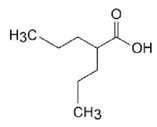
Potential for improving PBK predictions of foetal valproic acid exposure through consideration of placental influx/efflux transporters

Gopal Pawar[1], Beate Nicol[1], Ans Punt[1], Sandrine Spriggs[1], Iris Muller[1]

*Unilever, Safety and Environmental Assurance Centre (SEAC), Colworth Science Park, Sharnbrook, United Kingdom. *Gopal.Pawar@Unilever.com*

1. Background and Aim



Valproic acid (VPA)

- VPA is an established human teratogen with many published human clinical pharmacokinetic (PK) studies including sparse pregnancy PK studies.
- Little is known about the PK of VPA during the pregnancy trimesters and the mechanisms associated with the impact of physiological changes observed during the pregnancy.
- VPA may also change the expression of placental transporters at high and repeated dose (Rubinchik-Stern 2015), which might influence the overall levels of foetal exposure. So, it is essential to assess the effect of VPA on the foetus throughout the pregnancy.
- In clinical studies, generally only one blood sample (mother's serum and cord serum) is obtained per subject at the time of delivery, representing the last maternal dose taken which may not give a good estimate of levels of foetal exposure throughout the three trimesters of pregnancy.
- Physiologically based kinetic modelling (PBK) is a promising method for predicting the plasma exposure in mother and foetus during pregnancy. By simulating the interplay of placental transporters and metabolizing enzymes one can simulate foetal exposure at different trimesters.
- This study aimed to construct and validate a PBK model that simulates maternal plasma concentrations of VPA in a pregnant population across all the three trimesters. In addition, to better predict the foetal accumulation of VPA, this study also aimed to identify VPA relevant placental influx or efflux transporters and explore~~s~~ the expression and abundance of the transporters to be incorporated in the PBK model.

2. Methodology

Part 1: Construction and Validation of VPA healthy and pregnancy PBK model

A PBK model for VPA in humans was built in Gastroplus® based on absorption, distribution, metabolism, and excretion (ADME) properties derived from the literature and in silico calculations (Table 1).

	VPA	Reference
Mol wt (g/mol)	144.21	PubChem
Log P	2.75	PubChem
Solubility (mg/ml)	1.35	DrugBank (FDA label)
pKa (acid)	4.8	PubChem
Fraction unbound in plasma (Fup)	7-15%	Cloyd et al., 2003
Blood to plasma ratio (B/P) ratio	0.55	Soars et al., 2002
Human Caco-2 assay: Pappa (10E–4 cm/s)	0.22	Torii eta al., 2002
Human Intestinal effective permeability-Peff (10E–4 cm/s)	3.84	Conner et al., 2018
Clint (µl/min/10^6 cells)-Hepatocytes	0.9	Fortaner et al., 2021
CLrenal (L/hr)	0.0079	Conner et al., 2018

Table 1: Literature based phys. chem. and in vitro ADME parameters for VPA.

After validation of this model against published healthy human clinical data (Figure 1A-C), the model was extended to a pregnant population by considering pregnancy related ADME changes for VPA (Table 2).

	Physiological changes	Values	Reference
Absorption (fraction absorbed)	Gastric motility ↓; Delayed gastrointestinal emptying time, VPA highly water soluble ; rapidly absorbed from the gut and sodium salt from the intestine	No change in pregnancy	Nau et al., 1981
% Plasma protein binding (PPB)	Albumin & α1-acid glycoprotein ↓ correlates positively with ↑ with gestational age **VPA partially displaced from protein binding sites by circulating free fatty acids which ↑ during pregnancy** ↓ Protein binding capacity results in ↑ plasma clearance ↓ total serum levels	Normal or 1st trimester (TM): 93-85% (Fup: 7-15%) 2nd TM: 85-80% (Fup: 15-25%) 3rd TM: 75-65% (Fup: 25-35%)	Nau et al., 1981
Fraction unbound in plasma (Fup)	**Total VPA levels falls but free fraction during pregnancy ↑**	**Serum protein binding of VPA is sig. ↓ in preg women upto 2 fold ↑ in free fraction**	
Steady state volume of distribution (Vss)	Plasma volume ↑ by 50%; Cardiac output ↑ by 30% Total Body fluid ↑ with intravascular volume and extracellular fluid VPA distribute to all tissues & present in high conc. –blood, liver and kidney	Vd ↑ with ↑ in extracellular fluid , fat content and expanding foetal compartment Apparent Vd 0.14-0.20 L/kg in adults	Nau et al., 1981
Clearance (CL)	↑ progesterone -↑ hepatic enzymes; estrogens are inhibitors (large interindividual variations due to varying ratios of hormones among the individual) ↑ renal clearance and metabolic capacity and ↑ in tissue binding	Clearance increased significantly (approx. 3 times) from 1ST TM to 3rd TM	Koerner et al., 1989

Table 2: Pregnancy related ADME changes for VPA.

