Investigating Oral Absorption of Carbamazepine in Pediatric Populations

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Abstract. Prediction of the pharmacokinetics of orally administered drugs in children is of importance to optimize the efficacy and safety of pediatric medicines. Physiologically based pharmacokinetic (PBPK) models can be helpful for this purpose. However, application of these tools is limited by significant knowledge gaps regarding the physiological and anatomical changes which occur with age. This study aimed at investigating the age-dependent differences in oral absorption of a poorly soluble model compound, carbamazepine (CBZ) in children, infants, and neonates. We developed an oral absorption model in GastroPlus® and, after evaluation of the PBPK model for adults, extrapolation to younger ages was verified with clinical data and sensitivity analyses were applied for uncertain model parameters. We found that age-based scaling of physiological parameters, with clearance in particular, was important to obtain adequate simulation results. The sensitivity analysis revealed that CBZ absorption was influenced by solubility, particle radius, and small intestinal transit time depending on the pediatric age group and CBZ dose. However, *in vitro* dissolution testing using proposed pediatric biorelevant media suggested no major age dependency of dissolution kinetics. Such better understanding of oral absorption in pediatric patients is required to improve the prediction of exposure in children and the confidence in oral biopharmaceutical tools.

KEY WORDS: BCS2; *in vitro* dissolution; oral absorption; PBPK modeling; pediatric biopharmaceutics.

INTRODUCTION

The Best Pharmaceuticals for Children Act (BPCA) and the Pediatric Research Equity Act (PREA) encourage the pharmaceutical industry to increase drug safety and reduce off-label use in children by conducting clinical studies in pediatric populations (1,2). Drug development in pediatric patients requires careful attention due to ethical and logistical constraints associated with the conduct of clinical studies in these patient groups. Pediatric subjects are not small adults. They are a heterogeneous population that is undergoing significant physiological and anatomical alterations during developmental growth which can affect the rate and extent of drug absorption, as well as drug distribution, metabolism, and excretion. As all of these processes can influence safety and efficacy of drug compounds, it is extremely important to provide accurate predictions of pharmacokinetics in all age groups.

From an oral delivery perspective, the maturation of the gastrointestinal (GI) tract is of particular interest. The GI tract grows rapidly during the first years of life with an associated increase in luminal volumes and absorptive surface area. In addition, maturation of the GI tract affects its physical and biochemical environment, which can directly influence drug solubility, dissolution, and permeation across the intestinal membrane. For example, the stomach pH is known to be close to neutral at birth and decreases to more acidic conditions within hours after birth, before increasing again during the first month of life. Similarly, bile secretion is poor at birth and results in comparatively low concentrations of bile acids in the intestinal fluids during the first weeks of life (3,4).

Conventional oral dosage forms used for adult subjects are often not suitable for administration to children due to the impaired swallowing capabilities, sensory preferences, and physiological functions in the very young. For this reason, the development of age-appropriate formulations is strongly recommended and drives the need for bridging studies if more than one formulation is available for different age groups. Such studies are often conducted in adults to

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minimize the conduct of studies in children or may be entirely omitted if the compound is highly soluble and well-permeable, or if an *in vitro-in vivo* correlation has been established. Both cases imply that the biopharmaceutical performance of the compound and the drug product is comparable in adults and in children. However, such comparison is generally difficult to establish. While it would be important to apply age-specific biopharmaceutical prediction methods during pediatric drug development, such tools are currently limited by significant knowledge gaps regarding the physiological and anatomical changes which occur with age (3–8).

Biopharmaceutical tools such as *in silico* physiologically based pharmacokinetic (PBPK) models and *in vitro* biorelevant dissolution tests are routinely applied to predict the rate and extent of oral absorption in adults. Similarly, the biopharmaceutics classification system (BCS) is extensively used to guide formulation development. However, while they have been extensively validated in adults, validation in pediatric populations is still lagging behind due to limited knowledge of pediatric GI tract physiology and poor availability of pediatric clinical data. The BCS is a scientific tool that classifies the absorption behavior of oral compounds based on their solubility and permeability properties in adults. However, drug dose, intestinal membrane permeability, and GI fluid volumes and composition can be different between adults and children and, therefore, extrapolation of the BCS to pediatric populations is not straightforward (3,4,9,10). PBPK models are commonly used to study drug absorption processes in adults and some animal species by integrating drug material properties (e.g., ionization, particle size, solubility, dose) with physiological and biochemical processes occurring in the GI tract (e.g., GI transit, bile secretion, pH of GI fluids, active transport across the intestinal membrane, enterohepatic circulation). Pediatric physiologies are being implemented in PBPK software tools; however, verification of their predictive capability is still limited (3).

Although the prediction of drug absorption using pediatric PBPK models is still related to uncertainties, these models provide the possibility to study the complex interplay of processes involved in drug absorption and evaluate potential differences between adult and pediatric subjects (11,12). In a previous work, Villiger *et al.* studied the physiological factors affecting oral drug absorption in children using pediatric PBPK modeling and *in vitro* dissolution testing (11). These tools were applied for two highly soluble and well permeable model compounds, paracetamol and sotalol, and suggested that gastric transit was significantly slower in newborns and infants (< 2 years of age) compared to older children and adults. Recently, Batchelor *et al.* recommended that further research on pediatric biopharmaceutics should be undertaken with BCS class 2 and 4 compounds (9). Cristofoletti *et al.* highlighted the ability of advanced *in silico* absorption models to separate pre-absorptive from post-absorptive processes and to determine the rate-limiting steps in oral absorption of two weak bases in children (13). In line with these reports and while recognizing the need for further verification of drug absorption models in children, we conducted this study of pediatric PBPK absorption modeling for the BCS class 2 model compound carbamazepine (CBZ) to improve the understanding of oral absorption of a poorly soluble model compound in children. Clinical data in children are very limited and the selection of an ideal model compound is challenging. CBZ exhibits a complex pharmacokinetic behavior but has been extensively used to prevent epileptic seizures in pediatric patients and, therefore, the availability of clinical data is comparatively good. It has also been recommended as model compound by the Pediatric Biopharmaceutics Classification System Working Group of the National Institute of Child Health and Human Development (10). Moreover, Maharaj *et al.* recently found that the solubility of CBZ in media simulating the pediatric GI fluids fell outside an 80–125% range compared to solubility values in adult media, thereby indicating a potential age-related alteration in absorption (14).

To the best of our knowledge, no pediatric CBZ PBPK model has yet been published. CBZ pharmacokinetics is complex as it includes auto-induction of metabolism through the CYP3A4 pathway (15–18) and influence of frequent co-medication with other enzyme-inducing antiepileptic drugs (AEDs) such as phenobarbital (PB) and phenytoin (PT). The present study describes how we overcame these challenges and used modeling and *in vitro* tests to simulate CBZ absorption in children and investigate the sensitivity to dosing factors and age-dependent changes in GI physiology. While carbamazepine-epoxide is an active major metabolite and should also be considered in children, the modeling of this metabolite was beyond the scope of our work as the main aim was to explore oral absorption.

