Modeling of telmisartan disposition in sandwich-cultured rat hepatocytes. Does cryopreservation change disposition kinetics?

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INTRODUCTION

- Hepatocytes are commonly cryopreserved for long-term storage, providing a permanent and sufficient cell supply for future experiments.

- Cryopreservation might influence drug disposition kinetics, leading to unreliable conclusions.

- Telmisartan, a potent, long-acting, small-molecule antagonist of the angiotensin II type-1 receptor, was used as a model substrate to quantify differences in kinetic parameters between cryopreserved and freshly-isolated hepatocytes.

OBJECTIVES

- To develop a physiological model which describes hepatic uptake, metabolism and biliary excretion of the angiotensin II type-1 receptor antagonist telmisartan and its glucuronide metabolite in sandwich-cultured rat hepatocytes (SCRH).

- To utilize the model to quantify differences in estimated kinetic parameters between cryopreserved and freshly-isolated hepatocytes.

METHODS

- SCRH were used to study hepatobiliary disposition of telmisartan and its glucuronide.

- The experimental design for accumulation and efflux studies in SCRH is illustrated in Figure 1.

- Experiments were performed on both day-3 and day-4 of culture.
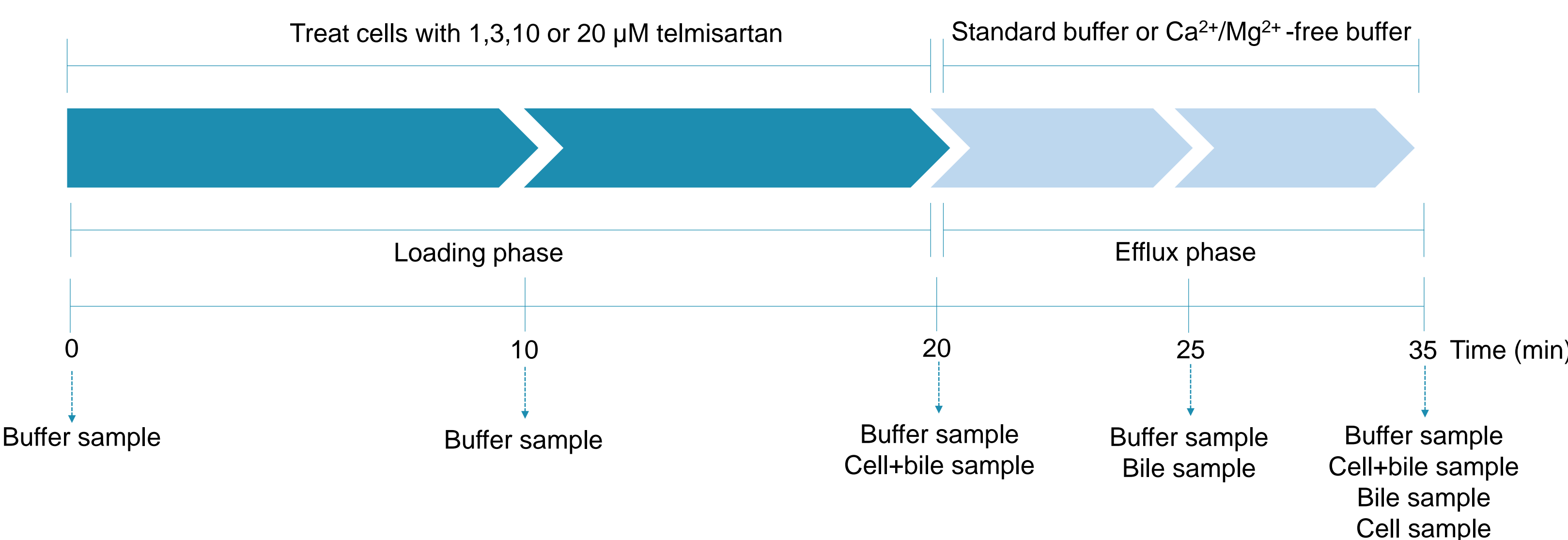
Figure 1. Experimental design for accumulation and efflux studies in sandwich-cultured rat hepatocytes (SCRH). Cells were treated with 1,3,10 or 20 µM telmisartan in standard buffer for 20 minutes. Following 10 and 20 minutes of loading, buffers containing telmisartan and telmisartan-glucuronide were collected, after which cells were lysed and total content (=cells+bile) was determined. Alternatively, standard buffer 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. Buffer and bile samples were collected after 5 minutes of efflux, whereas buffer, bile, cell and cell+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.

