

A mechanistic cellular disposition model of bile acid handling in sandwich-cultured human hepatocytes

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INTRODUCTION

- Over the past years, several mechanistic pharmacokinetic (PK) models of bile acid (BA) clearance have been successfully developed^{1,2,3}.
- In these models, the prototypical BA taurocholic acid (TCA) is being used to assess functional changes in bile acid transport.
- By doing so, important disposition pathways, like metabolic clearance, are being overlooked.
- This issue can be overcome by exposing cultured cells to primary BAs such as chenodeoxycholic acid (CDCA).

OBJECTIVES

- To develop a physiological model which describes hepatic uptake, metabolism and biliary excretion of the BAs CDCA and its glycine-conjugate glycochenodeoxycholic acid (GCDCA) in sandwich-cultured human hepatocytes (SCHH).
- The model was externally validated using *in vitro* disposition data of both CDCA and GCDCA obtained in a different batch of human hepatocytes.

METHODS

- SCHH were used to study the hepatobiliary disposition of CDCA and GCDCA.
- The experimental design for exposure and efflux studies in SCHH is illustrated in Figure 1.
- Experiments were performed on day-5 of culture time.

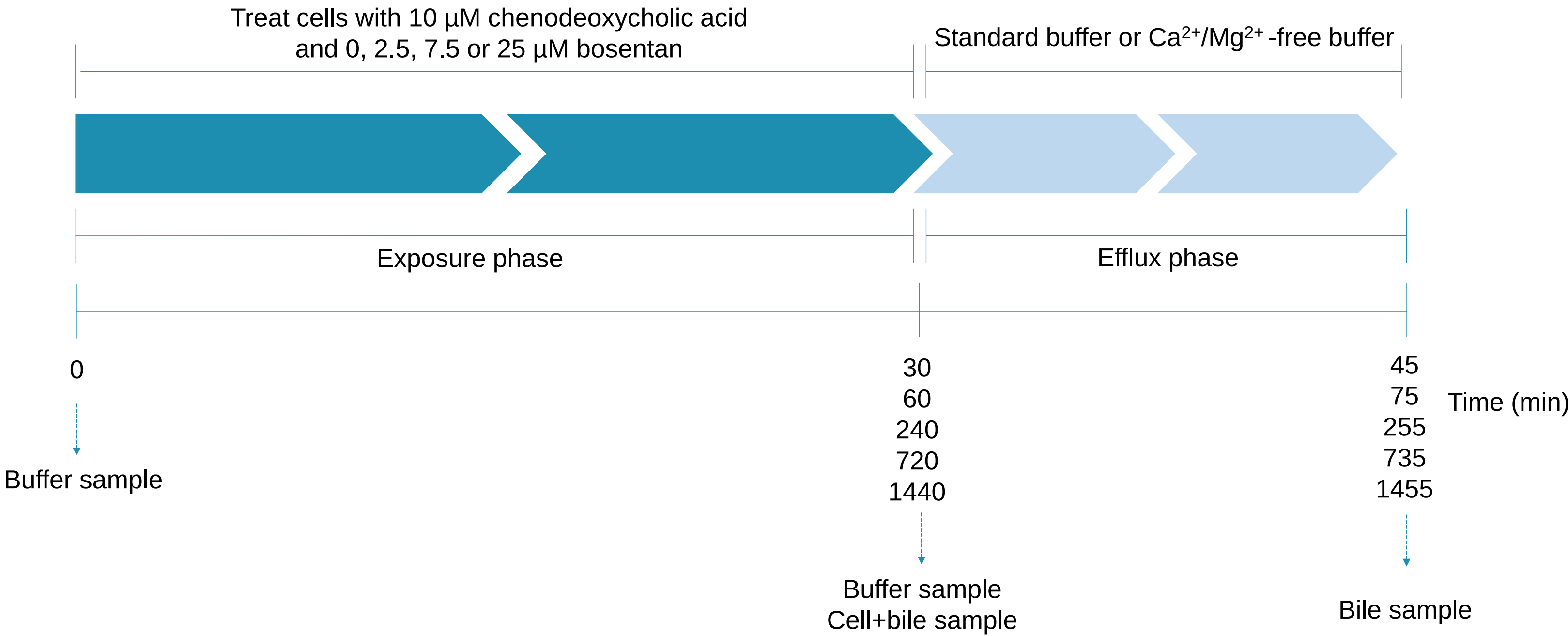