Parameters incorporated for pregnant and foetal PBK modelling:

- Based on Nau (1981) plasma protein binding (PPB) or Fraction unbound in plasma (Fup of VPA changes throughout pregnancy. 1st Trimester (TM): 93-85% (Fup- 7-15%); 2nd TM: 85-80% (Fup-15-25%); 3rd TM: 75-65% (Fup-25-35%) was considered. As a result, the systemic clearance (CL) and volume of distribution (Vd) calculated in the PBK model (GastroPlus®) also changed.
- For the VPA pregnancy model, a placental permeability limited tissue model was applied, and the estimated permeability surface area product (Pstc) of 15000 ml/s was used to capture the foetal plasma concentration profile.
- The VPA Pregnancy model was validated against observed individual maternal serum and foetal cord blood concentrations (Nau 1981).

Part 2: In silico predictions and in vitro evidences for potential VPA's transporters

For VPA, first in silico predictions for the Transporter Substrate Classification were performed using Gastroplus ADMET predictor.

Literature based in vitro, and in vivo human studies were searched in PubMed to find the evidence for type of placental transporters involved in the transport of VPA.

Part 3: Data mining for abundance of placental transporters during the pregnancy

Literature was searched for finding the abundance of placental transporters and their modulation during the pregnancy stages.

3. Results:

Part 1: Construction and Validation of VPA healthy and pregnancy PBK model

A) Nitsche et al., 1982 (IV 1000 mg) **B)** Georgoff et al., 2017 (IV 2151 mg) **C)** Nitsche et al., 1982 (Multiple Oral dose- 900 mg/12 hrs)

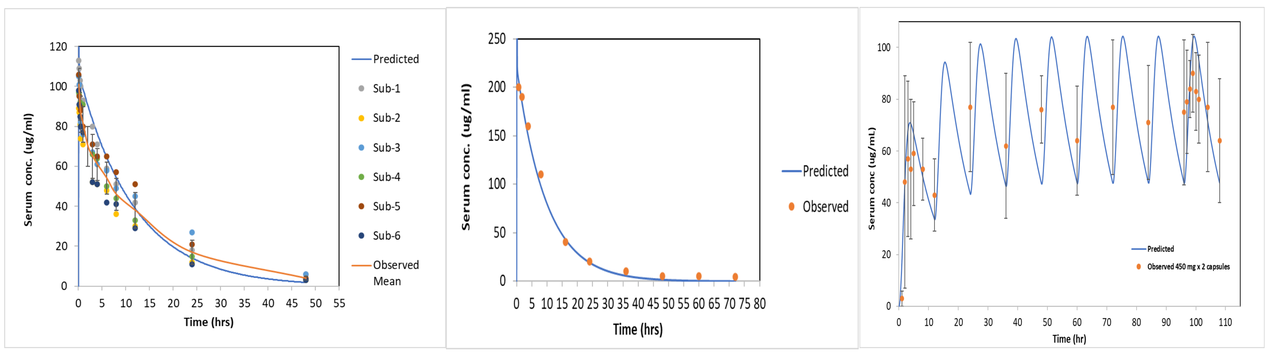


Fig.1 Comparison of VPA healthy volunteer PBK model predictions with clinical data. A healthy VPA PBK model was created using input parameters from table 1 and compared to clinical data for single IV exposure (A,B) as well as multiple oral dosage (C).

- A healthy volunteer VPA PBK model was created and compared to outcomes from clinical studies. The model predicted the observed AUC and Cmax seen in clinical studies very well (see Fig.1) with a total fold error within 0.9-2.3-fold.
- Similar results were seen for comparing pregnancy VPA PBK models with maternal serum concentrations in 1st and 2nd trimesters which are in good agreement with the observed clinical outcomes and are below a fold error of 2 (data not shown).
- A VPA pregnancy PBK model was created using GastroPlus which show good overlap with observed individual mother's serum concentration (Css) (see Fig.2A). However, this model underpredicted the foetal cord serum conc. (See Fig.2B and Table 3) estimating a foetal cord serum: mother serum (FM) ratio-approx. of 1 where the observed FM ratio is higher using clinical measurements (see Table 3). The reasons for these discrepancies may relate to a lack of understanding and parameterisation of placental transfer datasets in the model. It is possible the model may be improved by accounting for the abundance of placental metabolising enzymes and the interplay between VPA influx and efflux at the placental basolateral membrane.

