protein (4% Bovine Serum Albumin, BSA) and incubated for 10 minutes at 37°C. A separate study under identical conditions was performed to evaluate the effects of tolvaptan in the absence of 4% BSA. The hepatocytes were then washed three times with ice-cold Plus (+) Buffer. The plates were frozen at -80°C for bioanalysis.

Sample Preparation and Analysis. Lysates were filtered and evaporated to dryness under nitrogen. Dried samples were reconstituted, filtered and analyzed for d_8-taurocholate and tolvaptan. by LC-MS/MS, which employed reversed-phase HPLC and electrospray ionization.

Data Analysis. Total accumulation, cellular accumulation, intracellular concentration, biliary excretion index (BEI) and biliary clearance for d_8-taurocholate were determined using B-CLEAR® technology. In addition the intracellular concentration of tolvaptan was also determined.

RESULTS AND DISCUSSION

Inhibition of TCA Uptake and Efflux in SCHH by Tolvaptan

Inhibitor	Concentration (µM)	BSA	Total Accumulation Avg. (pmol/mg)	Total Accumulation Std. Dev.	Cellular Accumulation Avg. (pmol/mg)	Cellular Accumulation Std. Dev.	Intracellular Conc. Avg. (µM)	Intracellular Conc. Std. Dev.	BEI %	BEI Std. Dev.	CL (mL/min/kg)	CL Std. Dev.
Tolvaptan	0*	0%	215	27.0	43.3	4.45	5.64	0.579	79.8	12.8	20.3	3.25
	15		145	15.9	47.5	2.13	6.18	0.277	67.3	11.1	11.6	1.90
Tolvaptan	0*	4%	57.7	8.82	14.0	2.27	1.82	0.295	75.8	15.8	5.18	1.08
	0.15		50.3	7.43	13.0	1.35	1.68	0.175	74.3	15.0	4.43	0.895
	1.5		34.4	12.1	11.9	1.47	1.55	0.191	65.3	35.5	2.66	1.45
	15		31.8	6.21	14.7	3.66	1.91	0.476	53.8	22.6	2.03	0.854
	20		39.0	2.38	14.9	1.60	1.94	0.208	61.7	7.37	2.85	0.340
	50		36.0	2.71	17.7	4.98	2.31	0.648	50.8	15.7	2.17	0.672
Erythromycin Estolate	100	4%	37.1	3.40	12.9	6.22	1.68	0.809	65.3	19.1	2.87	0.840

Table 1. Effect of tolvaptan on the total accumulation, cellular accumulation, intracellular concentration, biliary excretion (BEI) and biliary clearance of d_8-taurocholate. Data represent mean ± SD of 3 replicates of a single human donor.

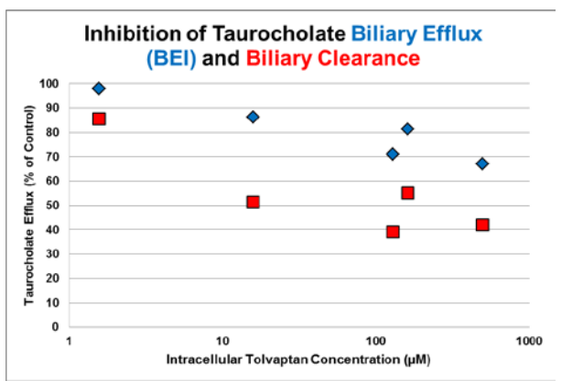

Figure 1. Effect of tolvaptan (intracellular concentration) on the biliary efflux (BEI) ♦, and biliary clearance ■ of d_8-taurocholate expressed as a % of control.

$$BEI = \frac{Accumulation_{total} - Accumulation_{cellular}}{Accumulation_{total}}$$

$$Cl_{biliary} = \frac{Accumulation_{total} - Accumulation_{cellular}}{AUC\ (Time \bullet Concentration_{media})}$$

CONCLUSIONS

- Increasing the dose concentration of tolvaptan in the presence of 4% BSA from 0.15 to 50 µM resulted in a decrease in the uptake of taurocholate to 62.4% of control, and a decrease in the biliary excretion index to 67% of control (Table 1).
- The combined effect of the observed inhibition of both uptake and efflux by tolvaptan was a decrease in the biliary clearance of taurocholate to 41.9% of control (Figure 1).
- The effect of the positive control inhibitor, erythromycin estolate (100 µM), was similar to tolvaptan and decreased the biliary clearance of taurocholate to 55% of control.
- Addition of 4% BSA reduced the intracellular concentration of taurocholate in proportion to the unbound concentration in the media.
- The combined inhibitory effects of tolvaptan on the uptake, and efflux of taurocholate resulted in decreased intracellular concentrations of taurocholate at lower tolvaptan exposures, followed by increased intracellular concentrations of taurocholate at tolvaptan exposures greater that 15 µM.
- At the highest dose of tolvaptan (50 µM), the intracellular concentration of taurocholate was increased to 127% of control. Erythromycin estolate (100 µM) had no effect on the intracellular concentration of taurocholate.