Figure 1. Experimental design for exposure and efflux studies in sandwich-cultured rat hepatocytes (SCHH). Cells were treated with 10 μM chenodeoxycholic acid (CDCA) and 0, 2.5, 7.5 or 25 μM bosentan in standard buffer for 1440 minutes. Following 30, 240 and 1440 minutes of exposure, buffers containing CDCA and glycochenodeoxycholic acid (GCDCA) were collected, after which cells were lysed and total content (=cells+bile) was determined. Subsequently, standard or Ca²⁺/Mg²⁺-free buffer was added to the cells in order to initiate the efflux phase in the presence or absence of tight junctions. Bile samples were collected after 15 minutes of efflux.

- An ordinary differential equation (ODE) model was fitted to concentration-time profiles using NONMEM v7.3.0.
- Bosentan treatment (0, 2.5, 7.5 or 25 μM) was implemented as a covariate in the dataset.
- Stepwise covariate modeling with backward exclusion (p<0.001) was performed on the base model.

RESULTS

- CDCA and GCDCA *in vitro* disposition data were best described using a five-compartment model which includes compartments representing the buffers, cells, bile and cells+bile (Figure 2).
- It is assumed that CDCA is instantaneously converted to GCDCA and therefore not secreted into the bile canaliculi, as it has been reported that the amidation rate of CDCA is very high⁴.
- Unbound intracellular fractions of CDCA and GCDCA were fixed to 0.048 and 0.058, values which were obtained using ADMET Predictor™ 8.
- The *in vitro* disposition data and model fit are shown in Figure 3.

