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ARTICLE

Physiologically based mechanistic insight into differential risk of valproate hepatotoxicity between children and adults: A focus on ontogeny impact

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Abstract

The anticonvulsant valproic acid (VPA) despite complex pharmacokinetics has been in clinical use for nearly 6 decades. Previous reports indicated neonates, infants, and toddlers/preschoolers had higher risk of valproate hepatotoxicity than adults. However, dosing recommendations for those less than 10 years of age are lacking. To decipher clinical puzzles, physiologically-based pharmacokinetic (PBPK) models of VPA and its hepatotoxic metabolite 4-ene-VPA were constructed and simulated with particularly integrated information of drug-metabolizing enzyme ontogeny. Adult and pediatric PK data of VPA ($n=143$ subjects) and 4-ene-VPA ($n=8$ subjects) collected from previous reports were used for model development and validation. Sensitivity analyses were performed to characterize ontogeny impacts of CYP2C9 and UGT2B7 on dispositions of VPA and 4-ene-VPA across age groups. Optimal VPA dosing for each pediatric age group was also predicted and objectively judged by ensuring VPA efficacy and avoiding 4-ene-VPA hepatotoxicity. The study revealed UGT2B7 ontogeny was quite influential on VPA clearance even in neonates and small children. Intrinsic clearance of CYP2C9 was the most prominent determinant for areas under the concentration-time curve of VPA and 4-ene-VPA in infants, and toddlers/preschoolers, reflecting higher hepatotoxicity risk due to noxious 4-ene-VPA accumulation in these groups. The ontogeny-based PBPK approach complements conventional allometric methods in dosing estimation for the young by providing more mechanistic insight of the processes changing with age. The established ontogeny-based PBPK approach for VPA therapy deserves further corroboration by real-world therapeutic data to affirm its clinical applicability.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Higher incidence of valproic acid (VPA)-associated hepatotoxicity is observed in small children than in adults. Enhanced role of cytochrome P_{450} (CYP)-mediated oxidation, as compared to the commonly recognized UDP-glucuronosyltransferase conjugation, for VPA elimination in small children might explain the discrepancy.

WHAT QUESTION DID THIS STUDY ADDRESS?

Can physiologically-based pharmacokinetic (PBPK) modeling and simulation provide mechanistic insights for elucidating the differential risk of VPA hepatotoxicity in children and adults? How applicable is the ontogeny-based PBPK approach in estimating VPA dosing for diverse pediatric age groups?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The study illuminates special roles of CYP2C9 ontogeny in VPA metabolism and the ensuing hepatotoxicant 4-ene-VPA formation. The long-awaited VPA dosing recommendations for small children (especially < 10 years old) are also provided by ensuring efficacious plasma VPA concentrations and minimizing 4-ene-VPA levels.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

By added deliberation of VPA formulation, toxic metabolite, and metabolizing-enzyme ontogeny, the established VPA PBPK model shall help optimizing VPA dosing in attaining precision medicine if substantiated by further real-world data.

INTRODUCTION

The serendipitously discovered anticonvulsant valproic acid (VPA; 2-propylpentanoic acid) has been of medical utility for almost 6 decades.[1,2] Its therapeutic effects against generalized tonic–clonic seizures and myoclonic seizures are deemed unsurpassed by other antiseizure medications (ASMs), and comparable to ethosuximide for absence seizures.[3,4] The broad spectrum, that is, indicated seizure type and patient age (from childhood to late adulthood), of VPA in anti-epileptic efficacy makes it among the most prescribed ASMs.[5] Nonetheless, teratogenicity and hepatotoxicity are still major concerns for physicians prescribing the drug.[4]

Early reports of VPA-associated fatal hepatotoxicity in children and adults had led to serial clinical, pathological, and mechanistic investigations.[6–9] The incidence of hepatic injury related to VPA was estimated to be 0.02% (1 in 5000 patients). Inexplicably, children appeared to be more susceptible than adults, with incidence increased to around 0.2% (1 in 500 patients) for those less than 2 years of age.[10] The onset of liver injury, often characterized by steatosis, was insidious. A metabolic mechanism rather than immunological mechanism was suspected. The 4-ene-valproic acid (4-ene-VPA), a product of VPA metabolism by cytochrome P_{450} (CYP), was further elucidated to be the hepatotoxic culprit.[10,11]

VPA is a branched short-chain fatty acid with rather complex pharmacodynamic (PD), pharmacokinetic (PK), and pharmacogenomic attributes.[12,13] VPA is not only used in the management of epilepsy but also mood disorders and migraine. The PD effects of VPA are attained probably through modulation of neurotransmitter transmission, ion channel, or even enzymatic functions.[12] Notable PK features of VPA include high protein-binding, intricate metabolic routes, and significant drug interactions.[13] For instance, there are three major routes of VPA metabolism, namely glucuronidation (accounting for 50% of dose; by UDP glucuronosyltransferase 2B7 [UGT2B7], UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, and UGT1A10), β oxidation in the mitochondria (40%), and CYP-mediated oxidation (~10%; by CYP2C9, CYP2A6, and CYP2B6) in adults.[13] Apparently, the quantitative contribution by these three metabolic routes in children may vary due to differential ontogeny of drug-metabolizing enzymes in various pediatric groups of distinct growth and development stages.[14]

Given the complexity of age-related dynamic changes of organ functions and assorted clinically available formulations of VPA, the study uses physiologically-based PK (PBPK) modeling and simulations to decipher the observation of higher incidence of VPA liver injuries in young children and to present the impacts in a quantitative manner.[15] The study objectives include: (1) to develop adult and pediatric PBPK models for VPA and 4-ene-VPA, (2) to investigate role of ontogeny in functions of major VPA-metabolizing enzymes using the established PBPK models; and (3) to examine

concordance of dosing estimation by PBPK approach and conventional allometric scaling methods (e.g., age-, weight-, and body surface area [BSA]-based methods).

METHODS

Data source

All PK parameters and clinically observed data shown herein were from published reports. A literature search was conducted through PubMed using search terms, including "valproic acid and (plasma concentration) and (pharmacokinetics)" and "4-ene-valproic acid and (plasma concentration) and (pharmacokinetics)." Literature containing concentration-time profiles was chosen. Because most of the observed human data in the literature were presented as graphs, WebPlotDigitizer (version 4.4; Pacifica) was used to digitize observed data of published articles.