MATERIALS AND METHODS

Materials

The marketed tablet formulation Tegretol® 200 mg was obtained from Novartis Pharma GmbH (Nuremberg, Germany). CBZ was obtained from Chemie Brunschwig AG (Basel, Switzerland). FaSSIF/FeSSIF/FaSSGF Instant powder® was from biorelevant.com (Croyden, UK), Bimbosan® Classic from Bimbosan AG (Welschenrohr, Switzerland), and standardized heat-treated and homogenized milk (UHT-milk) containing 3.5% fat was from Mopro Luzern AG (Luzern, Switzerland). Acetonitrile was purchased from Biosolve Chimie (Dieuze, France). Acetic acid, dimethyl sulfoxide, sodium hydroxide pellets, hydrochloric acid, sodium taurocholate, sodium oleate, maleic acid, and pepsin were from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Sodium dihydrogen phosphate monohydrate, methanol, sodium acetate, and water for chromatography were from Merck KGaA (Darmstadt, Germany). Lecithin was purchased from Lipoid AG (Ludwigshafen, Germany), sodium chloride from Fluka Chemie AG (Buchs, Switzerland), and glycerol monooleate from Gattefossé AG (Saint Priest, France).

Pharmacokinetic Data Collection

Pharmacokinetic (PK) data in adults and pediatric populations were collected from the literature. The PK data

in adults were used for model building and were included if the data were from healthy subjects, receiving a single CBZ dose formulated as immediate release (IR) suspension, solution, or tablet. In addition to the plasma concentration-time profiles, demographic and dosing information were extracted from the published reports. This resulted in a database of adult PK data from 34 different studies published between 1974 and 2004 (19–52), which included either single subject data or average data from studies in up to 24 subjects. The collected plasma concentration-time profiles were digitized using the software DigitizeIt® version 2.0.5 (I. Bormann, Braunschweig, Germany).

Weighted average plasma concentration-time profiles were calculated across the studies obtained after administration of IR Tegretol to healthy, fasted adults at either 200 mg (time points 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 h) or at 400 mg (time points 1, 2, 4, 6, 8, 12, 24, 48, and 72 h). These two profiles were based on data from 7 (19–25) and 10 (26–35) different studies involving a total of 76 and 94 patients, respectively.

One study in healthy children (53) and four different pediatric patient studies (54–56) were found, which reported plasma concentration profiles as well as details of the medication history and other clinically relevant information. After further examination, one of the patient studies was considered as not representative and therefore excluded because it was performed in critically ill children suffering from additional complications such as ileus and impaired renal and hepatic function (56). After classifying the remaining datasets into age groups according to the International Conference on Harmonisation (ICH) E11 guideline (57), data were available for 11 newborns, (0–28 days of age), 3 infants (1–24 months of age), 7 children (2–11 years of age), and 3 adolescents (12–18 years of age). However, as the literature reports did not group their studies according to the ICH age classification and did not provide individual data, we followed a categorization based on mean age and age range as reported in Table I. Table I also provides information on patient weight, CBZ dose, and co-medications. More detailed information of individual patient characteristics and co-medication can be found in the Supplementary Material (Table S1).

PBPK Modeling Software

PBPK modeling was performed using GastroPlus version 9.0 (SimulationsPlus Inc., Lancaster, USA). GastroPlus incorporates the Advanced Compartmental and Transit (ACAT) model for predicting oral drug absorption, separating the GI tract into nine compartments. A general description of GastroPlus is available in the user's manual (58) and in several publications (59–62).

Physiologically Based Scaling with Age

Adult and child physiologies, including age- and body weight-matched organ weights, volumes, and blood perfusion rates, were generated using the GastroPlus PEAR® module (Population Estimates for Age-Related Physiology). In brief, this module scales the physiological model framework based on published data for age-related

Table I. Patient Characteristics, Dose, and Co-medications Given for Each Study

Study	No. of patients	Mean age (range)	Mean weight (kg)	Mean dose (mg/kg)	Co-medications
MacKintosh (55) (newborns)	6	6.3 d (2–16 d)	2.6	5	Phenobarbital, penicillin, gentamicin, phenytoin, pancuronium, paregoric, ampicillin
Rey (54) (newborns)	7	20.9 d (7–42 d)	3.2	17	Phenobarbital
Rey (54) (infants/ children)	5	5.1 y (1.25–8 y)	16.6	19	Phenobarbital, phenytoin, clonazepam, primidone, valproic acid
Bano (53) (children/ adoles cents)	6	11.3 y (7–15 y)	31.3	9	No co-medication

d days, *y* years

changes in children (63,64). Thus, hepatic clearance was scaled to children accounting for changes in liver size and perfusion (65,66), protein binding and hematocrit (67–69), and enzyme expression maturation (e.g., for CYP3A (70)). Gut size in children was scaled according to data from Brown (71), and the GI transit times for children are based on reported data from Zwart *et al.* (72), Van Den Driessche *et al.* (73), Zhang *et al.* (74), and Bautista *et al.* (75). Due to lack of data, age dependencies in regional luminal solubility or in gut wall permeability were not considered in the model calculations. A list of anatomical and physiological parameters important for drug absorption and the basis for default values included in the GastroPlus pediatric absorption model is provided in the Supplementary Materials.

PBPK Modeling Strategy

The workflow of the modeling strategy is shown in Fig. 1. The general approach to developing a pediatric CBZ model was to first establish a robust adult model based on single-dose clinical data from healthy adults prior to extrapolation to pediatric age groups. CBZ clearance is complex because CBZ induces its own CYP450 3A metabolism and it is also commonly administered with other antiepileptic drugs (AEDs) which are also CYP450 3A inducers. Clearance scaling to children had to consider these effects because most of the pediatric plasma concentration profiles were obtained in patients receiving co-medications. The oral plasma concentration profiles in children were simulated using the default physiological age-based scaling of the GI tract after having adjusted the systemic clearance to children using empirical relationships taken from statistical analysis of clinical data (described in "Models for Clearance Estimation in Children" section). Simulated profiles were then compared to observed profiles and model parameter sensitivity analyses were employed to
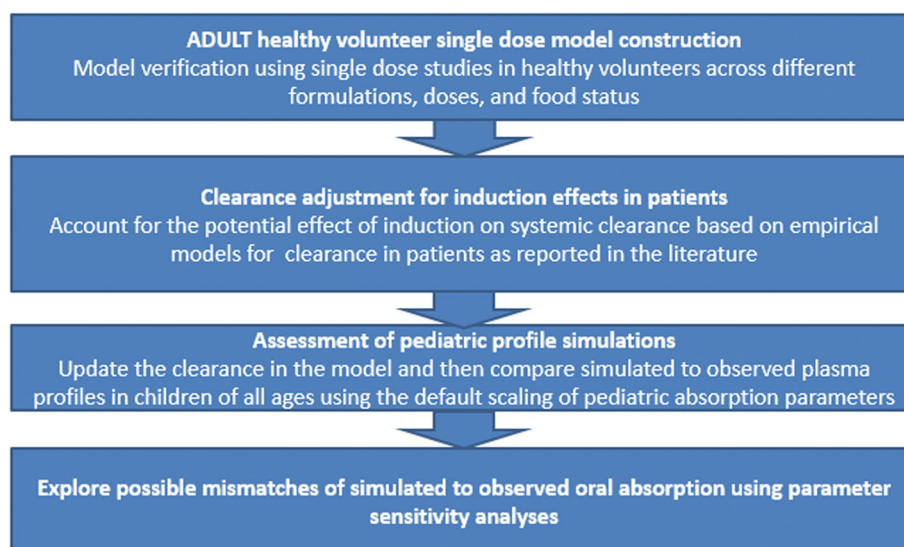
Fig. 1. Workflow and modeling strategy

scrutinize discrepancies between simulated and observed data.