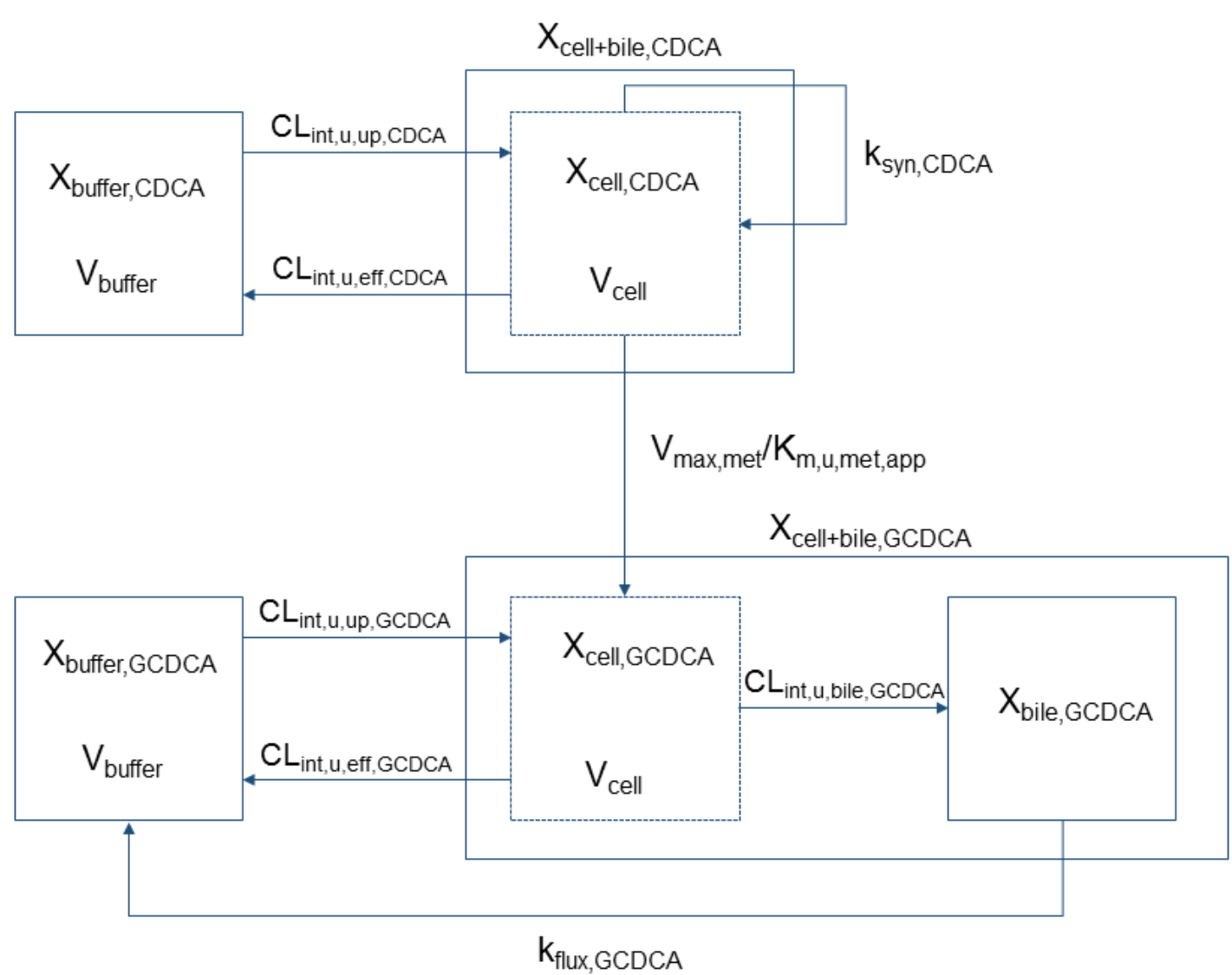


Figure 2. Schematic representation of the structural model. X represents the observed mass of chenodeoxycholic acid (CDCA) or glycochenodeoxycholic acid (GCDCA) in the corresponding compartment, whereas V denotes compartmental volume. *int*, *app*, *syn* and *u* represent intrinsic, apparent, synthesis and unbound. *In vitro* clearance values are designated as CL, where *up*, *eff*, *met* and *bile* represent total (passive+active) uptake, total (passive+active) efflux, metabolism and biliary excretion. *V*_{max} and *K*_m represent the maximal enzymatic velocity at saturating substrate concentration and the substrate concentration at which the reaction rate is half its maximal value. Boxes with solid lines represent observations.

