Iterative Development of a Physiologically Based Pharmacokinetic Model for Valproic Acid and Reverse Translation of *In Vitro* Toxicity Data

Ciarán Fisher, Barbara van Vugt, Richard Maclennan, Oliver Hatley, Iain Gardner
ciaran.fisher@Certara.com

PRELIMINARY PBPK MODEL DEVELOPMENT IN RAT

Table 1. Preliminary Rat Model Parameters.

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
ka	1/h	5.955	predicted
ELIMINATION			
CL_{iv}	mL/min	1.4	Kameya *et al* (2009)

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 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 model recovered the early observed concentration-time profile however, the model was not able to recover the extended terminal clearance phase; this is attributable to the significant enterohepatic recirculation (EHR) in rats (Dickinson *et al*. 1979). This preliminary model was used to generate initial predictions of the maximal plasma and liver concentrations (C_{max}) associated with repeat VPA dosing .

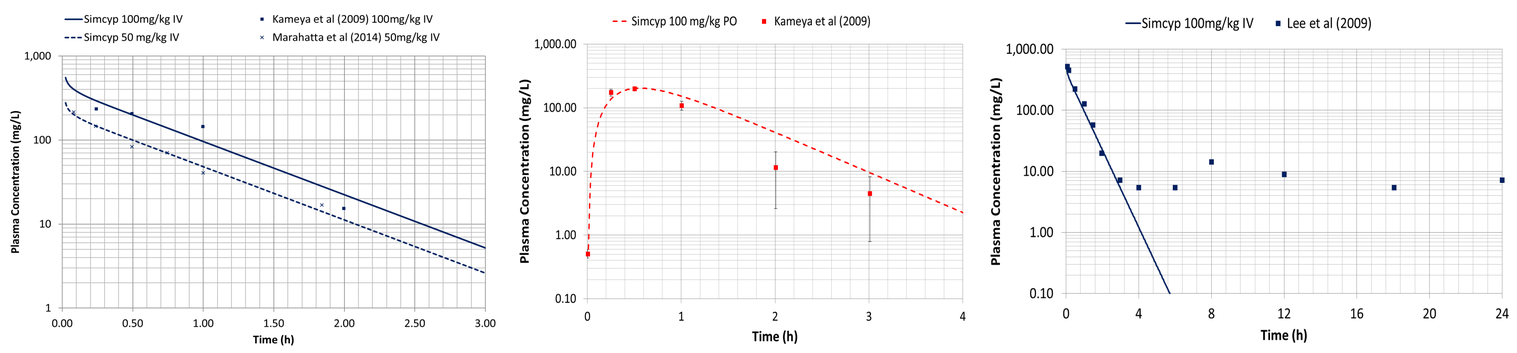


Figure 1. Performance verification of the rat model against published pharmacokinetic data following intravenous (left) and oral (middle) dosing. The preliminary model does not account for enterohepatic recirculation of valproic acid in rodents and so does not recapture the terminal phase of the concentration time profile (right).

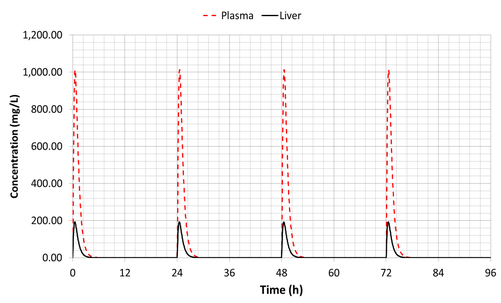


Figure 2. Simulation plasma and liver concentrations of repeat dose toxicity study (500 mg/kg oral) in rats.

Table 2. Repeat dose toxicity studies identified in rat.

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

Repeat dosing studies were identified by the case study team with a mean LOAEL of 530 mg/kg BW administered via intraperitoneal injection. This LOAEL was in agreement with that determined in 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, predicting maximal unbound plasma and unbound intrahepatic concentrations of 2.46mM and 2.47mM, respectively.

