Prediction of Altered Bile Acid Disposition by Drugs Using an Integrated Approach: Sandwich-Cultured Human Hepatocytes, Mechanistic Modeling and Simulation

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INTRODUCTION

- Inhibition of bile acid transport by drugs and/or metabolites may result in hepatocellular accumulation of bile acids and hepatotoxicity [1].
- Hepatocellular bile acid concentrations are influenced by transporters responsible for hepatic uptake clearance (CL_{Uptake}), biliary clearance (CL_{Bile}), and basolateral efflux clearance (CL_{BL}) of bile acid(Fig. 1).
- The impact of drugs on transporters is driven by local unbound concentrations according to the "free drug hypothesis" [2] .
- Data generated from B-CLEAR® sandwich-cultured human hepatocytes (SCHH) can inform modeling and simulation to predict altered hepatocellular bile acid concentrations due to impaired transport.

Abbreviations: BSEP (Bile Salt Export Pump); NTCP (Sodium-Taurocholate Cotransporting Polypeptide); MRP (Multidrug Resistance– Associated Protein)

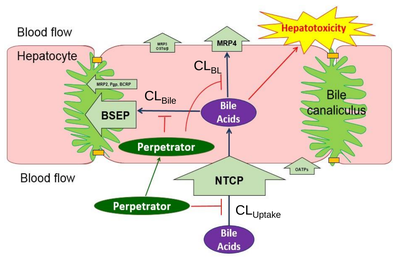


Fig. 1. Inhibition of bile acid transporters alters bile acid accumulation in SCHH and potentially results in hepatotoxicity.

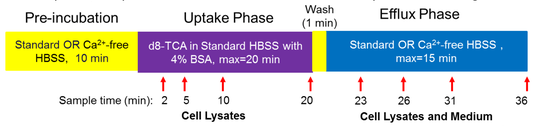
OBJECTIVES

The objectives of this study were to examine the disposition of d_8-taurocholic acid (TCA, a model bile acid) in SCHH, and to predict the effect of perpetrator drugs that inhibit bile acid transport (i.e. telmisartan, bosentan) on hepatocellular TCA concentrations. The specific aims were to:

- Estimate kinetic parameters for TCA disposition;
- Identify the most sensitive measurement from the SCHH assay to evaluate impaired bile acid clearance based on simulations;
- Develop methods to translate transport inhibition data from transfected systems (e.g., IC_{50} values) to hepatocytes for accurate predictions of altered cellular bile acid exposure.

METHODS

D_8-TCA Uptake and Efflux in SCHH. B-CLEAR®-HU, Transporter Certified™, cryopreserved hepatocytes (Lot number: HUM4045, HUM4061B, HUM4059 purchased from Triangle Research Labs, RTP, NC) were cultured in a sandwich configuration (overlaid with Matrigel®). On day 6 of culture, uptake and efflux studies were conducted as depicted by the following scheme. D_8-TCA concentrations in cells+bile, cells, and buffer during uptake (2, 5, 10, and 20 min) and efflux (2, 5, 10, and 15 min) phases were determined deterrmined using B-CLEAR® technology and analyzed by LC/MS. Bovine serum albumin (BSA; 4%) was added to mimic the effect of protein binding *in vivo*.



Pharmacokinetic Modeling of TCA Disposition in SCHH. A previously developed pharmacokinetic model [3] was fit to TCA cells+bile, cells, and buffer mass-time data during 20-min uptake and 15-min efflux phases using Phoenix (Pharsight, Mountain View, CA). The model scheme is depicted in Fig. 2. The estimated kinetic parameters were used for the simulations.

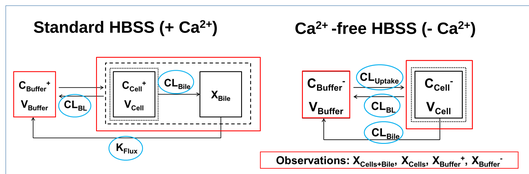


Fig. 2. Model schemes depicting disposition of d_8-TCA in SCHH using standard (Cells+Bile) and Ca^{2+}-free (Cells) HBSS. C_{Buffer}, buffer concentration; +, standard buffer; -, Ca^{2+}-free buffer; C_{Cell}, cellular concentration; V_{Buffer}, buffer volume, 0.3 mL; V_{cell}, cellular volume, 7.69 µL/mg protein; X_{Bile}, mass in bile; CL_{Uptake}, uptake clearance; CL_{BL}, basolateral efflux clearance; CL_{Bile}, biliary clearance; K_{Flux}, first-order rate constant for flux from bile compartment to medium; red and blue boxes represent observed and fitted parameters.

