Toward a More Predictive Model: Effect of Telmisartan on Taurocholate Disposition in B-CLEAR® Cryopreserved Sandwich-Cultured Hepatocytes Compared to BSEP-Expressing Membrane Vesicles

Tracy L. Marion, William R. Smith, Cassandra L. Hubert, Robert L. St. Claire III, Kenneth R. Brouwer
Qualyst Transporter Solutions, LLC, 2810 Meridian Parkway, Suite 100, Durham, NC 27713

INTRODUCTION

Inhibition of BSEP (bile salt export pump)-mediated bile acid transport has been implicated as a potential mechanism for drug-induced cholestasis. Initial correlations between BSEP inhibition and toxicity have been encouraging; however, there is significant room for improvement. Telmisartan (TEL), an angiotensin II receptor antagonist, reportedly demonstrates a low IC_{50} for BSEP inhibition in membrane vesicles (IC_{50} = 1.9 µM[1]), indicating that it is a potent BSEP inhibitor. However, there are no reports of clinical cholestasis/liver injury associated with TEL. Based on the BSEP membrane vesicle assay, TEL is classified as a False Positive.

Hypothesis: Application of an integrated, whole-cell system with hepatic uptake and efflux transporter functionality, *in vivo*-relevant intracellular concentrations, and metabolic capacity can correctly classify TEL as a True Negative, compared to BSEP membrane vesicles, which have limited transport function and no metabolic capacity.

RESULTS

TEL uptake was markedly decreased in incubations containing BSA compared to protein-free conditions (**Table 1**); however, the decrease was not in proportion to the extent of binding. TEL accumulated within hepatocytes to 90-fold higher than predicted (0.54 µM) based on its binding (**Table 1**). At clinically relevant extracellular concentrations (up to 1 µM) in the presence of BSA there were no changes in TC clearance (**Table 1**) despite the intracellular accumulation of TEL, indicating no inhibition of BSEP function. This is consistent with clinical ob-servations. Changes in TC disposition were seen only at the 10 and 20 µM extracellular exposures, which yielded high intracellular TEL concentrations that would not be observed clinically. When normalized to the intracellular concentration of TEL, the inhibition of TC clearance in the presence and absence of protein by TEL was identical (**Figure 1**), indicating the importance of the intracellular concentration in BSEP inhibition. Glucuronidation of TEL was <20% of total, consistent with clinical observations.

	TEL Dose (µM)	TEL Uptake (pmol/mg protein)	TEL [Intracellular] (µM)	TC BEI (%)	TC Clearance (mL/min/kg)
No BSA	0	4.1	0.6	62	22
	0.01	9.3	1.4	65	22
	0.1	90	17	66	22
	1	681	107	61	18
	10	3577	479	48	10
	20	5326	790	42	6.9
4% BSA	0	0	0	60	5.1
	0.01	4.2	0.6	63	5.1
	0.1	35	5.9	63	5.2
	1	310	49	62	4.9
	10	784	117	54	4.2
	20	801	130	52	4.1

Table 1: Uptake, intracellular concentration of TEL, and effect of TEL on TC BEI and *in vitro* biliary clearance in the absence and presence of 4% BSA.