Carbamazepine PBPK Model

The baseline model for CBZ in adults was constructed using input data listed in Table II. CBZ disposition was modeled using physiologies generated with PEAR with demographic parameters (gender, race, age) set to match the study specifications. If body weight, gender, and age were not specified in the source publication, a 70-kg and 30-year-old American male was assumed. CBZ is a neutral molecule and distributes well into tissues with a volume of distribution of approximately 1 L/kg. Drug distribution was predicted using perfusion-limited tissue models with tissue partition coefficients predicted using the Lukacova (Rodgers single) method (76). A good match to the observed volume was achieved using a logP of 2 which is within the range of reported measurements (Table II). CBZ metabolism by CYP450 3A4 was defined using metabolism rate data extracted from Kerr *et al.* (15) and, for single-dose studies, the clearance was scaled to different body weights and ages based on the physiological changes in liver size and enzyme expression.

For the simulations in children, except for one study in healthy subjects (53), the studies were for single doses in patients co-medicated with AEDs (54,55) and an adjustment to clearance was needed to account for enzyme induction (see below). Demographic data and dosing information for studies in children were set appropriately and when details were not reported, the following assumptions were made. Dose volume was set to 5 mL for children under 5 years of age and to 10 mL for children of 5 years and older (77). For a study in which the generic tablet formulation Mazetol® was administered (53) and for two studies in which a suspension was prepared from crushed Tegretol tablets (54,55), the same values of particle density, particle radius, particle radius standard deviation, and number of bins as for the Tegretol tablet formulation were used (Table II).

Models for Clearance Estimation in Children

The pediatric clinical data collected were all for a first dose of CBZ but the patients in four studies had been previously treated with CYP3A4 inducing AEDs and so induction of clearance needed to be accounted for. We conducted a literature search on published CBZ clearance models and four empirical models were retrieved based on patient characteristics and co-administered medications (78–81). The equations for these statistical models are provided below and the patient population characteristics for the studies used to derive the models are detailed in Table S2 (Supplementary Materials).

- *Reith et al.*(78) : $\text{CL (L/h)} = 2.24 \times Body\ surface\ area\ (\text{m}^2) + 0.047$
$$\times Dose\ (\text{mg/kg}) \tag{1}$$

- *Gray et al.*(81) : $\text{CL (L/h)} = \left(0.7 \times \text{BW}^{0.4}\right) \times 1.4^{\text{M}} \tag{2}$

- *Yukawa et al.*(80) : $\text{CL (mL/h/kg)} = 64.9 \times \text{BW}^{-0.336}$
$$\times Dose^{0.465}\ (\text{mg/kg/d}) \times 1.07^{\text{VPA}} \times 1.16^{\text{PB}} \times 1.27^{POLY} \tag{3}$$

- *Delgado Iribarnegaray et al.*(79) : CL (L/h)
$$= (0.0122 \times \text{BW} + 0.0467 \times Dose(\text{mg/kg/d}))$$
$$\times \text{Age}^{0.331} \times \left(1 + 0.289 \times 1.4^{\text{PB}}\right) \tag{4}$$

where BW is body weight in kilograms, M (= 1) is a general scaling factor for concomitant CYP3A-inducing medication (M = 0 in the absence of co-medication), VPA (= 1) and PB (= 1) are scaling factors for concomitant medication with valproic acid and phenobarbital, respectively (VPA and PB = 0 in the absence of co-medication), and POLY (= 1) is a scaling factor for concomitant mediation more than two AEDs

Table II. Input Data for the Carbamazepine Model

	IR suspension/solution	IR tablet	Source
MW (g/mol)	236.3		
logP	2		Optimized[&]
Aqueous solubility (mg/mL at pH 6.8)	0.12		(29,36)
pKa	Neutral		(36)
f_{up} (%)	22		(36)
B/P concentration ratio	1.21		(36)
Permeability (10^{-4} cm/s)	4.3		(36)
Diffusion coefficient (10^{-4} cm^2/s)	9.72		GastroPlus default value
Precipitation time (s)	900		GastroPlus default value
V_{max} CYP3A4 P450 (pmol/min/nmol)	1730		(15)
K_m CYP3A4 (μM)	442		(15)
Particle density (g/mL)	1.2	1.5	(36)
Mean particle radius (μm)	5	75	(39,40)
Particle radius standard deviation	0	20	(36)
Particle radius bin no.	1	5	(36)
Solubility in FaSSIF (mg/mL)	0.234		Experimental value (in-house)
Solubility in FeSSIF (mg/mL)	0.343		Experimental value (in-house)

MW molecular weight, *pKa* acid dissociation constant, f_{up} fraction unbound in plasma, *B/P* blood to plasma
[&] Reported literature values ranged from 1.5 to 2.9. We used the value of 2 based on a good match of simulated and observed plasma profiles in adult studies as shown in Figure S1

(POLY = 0 for co-medication with two or less AEDs). The conversion of body weight to body surface area for the calculation of CBZ clearance of the Reith model used tables from the National Institute for Health and Care Excellence Great Britain (82).

These empirical models are all based on clinical concentration measurements after repeated CBZ treatment and thus estimate the steady-state oral CBZ clearance, which includes both the bioavailable fraction and the systemic clearance. Since no data were available after intravenous dosing in children, a definitive separation of systemic clearance from bioavailability was not possible. However, since oral bioavailability is high in adults (most literature references report values > 0.9 (15,16,18)) and CBZ oral clearance, even after induction, remains very low compared to hepatic blood flow, we made the assumption that first-pass metabolism in gut and liver was negligible in children and the systemic clearance was derived from the predicted oral clearance according to $CL_{sys} = CL_{oral} \times F_a$, where the fraction of the dose absorbed (F_a) was estimated from simulations using the PEAR module in GastroPlus. The sensitivity of simulated profiles to the assumed systemic clearance was explored subsequently.