Sensitivity Analysis.

The optimal assay parameters [mass in cells (X_{Cells}), mass in cells+bile ($X_{Cells+Bile}$), mass in bile (X_{Bile}), ($X_{Cells}/X_{Cells+Bile}$), ($X_{Bile}/X_{Cells+Bile}$), and (X_{Bile}/X_{Cells})] was evaluated by impairing TCA CL_{Uptake} and efflux clearance (CL_{Efflux}=CL_{Bile}+CL_{BL}). $X_{Cells+Bile}$ and X_{Cells} were simulated by varying CL_{Uptake} and CL_{Efflux} from 0.01X to 0.1X of the baseline value, assuming CL_{Bile} and CL_{BL} were impaired to the same extent. Simulated assay parameters were plotted against fraction of inhibition, which was calculated as ($CL-CL_I$)/CL.

The necessity of obtaining experimental data on unbound fraction (fu) for perpetrator drugs was evaluated by simulating changes in TCA mass in cells (10-min uptake) in response to varying fu values in medium and cells. Values of fu were chosen within the literature reported ranges (Fig. 5).

Prediction of TCA Accumulation Based on Transport Inhibition Data. SCHH were incubated with low and high concentrations of perpetrator drugs (bosentan, telmisartan) for 20 min in a manner similar to that described above, except that the efflux phase was not included. Total concentrations (C_{total})of perpetrator drugs in cells and medium were measured. Altered cellular TCA concentrations in SCHH by perpetrator drugs were simulated based on CL_{Uptake}, CL_{Bile}, and CL_{BL} of TCA, which were assumed to be mediated by NTCP, BSEP and MRP4, respectively. CL values in the presence of inhibitors (CL_I) were estimated according to equation 1:

$$CL_I = CL/\left(1 + \frac{[I]}{IC_{50}}\right) \qquad \text{(Eq. 1)}$$

where [I] is the unbound concentration of inhibitors, calculated by C_{total} X fu. IC_{50} values are listed in Table 1, which were obtained from the literature except that telmisartan IC_{50} against NTCP was measured in house using NTCP-expressing CHO-K1 cells (SOLVO biotechnology, Hungary). Values for fu and C_{total} are presented in Table 2. Values of fu that were identified as sensitive parameters were measured experimentally using the following method.

Determination of fu of Drugs in Whole Cell Lysates (WCL) and Cytosol

SCHH were treated with perpetrator drugs as described above. Cells were fractionated and the cytosol was isolated as reported previously[4]. fu in whole cell lysates (WCL) and cytosol were determined using equilibrium dialysis..

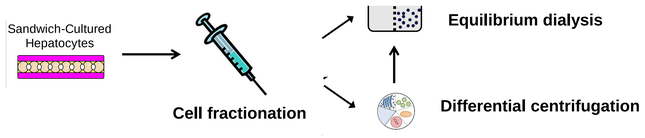


Table 1. Summary of IC_{50} (µM) data of perpetrator drugs for major TCA transporters.

	Bosentan	Telmisartan
NTCP	30	> 20
BSEP	23	1
MRP4	22	11
Ref.	[5, 6]	In-house, [6, 7]

RESULTS

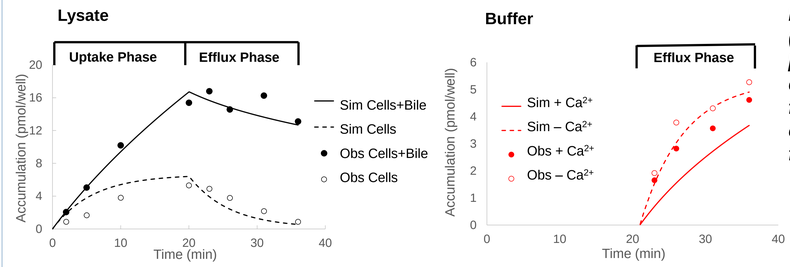
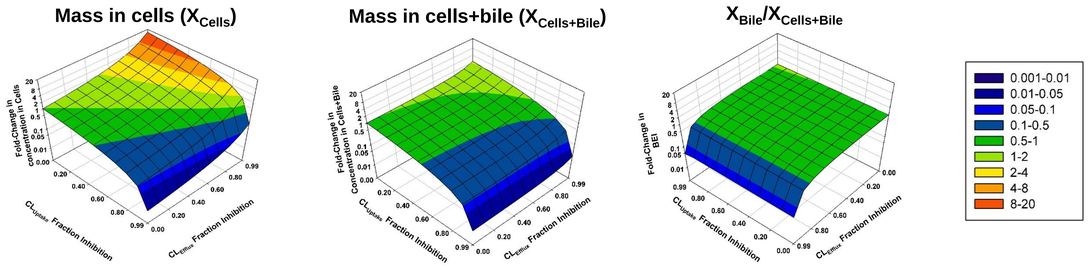