Software tool and study workflow

PBPK model was developed using the Simcyp population-based PBPK software Simcyp (version 21.1; Certara UK Limited). The strategy for establishing adult and pediatric PBPK models were to develop and validate the model first in adults and then translate to children using age-dependent anatomic and physiological changes. Finally, PK predictions in children were evaluated by comparing the results with clinical PK data taken from literature (Figure 1).

Adult PBPK model development and validation

The values and sources of all parameters for VPA and 4-ene-VPA are summarized in Tables S1 and S2.[16–24] The physicochemical and associated in vivo parameters of VPA, including molecular weight (MW), octanol–water partition coefficient ($LogP_{o:w}$), acid dissociation constant (pK_a), compound charge type, blood-to-plasma partition ratio (B:P), and unbound fraction (f_u) were collected. It is noteworthy to mention that the active pharmaceutical ingredient (API) of available formulations include VPA, sodium valproate (NaVPA), and divalproex sodium (DVS). The MW of VPA, NaVPA, and DVS are 144.21, 166.19, and 310.40 g/mol, respectively. The MW input required for the PBPK model in the study was that of VPA, the moiety measured in clinical PK studies. Therefore, when performing modeling and simulations of published articles involving either NaVPA or DVS, a dose correction was required to reflect VPA-equivalent dose administered (Table 1).[25] The VPA possesses nonlinear protein binding characteristics. A user defined concentration-dependent f_u profile, increased from 7% to 15% at VPA concentrations of 1.96 mg/L to 93.9 g/L, was applied.[17] First order model was used to describe oral absorption of VPA by fraction of drug absorbed and absorption rate constant (k_a) values. Different API demonstrated marked absorption differences.[19] The k_a for VPA was 2 h^{-1}, whereas the k_a for NaVPA and DVS was 4.1 h^{-1}. Lag-time (T_{lag}) for enteric-coated tablets was 2 h. The human jejunum effective permeability of VPA, predicted from polar surface area and hydrogen bond donors, was 5.8304×10^{-4} cm/s.

A minimal PBPK model with single adjusting compartment (SAC) was chosen for the VPA compound file. Conner et al. used the Phoenix WinNonlin program to assign the k_{in} (k_{12}), k_{out} (k_{21}), and V_{sac} (V_2) based on observed data from Nitsche and Mascher.[21,23] The volume of distribution at steady-state (V_{ss}) was predicted, with concentration-dependent volume function, by Rodgers and Rowland's method.[26] The predicted V_{ss} was 0.10843 L/kg which was in proximity to the observed value of 0.13 L/kg (weighted mean) in young adults (aged 20–35 years).[27]

Enzyme kinetics was applied to develop VPA compound file in the study. The in vivo intravenous clearance (Cl_{iv}) was assigned to hepatic metabolism mediated by UGT and CYP enzymes, additional hepatic metabolism (β-oxidation), and renal excretion. The maximum rate of the enzymatic reaction and Michaelis–Menten constant for VPA-metabolizing UGT enzymes were obtained from in vitro studies using recombinant UGT (Supersomes).[22] A recombinant UGT (rUGT) liver-tissue specific scalar for extrapolation of in vitro kinetic data was applied to all UGTs. The ω-oxidation mediated by CYP enzymes accounts for less than 10% of the VPA metabolism. It is mainly attributed to CYP2C9.[28] The Cl_{int} for CYP2C9 was predicted using Simcyp's built-in reverse translational tool, which was based on the input of Cl_{iv} and the in vivo fraction metabolized (f_m), and divided into 4-ene-VPA formation pathway ($Cl_{int,CYP2C9(4\text{-}ene\text{-}VPA)}$) and other metabolite pathways ($Cl_{int,CYP2C9(other\ metabolites)}$).[28] The additional hepatic clearance for β-oxidation ($Cl_{int,\beta\text{-}oxidation}$) and renal clearance ($Cl_R$) were set as 30%–40% and 1% of the Cl_{iv}, respectively.[13]

The intrinsic formation of 4-ene-VPA was derived from $Cl_{int,CYP2C9(4\text{-}ene\text{-}VPA)}$. The physicochemical parameters of 4-ene-VPA, including MW, $LogP_{o:w}$, and pK_a were obtained from respective literature. The metabolite B:P (0.55) was assumed to be the same as the parent compound as there was no measured data. The f_u (0.056704) value was predicted through Simcyp built-in prediction toolbox. A minimal PBPK model without SAC was chosen for the 4-ene-VPA compound file due to lack of observed data for k_{in} (k_{12}), k_{out} (k_{21}), and V_{sac} (V_2). The V_{ss} value of 0.209 L/kg was predicted by Simcyp built-in Small