Parameter Sensitivity Analysis

We conducted parameter sensitivity analysis (PSA) for critical input parameters to explore their impact on oral CBZ exposure. These parameters included physiological properties (stomach volume and transit time, fraction of small intestine fluid volume, small intestine length, radius, and transit time), compound-related properties (bile salt solubilization ratio, reference solubility, permeability, particle radius), and dosing variables (drug dose and dose volume). The baseline values as well as upper and lower bounds are detailed in Table S3. PSA was conducted for each of the four pediatric populations and assuming fasted conditions.

Preparation of Biorelevant Media

Adult fasted-state simulated gastric and fasted- and fed-state simulated intestinal fluids (FaSSGF, FaSSIF, and FeSSIF, respectively) were prepared according to Galia *et al.* (83) and the instructions provided by biorelevant.com (84). Pediatric fasted- and fed-state simulated gastric fluid representative of neonates (Pn-FaSSGF and Pn-FeSSGF, respectively), as well as pediatric fasted- and fed-state simulated intestinal fluids representative of neonates (P-FaSSIF and Pnc-FeSSIF, respectively), were prepared as proposed by Maharaj *et al.* (14). The composition of the simulated gastric and intestinal media is detailed in Tables S4 and S5.

Solubility Measurement

Solubility in Non-Milk-Based Media

For the assessment of CBZ solubility in non-milk-based media (FaSSGF, Pn-FaSSGF FaSSIF, P-FaSSIF 50%, P-FaSSIF 150%, FeSSIF, and Pnc-FeSSIF), 5 mL of each medium was filled into screw cap glass vials and an excess amount of crystalline CBZ was added. The experiments were conducted in triplicate. The samples were equilibrated at 37°C on a GFL 3025 rotation mixer at 20 rpm and aliquots of 200 μL were taken after 1, 2, 4, 6, and 21 h and centrifuged at 13,000 rpm for 90 s. The supernatant was removed and diluted 1:10 in a methanol-water mixture (60:40 *v/v*) prior to high-performance liquid chromatography (HPLC) analysis. Undissolved material was collected *via* centrifugation (13,000 rpm, 90 s) and this residual solid was analyzed *via* X-ray powder diffraction (XRPD). As a reference for XRPD, an excess amount of solid drug was suspended in fresh medium and immediately separated *via* centrifugation prior

to analysis. The patterns showed no polymorphic conversion during solubility testing.

Solubility in Milk-Based Media

Solubility measurements in milk-based media (FeSSGF and Pnc-FeSSGF, respectively) were conducted according to the procedure described by Maharaj *et al.* (14) after 24 and 48 h of equilibration. Briefly, milk-based media were filled into the donor and acceptor compartments of a Vivacon® 500 tube (molecular weight cutoff 500 kDa, Sartorius Stedim Biotech GmbH, Goettingen, Germany). An excess amount of solid drug was added to the donor compartment and the tubes were equilibrated on a rotation mixer at 20 rpm and 37°C. Samples were removed after 24 h from the acceptor compartment and diluted with methanol (1:2) to remove milk proteins and centrifuged (8000 rpm, 15 min). The supernatant was finally removed for HPLC analysis. Undissolved material was collected and prepared for XRPD analysis as described for non-milk-based media and XRPD patterns showed no polymorphic conversion. The experiment was conducted in four replicates. Drug recovery in milk-based media was tested as described in (14).

Dissolution Testing

Dissolution experiments were carried out using a μDISS Profiler® (Pion Inc., Billerica, MA). The total CBZ concentration in the dissolution test was kept constant in relation to the equilibrium solubility and corresponded to about 80% of the experimentally determined solubility value. Establishing real sink conditions in biorelevant media is generally challenging for poorly soluble compounds, and therefore, a constant dose-to-volume ratio was selected for direct comparison of potential influence of media composition on the dissolution rate. A fraction of Tegretol tablet was accurately weighted and added to 20 mL of the pre-heated dissolution medium (37°C). Samples were stirred at 100 rpm and the amount of dissolved drug was determined over 2 h. For Pn-FaSSGF, P-FaSSIF 50%, and P-FaSSIF 150%, the concentration of dissolved drug was measured using *in situ* UV analytics (path length 2 mm, wavelength 300 to 310 nm). *In situ* UV spectra were analyzed using AuPro® (Pion Inc., MA). For Pn-FaSSGF, FeSSGF, Pnc-FeSSGF, FaSSIF, FeSSIF, and Pnc-FeSSIF, drug quantification was done off-line *via* sample removal and HPLC analysis. Samples were removed at 2.5, 5, 10, 20, 30, 45, 60, 75, 90, 105, and 120 min. The milk-based media (FeSSGF and Pnc-FeSSGF) were centrifuged (5000 rpm, 2 min). A range of centrifugation conditions was evaluated to ensure complete separation of undissolved drug material. The supernatant was diluted with methanol (1:2 *v/v*) and centrifuged (5000 rpm, 45 s) to remove milk proteins. The sample was finally diluted 1:10 in methanol-water (60:40 *v/v*) prior to HPLC analysis. The non-milk-based media samples (Pn-FaSSGF, FaSSIF, FeSSIF, and Pnc-FeSSIF) were centrifuged (90 s, 13,000 rpm) and the supernatant was diluted 1:10 in methanol-water (60:40 *v/v*) prior to HPLC analysis.

HPLC Method

Chromatographic analysis was performed on a Waters HPLC system (2795 Seperations Module, Waters Corp., Milford, USA) with a reversed-phase C18 column (XTerra®, 280 × 4.6 mm, 5 μm) in connection with a photodiode array detector (Waters 2996, Waters Corp., Milford, USA). An injection volume of 10 μL at a flow rate of 1 mL/min was eluted with mobile phase (methanol:water, 60:40 *v/v*) and the compound was detected at a wavelength of 285 nm. HPLC samples were analyzed using Empower® 3 (Waters Corp., Milford, USA).

RESULTS

Adult PBPK Model

Simulations with the adult PBPK model were in good agreement with plasma concentrations measured after a wide range of CBZ doses (50 to 800 mg) in both the fasted and fed states, and for different oral IR formulations (solution, suspension, and tablet). A selection of simulated profiles is presented in Fig. 2. Goodness-of-fit plots and additional simulated profiles are provided in the Supplementary Material (Figures S1 and S6–12). A total of 86% of simulated plasma concentrations were within twofold of observed values and 85% of predicted area under the plasma concentration-time curve (AUC_{0-t}) values were within 1.25-fold of observed values.

Scaling of PBPK Model to Pediatric Population

The systemic clearances estimated using the four statistical models for the pediatric patients and by considering the default PEAR scaling for the healthy children are shown in Table III. The highest and lowest systemic clearance estimations from Table III were added to the GastroPlus model and the simulations were compared to the observed profiles. Figure 3a–d shows the simulated and observed plasma concentration profiles in pediatric subjects.

Simulated profiles in healthy children (Fig. 3a) and in child patients (Fig. 3b) were in quite close agreement with the observed profiles irrespective of the clearance used. For the healthy children, the clearance of 0.43 L/h based on default physiologically based scaling led to a slight underestimate of the terminal phase slope and a higher clearance of 0.75 L/h (shown as a dotted line) better matched the observed data. The clearances predicted for a 5.1-year-old child patient taking enzyme-inducing AEDs were higher (ranging from 1.5 to 2.2 L/h) and both values gave simulations close to the observed profile (Fig. 3b).