- Cell source (freshly-isolated or cryopreserved) was implemented as a covariate in the dataset.

- Stepwise covariate modeling with backward exclusion (p<.001) was performed on the base model.

RESULTS

- Telmisartan and telmisartan-glucuronide *in vitro* disposition data were best described using an eight-compartment model which includes compartments representing the buffers, cells, bile and cells+bile (Figure 2).

- Compound is distributed among the different compartments through both linear and non-linear processes.

- Unbound intracellular fraction of telmisartan was fixed to 0.01, based on previous literature reports[1].

- The *in vitro* disposition data and model fit are shown in Figure 3.
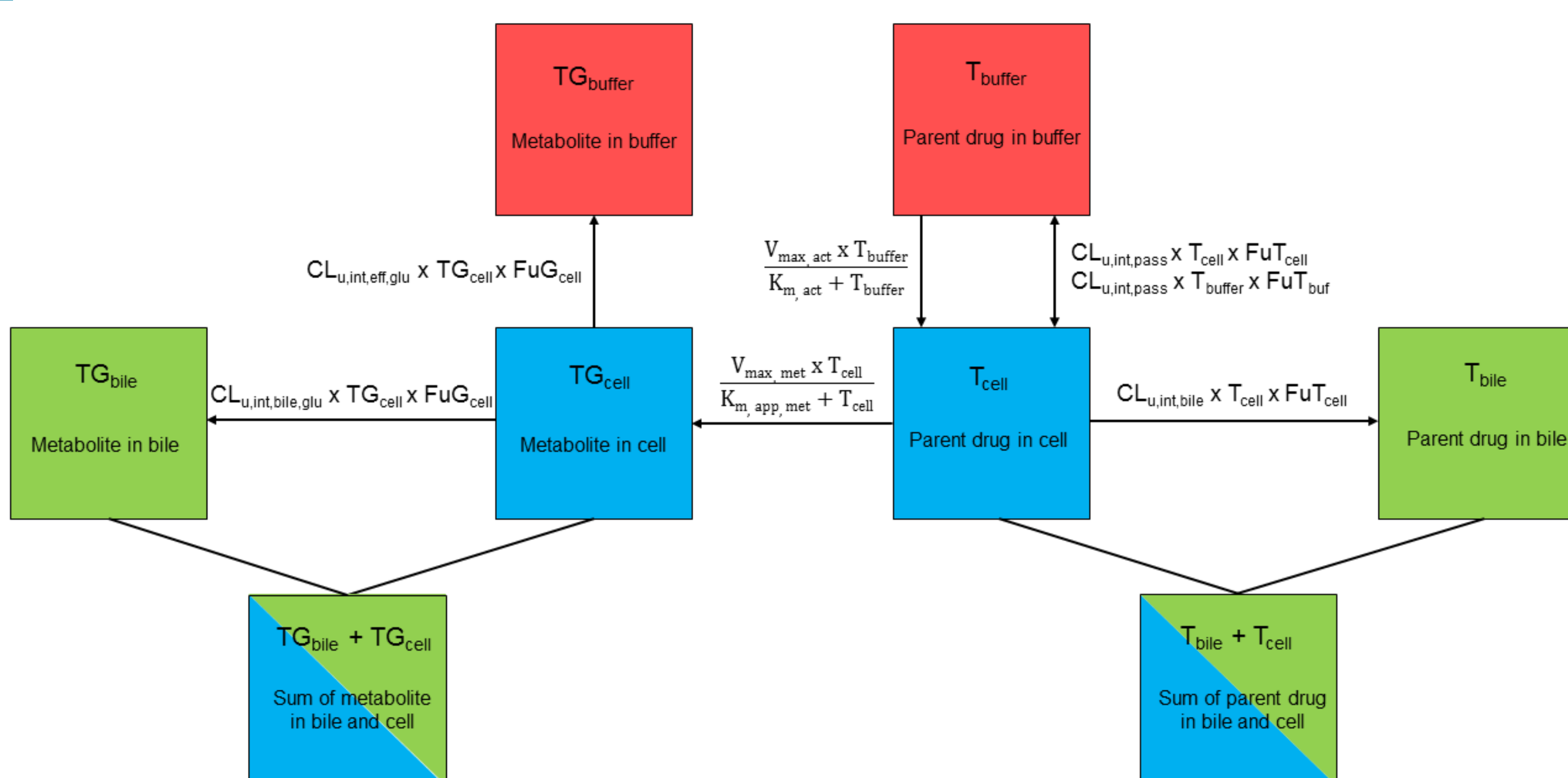
Figure 2. Eight-compartment model with cell, buffer and bile compartments for telmisartan (T) and its glucuronide metabolite (TG). *CL, int, u* and *app* represent intrinsic, unbound and apparent clearance; *act, eff, pass* and *bile* represent active uptake, total efflux, passive diffusion and biliary excretion; *FuTcell, FuGcell* and *FuTbuf* represent the unbound intracellular and buffer fraction of telmisartan and telmisartan-glucuronide. *Vmax* and *Km* represent the maximal enzymatic velocity at saturating substrate concentration and the substrate concentration at which the reaction rate is half its maximal value.
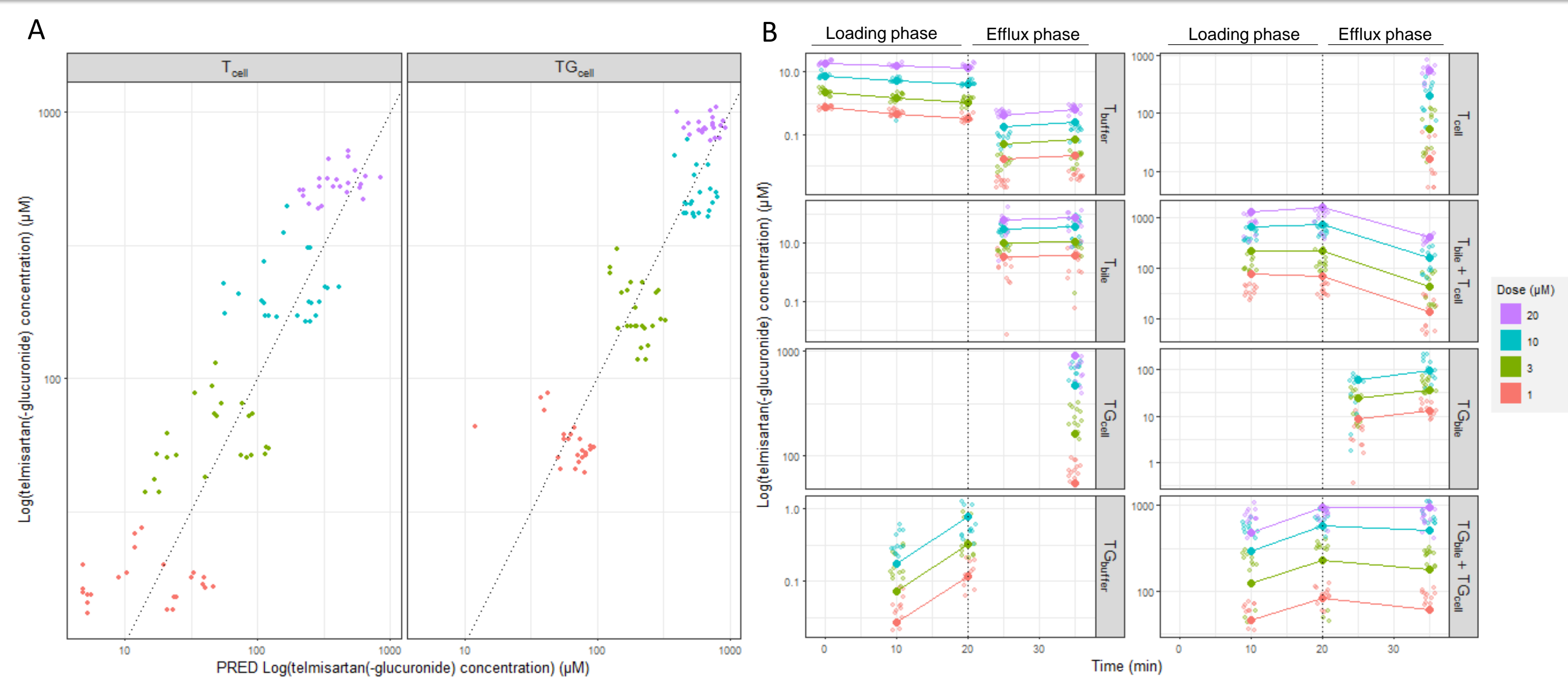
Figure 3. (A) Log observed values *versus* base model predicted Log concentrations (PRED) of intracellular telmisartan (T_{cell}) and intracellular telmisartan-glucuronide (TG_{cell}) for different initial doses telmisartan (20, 10, 3 and 1 µM). (B) Log observed values (open circles) and base model predicted Log concentrations (solid lines) *versus* time of telmisartan and its glucuronide in the buffer, bile and intracellular compartments for different initial doses telmisartan (20, 10, 3 and 1 µM).