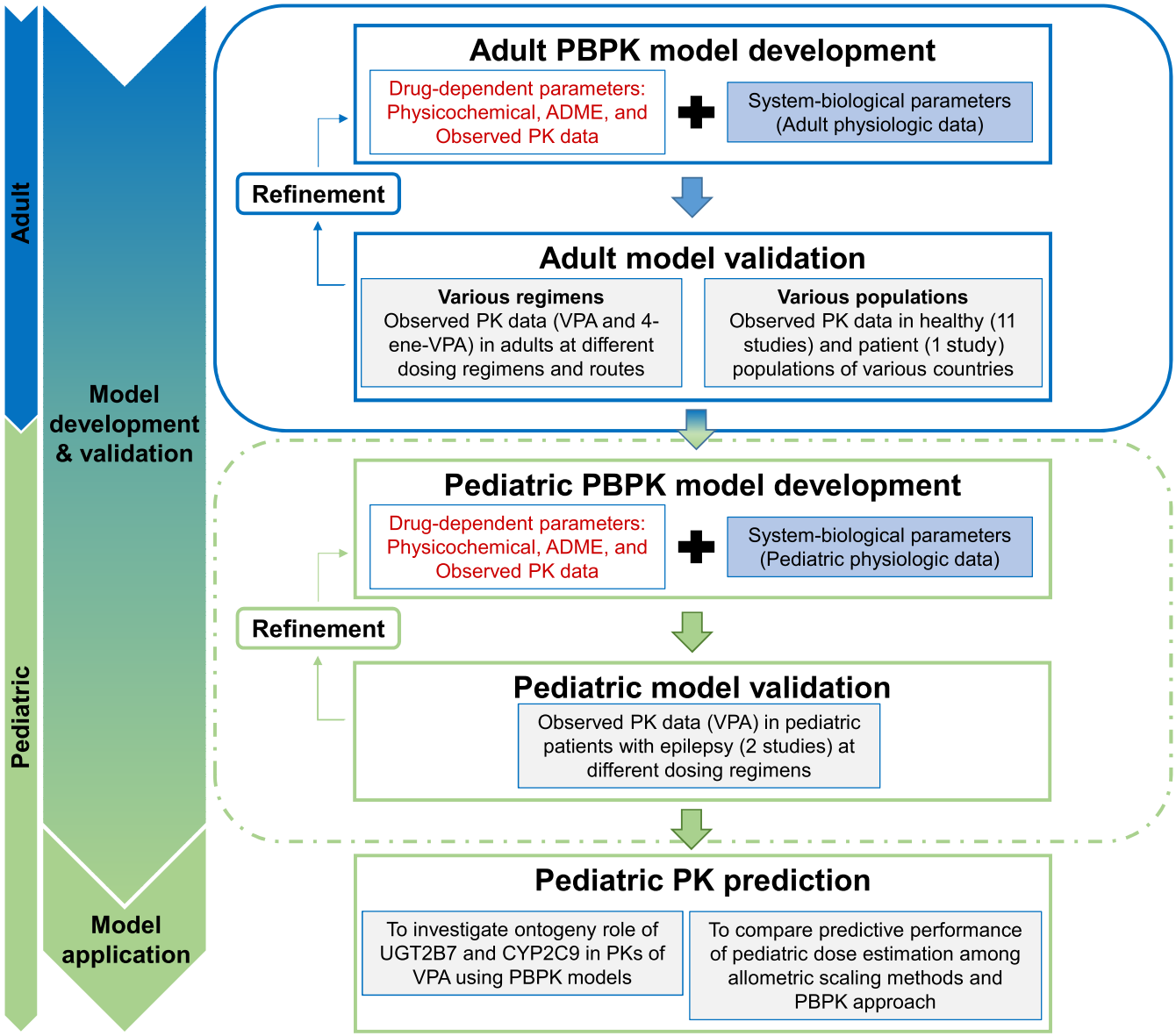


FIGURE 1 Schematic workflow of adult and pediatric PBPK model development. ADME, absorption, distribution, metabolism, excretion; CYP2C9, cytochrome P_{450} 2C9; PBPK, physiologically-based pharmacokinetic; PK, pharmacokinetic; UGT2B7, UDP-glucuronosyltransferase 2B7; VPA, valproic acid.

Molecular Allometry. The Cl_{iv} of 4-ene-VPA ($Cl_{iv,4\text{-ene-VPA}}$) was calculated from preclinical data to human clearance using the Simcyp built-in Small Molecular Allometry and was assigned to additional systemic clearance (Cl_{add}).[24] The human Cl_R was predicted from animal data using the Allometric Renal Clearance Calculator.[24]

The VPA and 4-ene-VPA plasma concentration-time profiles in adults were modeled, simulated, and validated utilizing the Simcyp Healthy Volunteer virtual population comprising of 100 subjects divided across 10 trials with 10 subjects each. For validation of the adult PBPK model, the predicted PK parameters for various dosing regimens were compared to those of the reported clinical studies by overlaying the observed systemic drug concentration-time profiles with the simulated profiles. Inputs for the demographics of the virtual populations were made to be as close as possible to the information in the literature with respect to age range, male–female ratio, and dosing regimens (Table 1).[20,23,29–40]

Pediatric PBPK model development and validation

For the development of pediatric PBPK model for VPA and 4-ene-VPA, the validated adult model was scaled down initially to the pediatric population using the Simcyp Pediatric population simulator. Some studies indicated that V_{ss} of VPA in pediatrics differs from that in adults.[27,41] A K_p

TABLE 1 Summary of adult and pediatric clinical pharmacokinetics studies on VPA and 4-ene-VPA.