Figure 3c, d represents data in neonatal patients taking enzyme-inducing co-medications. In both cases, the simulations with the lower clearance value were found to be in better agreement with the observed data. However, while the profile was well predicted in newborns receiving 5 mg/kg CBZ (Fig. 3d), C_{max} was underpredicted in the newborns
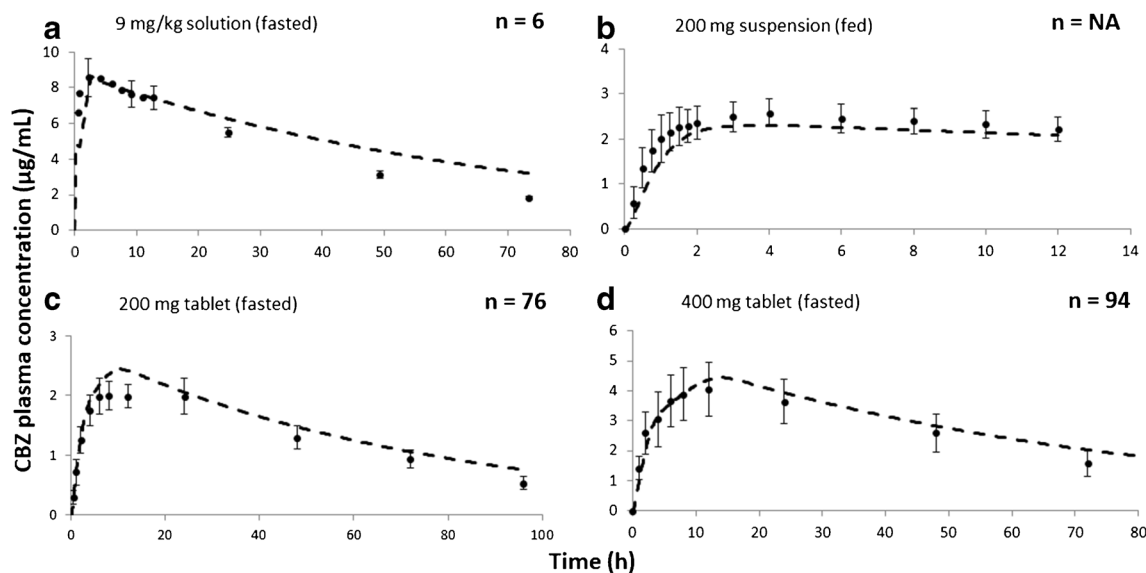
Fig. 2. A selection of simulated plasma concentration-time profiles (dashed line) in adult subjects receiving different doses and formulations in the fasted (**a**, **c**, **d**) and the fed states (**b**). Observed data from the selected studies are shown as circles with error bars (mean ± standard deviation). Source: **a** (37), **b** (36), **c** mean weighted profile (19–25), **d** mean weighted profile (26–35)

dosed 17 mg/kg CBZ (Fig. 3c), even when assuming the lower clearance.

The data in Fig. 3 were all simulated using the fasted-state physiology. However, with the exception of the Bano study (53), which reported drug administration in the fasted state, the published studies did not report any details on the food status. Due to this uncertainty, the simulations were also run assuming fed-state conditions and are shown in Fig. 4. The resulting oral fractions absorbed are provided in Table IV. When assuming fed-state conditions, a slightly higher systemic exposure was predicted in neonates and infants receiving comparatively high doses (> 17 mg/kg). In contrast, the difference was small in children and neonates administered low doses of CBZ (< 10 mg/kg). The fed-state model resulted in a better match between predicted and observed PK profiles in the neonates receiving high CBZ

doses, whereas oral exposures was overpredicted in infants dosed 19 mg/kg CBZ.

Analysis of Oral CBZ Absorption in Pediatric Age Groups

The pediatric PBPK models were used to study the extent and rate-limiting steps of CBZ absorption in the different age groups. Figure 5a illustrates the simulated fractions of drug dissolved and absorbed from the intestine following fasted-state administration for the four pediatric studies. There is very little separation between the dissolution and absorption profiles, suggesting that permeation is not a rate-limiting step for CBZ absorption as expected for the given BCS class 2 compound. No significant differences were observed in this regard between different age groups. Almost complete drug absorption within 4–6 h was predicted in the

**Table III.** Calculated Systemic Clearances for the Respective Estimation Models of the Pediatric Data Sets

Study	Mean age	Estimated steady-state systemic clearance with co-medications[#] (L/h)				Estimated single-dose systemic clearance[$] (L/h)
		Gray	Iribarnegaray	Yukawa	Reith	GastroPlus
MacKintosh (55) (newborns)	6.3 d	*1.34*	**0.08**	0.30	0.62	0.004
Rey (54) (newborns)	20.9 d	*1.19*	**0.31**	0.50	0.97	0.02
Rey (54) (infants/children)	5.1 y	*2.17*	1.63	**1.46**	1.72	0.51
Bano (53) (children/adolescents)	11.3 y	2.75	1.80	1.78	2.87	**0.43**