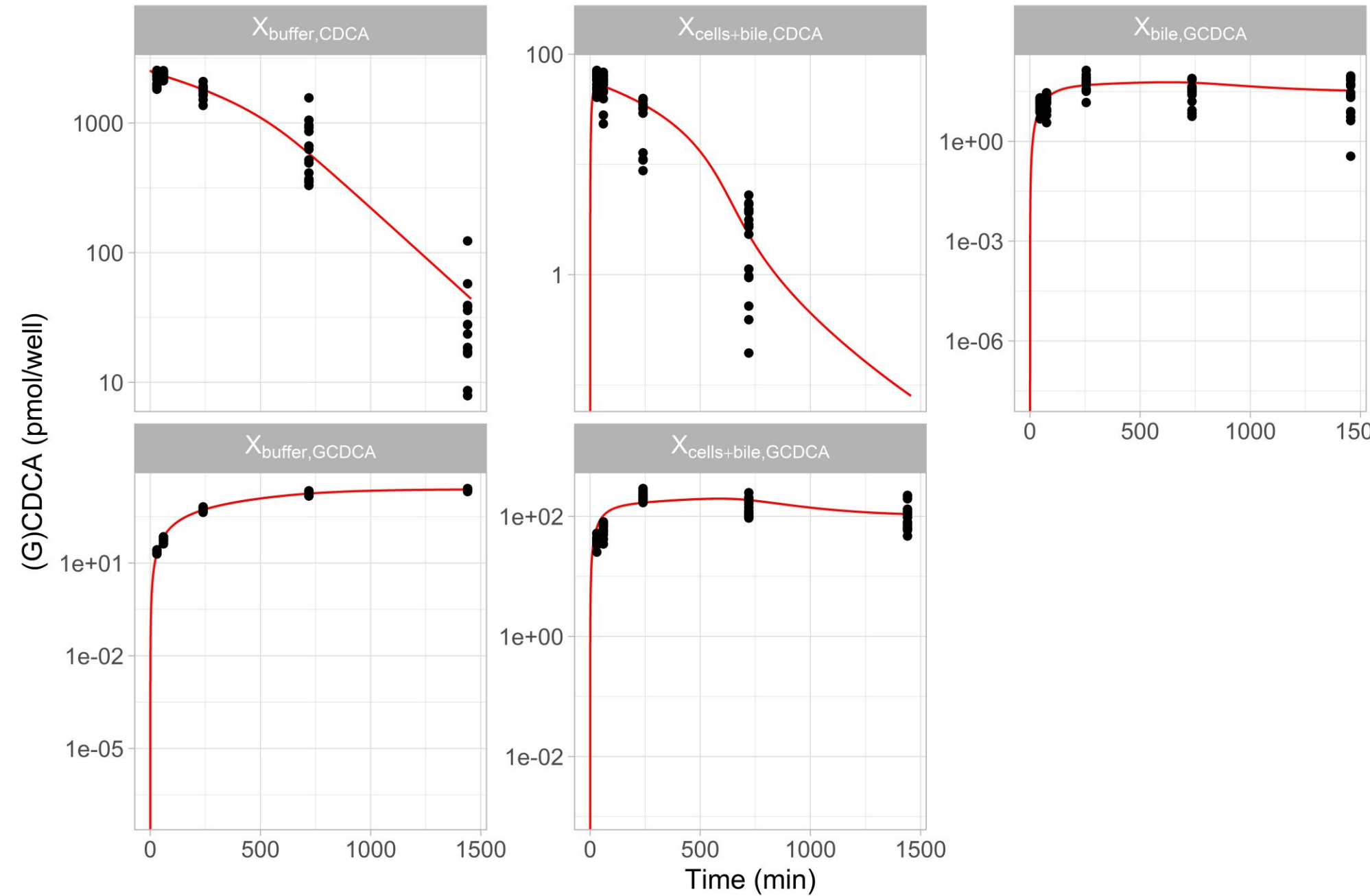


Figure 3. Measured amounts of chenodeoxycholic acid (CDCA) and glycochenodeoxycholic acid (GCDCA) in SCHH when cells are exposed to 10 μM CDCA. Red lines represent the predicted profile based on the five-compartment model illustrated in Figure 2.

- Estimates of the *in vitro* SCHH $CL_{u,int,up,CDCA}$, $CL_{u,int,eff,CDCA}$, $V_{max,met}$, $K_{m,u,app,met}$, $CL_{u,int,bile,GCDCA}$, $CL_{u,int,up,GCDCA}$, $CL_{u,int,eff,GCDCA}$ and $k_{flux,GCDCA}$ are shown in Table 1.
- Covariate analysis showed that bosentan had a significant effect on $CL_{u,int,up,CDCA}$, $CL_{u,int,up,GCDCA}$, $CL_{u,int,bile,GCDCA}$ and $k_{flux,GCDCA}$ (Table 1).

Table 1. Parameter estimates of the final model.

