Development of a Novel Maternal-Fetal Physiologically Based Pharmacokinetic Model I: Insights into Factors that Determine Fetal Drug Exposure through Simulations and Sensitivity Analyses^S

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ABSTRACT

Determining fetal drug exposure (except at the time of birth) is not possible for both logistical and ethical reasons. Therefore, we developed a novel maternal-fetal physiologically based pharmacokinetic (m-f-PBPK) model to predict fetal exposure to drugs and populated this model with gestational age-dependent changes in maternal-fetal physiology. Then, we used this m-f-PBPK to: 1) perform a series of sensitivity analyses to quantitatively demonstrate the impact of fetoplacental metabolism and placental transport on fetal drug exposure for various drug-dosing regimens administered to the mother; 2) predict the impact of gestational age on fetal drug exposure; and 3) demonstrate that a single umbilical venous (UV)/maternal plasma (MP) ratio (even after multiple-dose oral administration to steady state) does not necessarily reflect fetal drug exposure. In addition, we verified

the implementation of this m-f-PBPK model by comparing the predicted UV/MP and fetal/MP AUC ratios with those predicted at steady state after an intravenous infusion. Our simulations yielded novel insights into the quantitative contribution of fetoplacental metabolism and/or placental transport on gestational age-dependent fetal drug exposure. Through sensitivity analyses, we demonstrated that the UV/MP ratio does not measure the extent of fetal drug exposure unless obtained at steady state after an intravenous infusion or when there is little or no fluctuation in MP drug concentrations after multiple-dose oral administration. The proposed m-f-PBPK model can be used to predict fetal exposure to drugs across gestational ages and therefore provide the necessary information to assess the risk of drug toxicity to the fetus.

Introduction

Fetal exposure to drugs has become increasingly common. This can be attributed to the rising use of therapeutic drugs among pregnant women (Mitchell et al., 2011) as pre-existing maternal conditions (e.g., epilepsy, asthma) or conditions that developed during pregnancy (e.g., gestational diabetes and hypertension) must be treated to ensure the health and welfare of the mother and therefore her fetus. Sometimes, it

is the unborn child that is the target of the treatment [e.g., to prevent maternal-fetal human immunodeficiency virus (HIV) transmission] (McGowan and Shah, 2000). Consequently, the ability to quantitatively evaluate fetal exposure to drugs and risk of toxicity, not only at term but also earlier during pregnancy when the fetus is most vulnerable to teratogens, is needed.

Unlike the general population, fetal exposure to drugs ingested by the pregnant mother cannot be readily studied prior to birth for ethical and logistical reasons. Even at the time of birth, assessment of fetal exposure to drugs is limited to a single cord plasma concentration measurement and reported as the umbilical vein (UV)/maternal plasma (MP) drug concentration ratio. As shown here, in most clinical scenarios, this UV/MP ratio does not reflect the extent of fetal drug exposure relative to that in the mother. Nor does this ratio provide information on fetal drug exposure over time [i.e., fetal plasma area under the curve (AUC_f) or maximum fetal plasma drug concentration (C_{max,f}) that often drives drug efficacy and/or toxicity in the fetus. Further, since cord blood sampling is limited to term, fetal drug exposure during early gestation remains

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ABBREVIATIONS: AUC_f, fetal plasma area under the curve; AUC_m, maternal plasma area under the curve; AUC_p, placental area under the curve; C_{max,f}, maximum fetal plasma drug concentration; CL_{f0}, fetal metabolic clearance; CL_{MP}, placental apical uptake clearance; CL_{m0}, maternal systemic clearance; CL_{p0}, placental metabolic clearance; CL_{PM}, placental apical efflux clearance; CL_{PD}, transplacental passive diffusion clearance; CL_{PD,u}, unbound transplacental passive diffusion clearance; C-T, concentration-time; F_a, fraction absorbed; F_g, intestinal bioavailability; F/M, fetal/maternal plasma ratio; GA, gestational age; HIV, human immunodeficiency virus; k_a, first-order absorption rate constant; m-f-PBPK, maternal-fetal physiologically based pharmacokinetic; MP, maternal plasma; m-PBPK, maternal pregnancy physiologically based pharmacokinetic; PBPK, physiologically based pharmacokinetic; P-gp, P-glycoprotein; PK, pharmacokinetics; P/M, placental/maternal plasma ratio; steady state_{inf}, steady state after an intravenous infusion; UV, umbilical vein; V_{ss}, volume of distribution at steady state.

unknown. To overcome these gaps in knowledge, mechanistic understanding of the determinants of fetal drug exposure and noninvasive approaches to predict fetal exposure across gestational ages (GAs), such as physiologically based pharmacokinetic (PBPK) modeling and simulation, are needed.

Although a number of attempts to develop fetal PBPK models have been made (Clewett et al., 2007; Yoon et al., 2011; Loccisano et al., 2013), upon closer examination, except for a recently published model (De Sousa Mendes et al., 2016, 2017), the majority of these models are not intended to predict fetal exposure to therapeutic agents prescribed to pregnant women. Their limitations include the following: 1) incomplete inclusion of pregnancy-caused changes in maternal and fetal physiology; 2) not accounting for the alterations in maternal drug disposition; and 3) exclusion of fetal body compartments important for the disposition of therapeutic drugs. Recently, our laboratory has successfully refined and verified a mechanistic maternal pregnancy PBPK model (m-PBPK) that can predict the maternal disposition of drugs cleared by one or more cytochrome P450 enzymes during pregnancy (Ke et al., 2012, 2013, 2014). However, this model contains only a lumped tissue compartment representing the placenta and fetus. Therefore, the goals of the current investigation were as follows: 1) to develop a maternal-fetal PBPK (m-f-PBPK) model by incorporating a physiologically relevant fetal-PBPK into our previously verified m-PBPK; 2) to quantitatively demonstrate the impact of fetoplacental metabolism and placental transport on fetal drug exposure; 3) to quantitatively predict the impact of GA on fetal drug exposure; and 4) to show that the UV/MP ratio after a single dose or after multiple dosing (even at steady state) does not necessarily represent the extent of fetal drug exposure.

Materials and Methods

Model Structure and General Assumptions. Briefly, an m-f-PBPK model was built using MATLAB (R2014b; MathWorks, Natick, MA) by adding the placenta, the amniotic fluid compartment, and fetal organs important in drug disposition (e.g., liver and kidney) and distribution (e.g., brain) (Fig. 1) to our verified m-PBPK model (Ke et al., 2012, 2013, 2014). The remaining fetal organs were lumped into a single compartment referred to as “rest of body.” Our model also accounted for the marked differences in fetal circulation compared with that in the mother (Polin et al., 2004). For instance, it is the venous blood that carries oxygenated blood (via the UV) from the placenta to the fetus. The majority of this flow bypasses the fetal liver through the ductus venosus before perfusing fetal tissues.

General model assumptions include the following:

- The bidirectional maternal-placental and fetal-placental unbound trans-placental passive diffusion clearances across the placenta are equal and always present.
- For a given drug, the magnitude of $CL_{PD,u}$ is directly proportional to the placenta villous surface area, which increases with GA.
- The UV plasma drug concentration represents the systemic fetal plasma venous drug concentration.
- Fetal renal clearance is negligible during the first 20 weeks of gestation (Polin et al., 2004). After week 20, it consists of only glomerular filtration clearance, which can be estimated from fetal plasma protein binding and inulin clearance estimated in preterm (weeks 23–40) and term neonates (within first 14 days of life).
- Compared with fetal swallowing, the movement of amniotic fluid between the amniotic sac and maternal circulation is negligible (Gilbert and Brace, 1993). Therefore, fluid transfer between these two compartments was considered to be zero.