Fig. 3. Observed (circles) and simulated (lines) d_8-TCA accumulation in lysate [from cells+bile and cells] (black) and efflux buffer [Ca^{2+}-containing and Ca^{2+} -free] (red). Representative graph from one of n=3 experiments. Recovered estimates for CL (mean and SD):

(µL/min/mg protein)	Mean	SD
CL_{Uptake}	6.97	1.39
CL_{BL}	0.379	0.12
CL_{Bile}	0.827	0.30



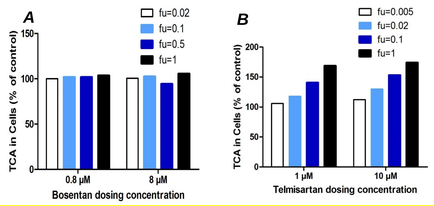
TCA mass in cells is most sensitive to inhibition of CL_{Uptake} and CL_{Efflux}

Fig. 4. Simulated effects of inhibition of d_8-TCA CL_{Uptake} and CL_{Efflux} on X_{Cells}, $X_{Cells+Bile}$ and $X_{Bile}/X_{Cells+Bile}$. The Z-axis represents the fold-change compared to baseline (shown in the colormap on the right), based on simulations of TCA accumulation at steady state. Methods were described in **Sensitivity Analysis**.

Table 2. Summary of total {unbound} concentrations (µM) of perpetrator drugs in medium and cell lysates after a 20-min incubation. Unbound concentrations were estimated based on fu.

Bosentan		Telmisartan	
Medium {fu=0.02}[a]	Cell {fu=0.096}[b]	Medium {fu=0.005}[a]	Cell {fu=0.024}[b]
0.800 {0.016}	2.99 {0.290}	1.00 {0.005}	36.4 {0.875}
8.00 {0.160}	25.4 {2.44}	10.0 {0.0500}	87.7 {2.10}

[a] data from protein binding in plasma
[b] data from protein binding in rat hepatocytes [8]

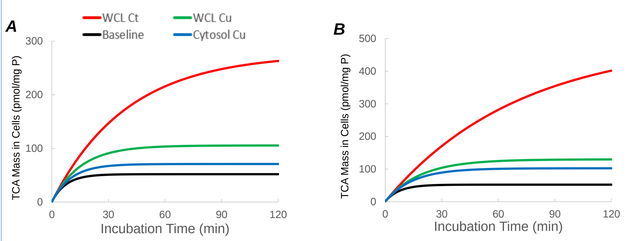


TCA mass in cells is sensitive to telmisartan fu

Fig. 5. Sensitivity analysis of bosentan fu (A) and telmisartan fu (B). Simulations were based on the total concentration presented in Table 2 and different fu values.

Table 3. Total concentration (C_t) and unbound concentrations (C_u) (µM) of telmisartan in whole cell lysates (WCL), and cytosol after a 20-min incubation. Unbound concentrations were estimated based on fu.

Dose (µM)	Sample	C_t	C_u
1	WCL	15.9	2.14
	Cytosol	11.2	0.60
10	WCL	39.8	3.58
	Cytosol	24.6	1.97



Whole cell lysate C_t predicts greater TCA mass in cells than cytosol C_u

Fig.6. Simulated effect of telmisartan (A: 1 µM ; B: 10 µM) on d_8-TCA mass in cells after 120-min uptake. Simulations were based on: whole cell lysates (WCL) total concentration (C_t), WCL unbound concentration (C_u), and cytosol C_u of telmisartan measured in this study (Table 3), telmisartan IC_{50} values (Table 1), and estimated CL_I using Eq. 1.

CONCLUSIONS

- **The mass of d_8-TCA in cells was the most sensitive measurement from the SCHH assay in response to changes in bile acid clearance, which was influenced by both CL_{Uptake} and CL_{Efflux} (Fig. 4).**
- **Measurement of telmisartan fu in cytosol is necessary because the mass of d_8-TCA in cells is sensitive to changes in fu of telmisartan, possibly due to low IC_{50} against BSEP (Fig. 5).**
- **Mechanistic modeling and simulations based on SCHH data are a useful approach to predict the impact of perpetrator drugs on cellular bile acid concentrations when multiple bile acid transporters are inhibited (Fig. 6).**

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