Authors, publication year (number of participants)		Study demographics and Valproate regimen								Plasma C_{max} (mg/L)			Plasma AUC_{0-t} (mg/L h)		
		Study cohort	Country	Male (%)	Age (year)	API	Formulation	Dose (mg)	VPA equivalent (mg)	Predicted	Observed	Pre/Obs ratio	Predicted	Observed	Pre/Obs ratio
VPA in adults															
i.v. (300 s)															
Perucca et al. (1978) ($n=6$)[29]		Healthy	Italy	100	22–38	NaVPA	–	800.0	693	115.2 ± 14.9	–	–	1289.4 ± 406.1	1372.0 ± 137.0	0.94
Nitsche and Mascher (1982) ($n=6$)[23]		Healthy	Austria	100	19–31	NaVPA	–	1000.0	866	143.9 ± 18.5	–	–	1459.0 ± 399.1	1251.0 ± 266.8	1.17
p.o. (single dose)															
Chun et al. (1980) ($n=9$)[30]		Healthy	USA	100	21–31	VPA	cap	250.0	250	26.7 ± 4.4	31.4 ± 5.0	0.85	436.0 ± 151.0	433.5 ± 59.0	1.01
Neuvonen et al. (1983) ($n=6$)[31]		Healthy	Finland	17	19–49	NaVPA	EC	300.0	260	36.2 ± 6.7	38.1 ± 3.2	0.95	493.8 ± 171.3	609.0 ± 71.0	0.81
Glazko et al. (1993) ($n=12$)[32]		Healthy	USA	100	23–48	VPA	cap	500.0	500	52.9 ± 9.9	58	0.91	827.3 ± 286.8	1049	0.79
Ibarra et al. (2013) ($n=14$)[33]		Healthy	Uruguay	50	19–35	DVS	EC	538.2	500	66.3 ± 13.9	–	–	860.38 ± 295.7	–	–
Ibarra et al. (2013) ($n=7$, male)[33]				100	19–35	DVS	EC	538.2	500	58.8 ± 10.5	35.6 ± 5.2	1.65	819.0 ± 280.8	496.6 ± 55.2	1.65
Ibarra et al. (2013) ($n=7$, female)[33]				0	19–35	DVS	EC	538.2	500	71.5 ± 12.2	56.3 ± 9.9	1.27	852.2 ± 315.0	872.4 ± 180.4	0.98
Lee et al. (2015) ($n=15$)[34]		Healthy	Korea	100	19–55	DVS	EC	538.2	500	58.9 ± 10.6	53	1.11	835.0 ± 283.8	889.6	0.94
Gugler et al. (1977) ($n=6$)[35]		Healthy	Germany	83	23–26	NaVPA	EC	600.0	520	63.4 ± 12.5	–	–	857.0 ± 297.3	1214	0.71
Perucca et al. (1978) ($n=6$)[29]		Healthy	Italy	100	22–38	NaVPA	tab	800.0	693	81.8 ± 14.3	81.9 ± 12.2	1.00	1116.8 ± 390.2	1366.0 ± 133.0	0.82
Bialer et al. (1985) ($n=6$)[36]		Healthy	Israel	100	20–35	NaVPA	tab	1000.0	866	102.3 ± 17.9	107.5 ± 8.1	0.95	1380.2 ± 485.3	2124.0 ± 457.0	0.65
Pokraiac et al. (1978) ($n=10$)[37]		Epileptic	Serbia	50	19–48	VPA	EC	900.0	900	119.9 ± 24.9	77.9 ± 11.4	1.54	1544.8 ± 530.0	1723.0 ± 546.0	0.90
p.o. (b.i.d.)															
Nitsche and Mascher (1982) ($n=6$)[23]		Healthy	Austria	100	19–31	VPA	cap	900.0 (300.0 × 3)	900	164.6 ± 45.7	–	–	1389.9 ± 494.1	–	–
Nitsche and Mascher (1982) ($n=6$)[23]		Healthy	Austria	100	19–31	VPA	cap	900.0 (450.0 × 2)	900	164.6 ± 45.7	–	–	1389.9 ± 494.1	–	–
Nitsche and Mascher (1982) ($n=6$)[23]		Healthy	Austria	100	19–31	VPA	cap	1000.0 (500.0 × 2)	1000	183.2 ± 50.9	–	–	1544.4 ± 552.8	–	–
4-ene-VPA in adults															
p.o. (b.i.d.)															
Addison et al. (2000) ($n=7$)[38]	VPA	Healthy	Australia	100	19–30	NaVPA	EC	200.0	173	33.8 ± 7.7	–	–	310.8 ± 104.4	316.3 ± 110.6	0.98
	4-ene-VPA									0.08 ± 0.1	–	–	0.9 ± 0.5	0.9 ± 0.4	1.00
Cheng et al. (2007) ($n=1$)[39]	VPA	Healthy	NA	NA (50)	NA (18–65)	NaVPA	tab	10 mg/kg	9 mg/kg	138.8 ± 41.9	–	–	1080.8 ± 407.4	–	–
	4-ene-VPA									0.3 ± 0.2	–	–	3.13 ± 2.1	–	–
VPA in children															
i.v. (300 s)															
Williams et al. (2012) ($n=10$)[20]		Epileptic	USA	NA (50)	1–17	NaVPA	–	15 mg/kg	13 mg/kg	79.2 ± 22.4	–	–	606.2 ± 180.2	–	–
Visudtibhan et al. (2011) ($n=11$)[40]		Epileptic	Thailand	27	1–15	NaVPA	–	19 mg/kg	16 mg/kg	98.5 ± 28.2	98.9	1.00	756.2 ± 227.7	–	–

Abbreviations: API, active pharmaceutical ingredient; AUC_{0-t}, area under the concentration-time profiles; b.i.d., twice daily; cap, capsule; C_{max}, maximum plasma concentration; DVS, divalproex sodium; EC, enteric-coated tablet; NA, not available; NaVPA, sodium valproate; Obs, observed; Pred, predicted; tab, tablet; VPA, valproic acid.

scalar of 2.254 was included in the pediatric PBPK model to adjust the predicted V_{ss} of 0.10843 L/kg by Rodgers and Rowland's method to 0.19001 L/kg (Table S1).[27]

The VPA and 4-ene-VPA plasma concentration-time profiles in children were modeled, simulated, and validated utilizing the Simcyp Pediatric population that takes age-dependent anatomic and physiological changes into account. To validate pediatric PBPK model for VPA, the predicted pediatric PK parameters for two intravenous dosing regimens were compared to those of the reported clinical PK studies by overlaying the simulated systemic drug concentration-time profiles with the observed profiles. Inputs for the demographics of the virtual populations were made to be as close as possible to the information in the literature with respect to age range, male–female ratio, and dosing regimens (Table 1). The simulations were performed for 100 subjects divided across 10 trials with 10 subjects each.

Characterization of impacts of ontogeny on hepatotoxicity likelihood

Previous study indicated that UGT2B7 demonstrated highest activity toward valproate glucuronide formation.[42] An in vitro study demonstrated that CYP2C9 was the principal enzyme (75%–80%) in the formation of 4-ene-VPA, 4-OH-VPA, and 5-OH-VPA.[28] The ontogeny of CYP enzymes has been reported to be the leading cause of age-related changes in the clearance of certain drugs, such as *S*-warfarin (a substrate of CYP2C9).[43] It has been demonstrated that each CYP/UGT isoform possesses unique maturational profile with respective PK consequences.[15] Moreover, maturation of most UGT enzymes emerges later than CYP enzymes. Equations for ontogeny profiles of CYP2C9 and UGT2B7 in the Simcyp PBPK platform are as follows:

$$\text{Fraction of adult CYP2C9 activity} = 0.17 + \frac{(0.98-0.17)\times \text{Age}^{0.53}}{0.0157^{0.53}+\text{Age}^{0.53}} \text{ (up to 5 years of age)}$$

$$\text{Fraction of adult UGT2B7 activity} = 0.113 + 0.0425\times \text{Age (up to 21 years of age)}$$

The impacts of CYP2C9 and UGT2B7 ontogeny profiles on age-dependent VPA apparent Cl_{oral} in virtual subjects after birth up to 25 years old were tested via the simulation analyses using the developed VPA PBPK model with modified pediatric systems parameters in four different scenarios (with or without the ontogeny profiles of CYP2C9/UGT2B7 activities). In the simulation, VPA was administrated (NaVPA tablet, 693 mg VPA p.o. b.i.d.) to 1000 subjects divided across 10 trials with 100 subjects each with an equal proportion of male and female subjects.