Collection and Analyses of Fetal Physiologic Parameters. To populate the m-f-PBPK model with fetal physiologic parameters, a systematic literature search was carried out using PUBMED to obtain these parameters (Table 1). The search strategy was aimed to identify cohort studies whereby the parameters of interest was longitudinally examined during gestation. Data

Fig. 1. Schematic diagram of the full m-f-PBPK model. Solid arrows indicate tissue blood flows, whereas dashed arrows indicate clearances. f/F, fetal; PD, passive diffusion; M, maternal; P, placental; A, amniotic fluid; met, metabolism; renal, renal excretion; reabsorp, amniotic fluid swallowing. $CL_{PF/FP}$ and $CL_{PM/MP}$ represent unidirectional transporter-mediated clearances.

TABLE 1
GA-dependent key fetal and selected maternal physiologic parameters

Parameter (units)	Formula	References	Graph ^a
Maternal placental blood flow (l/h) ^b	$1.71 + 0.207GA + 0.0841GA^2 - 0.0015GA^3$ ($R^2 = 0.991$, RSE = 2.54, $N = 5$; GA: 0–40 weeks)	(Abduljalil et al., 2012)
Fetal serum albumin (g/l)	$-31.7 + 4.35GA - 0.101GA^2 + 0.0009GA^3$ ($R^2 = 0.987$, RSE = 1.44, $N = 15$; GA: 10–40 weeks)	(Gitlin and Perricelli, 1970; Krauer et al., 1984; Moniz et al., 1985; Weiner et al., 1992)
Fetal serum α_1 -acid glycoprotein (g/l)	$0.02e^{0.0616GA}$ ($R^2 = 0.519$, RSE = 0.0669, $N = 19$; GA: 16–38 weeks)	(Krauer et al., 1984)
Fetal brain volume (ml)	$36.0 - 7.53GA + 0.400GA^2$ ($R^2 = 0.998$, RSE = 5.83, $N = 15$; GA: 12–41 weeks)	(Gruenwald and Minh, 1961; Cussen et al., 1990; Hansen et al., 2003; Archie et al., 2006)
Fetal liver volume (ml)	$16.6 - 2.92GA + 0.143GA^2$ ($R^2 = 0.996$, RSE = 2.93, $N = 15$; GA: 12–41 weeks)	(Gruenwald and Minh, 1961; Cussen et al., 1990; Hansen et al., 2003; Archie et al., 2006)
Fetal stomach volume (ml)	$0.127e^{0.101GA}$ ($R^2 = 0.962$, RSE = 0.184, $N = 18$; GA: 20–37 weeks) $25.3 - 0.548GA$ ($R^2 = 0.994$, RSE = 0.0572, $N = 3$; GA: >37 weeks)	(Nagata et al., 1990)

(continued)

TABLE 1—Continued

Parameter (units)	Formula	References	Graph ^a
Fetal small intestine volume (ml) ^c	$0.0203e^{0.194GA}$ ($R^2 = 0.998$, RSE = 0.0688, $N = 36$; GA: 12–25 weeks)	(FitzSimmons et al., 1988; Nagata et al., 1990; Parulekar, 1991; Archie et al., 2006)
Fetal large intestine volume (ml)	$0.078e^{0.169GA}$ ($R^2 = 0.866$, RSE = 7.44, $N = 44$; GA: 20–37 weeks)	(Rubesova et al., 2009)
Fetal total gut volume (ml) ^d	$-54.3 + 8.90GA - 0.479GA^2 + 0.0088GA^3$ ($R^2 = 0.998$, RSE ≈ 0 , $N = 28$; GA: 13–37 weeks)	(Nagata et al., 1990; Parulekar, 1991; Archie et al., 2006; Rubesova et al., 2009)
Fetal kidney volume (ml)	$2.37 - 0.619GA + 0.0335GA^2$ ($R^2 = 0.994$, RSE = 0.994, $N = 15$; GA: 14–41 weeks)	(Cussen et al., 1990; Hansen et al., 2003)
Fetal umbilical blood flow (l/h)	$0.647 - 0.227GA + 0.0179GA^2$ ($R^2 = 0.9984$, RSE = 0.235, $N = 23$; GA: 18–40 weeks)	(Sutton et al., 1990; Lees et al., 1999; Tchirikov et al., 1998, 2002; Kiserud et al., 2000; Boito et al., 2002; Acharya et al., 2004; Flo et al., 2010) weighted by study size
Ductus venosus blood flow (l/h) ^e	$2.05 - 0.297GA + 0.0116GA^2$ ($R^2 = 1.00$; GA: 20–38 weeks) ^f	(Bellotti et al., 2004; Kessler et al., 2008)

(continued)

TABLE 1—Continued

Parameter (units)	Formula	References	Graph ^a
Fetal portal vein blood flow (l/h)	$0.714 + 0.0489GA + 0.0008GA^2$ ($R^2 = 1.00$; GA: 20–38 weeks) ^f	(Bellotti et al., 2004; Haugen et al., 2004; Kessler et al., 2008)
Fetal brain blood flow (ml/min)	$5.56e^{0.0921GA}$ ($R^2 = 0.04$, RSE = 10.8, $N = 32$; GA: 10–20 weeks) ^g	(Rudolph et al., 1971; Kenny et al., 1986)
Fetal kidney blood flow (ml/min)	$2.18e^{0.0865GA}$ ($R^2 = 0.707$, RSE = 18.3, $N = 66$; GA: 10–41 weeks) ^g	(Rudolph et al., 1971; Kenny et al., 1986; Veille et al., 1993)
Fetal glomerular filtration clearance (l/h) ^h	$0.00046e^{0.15GA}$ ($R^2 = 0.69$, RSE = 0.03, $N = 16$; GA: 23–40 weeks)	(Arant, 1978; Hansen et al., 1983; Coulthard, 1985; van den Anker et al., 1995)
Fetal gut blood flow (ml/min)	$1.67e^{0.124GA}$ ($R^2 = 0.999$, RSE = 4.67, $N = 32$; GA: 10–20 weeks) ⁱ	(Rudolph et al., 1971; Veille et al., 1993; Kenny et al., 1986)
Fetal rest of body compartment volume (ml) ^j	$290.0 - 62.5GA + 3.22GA^2$ ($R^2 = 0.998$)	(Abduljalil et al., 2012)

(continued)

TABLE 1—Continued

Parameter (units)	Formula	References	Graph ^a
Syncytiotrophoblast volume (ml)	$-6.83 + 0.650GA + 0.0370GA^2$ ($R^2 = 0.757$, RSE = 3.86, $N = 6$; GA: 10–41 weeks)	(Mayhew et al., 2003)
Placental villous surface area (m ²)	$4.66 - 0.788GA + 0.0383GA^2 - 0.0004GA^3$ ($R^2 = 0.922$, RSE = 1.008, $N = 23$; GA: 12–41 weeks)	(Wang and Zhao, 2010)

^aIn the graphs above, the x-axes are GA in weeks, whereas the y-axes show the GA-dependent trends of the respective parameters in units, as indicated in the first cell of each row. The GA-specific average values (overall mean across studies $\pm$ overall S.D.) are overlaid when applicable. Residual standard error (RSE) and N (the number of overall means across GAs) are also provided in the formula column when relevant.