REFINEMENT OF THE RAT PBPK MODEL

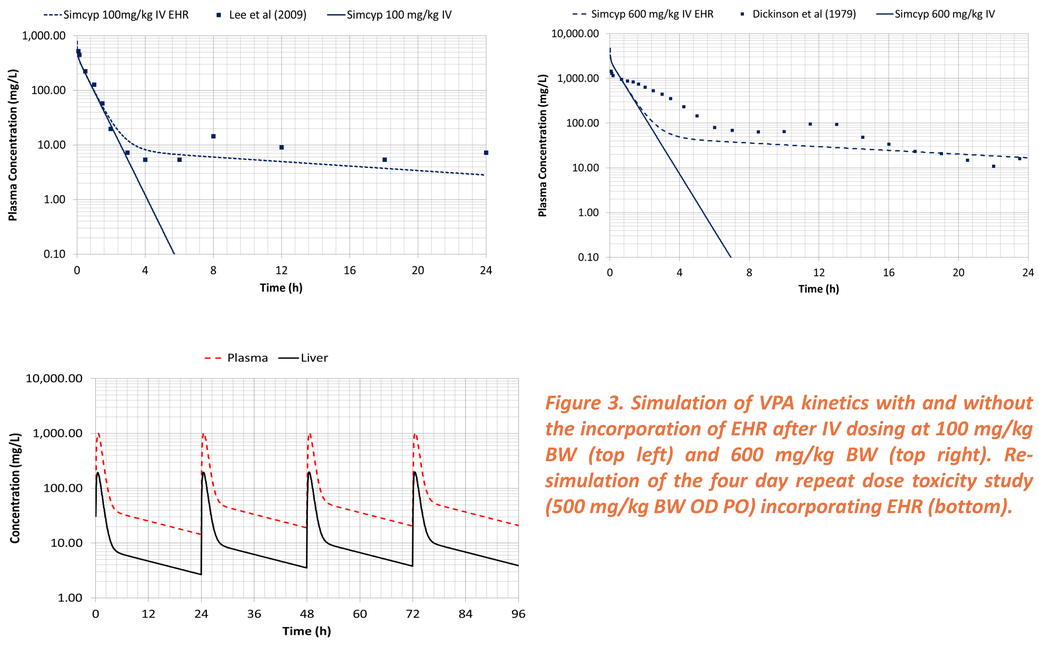


Figure 3. Simulation of VPA kinetics with and without the incorporation of EHR after IV dosing at 100 mg/kg BW (top left) and 600 mg/kg BW (top right). Re-simulation of the four day repeat dose toxicity study (500 mg/kg BW OD PO) incorporating EHR (bottom).

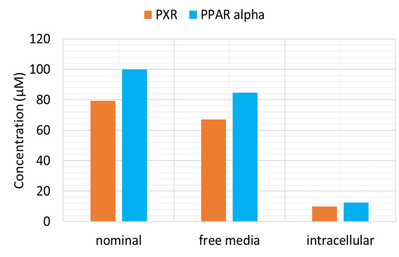
Table 3. Comparison of PBPK simulations with and without the inclusion of enterohepatic recirculation.

	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

The rat PBPK model was refined through the implementation of a semi-mechanistic model of deconjugation in the gastrointestinal tract of biliary cleared metabolites in the Simcyp Animal simulator. This allowed the deconjugation of glucoronidated VPA metabolites, and the subsequent EHR of VPA, to be incorporated within the model. Incorporation of this EHR mechanism resulted in better recovery of the pharmacokinetic profile of VPA in the rat (figure 3). Simulation of the 500 mg/kg repeat dosing study was repeated; this showed that although the preliminary and refined model predicted comparable plasma and tissue C_{max} in the both the plasma and the tissue, the preliminary model significantly under-predicted the systemic exposure to VPA.

IN VITRO AND INTER-SPECIES TRANSLATION

Using a verified human whole-body PBPK model of VPA distribution, a reverse dosimetry approach was applied to determine the human dose required to achieve equivalent hepatic C_{max} to those at the rat LOAEL (2.52 mM). In human, a dose of 275 mg/kg (~20,000 mg) was determined to result in a human liver C_{max} of 2.5mM. The steady-state biokinetic model developed within WP4 was used to predict the free media and intracellular concentrations achieved in *in vitro* reporter assays performed in the U2OS osteosarcoma cell line.



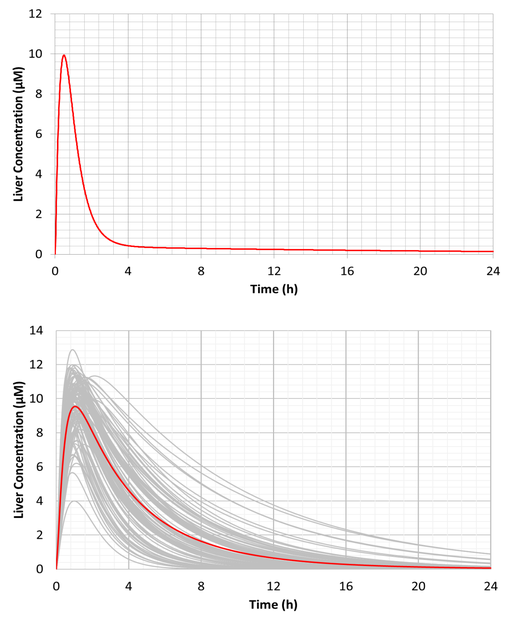


Figure 4. Biokinetic predictions for the free media and intracellular concentrations in in vitro reporter assays (top left); PBPK simulation of rat liver concentration profile following 3.7 mg/kg VPA PO (top right); PBPK simulation of human liver concentration (100 simulated individuals) following 1.7 mg/kg VPA PO, grey lines represent individual profiles, red line represents mean profile (bottom).

The intracellular concentration corresponding to the minimal VPA treatment concentration required to activate 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, a 3.7 mg/kg (0.93 mg) dose was determined to result in an intrahepatic concentration equivalent to those shown to activate PXR *in vitro*. Reverse dosimetry in human showed a 1.7 mg/kg (~125mg) dose resulted in equivalent hepatic concentrations. However, human simulations incorporating population variability (100 individuals) demonstrated that not all simulated individuals would reach this concentration. Conversely, some individuals would exceed the concentration associated with the activation of PXR and achieve hepatic concentrations identified as activating PPARα in the *in vitro* reporter assays based on the predictions of the biokinetic modelling (figure 4).

CONCLUSIONS

While the preliminary rat PBPK model was able to predict C_{max} in plasma and in liver, it was unable to recover the systemic exposure to VPA. Incorporation of a semi-mechanistic EHR mechanism into the model enabled the PBPK simulation to recover the extended VPA profile in rat. Using PBPK in conjunction with biokinetic models of *in vitro* systems facilitates the translation from *in vitro* to *in vivo* as well as between model species and human. While species differences in receptor binding affinity and expression must be considered, this approach complements the development of a quantitative AOP of steatosis in which the activation of PXR and PPARα represent critical molecular initiating events.

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