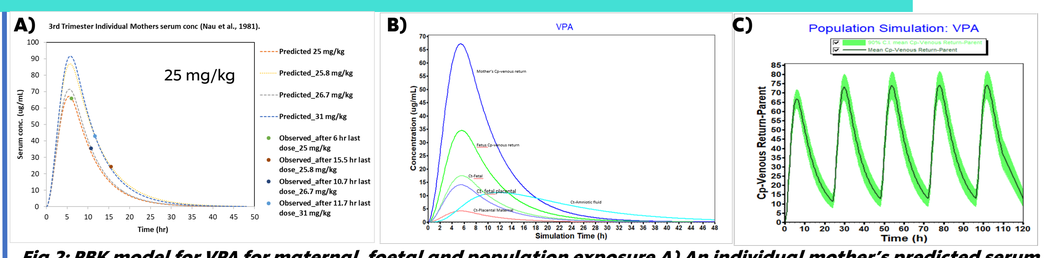


Fig.2: PBK model for VPA for maternal, foetal and population exposure A) An individual mother's predicted serum PK profile (at the time of delivery) superimposed with a single observed serum concentrations at various doses from Nau et al., 1981 study. B) A representative Gastroplus predicted mother and foetus (plasma, placental and amniotic fluids) full PK profiles at 25 mg/kg dose. C) Multiple dose mother's serum simulation output for VPA virtual pregnancy population.

Dose (mg/kg)	Time of blood collection after last dose (hr)	Obs Mother serum (µg/mL)	Pred Mother serum (µg/mL)	Fold error (Pred mother serum ÷Obs mother serum conc.)	Obs Cord serum conc. (µg/mL)	Pred: Cp-Fetal Arterial or venous Supply (µg/mL)	Fold error (Pred Cp- fetal arterial or venous ÷ Obs cord serum conc.)	Obs FM ratio	Pred FM ratio (Pred Cp- fetal arterial or venous ÷ Pred mother serum conc.)
31	11.7	43.2	42.9	0.99	70.5	22.7	0.32	1.63	0.53
25	6	62.7	65.8	1.05	88	34.2	0.39	1.4	0.52
26.7	10.7	27.1	35.6	1.31	80	19	0.24	2.95	0.53
25.8	15.5	25.1	24.3	0.97	31	13.0	0.42	1.23	0.53

Note: Individual Fup considered, 31 mg/kg=30; 25 mg/kg=35; 26.7 mg/kg=35; 25.8 mg/kg=25.

Table 3: Measured and predicted maternal serum and foetal cord serum concentrations for VPA.

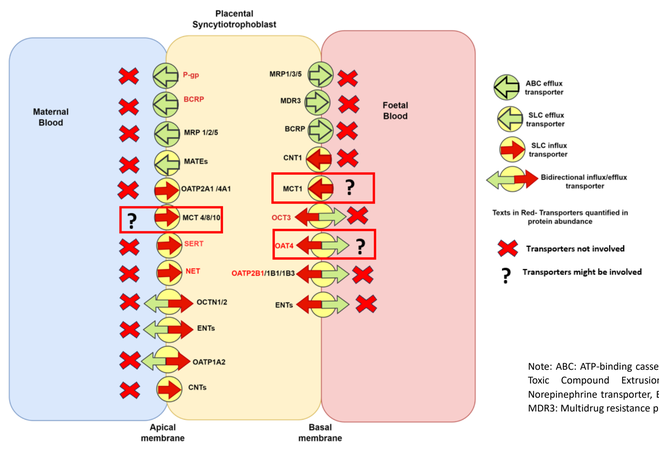
Part 2: In silico predictions and literature based in vitro evidences for potential VPA's transporters

G+ ADMET predictor's Transporter Substrate Classification system	VPA transporters based on literature based in vitro transport studies	Type	Localization in placenta	Remarks
OATP1B1		Influx	Basal membrane (facing foetal compartment)	
OCT1, OCT2		Influx	Basal membrane (facing foetal compartment)	
OAT1		Bidirectional: Influx/efflux transporter	Basal membrane (facing foetal compartment) ?	Not expressed in placenta (Dallman 2019)
P-gp	P-gp	Efflux	Apical membrane (facing maternal compartment)	VPA not a substrate based on MDCK II and LLC-PK1 cell lines (Baltes 2007)
MRP-1	MRP-1	Efflux	Apical membrane (facing maternal compartment)	
MRP-2	MRP-2	Efflux	Apical membrane (facing maternal compartment)	
	OAT4	Bidirectional: influx/efflux	Basal membrane (facing foetal compartment)	Possible substrate as VPA dissociates to form anion (Lloyds 2013, Daud 2017)
	MCT-1	Influx	Basal membrane (facing foetal compartment)	• Proton-dependent, saturable, and asymmetric transport system (BeWo) and may be a substrate of MCTs, Papp A to B>>B to A (Utoguchi 2000). • JEG-3 placental cell lines MCT-1 & MCT-4 did not contribute for VPA uptake (Ishiguro 2018)
	MCT-4			

Table 4: In silico based predicted and invitro based potential transporters for VPA.