^bAverage maternal placental blood flow values at various GAs and the resultant equation were those from the meta-analysis performed by Abduljalil et al. (2012).

^cCalculated using a reported fetal small intestine (SI) diameter formula (Parulekar 1991) and the reported fetal SI length (FitzSimmons, Chinn et al., 1988), assuming cylindrical SI and negligible gut wall thickness.

^dFetal gut consists of fetal stomach, small intestine, and large intestine. Unfortunately, there are no reported data on fetal small intestine volume during the second half of gestation. Because the reported data on these three organs overlapped between week 20 and week 25, the small intestine volume percentage (SI %) of the total gut volume for only this range of gestation was fitted to various models. A linear model ($SI\% = 0.652GA + 11.766$), best describing the GA dependency of SI% volume within this range, was used to estimate the SI% volume beyond week 25. Then, the total fetal gut volume after week 25 was calculated by dividing the sum of fetal stomach and large intestine volumes by their corresponding volume percentage (i.e., $1 - SI\%$).

^eCalculated as the difference between total liver venous blood flow and umbilical venous blood flow.

^fThe average values computed from published GA-dependent formulae were used as individual measurements were not available.

^gCalculated as the product of fetal combined cardiac output (CCO) (Rudolph et al., 1971) and the percentage CCO [before week 20 (Kenny et al., 1986) and after week 20 (Veille et al., 1993)] in respective organs.

^hBecause it is not possible to measure fetal glomerular filtration rate (GFR) or renal function in utero, inulin clearance measured in preterm and term newborns were collected as a surrogate for fetal GFR. It is worth pointing out that GFR value continues to increase after birth as a result of the drop in renal vascular resistance and increase in renal blood flow (Guignard et al., 1975). Consequently, only measurements taken within 7 days after birth were included in our literature search.

ⁱFetal rest of body compartment was back-calculated as fetal weight [based on published fetal volume formula (Abduljalil et al., 2012) assuming a 1 mg/ml density throughout gestation] minus the sum of fetal organ volumes and fetal blood volume [based on the reported average fetoplacental blood volume of 123 ml/kg fetal weight during the second and third trimesters (Pasman et al., 2009)].

from the control arm of case-control studies and healthy subjects of cross-sectional studies were considered for inclusion. Other inclusion criteria included the following: 1) human; 2) uncomplicated singleton pregnancies; and 3) otherwise uneventful pregnancies with conditions thought not to affect the parameter of interest (e.g., preterm birth data were used to estimate fetal renal function). If the data were not tabulated and only graphs were present, individual data points were digitized using Digitizer (a free MATLAB tool available on <http://www.mathworks.com/matlabcentral/>). When multiple qualified studies were available for a physiologic parameter of interest, data were pooled, stratified by GA (measured in weeks from the first day of the last menstrual cycle), and summarized using the approach previously published (Abduljalil et al., 2012). For a given GA, the overall sample size weighted mean parameter value $\bar{X}$ from different studies was calculated as follows: $\bar{X} = \frac{\sum_{i=1}^I n_i X_i}{\sum_{i=1}^I n_i}$, where n_i is the number of subjects in the i th study and X_i is the mean value from that study. In cases whereby both mean values and variability (S.D. or S.E.) of various GA groups were provided, overall S.D. was calculated as $\overline{SD} = \sqrt{\frac{\sum_{i=1}^I [(SD_i)^2 + (X_i)^2] \cdot n_i - N \cdot \bar{X}^2}{N}}$, where SD_i is the S.D. in the i th study and n_i is the number of subjects in the i th study, and X_i is the mean value from that study. When individual measurements associated with a given GA were not available, the average values computed from formulae across publications were used instead.

Data analysis was performed using Excel (2010; Microsoft, Redmond, WA). In general, polynomial, exponential, or power function equations were chosen to describe the longitudinal changes in parameters during development. The choice of the polynomial degree was determined by fitting various polynomials to data using nonlinear regression. If a higher order of polynomial equation did not reduce R^2 value and/or if it departed from the original data in comparison with a lower degree by visual check, then the lower one was chosen. Exponential and power

equations were chosen to describe parameters when polynomials did not adequately fit rapidly time-varying parameters.

Sensitivity Analyses to Identify Key Determinants of Fetal Drug Exposure. To identify the quantitative impact of key factors (e.g., fetal metabolic clearance) that influence maternal-placental/fetal plasma concentration-time (C-T) profiles of a drug at term (week 40), we conducted a series of simulations of two hypothetical drugs, X and Y (Table 2), using our newly developed m-f-PBPK model. Drug X was designed to be a neutral compound (e.g., the HIV nucleoside drugs zidovudine and didanosine, which are predominately un-ionized at physiologic pH) with intermediate permeability across the placenta and minimal plasma protein binding. Therefore, all the variables and parameters discussed here for drug X should be read as unbound values. A drug with these characteristics was chosen to quantitatively illustrate the impact of fetoplacental metabolism and placental transport on fetal exposure to drugs. Drug Y was designed to represent highly lipophilic, neutral drugs with high permeability across the biologic membrane. It was significantly bound to plasma albumin. Consequently, its maternal-fetal distributional equilibrium was affected by differences in maternal versus fetal plasma albumin concentrations. Relevant examples include protease inhibitors and many biopharmaceutics classification system class I and II drugs. These drugs are cleared by cytochrome P450 enzymes that have altered activity during pregnancy (Isoherranen and Thummel, 2013).

Additional assumptions made for the hypothetical drugs X and Y are as follows:

- Drug X is neutral, follows linear kinetics, and has negligible binding in the MP and fetal plasma and in the placenta. Therefore, all concentrations and clearances of drug X represent their corresponding unbound values.
- Drug Y is neutral, exhibits linear kinetics, and binds to plasma albumin. Its binding in the placenta tissue is the same as that in the MP.
- Maternal absorption of drug X or Y is first order and does not change during pregnancy [i.e., first-order absorption rate constant (k_a), fraction

absorbed (F_a), and fraction escaping gut metabolism or intestinal bioavailability (F_g).

- Maternal and fetal tissue-to-plasma partition coefficients are identical for both drugs and remain constant throughout pregnancy.
- Except where indicated, the fetal renal clearance of drug X or Y is negligible.
- Drug X or Y swallowed by the fetus (i.e., the amniotic fluid) is instantly and completely absorbed from the fetal intestine and is not metabolized there.

Although some of the above assumptions were made to simplify the simulations (e.g., neutrality thus zero ionization; unaltered maternal absorption during pregnancy), others were made (e.g., fetal renal secretion of drugs) because these values cannot be determined.

Except where indicated, the simulations were conducted using our m-f-PBPK model, where only one parameter was changed at a time (Tables 3 and 4) at week 40.

Effect of GA on the Fetal-MP Pharmacokinetics. We simulated the impact of GA on exposure of the maternal-fetal unit to drug X or Y under various scenarios (Table 5). Week 20 and week 40 were chosen, respectively, to represent the GA when fetal skin keratinization begins and when cord blood sampling is possible. Fetal metabolic clearance of drug X, when present, was assumed to be directly proportional to fetal body volume as the metabolism of HIV nucleoside drugs (i.e., phosphorylation) occurs throughout the body. Where invoked, placental apical efflux clearance (CL_{PM}) was assumed to be mediated by P-glycoprotein (P-gp), and the magnitude of this clearance was assumed to be proportional to the expression of P-gp in the placenta. At term, P-gp-mediated CL_{PM} was arbitrarily set at 20% of transplacental passive diffusion clearance (CL_{PD}). In the absence of placental P-gp expression data at week 20, we assumed that placental P-gp expression decreases by 5-fold based on our first-trimester data on placental P-gp expression (Mathias et al., 2005). This change in expression was then scaled up to the whole placenta based on change in the GA-dependent placental volume (Abduljalil et al., 2012).