Values in bold are the low clearance estimates shown as continuous lines in Fig. 3. Italicized values are the high clearance estimates shown as dashed lines in Fig. 3
d days, *y* years
[$] Used default physiologically based scaling of clearance from adults as implemented in GastroPlus PEAR module
[#] Details of models are given in "MATERIALS AND METHODS" section. Gray (81), Iribarnegaray (79), Yukawa (80), Reith (78). Systemic clearance (CL) was estimated from predicted oral clearance (CL/F) using simulated fraction absorbed to estimate F. As food status was not known except for the Bano study (fasted), fraction absorbed was taken as the mean of the fed and fasted states giving 93, 76, 72, and 99% for the four studies. Simulated fraction absorbed for children of different ages and administered dose is shown in Figure S2
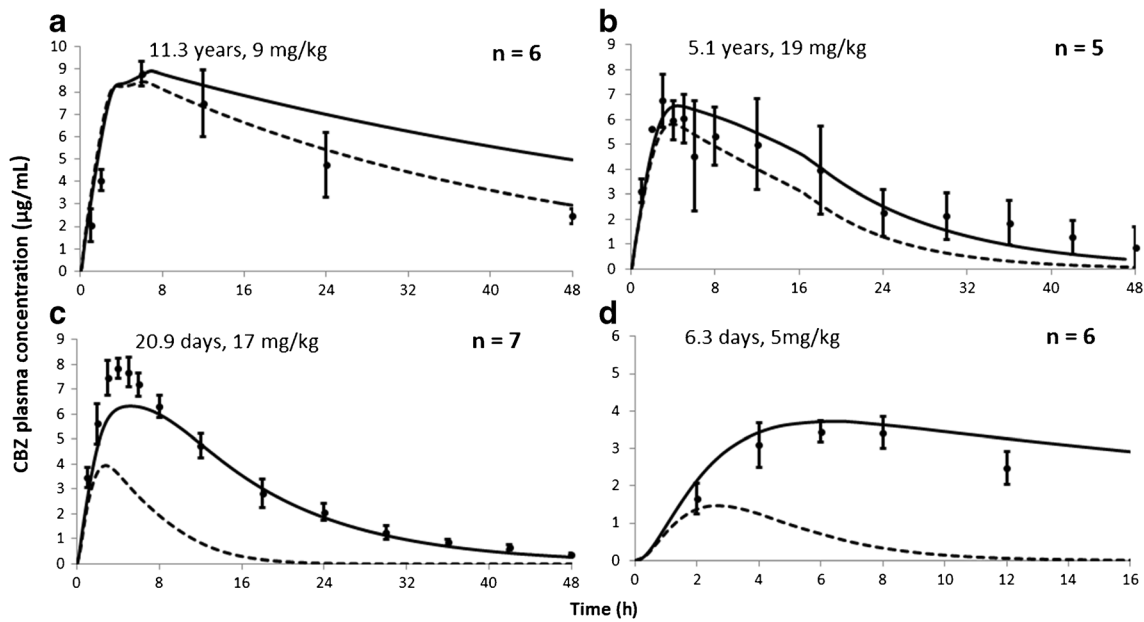
Fig. 3. Observed (black circle, mean ± standard deviation) and simulated CBZ plasma concentration-time profiles for four different studies in pediatric subjects. Mean ages and doses are specified on the figure. The formulations used were as follows: **a** Bano (Mazetol tablet) (53); **b** Rey (suspension) (54); **c** Rey (suspension) (54); **d** MacKintosh (suspension) (55). The dashed line shows the simulated high clearance scenario while the continuous line is the low clearance scenario. Clearances were estimated as described in "MATERIALS AND METHODS" section and values used are given in Table III. In **a**, the lower clearance (0.43 L/h) was based on default physiological-based scaling in GastroPlus (Table III) and the higher clearance value of 0.75 L/h was increased to match the terminal half-life of the observed data

older children and adolescents (mean age 11.3 years) receiving a mean dose of 9 mg/kg. In newborns receiving low CBZ dose (5 mg/kg), drug absorption was complete but comparatively slow. In contrast, absorption was incomplete in children and newborns receiving high CBZ doses (17 and 19 mg/kg).

Figure 5b shows the simulations for the fed-state conditions. The most significant difference in absorption with food was observed at the higher doses both in the newborns and children. At these doses, CBZ fasted-state absorption was incomplete at only 50–60% and food resulted in a 10–20% increase in the fraction absorbed in both age groups.
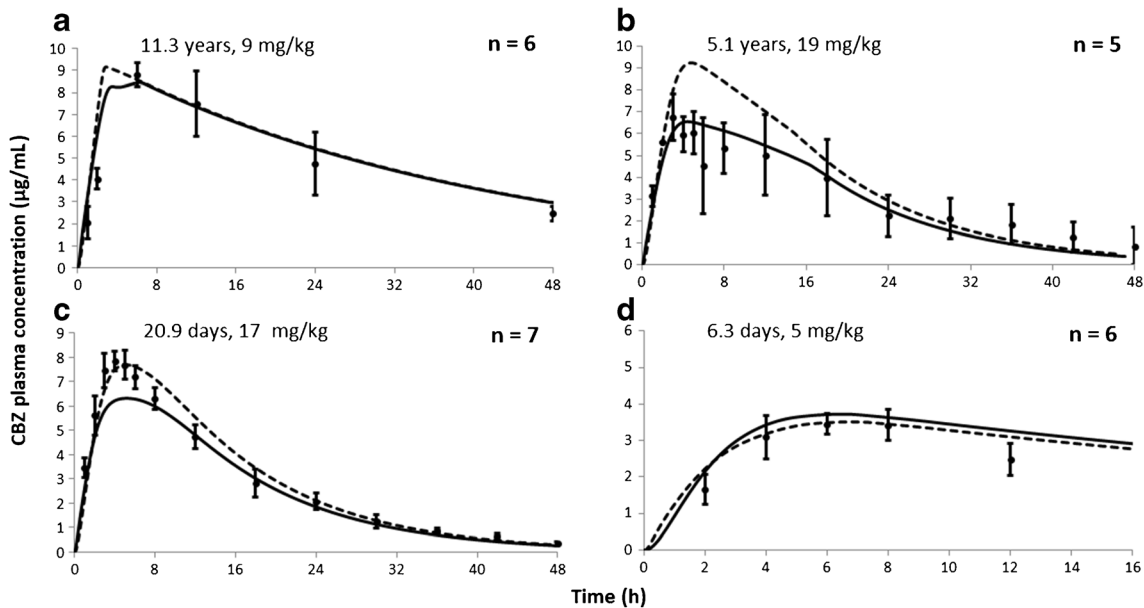
Fig. 4. Observed (black circle, mean ± standard deviation) and simulated plasma concentration profiles assuming either fed (dashed line) or fasted (continuous line) state in pediatric age groups. Source: **a** Bano study (children/adolescents; Mazetol tablet) (53); **b** Rey study (children; suspension) (54); **c** Rey study (neonates; suspension) (54); **d** MacKintosh study (neonates; suspension) (55). All simulations were run using the lower clearance values

Table IV. Comparison of Predicted Oral Fa (%) in the Four Pediatric Study Populations When Assuming Fasted- and Fed-State Physiologies

Study	Mean age	Dose (mg/kg)	Predicted Fa (%)	
			Fasted state	Fed state
MacKintosh (55) (newborns)	6.3 d	5	91	95
Rey (54) (newborns)	20.9 d	17	72	81
Rey (54) (infants/children)	5.1 y	19	66	80
Bano (53) (children/adolescents)	11.3 y	9	99	100

Fa fraction absorbed, *d* days, *y* years

While the predicted fraction absorbed was > 80% with low-dose CBZ (5–9 mg/kg) in both younger and older age groups, the extent of drug absorption was notably reduced in the high dose range (17–19 mg/kg) irrespective of age.

Parameter Sensitivity Analysis

Parameter sensitivity analysis was performed on critical parameters for oral drug absorption for the four pediatric models. The results depicted in Fig. 6a, b show that the fraction absorbed was most sensitive to variations in CBZ solubility and dose. The influence of small intestine transit time (Fig. 6c), particle size (Fig. 6d), fraction of small intestine fluid volume, small intestine radius and length, permeability, and bile salt solubilization ratio was more

Fig. 5. Predicted fraction of CBZ dissolved (dotted line) and absorbed (continuous line) from the intestine *versus* time for the four pediatric studies assuming **a** fasted state and **b** fed state. The corresponding oral doses are detailed in brackets

pronounced for high-dose CBZ, but was minor for low-dose CBZ, independent from the age group. In general, stomach volume, stomach transit time, and dose volume were less influential on drug absorption. The sensitivity analysis plots for all parameters are given in the Supplementary Materials (Figure S3).

