In Vitro to *In Vivo* Extrapolation of Valproic Acid Hepatotoxicity: a Biokinetic and Physiologically Based Toxicokinetic Informed Approach

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1. PBPK Model Development in Rat

Valproic acid (VPA) is used in the management of seizures, bipolar disorder and migraines however, it is associated with hepatic steatosis. A whole-body physiologically based pharmacokinetic (PBPK) model was developed to simulate the distribution of VPA in the rat based on physicochemical properties and published pharmacokinetic data (table 1; figure 1). The initial model recovered the early observed concentration-time profile following both intravenous (figure 1, top) and oral dosing (figure 1, bottom). However, the model was not able to recover the extended terminal clearance phase; this is attributable to significant enterohepatic recirculation (EHR) in rats (Dickinson *et al*. 1979). More specifically, biliary excreted, glucoronidated metabolites are deconjugated in the lumen of the gastrointestinal tract, resulting in the reabsorption of the regenerated parent compound. A semi-mechanistic model of deconjugation in the gastrointestinal tract of biliary cleared metabolites was implemented in the Simcyp Animal simulator (v17r1). This allowed the EHR and deconjugation of VPA and its metabolites to be incorporated within a refined PBPK model. Incorporation of this mechanism resulted in better recovery of the pharmacokinetic profile of VPA in the rat (figure 1, top).

Table 1. Summary of Rat PBPK model parameters; polar surface area (PSA), hydrogen bond donors (HBD)

Parameter	Units	Value	Reference
Physchem			
MW	g/mol	144.2	
Log $P_{o:w}$		2.75	Sangster, 1993
Compound type		monoprotic acid	
pKa		4.8	FDA label
Polar surface area	Å^2	37.3	ChemAxon
Hydrogen bond donor		1	ChemAxon
Protein Binding			
fu		0.35	Löscher, 1978
BP		0.74	Löscher, 1978
Absorption (ADAM)			
fa		0.997	predicted (PSA and
ka	1/h	5.955	HBD)
Elimination			
CL_{iv}	mL/min	1.4	Kameya *et al.*, 2009

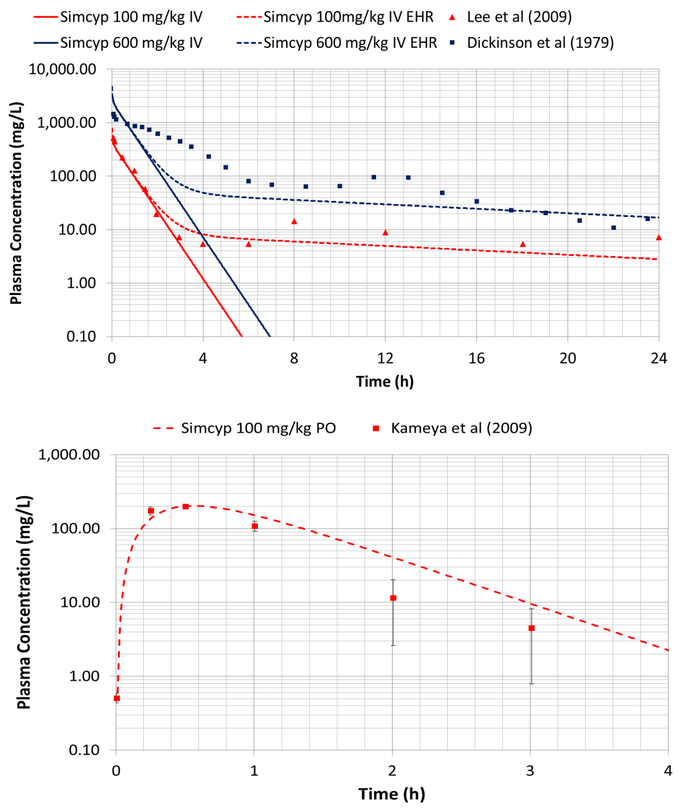


Figure 1. Performance verification of the rat model against published pharmacokinetic data following intravenous (top) and oral (bottom) dosing; simulation (lines), observed data (symbols). Simulations with model not including EHR (solid lines) do not recapture the terminal phase of the concentration-time profile. Simulations incorporating the EHR mechanism within the model better recover the concentration-time profile across a range of doses.

2. Hepatic Accumulation with Repeat Dosing

VPA repeat dosing studies in rat were identified with a mean LOAEL of 530 mg/kg BW administered via intraperitoneal injection (table 2). This LOAEL was in agreement with that determined in a two week oral dosing study (500mg/kg BW OD) in male Sprague-Dawley rats (n=6; Abdel-Dayem *et al*. 2014). A four day repeat dosing study (500 mg/kg OD PO) was simulated using the rat PBPK model incorporating EHR (figure 2; table 3). Simulations with the EHR-PBPK model show accumulation of VPA over the course of the simulated study. However, while simulations of the same study with the model not accounting for EHR predicted comparable C_{max}, they predicted much lower systemic and hepatic exposure (AUC) and did not predict VPA accumulation. This highlights the importance of model verification and incorporation of critical clearance/ distribution mechanisms.