Simcyp built-in global sensitivity analysis (GSA) was conducted to assess the potential impacts of CYP2C9 and UGT2B7 activities on areas under the concentration-time curve (AUCs) of VPA and 4-ene-VPA in virtual subjects aged from day 0 to 65 years old. In the simulation, VPA was administrated (NaVPA tablet, 693 mg VPA p.o. SD) to 8000 virtual individuals with an equal proportion of male and female subjects in each age group. The Sobol method is a variance-based type GSA method, which decomposes the variance of the model outputs into sums of variances for combinations of input parameters of increasing dimensionality. A total effect sensitivity index assessing each parameter's impact, including all possible interactions with others, was calculated to determine the importance of input parameters. Parameters with sensitivity index greater than 0.1 were regarded as critical parameters with a significant impact on model outputs.[44]

Dose estimation for young children

The pediatric population was grouped into newborn (0–0.02 years), neonate (0.02–0.08 years), infant (0.08–2 years), toddler and preschooler (2–6 years), school age (6–12 years), and adolescent (12–18 years). The adult dose of VPA, that is, 500 mg per os (p.o.) twice daily (b.i.d.), were simulated for various pediatric groups. Additionally, serial dose reduction of 6.25% (VPA 468.75 mg p.o. b.i.d.), 12.5% (437.5 mg), 25% (375 mg), 50% (250 mg), 75% (125 mg), 87.5% (62.5 mg), and 93.75% (31.25 mg) were also simulated and PK profiles and parameters recorded and compared among groups. The simulations were performed for 100 subjects divided across 10 trials with 10 subjects each with an equal proportion of male and female subjects. Finally, concordance of pediatric dose estimation by the PBPK approach of the study was compared to conventional allometric scaling methods as described below.[45]

Pediatric dose based on age, Fried's rule (for <1 year old):

$$\text{Pediatric dose} = \frac{\text{Age (months)}}{150} \times \text{Adult dose}$$

Pediatric dose based on age, Young's rule (for 1–12 years of age):

$$\text{Pediatric dose} = \frac{\text{Age (years)}}{\text{Age (years)} + 12} \times \text{Adult dose}$$

Pediatric dose based on body weight, Clark's rule:

$$\text{Pediatric dose} = \frac{\text{Weight (lb)}}{150} \times \text{Adult dose}$$

Pediatric dose based on BSA:

$$\text{Pediatric dose} = \frac{\text{BSA}\left(\text{m}^2\right)}{1.73} \times \text{Adult dose}$$

RESULTS

Adult and pediatric PBPK models for VPA and 4-ene-VPA

The compound files of VPA and 4-ene-VPA were developed (Table 1) and validated by PBPK simulations against diverse clinical PK reports of respective API, formulation, dosing regimens, routes of administration, age, sex, and race. Simulated VPA plasma concentration-time profiles overlaid with the observed data of a total of 1140 blood samplings from adults are presented in Figure S1. Most (79.3%) observed data fell within the 5th to 95th percentiles of the predicted range, indicating the modeling and simulations herein were reasonable. Moreover, ratios of the predicted to observed values for maximum plasma concentration (C_{max}) and AUC, that is, 0.85–1.65 and 0.65–1.65, were within the accepted twofold range (Table 1).

For the adult 4-ene-VPA PBPK model, ratios of the predicted to observed AUC values for VPA and 4-ene-VPA were 0.98 and 1.00, respectively (Table 1). The simulated VPA and 4-ene-VPA plasma concentration-time profiles in adults were in good agreement with the observed data (Figure S2). Thus, the adult PBPK models for VPA and 4-ene-VPA were deemed adequate and used for further translation into pediatric examinations.

Upon translating into pediatric cohorts, the simulated VPA plasma concentration-time profiles following i.v. doses were also in good agreement with the observed data (Figure S3). The ratio of the predicted-to-observed C_{max} of VPA was 1.00 (Table 1). Due to lack of 4-ene-VPA data in children, modeling and simulation of pediatric 4-ene-VPA plasma concentrations were not feasible.

Impacts of CYP2C9 and UGT2B7 ontogeny on VPA clearance

Distribution of the observed $Cl_{oral,observed}$ (△ and □) data, digitized from published studies for ages from 0.25 to 24 years,[46,47] is shown in Figure 2a. These data demonstrated that the younger the age, the more the divergence of the $Cl_{oral,observed}$ data. By overlaying the weight-adjusted estimates of VPA $Cl_{oral,predicted}$ (●) in four different scenarios (each representing respective combination of active/inactive CYP2C9 and active/inactive UGT2B7 ontogeny profiles), convergence (reduced width of 5th–95th percentiles) of predicted values could be discerned in Figure 2a1 (simultaneously activating CYP2C9 and UGT2B7 ontogeny profiles) and Figure 2a2 (only UGT2B7 ontogeny profile being activated). The observed convergence might indicate that UGT2B7 ontogeny plays important role for Cl_{oral} of VPA, especially in small children.

Large variance of clearance occurred in the neonate group (represented by 0.04 years of age – 14 days after birth). The $Cl_{oral,predicted}$ estimates at 0.04 years old could be described by the scenario of only UGT2B7 ontogeny profile being activated (0.01544 L/h/kg, 5th-95th percentiles: 0.0088–0.0221 L/h/kg; Figure 2a1,b1). Whereas at age 4 years, incorporation of CYP2C9 ontogeny profile would lead to slight increase of $Cl_{oral,predicted}$ (CYP2C9 vs. UGT2B7 ontogeny: 0.0098 vs. 0.0085 L/h/kg) but not better convergence (5th-95th percentiles: 0.0059–0.0188 vs. 0.0050–0.0157 L/h/kg; Figure 2a3,b2). The Figure 2a4, neither CYP2C9 nor UGT2B7 ontogeny profiles being incorporated, appeared to have largest variability (5th–95th percentiles: 0.0060–0.0189 L/h/kg) among the four scenarios. This phenomenon indicated the importance of incorporating ontogeny profiles in modeling and simulation for the young.