Results

Fetal Physiologic Parameters. The time-variant fetal physiologic parameters used to populate the m-f-PBPK model show that the GA-dependent changes in fetal physiologic parameters are pronounced (Table 1). For example, umbilical venous blood flow (i.e., fetal placental blood flow) increases by approximately 6.2-fold (from 3.3 to 20.2 l/h) from week 20 to week 40. Some fetal physiologic values change with GA in an opposite direction to the corresponding values in the mother. For example, the fetal plasma albumin concentrations increase with GA, whereas the reverse is true for the mother.

Impact of Maternal Metabolism and Placental Passive Drug Permeability on Fetal Exposure to Drug X or Y in the Absence of Placental/Fetal Metabolism or Placental Transport. As expected, after continuous intravenous infusion of drug X or Y, both steady state (steady state_{inf}) maternal venous plasma (MP) and fetal venous plasma (fetal plasma) concentrations and the time to reach steady state after an intravenous infusion (steady-state_{inf}) were inversely dependent on the maternal systemic clearance (CL_{m0}) when V_{ss} was held constant (Fig. 2, a and b; Fig. 3, a and b). In contrast, for both drugs, the MP C-T profile, but not the fetal plasma C-T profile, was independent of the CL_{PD} of the drug as were the maternal and fetal steady state_{inf} plasma concentrations (Fig. 4a; Fig. 5a). Although total fetal plasma steady-state_{inf} concentrations of drug Y were consistently higher than those in the mother (Fig. 3, a and b; Fig. 5, a and b), for both drugs the corresponding unbound steady-state_{inf} UV/MP ratio remained at unity irrespective of the magnitude of CL_{m0} or CL_{PD} values (Fig. 2b; Fig. 4b for drug X; data not shown for drug Y). However, for both drugs, the time to reach fetal steady-state_{inf} plasma concentration was prolonged with lower CL_{m0} or CL_{PD} values (Fig. 2b; Fig. 3b; Fig. 4b; Fig. 5b). Of note, the simulated UV/MP ratio of drug X (which is not bound to plasma proteins) or Y (after correcting for plasma protein binding) matched those predicted by our steady-state_{inf} model (see Supplemental Material).

TABLE 2

Summary of drug-specific parameters used in the simulations at term (week 40)

Parameter	Drug X	Drug Y
Molecular weight	236.23 ^a	325.8 ⁱ
logP	0.05 ^b	3.13 ⁱ
B/P ratio	1.17 ^c	0.66 ⁱ
V_{ss} (l/kg)	0.7 ^d	1.1 ⁱ
$f_{u,plasma}$	0.99 ^e	0.032 ⁱ
F_a	1.0 ^f	0.88 ⁱ
k_a (h ⁻¹)	1.5 ^g	4.0 ^j
F_g	0.78 ^f	0.58 ⁱ
CL_r (l/h)	18.1 ^d	0.085 ⁱ
CL_{iv} (l/h)	45.6 ^d	43.0 ⁱ
CL_{PD} (l/h)	1.80 ^h	22.7 ^k
f_m and f_c	$f_m = 61\%$, $f_c = 39\%$ ^d	$f_{m,3A} = 92\%$, $f_c \approx 0\%$ ⁱ
CL_{r0} (l/h)	0.9 ^h	0 ^m

The above values for drug X and drug Y at term (week 40) were based on the reported didanosine and midazolam PK parameters in the literature, respectively. B/P, blood/plasma concentration ratio; CL_{iv} , intravenous clearance; CL_r , renal clearance; f_c , fraction renally eliminated; f_m , fraction metabolized; $f_u,plasma$, unbound fraction in the plasma

^aExtracted from didanosine product monograph (<http://monographs.iarc.fr/ENG/Monographs/vol76/mono76-9.pdf>).

^bLiterature value (Tuntland et al., 1999).

^cCalculated from the reported blood/plasma didanosine AUC ratio (Barry et al., 1993).

^dPredicted by Simcyp (version 14) based on literature value (Knupp et al., 1991). V_{ss} increased slightly from 0.68 l/kg at week 20 to 0.71 l/kg to week 40.

^eReported plasma binding of didanosine is <5%. Minimal binding of 1% was assumed for ease of data interpretation.

^fReported average didanosine absolute bioavailability is 23.5% [range 14–33% (77)] (Knupp et al., 1991). Animal studies indicate that didanosine is rapidly and completely absorbed. Therefore, F_a was assumed to be 1. The reported intravenous nonrenal clearance (~30 l/h) (Knupp et al., 1991) does not fully explain the first pass effect. F_g of 0.78 was used to recover oral PK.

^gLiterature value (Velasque et al., 2007).

^hIrreversible human fetal drug X clearance at term was calculated as the product of fetal didanosine clearance in the macaque fetus (dam weight normalized) (Tuntland et al., 1999) and the average term body weight of 85 kg in human pregnant women (Abduljalil et al., 2012). The reported steady-state fetal/dam didanosine plasma concentration ratio is ~0.5 (Tuntland et al., 1999). Therefore, placental passive diffusion clearance was estimated as 1.8 l/h (Supplemental eq. 2), assuming that the same F/M AUC ratio holds true for drug X in human maternal-fetal pairs.

ⁱReported values of midazolam pregnancy PBPK model (Ke et al., 2012).

^jEstimated from midazolam oral data from term pregnant women (Kanto et al., 1983).

^kEstimated from the reported midazolam umbilical venous plasma concentrations (Kanto et al., 1983; Zhang and Unadkat, 2017).

^mAssumed.

After a single oral dose of drug X or Y, fetal drug exposure (AUC_f) was inversely proportional to CL_{m0} (Fig. 2c; Fig. 3c) but independent of CL_{PD} (Fig. 4c; Fig. 5c), although the fetal plasma time to reach maximum drug concentration (t_{max}) and $C_{max,f}$ were affected by both clearances. As expected, their UV/MP ratios varied over time until maternal-fetal distributional equilibrium was achieved (Fig. 2d; Fig. 3d; Fig. 4d; Fig. 5d). Interestingly, for both drugs (but drug X more than drug Y), its distributional equilibrium UV/MP ratio was greater than the expected value of unity in the absence of fetoplacental metabolism or drug transport (Supplemental eq. 1). For drug Y, this deviation from unity persisted after correcting for plasma protein binding (data not shown). Furthermore, for both drugs, the deviation from unity became significantly dampened with decrease in CL_{m0} (Fig. 2d; Fig. 3d) or increase in CL_{PD} (Fig. 4d; Fig. 5d).

After multiple oral doses, as expected, the time to reach steady state, and the accumulation and fluctuations in steady-state MP drug concentrations were inversely proportional to CL_{m0} (Fig. 2, e and g; Fig. 3, e and g) but were independent of changes in CL_{PD} (Fig. 4e; Fig. 5e). However, higher CL_{PD} resulted in greater fluctuations in steady-state fetal plasma drug concentrations within a dosing interval (Fig. 4e; Fig. 5e). Overall, the effect of CL_{m0} or CL_{PD} on the UV/MP ratio remained the same after single or multiple oral dosing.

