Extrapolation of midazolam disposition in neonates using physiological based pharmacokinetic/pharmacodynamic (PBPK/PD) modelling

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Abstract

Objective:

To explore the feasibility of model simulation research strategies for dose optimization in the neonatal populations. Using midazolam as a model drug, a PBPK/PD model was established to simulated and optimize the dosing regimen for sedation in the neonatal population.

Methods:

Firstly, an adult PBPK/PD model was established. Secondly, the research strategy of extrapolating adult drug use data to newborns was applied. The adult PBPK/PD model was extrapolated to the neonatal population according to the maturation formula of plasma albumin and metabolic enzyme CYP3A4/5. The robustness of the neonatal model was evaluated using clinical data from different age stratification. The neonatal PBPK/PD model was then used to simulate the dosage regimen of midazolam for sedation in newborns.

Results:

Individualized validation in adults showed that 95.1% of the predicted concentration values were within two-fold, and all the predicted AUC values were within two-fold; the extrapolated neonatal model showed that about 84.4% of the predicted concentration values were within two-fold, the AAFE value of the overall model was < 2, and the AFE value was between 0.5–1.5; the validated neonatal PBPK/PD model showed that virtual term neonates maintained a target plasma concentration range within 26 hours when using the dosage regimen recommended on the product label (0.06 mg/kg/h, iv infusion 12 hours), the optimal dose for premature infants to reach the target plasma concentration range may need to be slightly higher than the recommended dose on the product label (0.03mg/kg/h, iv infusion 12h).

Conclusion:

We successfully established a neonatal PBPK/PD model of midazolam by referring to extrapolated-based research strategy and integrating the influence of human physiological development on drug disposal. Finally, the model was validated with the dosage of midazolam in the product specification, and reliable results were obtained.

1. Introduction

In recent years, pediatric drug development has received extensive attention. The FDA has issued a series of industry guidelines (Best Pharmaceuticals for Children Act, Pediatric Research Equity Act, Pediatric Guidances) aimed at improving drug research in the pediatric population, however, the issue of drug

availability in children has not been solved(D'Errico et al.) (D'Errico et al., 2022). The problem is particularly acute in the neonatal population(Meng et al., 2022). In fact, in neonatal units, where the proportion of patients receiving at least one unlicensed or "off-label" drug varied from 80–100%, and 3.7% of newborns died from adverse drug reactions(Kaguelidou et al., 2016). The history of unexpected adverse reactions in neonates is alarming, such as chloramphenicol-related grey baby syndrome(Pauwels and Allegaert, 2016; Thyagarajan and Deshpande, 2014), sulfonamide-related nuclear jaundice(Thyagarajan and Deshpande, 2014), benzocaine-related methemoglobinemia(Arnold, 2021), and others. The root cause of the high incidence of adverse drug reactions in the neonatal population is the immaturity of the body's anatomy, physiological structure, and organ function, resulting in age-related differences in drug treatment and efficacy. This is the main reason why newborns cannot use adult doses directly(Ruggiero et al., 2019). In the early stage of liver and kidney development, bile acid metabolism decreased, metabolic enzyme expression showed different maturation patterns, renal blood flow decreased, renal tubule secretion and reabsorption limited, which all affected the metabolic kinetics of drugs(Ruggiero et al., 2019; Smits et al., 2012).

Because newborns are at the earliest stages of life development and the physiological characteristics of this group are highly complex, neonatal drug research faces particular challenges. Traditional dosing regimen adjustments are usually scaled by body weight (BW) or body surface area (BSA), which makes it challenging to describe changes in maturity and would result in incorrect calculations(Lack and Stuart-Taylor, 1997). Neonatal clinical trials involve vulnerable groups, increased morbidity and adverse reactions, and ethical issues. Moreover, the trial protocols and procedures applicable to adults and older children cannot be directly scaled to developing newborns, so there are specific difficulties in selecting enrollment groups, the design of drug administration protocols, and the selection of evaluation indicators(Coppini et al., 2016). These challenges make Model-Informed Drug Development (MIDD) an efficient and innovative tool for pediatric drug research.

Modeling methods commonly used in pediatric populations include PBPK model and popPK model. Compared with popPK model, PBPK model can use the physiological characteristics of model parameters to integrate age-related physiological differences to simulate the pharmacokinetics of specific age groups(Verscheijden et al., 2020). Nevertheless, as the most vulnerable and least studied group, neonatal PBPK modeling remains challenging, with biological physiological parameters, organ size and blood flow, gastrointestinal transit time and pH, protein binding, hepatic metabolic enzymes, renal function ontogenesis, all undergoing significant changes during the first weeks of life. The lack of understanding of pediatric development and related age-dependent changes makes it difficult to develop neonatal PBPK models. The complexity of clinical trials in practical application also limits the availability of neonatal PK data, and it is also necessary to consider whether the pathophysiological mechanism of the disease and the mechanism of drug action allow the extrapolation of the model to this special population(Lin et al., 2022).

This study used midazolam, a widely used intravenous anesthetic, as the model drug. Previous studies have been published on midazolam in neonatal populations (Abduljalil et al., 2020; Mansoor et al., 2019),

but no mechanically explored effects of physiological development changes on drug disposal in newborns. Therefore, we tried to establish a PBPK/PD model to characterize midazolam in adults and extrapolated this model to the neonatal population, then used the validated PBPK/PD model to evaluate the recommended dose of midazolam during neonatal sedation, providing a scientific basis for labeling midazolam for pediatric use, and also providing theoretical support for neonatal PBPK modeling and clinical application.

2. Materials and Methods

2.1 Clinical data for PBPK modeling

For the midazolam PBPK model, we systematically searched the "Pubmed" database using terms (midazolam and pharmacokinetics and intravenous injection) as well as filters (human adult or pediatric population). Finally, according to the appropriate route of administration and study subjects, we selected 16 intravenous injection studies in adult population and 8 pediatric population to establish and evaluate the established PBPK model. The dose ranges from 0.3-12.5mg for adults and 0.1–10 mg for the pediatric population. Tables S1 and S2 summarize the demographic data and study details for the adult and pediatric populations, respectively. Midazolam plasma concentration vs. time profiles from the literature were digitized using GetData Graph Digitizer (V2.26; Kogarah, Australia).

2.2 Adult PBPK Model Development

The PBPK model for midazolam was developed for adults using the software GastroPlus™ (V9.8; Simulation Plus Inc., Lancaster, CA, USA). The PBPK model was developed using data on midazolam's physicochemical and in vitro properties, as listed in Table 1.