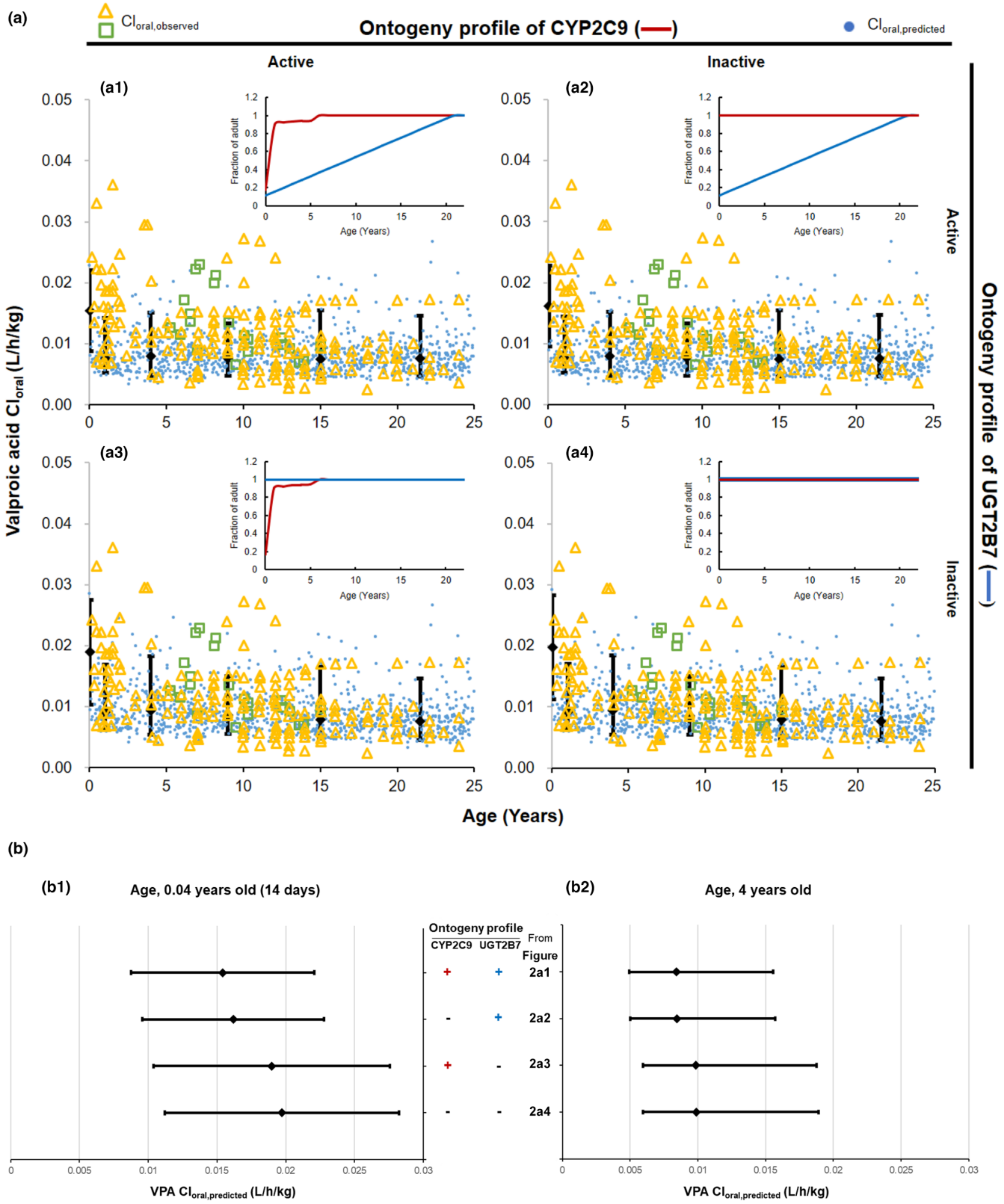
Sensitivity analyses of VPA clearance by CYP2C9/UGT2B7 on AUCs of VPA/4-ene-VPA across age groups

The variance-based GSA method by Sobol et al. was used to analyze ontogeny impacts of drug-metabolizing enzymes on AUCs of VPA and 4-ene-VPA through estimating sensitivity indices for differential $Cl_{int,CYP2C9}$ and $Cl_{int,UGT2B7}$ values across age groups. It is apparent that sensitivity indices for $Cl_{int,CYP2C9}$ on the AUC of VPA were incremental from newborns (sensitivity index: 0.20) up to 6 years (0.58) of age, consistent with the maturation process of CYP2C9 (Figure 3a). Correspondingly, the highest sensitivity index for $Cl_{int,CYP2C9}$ on 4-ene-VPA formation occurred in the age groups of infants (0.95) and toddlers/preschoolers (0.95), the most vulnerable age cohorts for VPA hepatotoxicity are reported elsewhere (Figure 3b).

The changes in sensitivity indices for $Cl_{int,UGT2B7}$ on AUCs of VPA and 4-ene-VPA were decremental from newborns up to 6 years of age (Figure 3a,b). These data indicated that $Cl_{int,CYP2C9}$ was the most influential parameter for 4-ene-VPA AUC, that is, formation of 4-ene-VPA.

Tailoring valproic acid dose for each pediatric age group

To fill in the gap of long-awaited dosing recommendation for those less than 10 years of age who may benefit



from VPA pharmacotherapy, the ontogeny-based PBPK approach developed herein was applied to estimate age-group-specific VPA doses, with resultant plasma concentrations of VPA and 4-ene-VPA predicted. Doses for simulation were initially set at 31.25 mg b.i.d. and stepwise increased to 500.00 mg b.i.d. (the adult usual dose). The predicted optimal dose(s) for each age group using the developed PBPK model were compared to those suggested by conventional allometric scaling methods (Tables 2 and S3). If estimated concentrations of VPA