Table 2. Valproic acid repeat dose studies

Study no	Route	Dosing	Study duration (days)	Effect	Effect LOAEL (mg/kg BW)
5577	intraperitoneal	once daily	4	fatty degeneration	500
5578	intraperitoneal	once daily	4	vacuolization	320
5579	intraperitoneal	once daily	4	vacuolization	650
5580	intraperitoneal	once daily	4	vacuolization	650

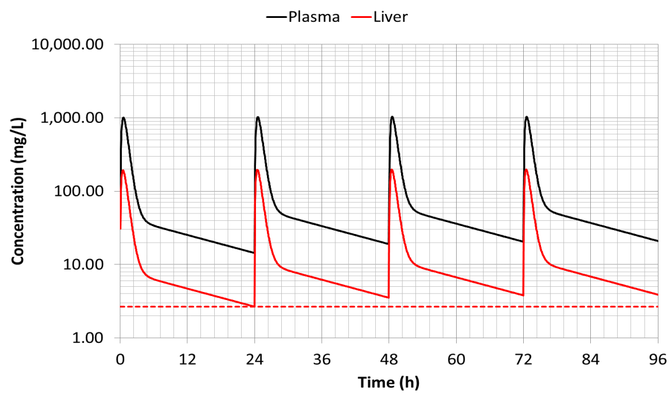


Figure 2. Plasma (black) and liver (red) concentration profiles from simulations of a four day repeat dose toxicity study (500 mg/kg oral) in male rats. The dotted red line indicates the C_{min} following the first dose. Simulated repeat dosing with VPA predicts accumulation within the liver, a possible contributor to the observed hepatotoxicity.

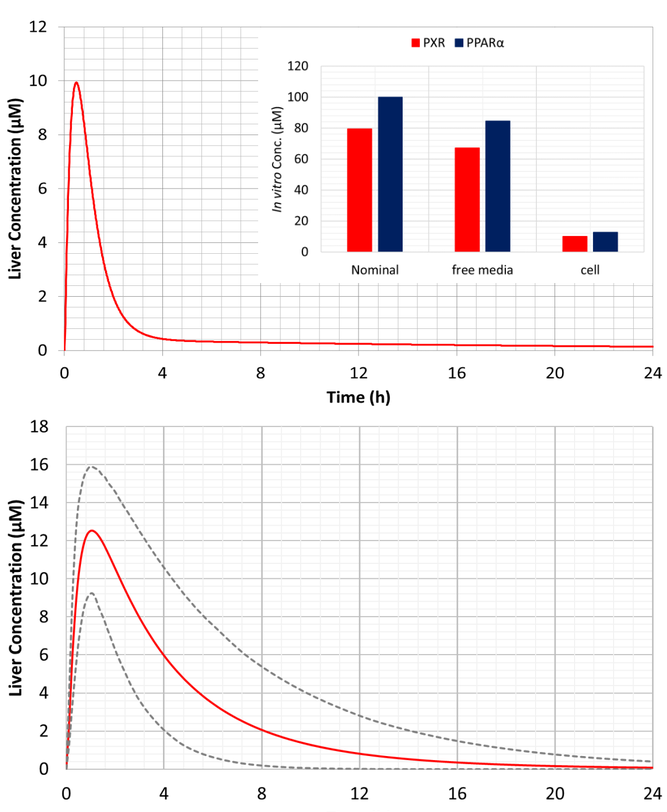
Table 3. Summary results of simulated repeat dose study (500 mg/kg)

	Plasma Unbound C_{max} (mM)	Plasma AUC (mg/L.h)	Liver Unbound C_{max} (mM)	Liver AUC (mg/L.h)
- EHR	2.46	1342.6	2.47	193.5
+ EHR	2.51	2184.8	2.52	407.3

3. *In vitro* and Inter-species Translation

Using a verified human PBPK model of VPA distribution, based on a published model (Ogunbenro *et al*., 2014), a reverse dosimetry approach was used to determine the human dose required to achieve equivalent hepatic C_{max} to those predicted at the rat LOAEL (2.52 mM); a dose of 275 mg/kg (~20 g) was predicted to result in a human liver C_{max} of 2.5mM. A biokinetic model, developed to calculate steady-state *in vitro* distribution, was used to predict the free media and intracellular concentrations achieved in *in vitro* reporter assays in the U2OS osteosarcoma cell line (figure 3, inset top). The intracellular concentration corresponding to the minimal VPA treatment concentration activating the PXR nuclear receptor (NR) was predicted to be ≈10µM; this was the lowest VPA concentration linked to reporter activation. Reverse dosimetry using the rat PBPK model predicted a 3.7 mg/kg (0.93 mg) dose to result in an intrahepatic C_{max} associated with PXR activation *in vitro* (figure 3 , top). Reverse dosimetry in human showed a 1.7 mg/kg (~125mg) dose resulted in an equivalent hepatic C_{max}. Simulations in a virtual population of healthy volunteers (n=1000) demonstrated that a dose of 2.25 mg/kg (~150mg), well within the therapeutic dosing range, would result in 37% of simulated individuals achieving an intrahepatic C_{max} activating PXR (>10µM), but not sufficient to activate PPARα. However, 57% of individuals would achieve an intrahepatic C_{max} sufficient to activate both PXR and PPARα (>12.5µM); both molecular initiating events (MIEs) in the hepatic steatosis adverse outcome pathway (AOP).

Figure 3. Biokinetic predictions for the free media and intracellular concentrations in in vitro reporter assays (top, inset); PBPK simulation of rat liver concentration profile following 3.7 mg/kg VPA PO (top); PBPK simulation of human liver concentration (1000 simulated individuals) following 2.25 mg/kg VPA PO, red line represents mean profile, dotted grey lines represent the 5th and 95th percentile (bottom).



4. Conclusions

Incorporation of a semi-mechanistic EHR/metabolite deconjugation mechanism enabled the PBPK model to recover the extended VPA profile in rat. Using PBPK in conjunction with a biokinetic model, activation of MIE associated NRs, identified *in vitro,* were translated to *in vivo* in both rat and human. While species differences in receptor binding affinity and expression must be considered, modelling and simulating facilitates *in vitro* to *in vivo* and cross-species translation of toxicity, and suggests that repeat dosing at therapeutic levels could result in sustained activation of MIEs associated with adverse hepatic outcomes.

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