Estimates of the *in vitro* SCRH $CL_{u,int,pass}$, $V_{max,act}$, $K_{m,act}$, $CL_{u,int,bile}$, $CL_{u,int,bile,glu}$, $CL_{u,int,eff}$ $K_{m,app,met}$, $V_{max,met}$ and FuG_{cell} are shown in Table 1.

Table 1. Pharmacokinetic parameter estimates of the final model.

Parameter	Estimate	RSE(%)	95% CI
$CL_{u,int,pass}$	2.86 µL/min/well	20	1.53 - 4.19
$V_{max,act}$	287 pmol/min/well	28	132.16 - 441.84
$K_{m,act}$	11 µM	33	3.85 - 18.27
$CL_{u,int,bile}$	0.138 µL/min/well	15	0.099 - 0.177
$CL_{u,int,bile,glu}$	0.006 µL/min/well	6	0.006 - 0.007
$CL_{u,int,eff}$	0.06 µL/min/well	9	0.052 - 0.073
$K_{m,app,met}$	36 µM	15	25 - 46
$V_{max,met}$	243 pmol/min/well	13	183.02 - 302.98
FuG_{cell}	0.689	10	0.481 - 0.897

CI: confidence interval; RSE: residual standard error.

Covariate analysis showed that cryopreservation had a significant effect on $CL_{u,int,bile}$, $CL_{u,int,bile,glu}$, $V_{max,met}$ and $V_{max,act}$ (Table 2).

Table 2. Pharmacokinetic parameter estimates of both freshly-isolated and cryopreserved hepatocytes.

Parameter	Freshly-isolated hepatocytes		Cryopreserved hepatocytes	
	Estimate	RSE(%)	Estimate	RSE(%)
$CL_{u,int,bile}$	0.86 µL/min/10⁶ cells	21	0.77 µL/min/10⁶ cells	49
$CL_{u,int,bile,glu}$	0.033 µL/min/10⁶ cells	8	0.045 µL/min/10⁶ cells	9
$V_{max,met}$	1190 pmol/min/10⁶ cells	21	2240 pmol/min/10⁶ cells	22
$V_{max,act}$	1495 pmol/min/10⁶ cells	30	2720 pmol/min/10⁶ cells	34

RSE: residual standard error.

- Maximal metabolic ($V_{max,met}$) and uptake ($V_{max,act}$) velocity are increased two-fold after cryopreservation.

- Biliary clearance of telmisartan ($CL_{u,int,bile}$) and telmisartan-glucuronide ($CL_{u,int,bile,glu}$) are affected by cryopreservation. However, the estimate of $CL_{u,int,bile}$ for cryopreserved hepatocytes is deemed to be less reliable due to high residual standard error.