FIGURE 2 Observed versus predicted oral clearances of VPA, aged 0–25 years old. (a) Estimated by four different scenarios. (a1) Active CYP2C9 and UGT2B7 ontogeny profiles, (a2) active UGT2B7 ontogeny profile only, (a3) active CYP2C9 ontogeny profile only, (a4) inactive CYP2C9 and UGT2B7 ontogeny profiles; (b) estimated at 0.04 years (14 days, b1) and 4 years of age (b2) by different scenarios of UGT2B7/CYP2C9 ontogeny profiles. Insert figure depicts specific ontogeny profile(s) simulated in the scenario (Simcyp Simulation version 21.1; Simcyp Limited). Cl_{oral}, apparent oral clearance; VPA, valproic acid; blue dot (●), predicted individual Cl_{oral} (10 trials × 100 subjects); black diamond (◆), mean Cl_{oral} of age groups 0.04, 1.04, 4, 9, 15, and 21.5 years old; open triangle (△), Bondareva et al. (2004) ($n = 42$)[46]; open square (□), Chiba et al. (1985) ($n = 21$)[47]; red line (▬); CYP2C9 ontogeny profile; blue line (▬), UGT2B7 ontogeny profile. Equations for ontogeny profiles of CYP2C9 and UGT2B7 in Simcyp: Fraction of adult CYP2C9 activity = $0.17 + \frac{(0.98 - 0.17) \times Age^{0.53}}{0.0157^{0.53} + Age^{0.53}}$ (up to 5 years of age). Fraction of adult UGT2B7 activity = $0.113 + 0.0425 \times Age$ (up to 21 years of age).

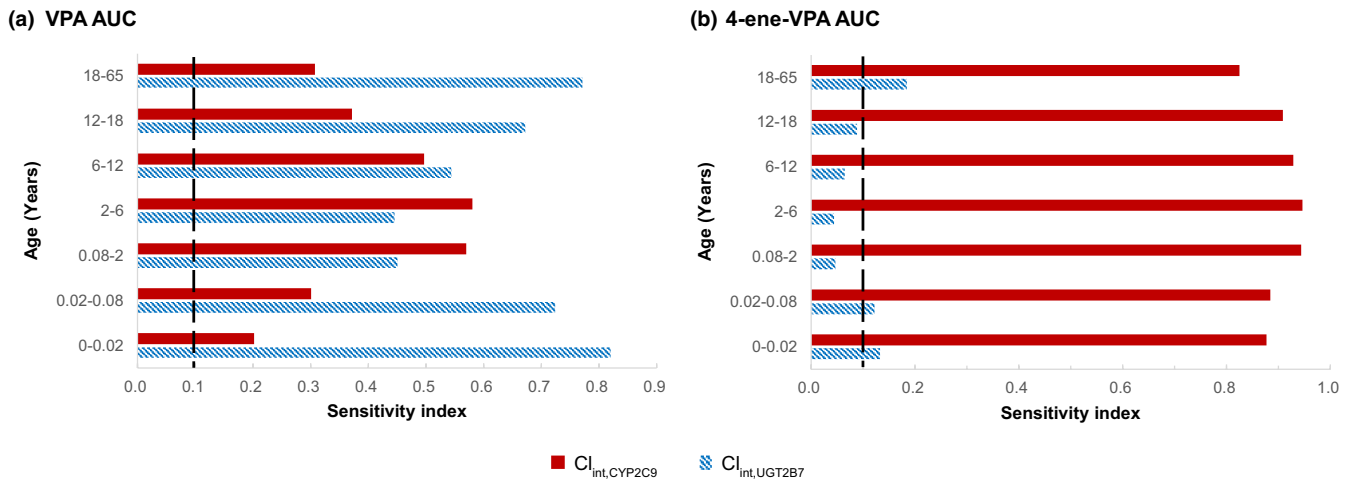


FIGURE 3 Global sensitivity analyses comparing impacts of $Cl_{int,CYP2C9}$ and $Cl_{int,UGT2B7}$ of designated age groups on simulated AUCs of (a) VPA and (b) 4-ene-VPA, following single oral dose (693 mg) of VPA. The higher the sensitivity index, the more influential the input parameter is. Vertical dashed line (|) represents sensitivity index of 0.1, a threshold indicating importance. AUC, areas under the concentration-time curve; Cl_{int}, intrinsic clearance; VPA, valproic acid.

and the 4-ene-VPA were within the recommended therapeutic range (50–100 mg/L) and below the toxic level (>0.5 mg/L),[48] respectively, relevant doses predicted were regarded as acceptable in the study (Table 2).

As shown in Tables 2 and S3, the estimated doses using the PBPK method were analogous to those predicted by using the Clark's rule (weight-based). Of note, doses predicted using Fried's or Young's rules (age-based) would lead to underdose in neonates, infants, and adolescents; whereas doses predicted using the BSA-based method might lead to overdose in neonates, infants, and toddlers/preschoolers.

DISCUSSION

The study developed ontogeny-based PBPK models for both VPA and its hepatotoxic metabolite 4-ene-VPA by which the simulated adult and pediatric PK profiles adequately reproduced clinical data published elsewhere. The PBPK methodology was then applied to differentiate ontogeny roles of drug-metabolizing enzymes (CYP2C9 vs. UGT2B7) in young age. The study revealed that UGT2B7 ontogeny was quite influential on the Cl_{oral} of VPA even in neonates and small children. The $Cl_{int,CYP2C9}$ was the most prominent determinant for AUCs 4-ene-VPA in infants, and toddlers/preschoolers, the age groups with evidence-based greater risk of VPA hepatotoxicity – probably attributable to higher noxious 4-ene-VPA accumulation.[10,11]

Optimal VPA dosing for each age group was also predicted and objectively judged by ensuring efficacy (desired $C_{trough,VPA}$: 50–100 mg/L) and avoiding hepatotoxicity risk (desired $C_{max,4\text{-}ene\text{-}VPA}$: <0.5 mg/L).[48,49] By far, the study is the first in using mechanistic PBPK approach to provide VPA dosing recommendations for children younger than 10 years of age. Concordance of dose estimates for each age group between the ontogeny-based PBPK approach and the three readily available allometric scaling (age-, weight-, and BSA-based)[45] methods was also probed. The study revealed that dosing suggestions by ontogeny-based PBPK approach and weight-based Clark's rule were comparable. Nonetheless, the ontogeny-based PBPK approach has the flexibility of considering systems- (e.g., organ dysfunction or genetics) and drug-factors (e.g., regimen adjustment or co-medications) for achieving precision dosing when needed.[15] The weight-based Clark's rule, however, lacks similar flexibility. As aforementioned, the applicability of the age-based (Fried's or Young's rules) or the BSA-based