Table 1
Midazolam physiologically based pharmacokinetic model parameters

Parameter	Unit	Value
MW	g/mol	325.77
Solubility	mg/mL	0.055@pH = 8.57
ccLogP	-	2.7
PKa1 (Base)	-	3.48[a]
PKa2 (Base)	-	5.93[a]
ABSCa Peff	cm/s*10^5	3.33[b]
Rbp	-	0.55[c]
fu,p	%	4
CYP3A4/5 Km	μM	1.88
CYP3A4/5 Vmax	pmol/min/mg protein	852.3
[a]From literature, and fit model by GastroPlus™ software		
[b]Predicted values by MembranePlus™ software		
[c]Adjusted by GastroPlus™ software		

We established the intravenous PBPK model, mainly considering the influence of physicochemical parameters on drug distribution and clearance. The key parameters affecting the distribution of midazolam in vivo are tissue type, tissue distribution (Kp) calculation method, Oil-water distribution coefficient (LogP), negative base-10 logarithm of the acid dissociation constant (pKa), blood plasma ratio (Rbp), Plasma protein unbinding fraction (fu,p), etc.; clearance is related to the effects of hepatic metabolic enzymes. Midazolam is a Biopharmaceutical Classification System (BCS) class I drug(Liu et al., 2020), and it has high permeability and solubility. Combined with its lipophilic properties(Erstad and Barletta, 2020), the amount of medicine that partitions into the tissue will be limited by the blood flow rate (perfusion rate) through the tissue. Therefore, we hypothesize that the dynamics of the drug in tissue can be described by perfusion rate limiting kinetics(Jones and Rowland-Yeo, 2013). Predictions for Kp were calculated as Rodgers, Leahy and Rowland described to incorporate electrostatic interactions, which might result in tissue binding. The LogP and fu,p parameters were selected as the experimental values in the literature(de Vries et al., 1990; Mulla et al., 2003). The pKa and Rbp parameters were optimized according to the built-in algorithm of Gastroplus™ software and the physicochemical properties of the drug. The in vitro to in vivo extrapolation (IVIVE) module in GastroPlus™ was chosen to predict

midazolam's enzymatic kinetic parameters (Km and Vmax) in vivo(Poirier et al., 2009). Other settings have defaulted in the software.

2.3 Adult PBPK Model Evalution

Based on the established PBPK model, we used the physiological module of Gastroplus™ to create a virtual adult with age, height, weight, and body mass index (BMI) of 30 years old, 176.3cm, 70kg, and 22.5 kg/m2, respectively, to simulate the typical values in the healthy adult population. This individual will represent the average individual in the created population. At the same time, the "population simulator" module of the software was used to build 10 clinical trials with ten individuals in each trial. 100 virtual groups (1:1 male to female ratio) of individuals aged between 18 and 55 were used to characterize midazolam PK behavior in the population. All virtual populations were established based on the dosing regimen, and the model's predictive performance was evaluated by visually inspecting the observed values, the predicted mean values of the population, and the concentration ranges from 5th–95th. Model fitting was evaluated by comparing the predicted plasma concentration-time profiles and observed in vivo adult data(Yan et al., 2012), and by using the average folding error (AFE) and the absolute average folding error (AAFE), the predicted PK curves are compared with the observed obtained in the literature(Sun et al., 2018) (as shown in equations 1 and 2).

$$AFE = 10^{\frac{1}{n}*\sum \log(\frac{Predicted_i}{Observed_i})} \quad \text{eq1}$$

$$AAFE = 10^{\frac{1}{n}*\sum \left|\log(\frac{Predicted_i}{Observed_i})\right|} \quad \text{eq2}$$

Where n is the number of time points at which the sample collecting, Predictedi, and observedi are the predicted and observed concentrations at a given time point i. Overall, the PBPK model performance was deemed acceptable if the AFEs were between 0.5 and 2, AAFEs <2, and all predicted values of PK parameters were within the 2-fold range(Sjöstedt et al., 2021).

2.4 Adult PBPK Model Extrapolates the Pediatric Population

2.4.1 Physiological Parameters in the Pediatric Population

We used the age-dependent algorithm built into Gastroplus to generate basic anatomical and physiological parameters, including organ weights, composition, regional blood flow, volume, cardiac output, lipids, moisture, respiratory parameters.

2.4.2 Scaling Unbound Fraction

Midazolam mainly binds to plasma albumin and belongs to high plasma protein binding drugs. Its plasma-free drug fraction (fu,p) is about 3–4%(Franken et al., 2016). Combined with the previous literature describing the mature function of age-related individuals with albumin(Johnson et al., 2006), we

can extrapolate the unbound portion of midazolam in the pediatric population from the unbound fraction value in adults (as shown in equations 3 and 4).

$$\mathrm{Alb}\ (\mathrm{g/L}) = 1.1287 * \ln(\mathrm{Age}) + 33.746 \qquad \text{eq3}$$

$$\mathrm{fu}_{\mathrm{pediatric}} = \frac{1}{(1+(1-\mathrm{fu}_{\mathrm{adult}}) * \frac{[\mathrm{P}]_{\mathrm{pediatric}}}{[\mathrm{P}]_{\mathrm{Adult}} * \mathrm{fu}_{\mathrm{adult}}}} \qquad \text{eq4}$$

where Age is measured in days, [P] is the plasma protein concentration, and fu_{Adult} is the average unbound fraction of the drug in healthy adults.

2.4.3 Scaling Hepatic Clearance

We used a physiologically based approach to calculate hepatic clearance of midazolam. Physiological scaling of hepatic clearance is based on the following assumptions(Edginton et al., 2006): children and adults have the same clearance pathway; hepatic clearance model uses the well-stirred model; enzyme metabolism follows the first-order kinetic theory.

As a significant probe substrate for CYP3A4/5, midazolam can be assumed that the metabolic pathway of both adult and pediatric populations is mainly hepatic microsomal enzyme CYP3A4/5 metabolism. In the neonatal population, CYP3A7, which occupies the dominant role of hepatic enzyme during the fetal period, gradually decreases in the third trimester(Hines, 2007). The level of hepatic CYP3A7 in neonates decreased significantly after birth, while CYP3A4 gradually increased after birth, becoming the major CYP3A subtype(Li and Lampe, 2019). CYP3A4 and CYP3A7 appear to mirror each other from embryo maturation to birth and growth. This pattern of maturation allows our hypothesis to be valid. The individual intrinsic clearance of midazolam and the individual maturation function of the related metabolic enzyme CYP3A4/5 have been previously reported(Strougo et al., 2014) (as shown in Eq. 5).