- Linear regression analysis of the observed values in the bile compartment of telmisartan and its glucuronide show that biliary excretion of telmisartan-glucuronide is slightly increased in cryopreserved hepatocytes compared to freshly-isolated hepatocytes (Figure 4). This finding is in concordance with our model-based predictions.
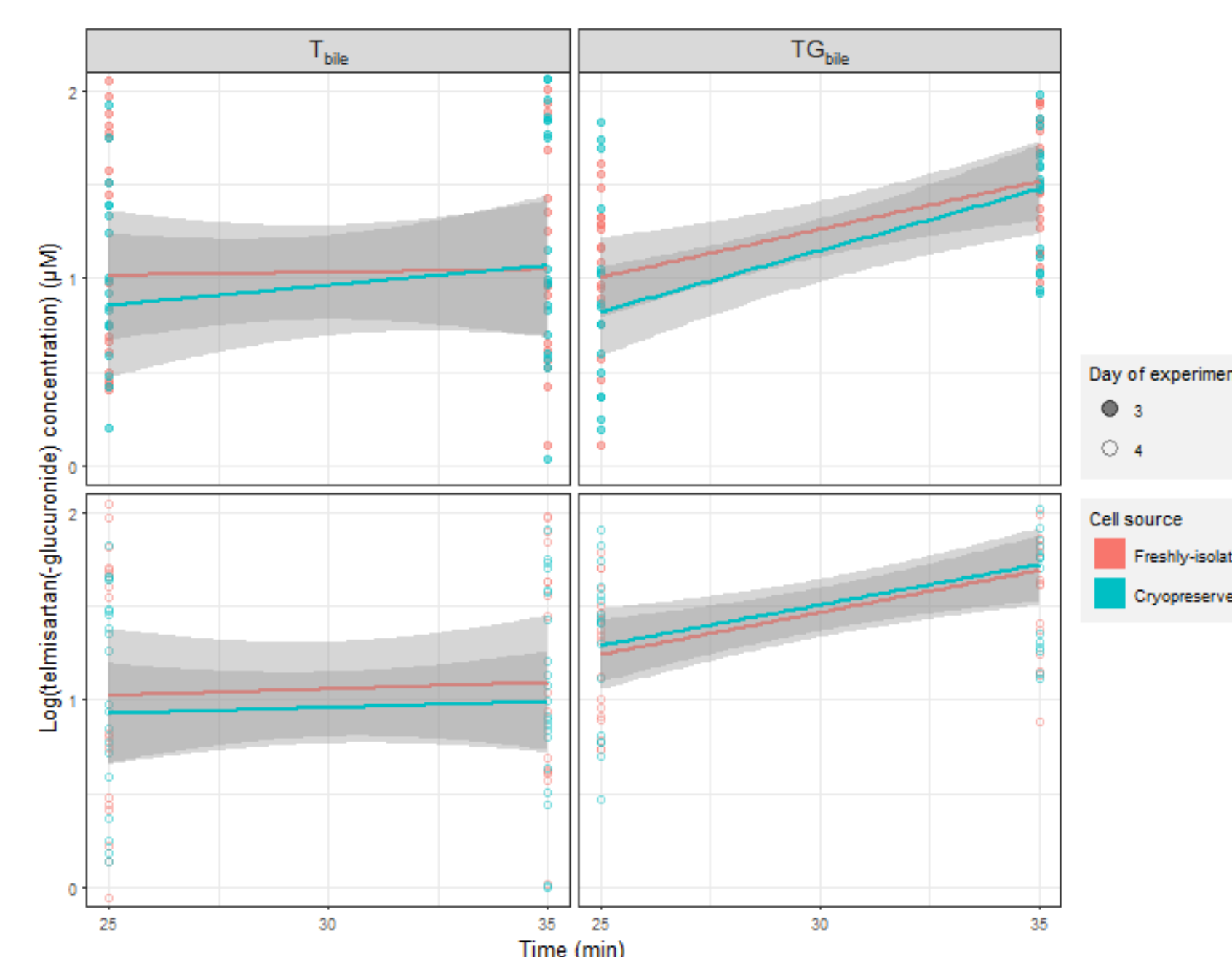
Figure 4. Log observed values *versus* time of both telmisartan (T_{bile}) and telmisartan-glucuronide (TG_{bile}) in the bile comparment. The red and blue lines are linear regression lines. The grey shaded area represents the 95% confidence interval.

CONCLUSIONS

- A mechanistic eight-compartment model has been developed which adequately describes the hepatobiliary disposition of both telmisartan and telmisartan-glucuronide in SCRH.

- Covariate analysis suggests that cryopreserving rat hepatocytes has a significant effect on biliary excretion, metabolism and/or uptake of telmisartan and its glucuronide in SCRH.

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[1] Li R. *et al*, Physiologically based pharmacokinetic prediction of telmisartan in human. Drug Metab. Dispos. Biol. Fate Chem. 42, 1646–1655 (2014)