Parameter	Estimate	RSE(%)	95% CI
$V_{max,met}$	14.4 pmol/min/10 ⁶ cells	4.3	13.0 - 15.5
$K_{m,u,app,met}$	0.6 μM	64.6	0 - 7.0
$CL_{u,int,bile,GCDCA}$ (0 μM bosentan)	0.3 μL/min/10 ⁶ cells	18.1	0.2 - 0.4
$CL_{u,int,bile,GCDCA}$ (2.5 μM bosentan)	0.5 μL/min/10 ⁶ cells	30.1	0.2 - 1.0
$CL_{u,int,bile,GCDCA}$ (7.5 μM bosentan)	0.3 μL/min/10 ⁶ cells	10.5	0.2 - 0.3
$CL_{u,int,bile,GCDCA}$ (25 μM bosentan)	0.3 μL/min/10 ⁶ cells	19.4	0.2 - 0.4
$CL_{u,int,up,CDCA}$ (0 μM bosentan)	6.7 μL/min/10 ⁶ cells	15.5	4.5 - 8.5
$CL_{u,int,up,CDCA}$ (2.5 μM bosentan)	4.2 μL/min/10 ⁶ cells	6.8	3.5 - 4.5
$CL_{u,int,up,CDCA}$ (7.5 μM bosentan)	5.7 μL/min/10 ⁶ cells	4.7	5.0 - 6.0
$CL_{u,int,up,CDCA}$ (25 μM bosentan)	5.7 μL/min/10 ⁶ cells	4.4	5.0 - 6.0
$CL_{u,int,up,GCDCA}$ (0 μM bosentan)	1.5 μL/min/10 ⁶ cells	11.4	1.0 - 2.0
$CL_{u,int,up,GCDCA}$ (2.5 μM bosentan)	0.8 μL/min/10 ⁶ cells	16.2	0.5 - 1.0
$CL_{u,int,up,GCDCA}$ (7.5 μM bosentan)	0.7 μL/min/10 ⁶ cells	14.5	0.5 - 1.0
$CL_{u,int,up,GCDCA}$ (25 μM bosentan)	0.4 μL/min/10 ⁶ cells	8.3	0.3 - 0.4
$CL_{u,int,eff,CDCA}$	8.9 μL/min/10 ⁶ cells	10.9	7.0 - 11.0
$CL_{u,int,eff,GCDCA}$	0.4 μL/min/10 ⁶ cells	5.2	0.5 - 1.0
$k_{flux,GCDCA}$ (0 μM bosentan)	0.07 min ⁻¹	20.1	0.04 - 0.1
$k_{flux,GCDCA}$ (2.5 μM bosentan)	0.1 min ⁻¹	28.7	0.04 - 0.15
$k_{flux,GCDCA}$ (7.5 μM bosentan)	0.06 min ⁻¹	28	0.025 - 0.10
$k_{flux,GCDCA}$ (25 μM bosentan)	0.1 min ⁻¹	24.5	0.50 - 0.15

CI: confidence interval; RSE: residual standard error.

- Simulated concentration-time profiles with other doses of CDCA (2.5 μM, 10 μM, 25 μM and 50 μM) are shown in Figure 4, overlaid with observed data from another batch of human hepatocytes (i.e. external validation).
- The simulations show good agreement with the observed data of both CDCA and GCDCA in the buffer and biliary compartment.