Drug clearance of hepatically cleared compounds depends on several factors, including intrinsic clearance, hepatic blood flow, the extent of protein binding, and transport processes (e.g., biliary excretion, transporters)(Edginton et al., 2006). Firstly, the IVIVE method was used to convert the CYP3A4/5 enzymatic kinetics value obtained from in vitro experiments into the intrinsic clearance of adults (CLint, Adult)(Xiao et al., 2019). Combined with physiological parameters such as liver weight (ICRP, 2002), hepatic microsomal protein content(Bunglawala et al., 2020) in adult and pediatric, and maturation function that characterizes ontogenesis of CYP3A4/5 enzyme activity, calculated the intrinsic clearance in the pediatric population (CLint, child) (as shown in Eqs. 6 and 7). Next, the plasma clearance of pediatrics needs to be calculated according to the data of intrinsic hepatic clearance (CLint, H), hepatic blood flow (QH), fu,p, and Rbp(Brussee et al., 2018) (as shown in Eq. 8).

It should be noted here that when characterizing hepatic blood flow in pediatric populations, assumptions need to be made that: blood flow per unit of liver weight in children and adults is the same(Edginton et al.,

2006); the percentage of cardiac output to the liver remains constant with age(Arya and Ramji, 2001). On this basis, since the hepatic blood flow of the pediatric population is difficult to obtain, referring to the physiological values of hepatic blood flow and liver weight, we can replace QH in the formula with liver weight here, which has negligible influence on the results. Finally, the in vivo disposal of midazolam does not necessitate active transportation.

$$\text{CYP3A4/5(Relative expression)} = \frac{0.2953 * \text{Age}}{0.1045 + \text{Age}} + \frac{0.7290 * \text{Age}}{1.135 + \text{Age}} \quad \text{eq5}$$

$$\text{MPPGL (mg/g)} = 10^{1.407 + 0.0158 * \text{Age} - 0.00038 * \text{Age}^2 + 0.000002 * \text{Age}^3} \quad \text{eq6}$$

$$\text{CL}_{\text{H,int,child}} = \frac{\text{CL}_{\text{H,int,adult}}}{\text{MPPGL}_{\text{adult}} * \text{LW}_{\text{adult}}} * \text{MPPGL}_{\text{child}} * \text{LW}_{\text{child}} * \text{f (Age)} \quad \text{eq7}$$

$$\text{CL}_{\text{plasma}} = \frac{\text{Q}_{\text{H}} * \text{CL}_{\text{H,int}} * \text{f}_{\text{u,p}}}{\text{Q}_{\text{H}} + (\text{CL}_{\text{int}} * \text{f}_{\text{u,p}}) / \text{B : P Ratio}} \quad \text{eq8}$$

Where MPPGL is the microsomal protein amount per gram of liver, CLH, int, child and CLH, int,adult were intrinsic hepatic clearance in the pediatric population and adults, respectively. f(Age) is the function of age representing the maturation function that was used to characterize the ontogeny of the CYP3A4/5 enzyme activity. In Eq. 5, Age is in years, and in Eq. 6, Age is in days.

2.5 Pediatric PBPK Model Evaluation

Referring to the evaluation strategy of the adult PBPK model and the age stratification of the pediatric population specified by ICH, we created an adolescent individual aged 15 years, a child individual aged 7 years, a 12-month infant individual, a full-term newborn individual with gestational age of 40 weeks and 5 days of birth, and a premature newborn individual with gestational age of 33 weeks and 5 days of birth through the "PBPK" module of the Gastroplus™ software to simulate typical values in the pediatric population. Each individual's height, weight and BMI were predicted by the software. Using the "population simulator" module of the software, a virtual population of 100 individuals (1:1 male to female ratio) was obtained by creating 10 individuals in each of the 10 clinical trials, respectively simulating the preterm infants with gestational age of 27–37 weeks and within 0–27 days of birth, the full-term newborns with gestational age of 40 weeks and within 0–27 days of birth, the infants aged 2–23 months and children aged 2–12 years. The AFE and AAFE method was used to evaluate the model fit.

2.6 PBPK/PD Model Development

A comprehensive literature review was undertaken to identify pharmacokinetic- pharmacodynamic (PKPD) studies on midazolam. We systematically searched PubMed with the terms (Midazolam AND Pharmacokinetic AND bispectral index), and the filters applied "Human adults." Titles and abstracts were screened to identify studies detailing midazolam use alone as a sedative. The bispectral index (BIS) is a scale derived from measuring cerebral electrical activity in anesthetized patients, and also the most widely used measure among the various anesthetic depth indices. BIS values were divided into four deep

sedation categories: 81–100 were defined as light sedation, 61–80 as moderate sedation, 41–60 as deep sedation, and ≤ 40 as deep sedation(Johnson et al., 2005).

We extracted data from the study of Miyake W. et al. (BIS scores over time under different midazolam dosage regimens) (Miyake et al., 2010). Using the PBPK model established before and the dosing scheme mentioned in the literature, the simulated drug concentration-time curve was obtained. Therefore, combined with the PBPK model, the Sigmoidal Emax model of the BIS score with time was derived using the direct-effect model (as shown in Eq. 9). The target was assumed to be a BIS score of approximately 60–70 (Miyake et al., 2010).

$$E_{(BIS)_{adult}} = E_0 - \frac{E_{max} * C_e^{Hill}}{EC_{50}^{Hill} + C_e^{Hill}} \qquad eq9$$

Where E0 is the baseline response, Emax is the maximum possible midazolam effect (equal to 100 on the BIS scale), Ce is the concentration in the effect compartment, EC50 is the concentration at which 50% of the maximum response is observed, and the Hill coefficient to represent the typically steep dependence of the pharmacological effect on concentration changes in the effect compartment.

Midazolam is commonly used for sedative anesthesia during mechanical ventilation in pediatric populations, especially neonates, often intravenously and intranasally(Arbour, 2004). The indications of the drug in the pediatric population coincide with those in the adults. Jacqz-Aigrain et al(Jacqz-Aigrain et al., 1994), Anand et al.(Anand et al., 1999), and Sheridan et al.(Sheridan et al., 1994) used midazolam to sedate neonates undergoing mechanical ventilation. Midazolam has resulted in safe and effective sedation. de Wildt SN et al.(de Wildt et al., 2005) investigated the possible pharmacokinetic-pharmacodynamic relationship of midazolam in pediatric intensive care patients. Twenty-one pediatric intensive care patients (2 days to 17 years) received a midazolam infusion (0.05–0.4 mg/kg/h, 3.8 hours to 25 days). Sedation levels were determined using the COMFORT scale as well as plasma concentrations of midazolam and metabolites. An evident PK-PD relationship was not found. Swart et al. (Swart et al., 2012) stated that from the clinical studies it is clear that there is a large variability in response at a given concentration of midazolam. The pharmacodynamic results of midazolam cannot be well quantified at this time, so we hypothesize that adults and children here have similar exposure responses to midazolam (Germovsek et al., 2019).