Impact of Dosing Interval (τ) on UV/MP Ratio of Drug X or Y in the Absence of Placental/Fetal Metabolism or Placental Transport. As expected, shortening τ resulted in greater drug accumulation (Fig. 2, e versus g; Fig. 3, e versus g) but fewer fluctuations in the UV/MP ratio within a dosing interval (Fig. 2, f versus h; Fig. 3, f versus h). As in the

TABLE 3
Drug X clearance values used in various scenarios

Drug X	Dosing Regimen	τ	CL_{m0}^a	CL_{PD}^b	CL_{p0}	CL_{f0}	CL_{MP}	CL_{PM}
						l/h		
Figure 2, a and b	Week 40, 16.7 mg/h continuous i.v. infusion	N/A	4.5 vs. 45	1.8	0	0	0	0
Figure 2, c and d	Week 40, 400 mg single oral dose	N/A	4.5 vs. 45	1.8	0	0	0	0
Figure 2, e and f	Week 40, 133.3-mg multiple oral doses	8 h	4.5 vs. 45	1.8	0	0	0	0
Figure 2, g and h	Week 40, 400-mg multiple oral doses	24 h	4.5 vs. 45	1.8	0	0	0	0
Figure 4, a and b	Week 40, 16.7 mg/h continuous i.v. infusion	N/A	45	1.8 vs. 18	0	0	0	0
Figure 4, c and d	Week 40, 400-mg single oral dose	N/A	45	1.8 vs. 18	0	0	0	0
Figure 4, e and f	Week 40, 400-mg multiple oral doses	24 h	45	1.8 vs. 18	0	0	0	0
Figure 6a	Week 40, 400-mg single oral dose	N/A	45	1.8	0, 0.18, 0.90, 1.8	0	0	0
Figure 6b					0	0, 0.18, 0.9, 1.8	0	0
Figure 6c					0, 0.18, 0.9, 1.8	0, 0.18, 0.9, 1.8	0	0
Figure 6d					0	0	0, 0.18, 0.9, 1.8	0
Figure 6e					0	0	0	0, 0.18, 0.9, 1.8
Figure 8, a and b (scenario 1)	400-mg single oral dose	Week 20	N/A	44	0.21	0	0	0
		Week 40		45	1.8	0	0	0
Figure 8, c and d (scenario 2)		Week 20		44	0.21	0	0.11	0
		Week 40		45	1.8	0	0.90	0
Figure 8, e and f (scenario 3)		Week 20		44	0.21	0	0	0.70
		Week 40		45	1.8	0	0	0.36
Figure 8, g and h (scenario 4)		Week 20		44	0.21	0	0.11	0.70
		Week 40		45	1.8	0	0.90	0.36

N/A, not applicable; week, gestational week.

^aAt 40 weeks, CL_{m0} was set at 45 l/h based on the published didanosine clearance value (see Supplemental Table 1; rounded down to the nearest integer).

^b CL_{PD} was extrapolated from the reported didanosine transplacental passive diffusion clearance in pregnant macaques based on body weight (for details, see Supplemental Table 1).

above scenarios, the observed distributional equilibrium UV/MP ratio of drug X and Y remained higher than the expected unbound steady-state_{inf} UV/MP ratio of 1.0, and the extent of this deviation was inversely related to CL_{m0} (Fig. 2, f and h; Fig. 3, f and h).

Impact of Placental Drug Transport, Fetal Metabolism, and Placental Metabolism on Fetal Exposure to Drug X or Y. Overall, variations in these pathways (Table 3) produced significant changes in fetal plasma C-T profiles of drug X without affecting maternal pharmacokinetics (PK) (data not shown). As expected, after a single oral dose of 400 mg of drug X, fetal plasma concentrations, $C_{max,f}$, and placental concentrations (data not shown) of drug X were inversely correlated with its metabolism in the placenta [placental metabolic clearance (CL_{p0})] (Fig. 6a1), in the fetus [fetal metabolic clearance (CL_{f0})] (Fig. 6b1), or both (Fig. 6c1). Irrespective of the magnitude of CL_{p0} relative to CL_{PD} , the effect of change in CL_{p0} on the magnitude of reduction in AUC_f and placental AUC (AUC_p) were identical (Fig. 6a2). In contrast, an increase of the same magnitude in fetal metabolism (CL_{f0}) resulted in a greater reduction in AUC_f compared with AUC_p (Fig. 6b2). For example, when CL_{p0} equaled CL_{PD} , AUC_p and AUC_f were both reduced by 50%, whereas when CL_{f0} equaled CL_{PD} , a greater reduction in AUC_f was seen compared with AUC_p (67% versus 33%) (Fig. 6, a2 versus b2). When both metabolic processes were present (CL_{p0} and CL_{f0}) and equal to CL_{PD} , the reduction in the $C_{max,f}$ (Fig. 6c1), AUC_p (60%), and AUC_f (80%) was even greater (Fig. 6c2). The addition of placental apical uptake clearance (CL_{MP}) or CL_{PM} on the apical side of the placenta altered fetoplacental exposure to drug X in opposite directions (Fig. 6, d versus e). Although increasing CL_{MP} resulted in

higher fetal plasma drug concentrations and $C_{max,f}$ (Fig. 6d1) as well as increased fetal/MP (F/M) and placental/MP (P/M) AUC ratios (Fig. 6d2), increasing CL_{PM} lowered $C_{max,f}$ (Fig. 6e1) and reduced F/M and placental/MP (P/M) AUC ratios (Fig. 6e2). The predicted F/M and P/M AUC ratios quantitatively matched those predicted by our steady-state_{inf} model (Supplemental eqs. 1 and 2, respectively). However, even under fetal-maternal distributional equilibrium the UV/MP ratio (Fig. 6, a3–e3) deviated from its respective steady-state_{inf} F/M drug concentration ratio and therefore did not represent the F/M AUC ratio.

Overall, after a single oral dose of drug Y, within the test range, variations in the same set of drug Y clearance pathways (Table 4) produced similar changes in fetal plasma drug concentrations (Fig. 7, a1–e1) without altering the MP C-T curve of drug Y (data not shown). As was the case with drug X, both P/M and F/M AUC ratios decreased as these clearance pathways became larger with the exception of CL_{MP} , which was positively correlated with P/M and F/M ratios. After accounting for binding, the impact of fetal clearance was larger than that of placental clearance on F/M and P/M AUC ratios (Fig. 7, a2–e2). In addition, the predicted drug Y UV/MP ratio at distributional equilibrium (Fig. 7, a3–e3) was consistently higher than the expected steady-state_{inf} UV/MP ratios (Supplemental eq. 1).