TABLE 2 Estimation and comparison of optimal VPA doses across pediatric age groups using ontogeny-based PBPK approach and conventionally allometric scaling methods.[a]

	The study (ontogeny-based PBPK approach)			Conventional allometric scaling methods									
				Fried's rule or Young's rule				Clark's rule			BSA-based		
		Predicted C_{max}				Predicted C_{max}			Predicted C_{max}			Predicted C_{max}	
Pediatric age groups (years old)	Dose input (mg, b.i.d.)	VPA	4-ene-VPA (mg/L)	Age input (years)	$Dose_{calculated}$ (mg, b.i.d.)	VPA	4-ene-VPA (mg/L)	Weight input (lb)	$Dose_{calculated}$ (mg, b.i.d.)	VPA	4-ene-VPA (mg/L)	BSA input (m^2)	$Dose_{calculated}$ (mg, b.i.d.)	VPA	4-ene-VPA (mg/L)
Neonate (0.02–0.08)	**31.25**	66.92	0.10	0.08	3.3	7.54	0.01	8.8	**29.3**	58.46	0.09	0.24	69.4	141.74	0.22
Infant (0.08–2)	**62.50**	61.46	0.10	1	38.5	36.51	0.06	19.8	**66.0**	62.62	0.10	0.43	124.3	119.48	0.20
Toddler and preschooler (2–6)	**125.00**	75.24	0.13	5	**147.1**	80.98	0.14	39.7	**132.3**	72.67	0.13	0.74	213.9	118.59	0.21
School age (6–12)	**250**.00	87.7	0.18	12	**250.0**	64.48	0.14	90.4	**301.3**	78.05	0.17	1.31	**378.6**	98.56	0.21
Adolescent (12–18)	**375–500**	71.69–96.25	0.17–0.23	18	300.0	49.45	0.11	136.7	**455.7**	75.82	0.16	1.71	**494.2**	82.37	0.17

Note: Doses in bold within the "**Dose** input" or "**Dose**$_{calculated}$ "columns indicate optimal dosing suggested from the study. The predicted C_{max} values, data extracted from Table S3, of VPA (recommended therapeutic range: 50–100 mg/L) and its metabolite 4-ene-VPA (reported toxic level: > 0.5 mg/L[48]) are presented in the column with gray background. Concentrations below or above recommended therapeutic ranges are noted in orange or red, respectively.

Abbreviations: BSA, body surface area; C_{max}, maximum plasma concentration; PBPK, physiologically-based pharmacokinetic; VPA, valproic acid.

[a]Based on adult usual dose of VPA, 500 mg b.i.d..

methods is somewhat limited because of possible VPA under- or over-dose in respective age groups.

The ontogeny profiles of CYP2C9 and UGT2B7, in addition to age-related developmental changes, such as stature, were particularly integrated into the present study model to predict VPA Cl_{oral} in different age groups. The inclusion of UGT2B7 ontogeny profile led to the most adequate prediction of VPA Cl_{oral} per unit weight. The neonate group presented the highest, although somewhat disperse, simulated value for VPA Cl_{oral}. The findings were in agreement with previous clinical studies enrolling small children, indicating robustness of the ontogeny-based VPA PBPK model developed herein.[46,47]

To avoid model over-parameterization, GSA was attempted to reduce unnecessary parameters in the study.[44] The PBPK model considering only CYP2C9 enzyme performed better than all three VPA-associated CYP isozymes (CYP2C9, CYP2A6, and CYP2B6) incorporated. Hence, only CYP2C9 remained in the study model for further investigation. The computed sensitivity indices of $Cl_{int,CYP2C9}$ for both VPA AUC and 4-ene-VPA formation coincidentally reached highest in the age cohorts of infants and toddlers/preschoolers, consistent with the most vulnerable age for VPA hepatotoxicity.[10,11] Monostory et al.[50] recommended that valproate dosing in children should take into consideration of CYP2C9 functional status, including individual's genotype and mRNA expression levels, to proactively avoid drug misadventures. Although CYP2C9 metabolism is generally regarded as a minor pathway for VPA elimination,[13,28] Monostory and colleagues' report and the present study coherently pointed to the significant implication of CYP2C9 functional status for VPA dosing in small children. An increase in f_m by CYP2C9 due to lower expression or activity of UGT at young age is most likely.[15]

It is worth mentioning that the present study used the virtual Simcyp Healthy Volunteer population to simulate PK measured in healthy (11 studies) and disease (1 study) populations of different ethnicities without accounting for the potential impact of disease and ethnicity differences on drug exposure. The study was also limited by scarce clinical PK studies of VPA in small children. In particular, 4-ene-VPA levels in relation to VPA dosing in children was unobtainable for obvious reasons. Appraisal of 4-ene-VPA model in children was, thus, not feasible. Nonetheless, simulations of either pediatric or adult VPA plasma concentrations in the present study were in good agreement with respective real-world data.[20,23,29–37] Use of the observed data-verified adult 4-ene-VPA model established herein would be a justified approach in estimating pediatric 4-ene-VPA levels.[38,39] Certainly, unidentified

cryptic factors affecting absorption, distribution, metabolism, and excretion of VPA or 4-ene-VPA in small children remain to be uncovered through clinical studies and real-data collection to facilitate precision dosing.

In conclusion, the study not only developed ontogeny-based PBPK approach for elucidating differential risk of VPA hepatotoxicity across age groups, but also demonstrated clinical utility of the approach in providing long-awaited dosing recommendation for those younger than 10 years of age. If further substantiated by real-world data, the established VPA PBPK models that specifically integrated drug formulation, VPA toxic metabolite, and metabolizing-enzyme ontogeny information would become a robust clinical tool in patient care and a good exemplar for comprehending a pharmaceutical of decades-long clinical use.

AUTHOR CONTRIBUTIONS

Y.-T.H. and Y.-F.H. wrote the manuscript. Y.-T.H., Y.-M.H., C.-J.L., F.L.K., T.J., and Y.-F.H. designed the research. Y.-T.H. performed the research. Y.-T.H., Y.-M.H., C.-J.L., and Y.-F.H. analyzed the data. Y.F.H. provided analytical tools.

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CONFLICT OF INTEREST STATEMENT

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SUPPORTING INFORMATION

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