2.7 PBPK/PD Model Application

The final pediatric PBPK model was used to assess the probability of achieving sedation goals in virtual newborns using the recommended dose of midazolam product labels. The "Population Simulator" module built-in Gastroplus™ software was used to construct 100 virtual groups of full-term newborns with gestational ages of 40 weeks and 0-27days and 100 virtual groups of premature newborns with gestational ages of 27-37weeks and 0-27ddays, respectively, to predict the blood concentration of

midazolam under a given regimen. Plasma concentration profiles were plotted using GraphPad (Version 6.1) to determine if therapeutic levels would likely be achieved.

3. Result

3.1 Adult PBPK model prediction

Based on the drug parameters and the human physiological information, we successfully established an adult PBPK model. As shown in Fig. 1, the midazolam PBPK model adequately simulates the PK profiles of single intravenous injection at 0.3-12.5mg dose. Most visually observed values for plasma drug concentrations in midazolam were in the 5th-95th percentile of the simulation.

The goodness-of-fit plot showed that approximately 57.2% of the observed drug concentrations were within a 1.25-fold error of the predicted values, and 95.1% of the observed drug concentrations were within a two-fold error (Fig. 2a). Approximately 79.0% of the observations for the area under curve (AUC) were within a 1.25-fold error of the predicted values. All AUC observations were within a two-fold mistake (Fig. 2b). In addition, comparing the observed and predicted drug concentration data, the AFE values were within 0.5–1.5, and the AAFE values of all datasets were < 2 (Table S1). In summary, the virtual adult PBPK model is well constructed and can be used for follow-up studies.

3.2 Pediatric PBPK model prediction

After scaling physiological parameters such as plasma protein binding fraction and liver clearance, the established pediatric PBPK model was first evaluated using the published plasma concentration and time data (Table S2). The evaluation results were shown in Fig. 3, and about 84.4% of the observed drug concentration errors were within a two-fold error. The overall model AAFE values were < 2, and the AFE values were within 0.5–1.5 (Table S2).

Simultaneously, we simulated the method of establishing a population model for different pediatric subgroups by simulating clinical subject information, using the demographic information from publications to generate individual information randomly. Then, we used the simulated individual information to construct a model. After importing the blood drug concentration data into the population simulation, we observed that the consistency is within the 95% prediction interval. The simulated concentration vs. time profiles adequately captured the observed data. The majority of visually observed values for plasma drug concentrations in midazolam were in the 5th-95th percentile of the simulation (Fig. 3).

The main reason Cmax data were not analyzed was that the PBPK model used intravenous administration data. In case of intravenous bolus, the drug concentration at the first sampling point after administration was Cmax. If intravenous infusion is used, the Cmax value in the literature usually appears at the first sampling point after infusion. However, during the actual operation, there may be an error within a certain period of time, resulting in the fact that the peak concentration in the body is not

measured in time. The model will simulate from "0 moment" when predicting. Therefore, the Cmax value predicted by the model is usually much higher than the value in the literature. This siuation does not mean that the model fits poorly. In summary, the PBPK model of pediatric population is well established.

3.3 PBPK/PD model prediction

The PD model of midazolam was based on its PBPK model, using its predicted PK data. The sigmoid E_{max} equation was used in the PD models, as shown in Eq. 9. The E_0 was set as the optimized clinical trial baseline of 95.27. The E_{max} is the maximum possible midazolam effect (equal to 100 on the BIS scale). The EC_{50} was estimated by PBPK/PD modeling analysis of 6.41 ng/mL as the unbound midazolam concentration, and the Hill factor was 0.27. The negative sign of E_{max} indicates the PD response is the inhibition of an effect. Figure 4(a,b) shows the experimental data and the model predictions of BIS measurements versus time. Figure 4(c) shows that target-free drug concentrations of 3, 8, or 19 ng/mL correspond to a BIS score of 70, 65, and 60, respectively.

3.4 PBPK/PD Model Application

The recommended dosage for preterm and full-term newborns on the midazolam product label is 0.03mg/kg/h and 0.06mg/kg/h, respectively. In the virtual population created by the model, the BIS value of full-term newborns can be maintained between 60–70 for 26 hours under the condition of continuous intravenous infusion of this dose for 12 hours; studies of preterm neonates have shown that for optimal sedation and hypnosis, doses higher than 0.03mg/kg/h are needed, preferably increased to the applied dose of full-term infants (Fig. 5). Model results support the use of midazolam in preterm and term neonates.

4. Discussion

In this study, we built the mechanism PBPK/PD model of midazolam using in vivo and in vitro data (Table S1, Table 1), validated our adult model (Fig. 1; Fig. 2), then incorporated maturity into the pediatric patient model and validated the model using clinical observation data from 96 pediatric populations aged 0–8 years (Table S2). The validation clearly showed that our model could recapitulate the configuration of midazolam in both adult and pediatric populations. The validated model is then applied to generate simulations in a virtual population with a broad representation of all age groups (Fig. 3). The final PBPK/PD model was used to evaluate the recommended dose on the product label for preterm and term newborns(Fig. 5). The results showed that the drug effect on full-term newborns could be well exerted when administered at 0.06mg/kg/h. The preterm population may require higher doses than 0.03mg/kg/h, and overall, our study supports midazolam in the neonatal population.

Pediatric population medication has always had various situations, such as drug label irregularities, drug abuse, or off-label use, and this irrational drug use is more prominent in the neonatal population because some of the problems associated with clinical trials in this age group are further amplified due to economic or ethical circumstances(D'Errico et al., 2022; Hudgins et al., 2018; Kaguelidou et al., 2016). A

study of 5,118 hospitalized children by Thyssen et al. reported that 7.48% of all children hospitalized for more than 17 hours experienced at least one ADR. Newborns occupy a unique subgroup of pediatric patients with a higher risk of ADR and even more severe ADR(Arnold, 2021). Pharmacovigilance reports associated with children indicate that many ADRs occur in the first few years of life(Kaguelidou et al., 2016).