Impact of GA on Fetal Disposition of Drug X. Maternal and fetal plasma C-T profiles after a single 400-mg oral dose of drug X at weeks 20 and 40 were simulated using the m-f-PBPK model under various scenarios outlined in Table 3. GA significantly altered fetal plasma C-T profile while minimally affecting maternal PK (Fig. 8). Furthermore, the impact of GA on fetal exposure to drug X depended on the clearance

TABLE 4
Drug Y clearance values used in various scenarios

Drug Y	Dosing Regimen	τ	CL_{m0}^a	CL_{PD}	CL_{p0}	CL_{f0}	CL_{MP}	CL_{PM}
Figure 3, a and b	Week 40, 0.63 mg/h continuous i.v. infusion	N/A	12 vs. 43	22.5	0	0	0	0
Figure 3, c and d	Week 40, 15-mg single oral dose	N/A	12 vs. 43	22.5	0	0	0	0
Figure 3, e and f	Week 40, 2.5-mg multiple oral doses	4 h	12 vs. 43	22.5	0	0	0	0
Figure 3, g and h	Week 40, 15-mg multiple oral doses	24 h	12 vs. 43	22.5	0	0	0	0
Figure 5, a and b	Week 40, 0.63 mg/h continuous i.v. infusion	N/A	43	2.25 vs. 22.5	0	0	0	0
Figure 5, c and d	Week 40, 15-mg single oral dose	N/A	43	2.25 vs. 22.5	0	0	0	0
Figure 5, e and f	Week 40, 15-mg multiple oral doses	24 h	43	2.25 vs. 22.5	0	0	0	0
Figure 7a	Week 40, 15-mg single oral doses	N/A	43	22.5	0, 2, 3, 11.3, 22, 5	0	0	0
Figure 7b					0	0, 2, 3, 11.3, 22, 5	0	0
Figure 7c					0, 2, 3, 11.3, 22, 5	0, 2, 3, 11.3, 22, 5	0	0
Figure 7d					0	0	0, 2, 3, 11.3, 22, 5	0
Figure 7e					0	0	0	0, 2, 3, 11.3, 22, 5
Figure 9, a–c (Scenario 1)	400-mg single oral dose	Week 20	N/A	34.8	2.2	0	0	0
		Week 40		43	22.5	0	0	0
Figure 9, c–e (Scenario 2)		Week 20		34.8	2.2	0	0	0
		Week 40		64.1	22.5	0	0	0
Figure 9, e–g (Scenario 3)		Week 20		34.8	2.2	0	0	4.5
		Week 40		43	22.5	0	0	7.4
Figure 9, h–j (Scenario 4)		Week 20		34.8	2.2	0	0	4.5
		Week 40		64.1	22.5	0	0	7.4

$CL_{MP,u}$, unbound placental efflux clearance; $CL_{PM,u}$, unbound placental uptake clearance; $CL_{p0,u}$, unbound placental metabolism; $CL_{f0,u}$, unbound fetal metabolism; N/A, not applicable; week, gestational week.

^aAt week 20, baseline value CL_{m0} was set at 43 l/h based on the published midazolam clinical data at term (Ke et al., 2012), whereas $CL_{PD,u}$ was estimated through sensitivity analysis to match reported fetal UV plasma midazolam drug C-T profile (Zhang and Unadkat, 2017). At week 20, CL_{m0} was slightly decreased primarily resulting from more plasma albumin binding.

mechanisms within the fetoplacental unit. In scenario 1 (Fig. 8, a and b), where drug X was assumed to passively diffuse across the placenta without placental drug transport or irreversible clearance in the fetoplacental unit (e.g., metabolism), despite a modest 16% decrease in $C_{max,f}$, the F/M plasma AUC ratio remained at unity and did not change with advancing GA. In scenario 2 (Fig. 8, c and d), the addition of fetal metabolism produced significant reductions in both the $C_{max,f}$ and AUC_f , resulting in F/M AUC ratio of 0.48 and 0.50 at weeks 20 and 40, respectively. In scenario 3 (Fig. 8, e and f), where P-gp-mediated CL_{PM} was assumed to be 20% of CL_{PD} with no fetal metabolism, advancing GA from week 20 to week 40, and the associated decrease in CL_{PM} resulted in substantial changes in the shape of the fetal C-T curve

as well as in increase in $C_{max,f}$ (by 118.5%) and fetal AUC (by 3.6-fold). Finally, in scenario 4 (Fig. 8, g and h), the combination of both fetal metabolism and placental efflux resulted in the lowest $C_{max,f}$ and AUC_f at both GAs.

Impact of GA on Fetal Disposition of Drug Y. Overall, GA had a marked effect on maternal-fetal plasma C-T curves of drug Y. In scenario 1, where neither fetoplacental metabolism nor placental drug transport was present and maternal hepatic unbound intrinsic clearance remained independent of GA, increasing GA resulted in slightly lower MP concentrations of drug Y (Fig. 9a), as evidenced by a 19% reduction in MP AUC from week 20 to week 40. Over the same period, fetal plasma AUC increased significantly by 67% (Fig. 9b). This decrease in

TABLE 5
GA-dependent changes in the fetal metabolic (CL_{f0}) and placental efflux (CL_{PM}) clearances of drugs X and Y

	Drug X Clearance			Drug Y Clearance		
	GA Week 20	GA Week 40	Week 40/Week 20 Ratio	GA Week 20	GA Week 40	Week 40/Week 20 Ratio
	l/h					
CL_{PD}^a	0.21	1.80	8.6	2.2	22.5	9.
CL_{f0}	0.11 (53% of CL_{PD})	0.90 (50% of CL_{PD})	8.2	0	0	N/A
CL_{PM}^b (P-gp mediated)	0.70 ^c (330% of CL_{PD})	0.36 (20% of CL_{PD})	0.51	7.4 (336% of CL_{PD})	4.5 (20% of CL_{PD})	0.6

^aDenotes transplacental passive diffusion clearance after accounting for binding.

^bDenotes efflux clearance mediated by P-gp located on the apical side of placenta; assumed to be 20% of CL_{PD} at week 40.

^cBack-extrapolated based on the assumed 5-fold decrease in placental P-gp expression and the reported 2.6-fold increase in the placenta volume (Abduljalil et al., 2012).

Fig. 2. Impact of changes in CL_{m0} on fetal and maternal drug X plasma concentration and UV/MP ratio. Changes in CL_{m0} of drug X significantly influenced maternal-fetal drug X plasma C-T profiles at week 40. (a) After infusion (16.7 mg/h, i.v.) at week 40, decreasing CL_{m0} from 45 l/h (red) to 4.5 l/h (blue) increased the steady-state maternal (solid lines) and fetal (dashed lines) plasma concentration of drug X as well as the time to reach steady state. Inset shows the curves on a semilogarithmic scale. (b) The corresponding UV/MP ratios indicate that at steady state, these ratios do not change with changes in CL_{m0} (45 l/h, red; 4.5 l/h, blue). (c) After a single oral dose (400 mg), increasing CL_{m0} from 4.5 l/h (blue) to 45 l/h (red) resulted in lower MP drug concentrations (solid lines) and subsequently lower fetal plasma drug X concentrations (dashed lines). (d) Corresponding changes in UV/MP ratio indicate that higher CL_{m0} (red) led to greater time-dependent fluctuations in the UV/MP ratio as well as a larger UV/MP ratio at distributional equilibrium. (e) Under a multiple oral dosing regimen (133.3 mg; τ = 8 hours) lower CL_{m0} (blue) not only prolonged the time to reach steady state but also resulted in greater extent of drug accumulation. (f) In addition, lower CL_{m0} (blue) led to fewer fluctuations in UV/MP ratio within a dosing interval compared with higher CL_{m0} (red). When the dosing interval was increased (400 mg; τ = 24 hours), the effect of reduction in CL_{m0} on drug accumulation (g) or variation in UV/MP ratio (h) was dampened. In panels (e) and (g), the predicted F/M AUC ratio remained at unity despite the changes in CL_{m0} . See Table 3 for the clearance values used in these simulations.