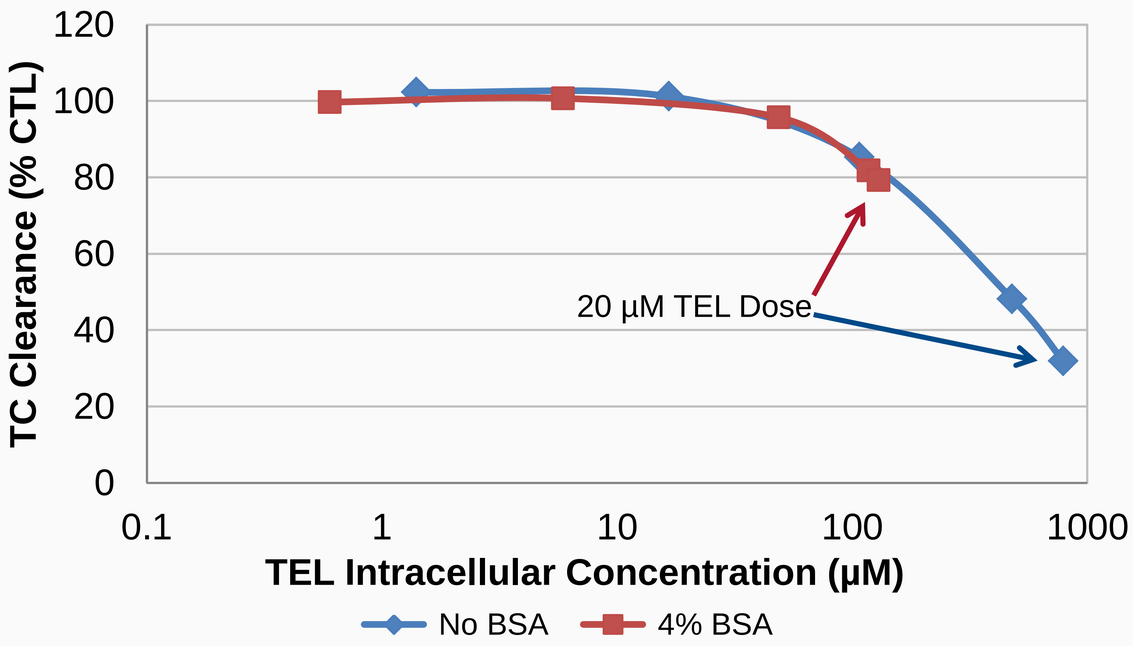

Figure 1: Effect of TEL intracellular concentration on the *in vitro* biliary clearance of TC (% CTL) in the absence and presence of 4% BSA.

METHODS

Transporter Certified™ cryopreserved human hepatocytes cultured in sandwich configuration were treated with increasing concentrations of TEL (0.01-20× $C_{max,total,ss}$ [1 µM]) in the presence or absence of 4% bovine serum albumin (BSA) to approx-imate physiologic conditions (*in vivo*, TEL is 99.5% bound to plasma proteins) and the conditions of BSEP membrane vesicles experiments (no protein in-cluded). The uptake, intracellular accum-ulation, and biliary excretion of the BSEP probe substrate d_8-taurocholate (TC) were determined using B-CLEAR® technology.

Sample Preparation and Analysis. The accumulation of TC, TEL, and TEL-glucuronide (TEL-GLUC) in cell lysates was analyzed by LC-MS/MS using reversed-phase HPLC and electrospray ionization.

Data Analysis. Total TC accumulation (cell + bile pocket contents), intracellular TC accumulation (cell contents only), and TC biliary excretion index (BEI; the per-centage of substrate taken up into the cells and effluxed into the bile pockets) were determined using B-CLEAR® tech-nology. BEI and *in vitro* biliary clearance were calculated using the equations below. Intracellular concentrations of TEL and TEL-GLUC were calculated using an estimate of hepatocyte volume (QTS internal Technical Application Bulletin TAB Biol 007). Data are expressed as mean and/or percent of control of duplicate samples in n=1 human liver.

$$BEI = \frac{Accumulation_{total} - Accumulation_{cellular}}{Accumulation_{total}} \times 100$$

$$Cl_{biliary} = \frac{Accumulation_{total} - Accumulation_{cellular}}{AUC_{medium}} \times 100$$

CONCLUSIONS

Consistent with clinical data, under physiologically-relevant conditions (in the presence of protein and at extracellular exposures approximating $C_{max,total,ss}$), TEL did not inhibit BSEP-mediated TC transport. BSEP inhibition was observed, but only at extracellular and intracellular concentrations that would not be achieved with clinical doses. The disproportional hepatic uptake observed in the presence of protein suggests that inclusion of protein may be an important parameter in these types of assays. An integrated system using B-CLEAR® sandwich-cultured hepatocytes correctly predicts that TEL would be a True Negative, and may offer a better model than membrane vesicles for predicting the potential for a compound to interact with BSEP *in vivo*.

REFERENCES

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