The physiological development of the pediatric population is in dynamic change, and different subgroups of the pediatric population show different developmental characteristics. Current studies typically refer to ICH's classification of the pediatric population: premature newborns (gestational age less than 37 weeks, 0–27 days old), full-term newborns (gestational age 37–40 weeks, 0–27 days old), infants and toddlers (28 days to 23 months), children (2 to 11 years), adolescents (12 to 16–18 years, dependent on region) (Agency, 2017). The difficulties of drug use in the neonatal population are primarily due to the population itself. They are in the most notable period of growth and development, and the body's response to drugs is naturally different from that of adults.

In terms of pharmacokinetics(Batchelor and Marriott, 2015; de Wildt et al., 2014; Lim and Pettit, 2022; Michelet et al., 2017), the pH in the stomach of newborns is higher, and the degradation of acid-unstable drugs is limited, resulting in increased concentrations; the level of albumin is proportional to gestational age (GA), and the low protein binding rate leads to the increase of free drugs in plasma; liver metabolic enzymes have unique developmental patterns in newborns, including prenatal pattern (high activity during fetal life: CYP3A7, SULT1A3), constant pattern (stable activity from early fetal to adult life: CYP3A5, SULT1A1, TPMT) and postnatal pattern (increased activity after birth: CYP3A4, CYP2C9, CYP2D6, CYP2E1, most UGTs); kidney function (glomerular filtration, tubular secretion and reabsorption) in newborns is a gradual process, reaching adult levels at about 1–3 years old.

In terms of pharmacodynamics(Anderson, 2017; van den Anker et al., 2018), due to limited data on receptor expression and sensitivity, it is usually only qualitatively determined that receptor-related differences are related to changes in the exposure-response relationship, and this correlation cannot be well quantified, for example, increased neonatal sensitivity to morphine is associated with increased expression of μ opioid receptors after birth. And a study of sontalol in children with supraventricular tachycardia that neonates are more sensitive to QTc interval prolongation than older children.

The PBPK model integrates information related to organism physiology, compound physicochemical properties, clinical trial design, etc., and uses mathematical models to simulate blood flow in the circulatory system, thereby connecting tissues or organs of the body to predict drug disposal. Ultimately, it could assist in developing and adjusting drug regimens for different populations(Lin et al., 2022). Integrating changes in physiological development in PBPK models has been shown to successfully predict dose ranges across pediatric age spans(Verscheijden et al., 2020). Regulatory agencies also recognize the research value of these models and support their use in pediatric drug development (Administration).

The extrapolation and model simulation strategies are not widely used in neonatal population drug studies, but they have also been published in recent years. Ganguly, S. et al.(Ganguly et al., 2020)established the neonatal PBPK model of meropenem, considering the ontogenetic effects of albumin, glomerular filtration and renal tubular secretion on drug disposal when extrapolating the adult PBPK model to the neonatal population. Still, due to the lack of data on certain physiological processes, tubular secretion was defined as a transport process mediated by OAT3 and hypothetical efflux transporters. The scientificity of the model needs to be further verified.

The antiretroviral drug dolutegravir is commonly used in the perinatal HIV-exposed neonatal population, Bunglawala, F.et al.(Bunglawala et al., 2020) integrated age-related changes, such as maturation of plasma protein binding and ontogenesis of CYP3A4 and UGT1A1, established a neonatal PBPK model of dolutegravir. Although the validation results of the model were within the acceptable range, the model did not consider maternal transfer of drugs through breast milk or placenta, which may lead to an increase in the actual exposure of newborns. For neonatal drug development, the model's performance will continue to improve.

Research on midazolam in model simulation or neonatal applications has been of interest. In the study, Mansoor N et al.(Mansoor et al., 2019) used relevant physiological parameters in the literature to establish a virtual whole-body PBPK model for a preterm newborn with a gestational age of 29 weeks. However, the model did not systematically consider the impact of individual growth and development on drug disposal. Pan-Pan Ye et al.(Ye et al., 2020) developed a population PK model of midazolam in juvenile mice. Using the allometric method of maximum lifespan potential correction factor, the PK parameters of juvenile mice were bridged to newborn PK parameters to predict the first dose of midazolam in newborns, providing another research strategy.

To our knowledge, the study is the first to combine physiological data with anatomical and developmental expertise in a PBPK/PD model. It is the first to evaluate the feasibility of recommended doses on the product label for the neonatal (preterm and full-term) population. It’s improved our understanding of midazolam metabolism in this susceptible age group in the pediatric population, and our model was validated with clinical PK data from different dosing regimens, increasing the robustness of PK simulations.

Although the proposed model predicts the PK of midazolam in newborns, it is not without limitations. We attempted to mechanistically explain the exposure-effect relationship of midazolam in the neonatal population. However, due to the lack of data on specific physiological processes in preterm infants, we used the same metabolic enzyme maturation equation in both preterm and full-term populations. Although we made this assumption, an acceptable agreement remained between the observed and simulated PK parameters (blood concentration and AUC).

It's just that the clearance of preterm infants early in life may be overestimated. Our final dose simulations show that slightly higher doses may be required to achieve the desired efficacy compared to

the product label. Not surprisingly, the model's dose simulations in the term neonatal population were consistent with the recommended dose on the product label, and the model was generally acceptable.

Secondly, in the pharmacodynamic model of the newborn population, According to Anderson B. et al. (Anderson, 2017), the neonatal cortical function is relatively immature, dendrites are underdeveloped, and GABA receptors are relatively few. Still, there are no studies to support or refute this premise. Arbour R et al.(Arbour et al., 2009) said that the best measures for monitoring pharmacodynamic endpoints of midazolam remain to be determined. Dosing regimens in children are usually based on empirical inferences from adult dosing regimens.

Quantitative studies on the pharmacodynamics of midazolam are not fully mature in this regard. We adopted a reasonable hypothesis based on an extrapolation strategy. Due to the lack of quantitative pharmacodynamic evaluation data in newborns, the model needs to be verified.

5. Conclusion

In summary, we successfully established midazolam neonatal PBPK/PD model based on extrapolation strategy. The model was validated using the dosage for newborns on the product label, and reliable results were obtained. If the established neonatal PBPK model is considered accurate and valid, simulations can be performed to optimize the dosing regimen. The extrapolation strategy and modeling simulation research methods provide valuable references for future drug development and clinical rational drug use in the neonatal population.