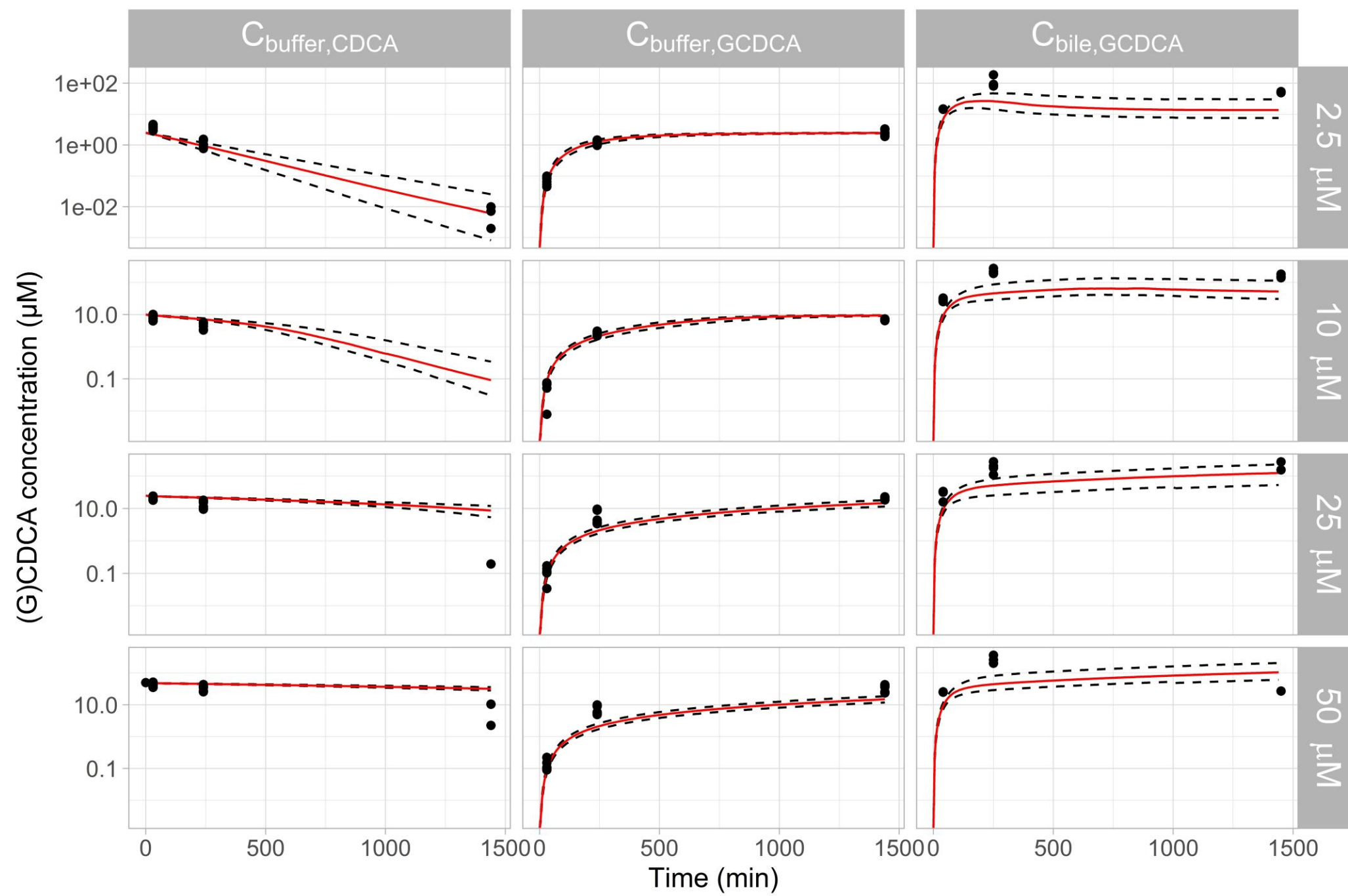


Figure 4. Model predicted vs. observed concentration-time profiles of chenodeoxycholic acid (CDCA) and glycochenodeoxycholic acid (GCDCA) in sandwich-cultured human hepatocytes (SCHH). Simulations of CDCA and GCDCA concentration-time profiles were conducted for 4 different doses of CDCA (2.5 μM, 10 μM, 25 μM and 50 μM). The mean (red line) and 5th and 95th percentiles (dotted lines) are represented. Simulation results were overlaid with observed data (closed circles) obtained in another batch of human hepatocytes. C represents the concentration of CDCA and GCDCA in the corresponding compartment.

CONCLUSIONS

- A mechanistic five-compartment model has been developed which adequately describes the hepatobiliary disposition of both CDCA and GCDCA in SCHH.
- External validation using *in vitro* disposition data obtained from another batch of human hepatocytes showed that the model was able to adequately predict concentration-time profiles of higher doses CDCA.

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[1] Kaschek D. *et al*, A dynamic mathematical model of bile acid clearance in HepaRG cells. *Toxicological Sciences*, 161(1), 48-57 (2018)
 [2] Guo C. *et al*, FXR agonists obeticholic acid and chenodeoxycholic acid increase bile acid efflux in sandwich-cultured human hepatocytes: functional evidence and mechanisms. *J. Pharmacol. Exp. Ther.* 365(2), 413-421 (2018)
 [3] Yang K. *et al*, Species differences in hepatobiliary disposition of taurocholic acid in human and rat sandwich-cultured hepatocytes: implications for drug-induced liver injury. *J. Pharmacol. Exp. Ther.* 353(2), 415-423 (2015)
 [4] Falany. *et al*, Glycine and taurine conjugation of bile acids by a single enzyme. *J. Biol. Chem.* 269(30), 19375-19379 (1994)