In Vitro Dissolution Testing

In vitro dissolution of CBZ was generally fast with almost 80% dissolved within 20 min. No difference in dissolution kinetics was observed between pediatric and adult simulated gastric and intestinal fluids, suggesting similar dissolution behavior of CBZ in adults and pediatric subjects. The results of the *in vitro* dissolution tests of CBZ in biorelevant media are displayed in Figure S4.

DISCUSSION

In this study, PBPK modeling and *in vitro* dissolution testing were employed to investigate the age dependency in oral absorption of the poorly water-soluble compound CBZ. A good match of simulations to observed data in healthy adults was seen for solution, suspension, and tablet formulations in both fasted and fed states which is consistent with previous GastroPlus modeling of immediate and extended release CBZ (36).

The verified adult model was extrapolated to simulate the single oral dose concentration *versus* time profile obtained in a study in healthy children/adolescents of average age 11 years (53) by using the default GastroPlus physiologically based age-dependent scaling. The simulated profile was in excellent agreement to observations for the absorption phase, but a slight underestimate of the terminal half-life was seen and an increase in clearance by a factor of 1.7 was necessary to match the observed profile. The current CYP3A ontogeny profile in GastroPlus is based on work of Johnson (70) which relies on *in vitro* measurements of enzyme levels in children. More recently, Upreti *et al.* (85) reported a method to derive *in vivo* ontogenies and showed higher *in vivo* CYP3A activities than has been estimated from *in vitro* data. Indeed in Upreti's study with the probe substrate sufentanil, the CYP3A activity in the infant and child exceeded adult values. Our example of CBZ seems to support this finding although other reasons for the apparent under prediction of CBZ clearance in these children are possible and further analysis with different CYP3A substrates is needed to fully understand the ontogeny of this enzyme. The estimated single-dose
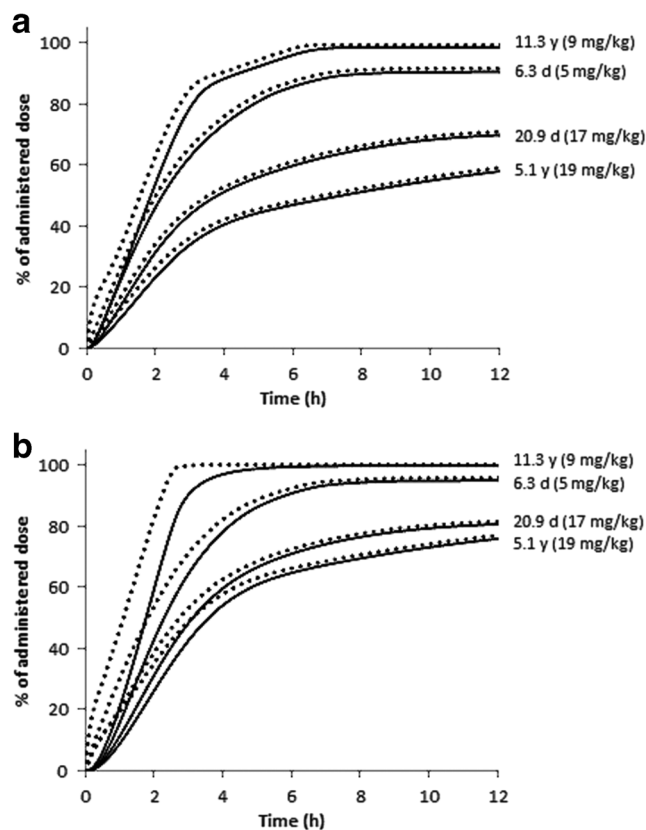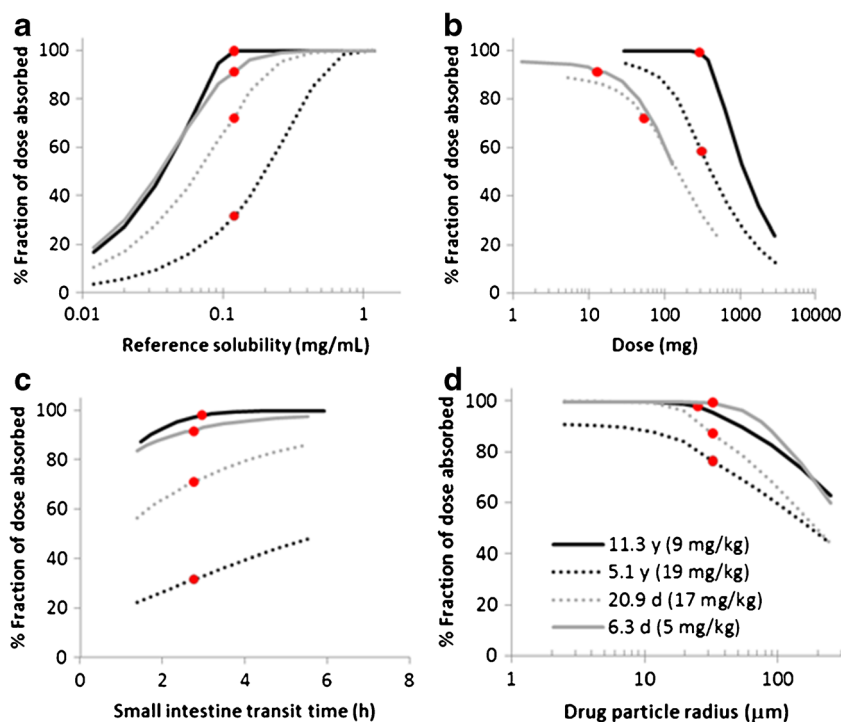
Fig. 6. Parameter sensitivity analysis results for **a** reference solubility, **b** dose, **c** small intestine transit time, and **d** drug particle radius. Gray-colored profiles represent data in neonates; black profiles are data in older children; low doses are illustrated as continuous lines and high doses as dotted lines. The baseline values used in the models are shown as a red circle

clearance of 0.75 L/h is 2.4 to 3.8 times lower than the estimated steady-state clearance for an 11-year-old child taking AEDs as predicted by the four models used in this study (Table III). This is consistent with the increase which is expected due to a combination of auto-induction of CBZ (estimated as approximately twofold in adults (86) and in children (87)), and the additional induction due to co-medications (predicted to be less than ~2-fold based on the four models used in this study).