Declarations

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Availability of data and material: The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Credit Author Statement

Tangping Zhao: Writing - original draft, Formal analysis, Conceptualization, Data curation, Validation, Methodology, Visualization; **Sufeng Zhou:** Formal analysis, Software, Supervision; **Lu Wang:** Formal analysis, Software, Validation; **Tongtong Li:** Conceptualization, Investigation, Investigation; **Jinying Zhu:** Conceptualization, Methodology, Investigation; **Feng Shao:** Writing - review & editing, Data curation, Software, Supervision

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Figures

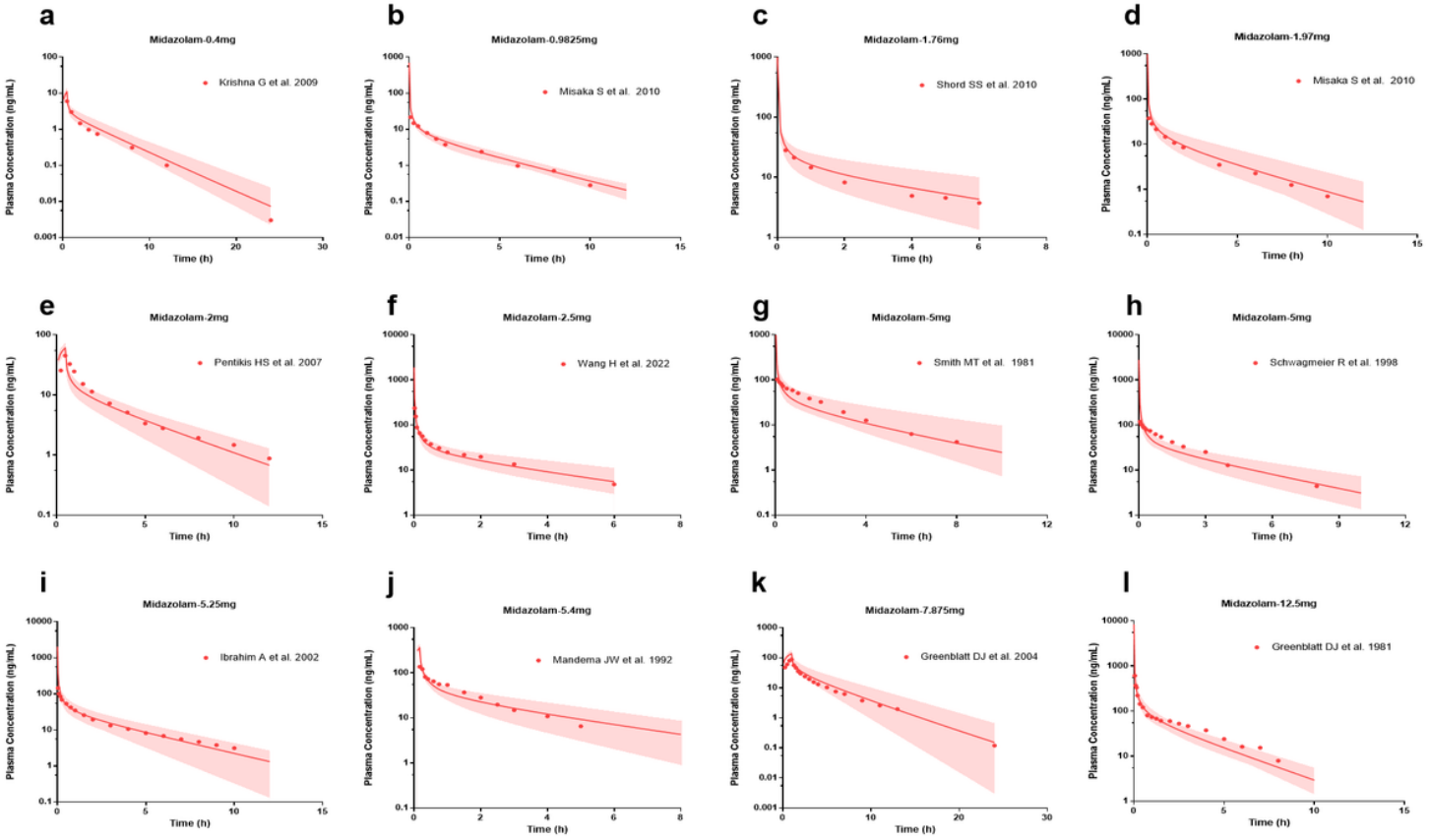

Figure 1

Simulation of midazolam concentration vs. time for one hundred virtual adults. The adult midazolam PBPK model was developed and evaluated using published plasma midazolam concentration vs. time data digitized for intravenous (IV) administered (0.4-12.5mg). The observed concentration values are arithmetic means determined from the references and indicated as solid circles. (a)-(l) The solid line indicates the arithmetic mean of the population simulation; the shaded area indicates the 5%-95%.