G+ ADMET predictor's Transporter Substrate Classification system indicated that VPA is a substrate for OATP1B1, OCT1, OCT2, OAT1, P-gp and MRP1 /MRP2 (Table 4).

From in vitro studies MCT isoforms (not 1 & 4) & OAT4 potential transporters for VPA (Table 4, Fig.3)



Note : OATP1B1: Organic Anion Transporting Polypeptide 1B1, OCT1/2: Organic Cation Transporter, OAT1: Organic Anion Transporter, P-gp: P-glycoprotein, MRP-1/2: Multidrug resistance associated protein

Note: ABC: ATP-binding cassette, SLC: Solute carrier: BCRP: Breast Cancer Resistance Protein, MATEs: Multidrug and Toxic Compound Extrusion, MCT: Monocarboxylate Transporters, SERT: Serotonin transporters, NET: Norepinephrine transporter, ENTs: Equilibrative nucleoside transporters, CNTs: Concentrative nucleoside transporter, MDR3: Multidrug resistance protein 3, CNT1: Concentrative nucleoside transporter.

Fig 3: Figure showing various types of ABC and SLC class of efflux/influx/bidirectional transporters usually expressed and localized on either the apical (facing mother's side) and basolateral membrane (facing foetal side) of placental syncytiotrophoblast. The figure also highlights some of the key influx type of transporters that are believed to be involved in the VPA transport from mother to foetus direction and those transporters that are not involved in the transport of VPA are highlighted in red cross symbol (X).

Part 3: Data mining for abundance of placental transporters during the pregnancy

Transporter	Protein abundance (pmol/g placental tissue) -Trimester 1; n=15 (% CV)	Protein abundance (pmol/g placental tissue) -Trimester 2; n=19 (% CV)	Protein abundance (pmol/g placental tissue) -Term; n=15 (% CV)	Remarks	Reference
P-gp	14.7 ± 8 (54.4)	9.57 ± 5.3 (55.4)	4.57 ± 2.67 (58.4)	Decreases 69% (T1 to term)	Anoshchenko 2020
OAT4	17.1 ± 7.11 (41.6)	12.8 ± 7.15 (55.9)	20.7 ± 11.6 (56.0)	Increases 2-fold from T1-Term and 1.6-fold from T2 to term	
MCT-8	NA*	NA*	NA*	* mRNA levels of MCT 8 significantly increased in placenta with advancing gestation	Chan 2006

During pregnancy, the abundance of OAT4 and mRNA expression of MCT-8 increased significantly in comparison to non-pregnant adults (Table 5).

Table 5: Protein abundance and mRNA levels of VPA's potential transporters during the pregnancy stages. Note: mRNA levels for MCT-8 are provided here due to lack of abundance data on MCT-1 & MCT-4.

4. Conclusion

- Validating the VPA pregnancy model against observed individual maternal serum and foetal cord blood concentrations (Nau 1981) showed that the model predicted mother serum concentrations very well across all three trimesters but underpredicted the foetal cord serum concentration, resulting a foetal cord serum: mother serum (FM) ratio approx. 2 to 6-fold lower than the observed FM ratio derived from clinical studies.
- In silico predictions for VPA transporters did not completely match the vitro transporters evidence except the OAT class. Literature studies indicated that VPA might be a substrate for the influx type of proton dependent MCT and OAT class of transporters. Interestingly in pregnancy, MCT and OAT transporters are upregulated which might influx VPA significantly from mother to foetus compartment.

5. Future studies

- Due to uncertainty over the relevant transporters for VPA, more in vitro work is needed to identify relevant influx transporters which could explain VPA's foetal accumulation.
- Further advanced cellular test system (placenta-on-chip models, or BeWo cell lines) transfected with the transporters is required to characterise the kinetics (Vmax/Km) of placental transfer.
- More research work is required to incorporate the abundance or expression (with turnover rate) of relevant transporters and then perform scaling (e.g., relative expression factor (REF) or the relative activity factor (RAF)) approaches within PBK modelling to quantitatively predict in vivo placental transporter mediated clearance to optimize the prediction of levels of foetal exposure.

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