AUC_m , in conjunction with the increase in AUC_f , resulted in a pronounced $\sim 100\%$ increase in F/M AUC ratio from week 20 to 40. However, after correcting for plasma protein binding, unbound F/M AUC ratios remained unity at both GAs (Fig. 9c). In scenario 2 when a 100% induction in maternal $CL_{int,u,hep}$ at week 40 relative to that at week 20 was assumed, the resultant higher CL_{m0} gave rise to lower the maternal plasma AUC (AUC_m) and thus a 34% decrease in term AUC_f (Fig. 9, d and e) compared with scenario 1. Of note, F/M AUC ratios, both total and unbound, were identical to those in scenario 1 (Fig. 9f) with unbound F/M AUC ratios being unity. Like drug X, the introduction of CL_{PM} (20% of CL_{PD} at term) in scenario 3 produced markedly lower fetal plasma drug concentrations and hence much smaller AUC_f at week 20 compared with week 40 (Fig. 9h). Unbound F/M AUC ratios reduced to lower-than-unity values (~ 0.2 and ~ 0.8 at week 20 and 40, respectively) (Fig. 9i). Last, in scenario 4, the combination of placental efflux and increasing CL_{m0} with GA resulted in a pronounced difference in maternal-fetal plasma drug C-T curves

between week 20 and week 40. The increase in CL_{m0} from week 20 to week 40 in GA led to significantly lower maternal drug Y concentrations and a greater than 50% reduction in AUC_m at week 40. When compared with scenario 3, this scenario had much lower $C_{max,f}$ and AUC_f values at week 40 (Fig. 9, j and k), whereas the F/M AUC ratios were of the same magnitude (Fig. 9l).

Discussion

Here we present a novel maternal-fetal PBPK model that, to our knowledge, allows for the first time the capability to predict the fetal disposition of pharmaceutical drugs at various GAs. The model incorporated the unique fetal vascular physiology and allows future incorporation of placental transport and metabolism within the fetoplacental unit (in progress in our laboratory). This model has been verified by us, at term, where the predicted and observed maternal and fetal plasma concentrations of theophylline and

Fig. 3. Impact of changes in CL_{m0} on fetal and maternal drug Y plasma concentration and UV/MP ratio. Changes in CL_{m0} of drug Y significantly influenced maternal-fetal drug Y plasma C-T profiles at week 40. After infusion (0.625 mg/h, i.v.) at week 40, a 10-fold decrease in maternal hepatic intrinsic clearance (from 3327 to 332.7 l/h) resulted in a decrease in CL_{m0} from 43 l/h (red) to 12 l/h (blue). (a) The resultant steady-state_{inf} maternal (solid lines) and fetal (dashed lines) plasma concentration of drug Y as well as the time to reach steady state increased. (b) The corresponding UV/MP ratios indicate that at steady state_{inf} these ratios stay at 1.2 and do not change with varying CL_{m0} values (43 l/h, red; 12 l/h, blue). (c) After a single oral dose (15 mg), increasing CL_{m0} from 12 l/h (blue) to 43 l/h (red) resulted in lower MP drug concentrations (solid lines) and subsequently lower fetal plasma drug X concentrations (dashed lines). (d) Corresponding changes in UV/MP ratio indicate that higher CL_{m0} (red) led to greater fluctuations in the UV/MP ratio as well as a larger UV/MP ratio at distributional equilibrium. (e) After multiple oral doses (3.75 mg; $\tau = 4$ hours), lower CL_{m0} (blue) not only prolonged the time to reach steady state but also resulted in much greater drug accumulation. (f) In addition, lower CL_{m0} values (blue) led to fewer fluctuations in UV/MP ratio (within a dosing interval) when compared with higher CL_{m0} values (red). When the dosing interval was increased (15 mg; $\tau = 24$ hours), the effect of reduction in CL_{m0} on dose accumulation (g) or variations in UV/MP ratio (h) was dampened. The inset shows the curves on a semilogarithmic scale. In panels (e) and (g), the predicted unbound F/M AUC ratio remained at unity despite the changes in CL_{m0} . See Table 4 for the clearance values used in these simulations.

zidovudine were in a good agreement (Zhang and Unadkat, 2017). However, since such verification data can only be collected at term and does not speak to which factors might affect this ratio (after a single dose or at steady state), we have used this novel m-f-PBPK model to quantitatively demonstrate the factors that can affect this ratio and when this ratio can and cannot be used to estimate fetal exposure to drugs (Figs. 2–9). Although some of these results are obvious from fundamental pharmacokinetic principles, others are not. These unexpected results are highlighted below and summarized in Supplemental Table 2. Also, note that all fetoplacental clearance pathways below refer to clearance values after accounting for binding.

When Does UV/MP Ratio Reflect Fetal Drug Exposure?

Contrary to a widely held belief, the UV/MP ratio, even at distributional equilibrium, does not indicate fetal drug exposure relative to that in the mother (AUC_f/AUC_m) (Figs. 2–7). The only exception is when the drug

is administered to steady state via an infusion or when MP concentrations, at steady state, do not fluctuate much after multiple oral administration situations that occur infrequently in the clinic. The same UV/MP ratio is often deemed to infer the mechanisms by which the drug crosses the placenta. A greater than unity ratio is often interpreted as accumulation of the drug in the fetal compartment (Else et al., 2011). In contrast, a ratio of less than unity is interpreted as low fetal exposure and is attributed to low maternal drug concentrations (Chappuy et al., 2004a,b), fetal metabolism (Ngan Kee et al., 2009), and/or placental efflux (Marzolini and Kim, 2005; Else et al., 2011). However, as shown by our simulations, such interpretations can be false. Although fetal/placental metabolism or placental transport processes cannot be discounted based on a single UV/MP ratio, such deviation from unity is more likely due to the time-dependent distributional kinetics of the drugs across the placenta.

Fig. 4. Impact of changes in CL_{PD} on fetal and maternal drug X plasma concentration and UV/MP ratio. Changes in CL_{PD} of drug X significantly influenced the fetal (dashed lines) but not the maternal (solid lines) drug X plasma C-T profile at week 40. (a) After infusion (16.7 mg/h, i.v.) at week 40, decreasing CL_{PD} from 18 l/h (red) to 1.8 l/h (blue) did not affect the maternal (the red and blue solid lines overlap) or fetal steady-state_{inf} plasma concentrations of the drug. (a and b) The corresponding UV/MP ratios indicate that at steady state_{inf} these ratios do not change with alterations in CL_{PD} (18 l/h, red; 1.8 l/h, blue), but the time to reach the steady-state ratio was prolonged. (c) After a single oral dose (400 mg), increasing CL_{PD} from 1.8 l/h (blue) to 18 l/h (red) significantly modified the shape of fetal plasma C-T curves (dashed lines) but not MP drug concentrations (solid lines; blue and red lines overlap). (d) Corresponding changes in UV/MP ratio indicate that higher CL_{PD} values (red) not only shortened the time to reach distributional equilibrium but also reduced distributional equilibrium UV/MP ratio compared with lower CL_{PD} (blue). After multiple oral doses (400 mg; $\tau = 24$ hours), alterations in CL_{PD} did not affect the MP drug X C-T curve but significantly changed the shape of fetal plasmas in the drug X C-T profile. (e) Higher CL_{PD} values (red) resulted in greater fluctuations in fetal plasma drug X concentration within a dosing interval compared with lower CL_{PD} values (blue). (f) In contrast, higher CL_{PD} (red) produced fewer fluctuations in UV/MP ratio within a dosing interval compared with lower CL_{PD} (blue). Insets in (d) and (f) show the F/M AUC ratios at lower CL_{PD} (blue) or higher CL_{PD} (red). The predicted F/M AUC ratio remained at unity despite changes in CL_{PD} after single (c) or multiple (e) oral doses of drug X. See Table 3 for the clearance values used in these simulations.