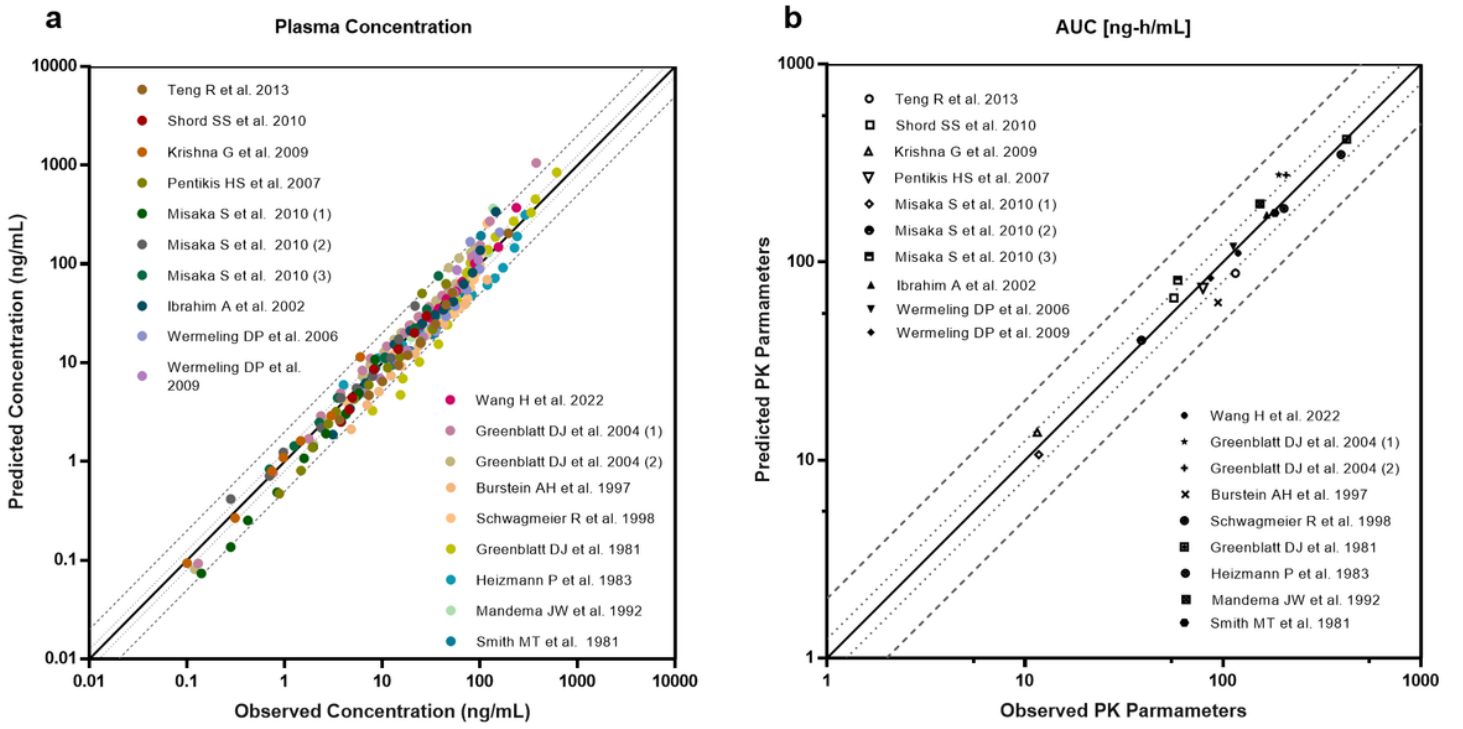

Figure 2

Validation of PBPK model for midazolam in adult populations. (a) The goodness-of-fit plot for the simulated midazolam plasma concentrations in adult population after intravenous administration. (b) The goodness-of-fit plots for the predicted values of AUC for adult population. The solid black line indicates unity line, the thin dashed black line indicates 1.25-fold change, and the thick dashed black line indicates 2-fold change.

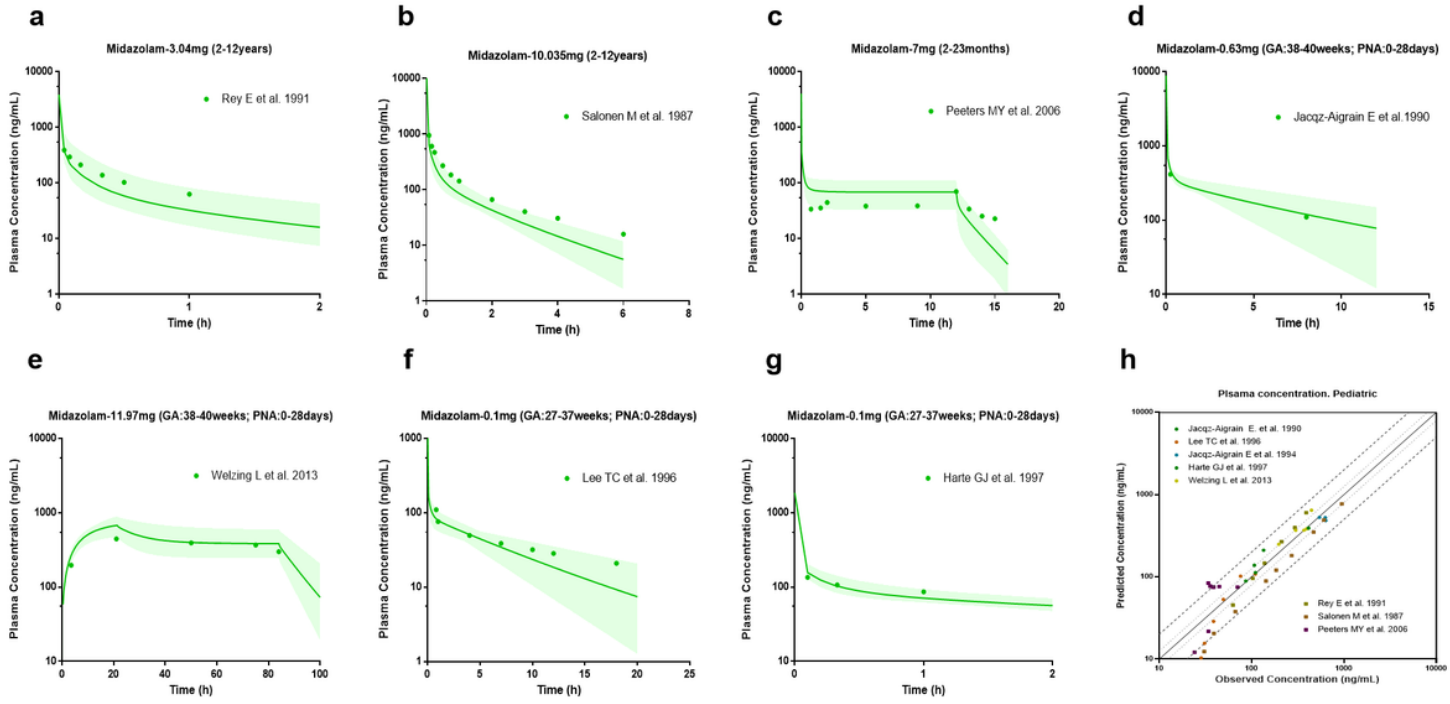

Figure 3

Simulation of midazolam concentration vs. time for one hundred virtual adults. The adult midazolam PBPK model was developed and evaluated using published plasma midazolam concentration vs. time data digitized for intravenous (IV) administered (0.1-10mg). (a,b) 100 virtual children with aged 2-12 years. (c) 100 virtual infants aged 2-23 months. (d,e) 100 virtual full-term newborns with gestational age of 40 weeks and within 0-27 days of birth. (f,g) 100 virtual preterm infants with gestational age of 27-37 weeks and within 0-27 days of birth. The observed concentration values are arithmetic means determined from the references and indicated as solid circles. (a)-(g) The solid line indicates the arithmetic mean of the population simulation, the shaded area indicates the 5%-95% concentration range. (h) The goodness-of-fit plot for the simulated midaozlam plasma concentrations in pediatric population after intravenous administration. The solid black line indicates unity line, the thin dashed black line indicates 1.25-fold change, and the thick dashed black line indicates 2-fold change.