All other pediatric studies in our analysis were from children being co-medicated with enzyme-inducing AEDs and so it was necessary to estimate the induced CBZ clearances before simulating single-dose oral profiles. To obtain full plasma concentration profiles showing the effect of enzyme-inducing co-medications on CBZ proved to be difficult. However, we did find four different statistical models for prediction of steady-state oral CBZ clearance in patients taking AEDs. These models gave consistent clearance estimates in a 5-year-old child with a range of only 1.5-fold between the model predictions (Table III). However, a much wider range of prediction was obtained in very young children with a fourfold range in a 21-day-old and 16-fold range in a 6-day-old (Table III). This uncertainty is not unexpected since none of the data sets used to build these clearance models included data in neonates (Table S2). Indeed, the ages of the children on which these models are based are biased towards older ages and only the Iribarnegaray model includes age as an independent predictor of clearance while the other models are based on body size (either body weight or body surface area). As CYP3A activity is low at birth and develops rapidly, an over

prediction of clearance in neonates is to be expected with these models. This was indeed seen when using the range of predicted clearances in the PBPK absorption models. Simulated oral profiles were close to the observed clinical data (54) in the 5-year-old child independent of the systemic clearance used. However, simulated oral profiles in neonates were in much better agreement to observed data when using the lowest clearance estimates. Overall, it seems likely that the development in enzyme expression is not captured in these models due to the low number of data in very young children. However, as simulations with the lowest clearance were close to observations and the simulated and observed half-life for the neonates were in good agreement where concentrations were measured up to 48 h (54), we were reassured that the predicted clearance was appropriate. Interestingly, these estimated CBZ clearances in neonates are significantly higher (15- to 20-fold) than predicted with the default scaling in GastroPlus (Table III). This default scaling has assumed that CYP3A4 metabolism is the sole clearance route whereas other enzymes including CYP3A5 and CYP2C8 are involved (88,89). Furthermore, the assumed ontogeny of CYP3A starting from zero activity at birth and reaching only 4% of adult levels by the age of 6 days is also questionable based on recent *in vivo* analyses (85).

Simulation of fraction of the dose absorbed was almost complete in adults (96% of a 200-mg dose and 91% of a 400-mg dose) while in children it was reduced and showed a clear dose dependency. Neonates receiving a dose of 5 mg/kg showed almost complete simulated absorption (> 90%) but this was reduced in the same age group after administration of 17 mg/kg. A similar behavior was predicted in older

children, with complete absorption at 9 mg/kg, while 19 mg/kg resulted in a fraction absorbed of only 66%. These observations are comparable with the reduced fraction of dose absorbed in children compared to that in adults reported for another BCS class 2 molecule, montelukast, using the SimCYP pediatric module (12). Further work on additional molecules from different BCS classes is needed before conclusions on behavior of different BCS classes in pediatrics can be drawn.

It must be considered that the currently available pediatric PBPK models are based on certain assumptions (Table S7, Supplementary Materials) and their predictive capability needs more thorough verification. Although good predictions of oral CBZ exposure were obtained with the current datasets, sensitivity analyses were conducted to scrutinize how age-dependent changes in the GI tract may affect oral exposure. At high dose levels, the oral fraction absorbed was sensitive to the intestine length and transit time, suggesting that, due to the smaller GI tract, the residence time in the gut is critical for complete absorption of CBZ. In contrast, this effect was less pronounced for lower doses (e.g., 5 mg/kg in neonates), in which case almost complete absorption was predicted for a broad range of small intestine transit times and lengths. Similarly, use of fed-state conditions had almost no impact on the extent of absorption simulated at low dose, but showed a significant increase for high CBZ doses.

Aqueous solubility was another sensitive parameter for oral exposure. This parameter showed the most pronounced influence on the oral fraction absorbed in high-dose patients (also illustrated in Figure S5), along with the physiological and anatomical parameters determining the residence time in the intestine (length, radius, transit times). This observation highlights the importance of accurate solubility prediction for reliable simulations with poorly water-soluble compounds. As newborns and infants are fed in regular, short intervals, there is likely to be a prolonged presence of milk in the intestine which could facilitate drug dissolution and increase the fraction absorbed. These considerations might explain the more accurate simulations seen when assuming fed-state physiology in neonates. A similar observation was made for two BCS 1 compounds where a prolonged gastric emptying improved simulations (11). The differences in gastric and intestinal fluids between adults and children (e.g., the presence of milk due to frequent infant feeding) may not only affect the overall solubility of the compound but as well its dissolution rate. The PBPK model assumed no age-dependent difference in dissolution rate and, to test this model assumption, we conducted dissolution studies using the proposed pediatric (14) and adult biorelevant media. The *in vitro* studies suggested no influence of media composition on the dissolution rate of CBZ from immediate release tablets. It must be noted that the simulated pediatric gastric and intestinal media used for dissolution testing were based on age-specific changes in GI fluid parameters which were obtained from the literature (e.g., pH, osmolarity, concentration of bile salts) (14). However, there still exist significant knowledge gaps in this regard, and therefore, the media represent a rough estimate of GI fluid composition. Prospective studies are still required to confirm their biorelevance.

Some uncertainty was related to the drug particle size parameter, which was found to be a sensitive parameter at high doses. The uncertainty in this parameter value could not be fully ruled out due to the lack of product-specific particle size data. However, dissolution data showed that the dissolution rate from Tegretol tablets was generally fast, with almost 80% dissolved within 20 min, and suggested no influence of medium (with or without milk) on the kinetics of drug release.

The impact of age-specific differences in GI tract physiology on oral exposure have been discussed by several authors recently who have provided theoretical considerations supporting the need for a pediatric BCS for drug development and regulatory purposes (3,4,9,10,13,90–92). However, clinical data in children are very limited and the challenge of isolating pre-absorptive from post-absorptive processes has restricted a clear assessment of the *in vivo* relevance of the proposals. Mechanistic absorption modeling, as presented in the current study, provides valuable insights into the oral absorption process in children and shows that age can influence both the rate and extent of oral absorption. Furthermore, the fraction absorbed of CBZ was strongly dose-dependent which also needs to be carefully considered as children may sometimes require higher doses per unit body weight than adults. Differences in dose and physiology should also be considered when conducting relative bioavailability studies to compare different formulations since the rate and extent of oral absorption might be limited by different factors depending on the dose and age of the patient population. The relevance of studies conducted in adults for data extrapolation to children needs careful consideration, and oral absorption modeling should be an important component in the toolkit for pediatric drug development and regulatory questions.

CONCLUSIONS

This work has shown that if a poorly soluble drug like CBZ is given at high doses, the reduced GI size and transit times may lead to an age-dependent reduced fraction absorbed in pediatric patients. Furthermore, assuming fed-state conditions showed an improvement in simulation results in neonates, believed to be due to slower gastric emptying rates and higher solubility caused by presence of milk. Overall, the simulation results in this study supported the GastroPlus age-based scaling of absorption in pediatric populations and work on additional molecules from different BCS classes is needed to further verify the predictive capability of pediatric PBPK models for compounds of different BCS classes. Care is needed when extrapolating the biopharmaceutical performance of orally administered compounds from adults to pediatric patients. There still exist significant knowledge gaps with regard to the characteristics of the pediatric GI tract which requires further research. Such knowledge will also help in the design of more biorelevant *in vitro* and *in silico* tools to predict the biopharmaceutical performance of drug products.

This study further demonstrates the importance of research to develop a pediatric relevant BCS and improve pediatric absorption modeling tools which can aid the design of formulations and support appropriate clinical studies in children.

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