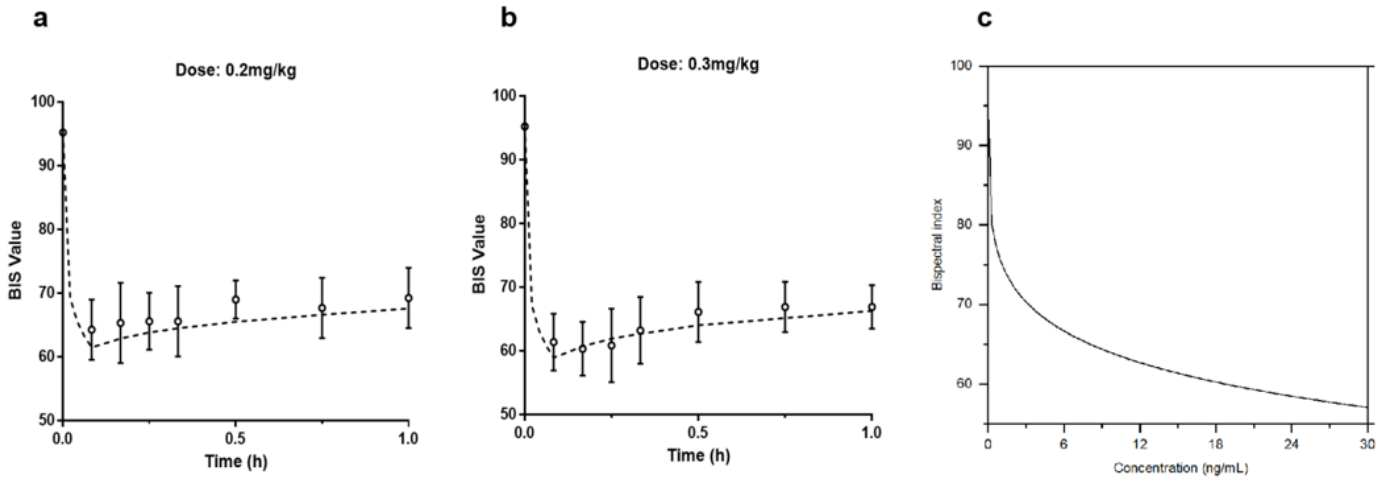

Figure 4

Midazolam adult PD model. (a,b) Plot of the observed and population predicted BIS score versus time for the dose levels given by Miyake, W. et al (0.2 and 0.3 mg/kg iv bolus). Circles are observed BIS data from Miyake, W. et al and the dashed line is the model prediction; (c)The Model predicted change in BIS index with midazolam concentration.

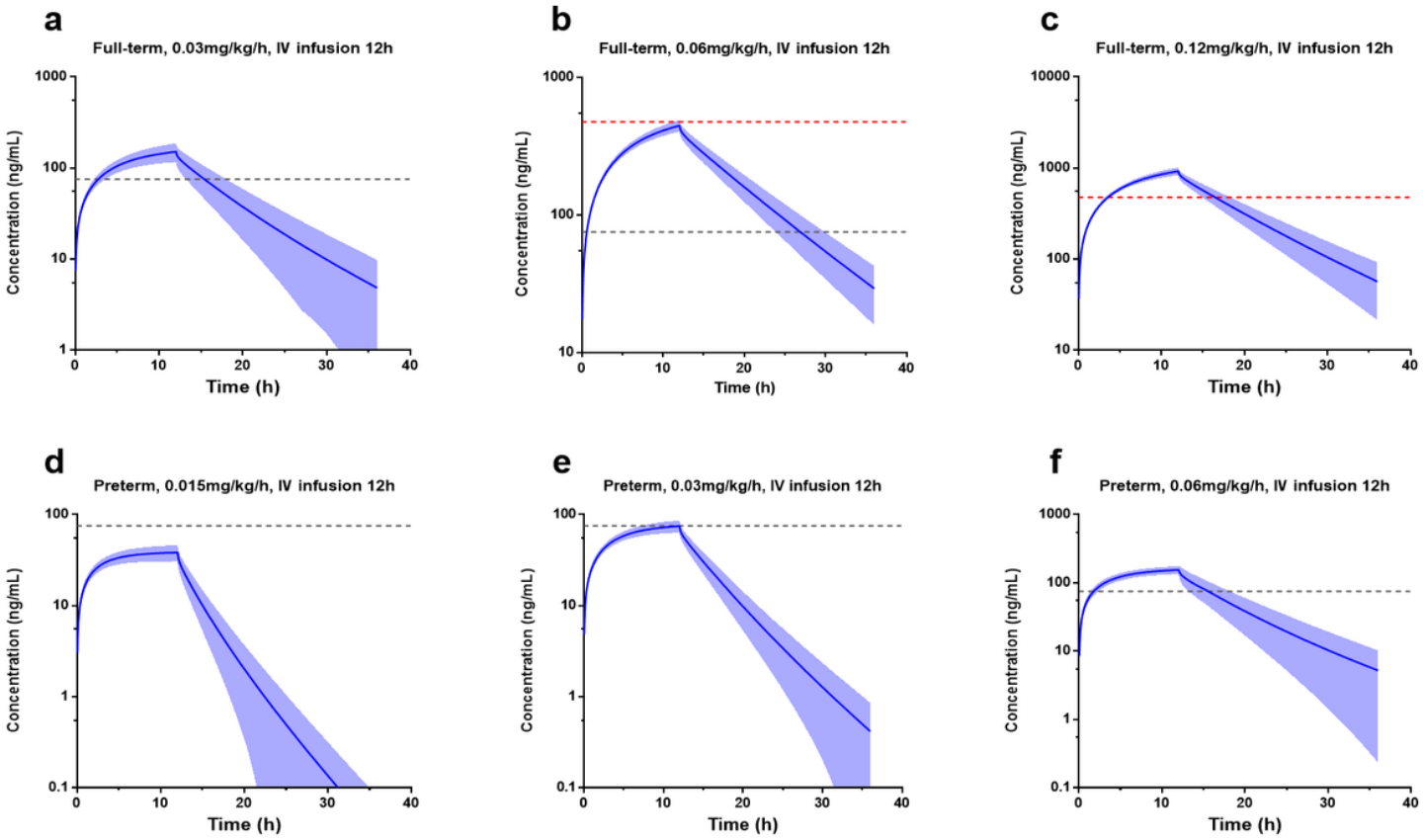

Figure 5

Simulated drug concentrations of (a-c) full-term infants and (d-f) preterm infants at low, medium, and high doses. Red dashed line: drug concentration when BIS value is 70; gray dashed line: drug concentration at BIS value of 60 the solid line indicates the arithmetic mean of the population simulation; the shaded areas represent 90% confidence intervals.

Supplementary Files

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