In the absence of fetal/placental metabolism or placental transport, the unbound steady-state_{inf} UV/MP ratio and therefore the unbound F/M AUC ratio after single or multiple doses (assuming linearity) should be unity (Supplemental eq. 1). Indeed, it is (Figs. 2-7). In all other cases, the degree of deviation of this ratio from unity (sometimes by an order of magnitude) not only varied with time but also depended on the magnitude of CL_{PD} (Figs. 4 and 5) and CL_{m0} (Figs. 2 and 3). The extent of this deviation decreased when the fetal compartment was allowed to rapidly equilibrate with the maternal compartment. Even in these cases where no active placental uptake was invoked, the UV/MP ratio at distributional equilibrium still exceeded unity (Fig. 2d, ~1.7; Fig. 3d, 1.5). Although puzzling at first glance, these observations can be explained by the multicompartmental PK of drugs X and Y. During the postdistributional phase, the peripheral/central compartment drug concentration ratio increases as the central compartment clearance is increased or as the intercompartmental clearance is decreased (for details, see Supplemental Material). The above conclusions also hold true for multiple dosing regimens (Figs. 2-5). Our simulations are consistent with literature reports. For example, after the last maternal dose, the UV/MP ratio of zidovudine (half-life 1.1 hours) (Collins and Unadkat, 1989) range from 0.18 to 17.2 (Chappuy et al., 2004a), whereas that of theophylline (half-life ~8 hours) is much less variable (from 0.98 to 1.59) (Ron et al., 1984). Clearly, in the case of zidovudine, interpreting UV/MP ratios of much greater than 1.0 as indicative of active maternal-fetal

transport would be incorrect. Similarly, another frequently reported ratio, the amniotic fluid/UV drug concentration ratio, varied with time and did not reflect fetal drug exposure (see Supplemental Material).

Unbound F/M AUC Ratio Is Determined by the Magnitude of Placental Clearance Relative to Fetoplacental and/or Placental Transport Clearance. Another common misconception about fetal drug exposure (i.e., AUC_f) is that AUC_f is mainly driven by MP drug concentrations and not by passive placental transfer and/or fetoplacental metabolism. The reasoning behind it is that fetal/placental clearances are thought to be minor compared with maternal clearance of the drug, due to the small size of the fetal liver [only about 9.5% of maternal liver weight at term (Abduljalil et al., 2012)] or the limited metabolic capacities of the placenta and the fetal liver. Therefore, it is often assumed that when active transport across the placenta is absent, the unbound F/M AUC ratio will approximate unity. Although MP drug concentrations do drive fetal plasma drug concentrations, the above reasoning about F/M AUC ratio is false because it fails to recognize an additional critical factor, CL_{PD} . It is the ratio of these two clearances (i.e., the magnitude of fetoplacental clearances relative to CL_{PD} and not relative to CL_{m0}) that determines the unbound F/M AUC ratio. These determinants of fetal drug exposure, however, have been largely overlooked by others (Hill and Abramson, 1988; Marzolini and Kim, 2005; Myllynen et al., 2007; Bernick and Kane, 2012) and are discussed below with examples (Figs. 6 and 7).

Fig. 5. Impact of changes in CL_{PD} on fetal and maternal drug Y plasma concentration and UV/MP ratio. Changes in CL_{PD} of drug Y significantly influenced drug Y fetal (dashed lines), but not maternal (solid lines), plasma C-T profile at week 40. (a) After infusion (0.625 mg/h, i.v.) at week 40, decreasing CL_{PD} values from 22.5 l/h (red) to 2.25 l/h (blue) did not affect the maternal (the red and blue solid lines overlap) or fetal steady state_{inf} plasma concentration of the drug. (b) The corresponding UV/MP ratios indicate that at steady-state_{inf} these ratios do not change with alterations in CL_{PD} (22.5 l/h, red; 2.25 l/h, blue), but the time to reach the steady-state ratio was prolonged. (c) After a single oral dose (15 mg), decreasing CL_{PD} from 22.5 l/h (red) to 2.25 l/h (blue) significantly modified the shape of fetal plasma C-T curve (dashed lines) but not MP drug concentrations (solid lines; blue and red lines overlap). (d) Corresponding changes in UV/MP ratio indicate that higher CL_{PD} values (red) not only shortened the time to reach distributional equilibrium but also reduced distributional equilibrium UV/MP ratio compared with lower CL_{PD} (blue). Under a multiple oral dosing regimen (15 mg; $\tau = 24$ hours), alterations in CL_{PD} values did not affect the MP drug Y C-T curve but significantly changed the shape of the fetal plasma drug Y C-T profile. (e) Higher CL_{PD} values (red) resulted in greater fluctuations in fetal plasma drug Y concentration within a dosing interval compared with lower CL_{PD} values (blue). (f) In contrast, higher CL_{PD} values (red) produced fewer fluctuations in UV/MP ratio within a dosing interval compared with lower CL_{PD} values (blue) as transplacental distributional equilibrium of drug Y was quickly attained after an oral dose. Insets in (d) and (f) show the unbound F/M AUC ratios at lower CL_{PD} (blue) or higher CL_{PD} (red). The predicted unbound F/M AUC ratio remained at unity despite changes in CL_{PD} after single (c) or multiple (e) oral doses of drug Y. See Table 4 for the clearance values used in these simulations.

For relative polar drugs with intermediate or low CL_{PD} (e.g., drug X), the introduction of fetal/placental metabolism and/or placental transport can significantly alter fetal drug exposure if their magnitude is significant relative to CL_{PD} (Fig. 6). It is important to stress here that the reference point is CL_{PD} and not CL_{mo} . Consistent with our simulations, due to fetal metabolism, the unbound steady-state_{inf} F/M drug concentration ratios of two predominantly polar dideoxynucleoside HIV drugs, didanosine and zalcitabine, were 0.48 and 0.63 in the chronically catheterized maternal-fetal macaques (Pereira et al., 1994; Tuntland et al., 1996). Furthermore, our simulations yielded another novel insight. The site of drug clearance (placental, fetal, or both) had a differential impact on AUC_f and AUC_p (Fig. 6; Supplemental eqs. 1 and 2). Because any drug taken by mother has to first traverse the placenta to reach fetal circulation, the placenta essentially behaves like the “fetal gut.” If metabolism or drug efflux/influx occurs only in the placenta, then the change in placental and fetal drug exposure will be identical and will depend on the degree of presystemic “first pass” (Fig. 6, a, d, and e). Instead, if metabolism (of equal magnitude) occurs in the fetus, this will result in a greater reduction in AUC_f compared with AUC_p (Fig. 6, b and c). In essence, metabolism in the fetus magnifies the reduction in fetal drug exposure caused by placental clearance. This “site effect” may have clinical implications as fetal toxicity can occur via toxicity to the placenta (e.g., formaldehyde) (Pidoux et al., 2015). In these cases, fetal toxicity cannot be readily ruled out even when fetal exposure to the

toxin/drug is low. In contrast, when the CL_{PD} of drugs is much greater than fetal/placental metabolism or placental transport (e.g., lipophilic drugs such as drug Y), the placenta becomes “transparent.” In other words, fetal and maternal compartments rapidly equilibrate. Therefore, AUC_f will approach or equal AUC_m after single-dose or multiple-dose administration of the drug (assuming linearity) (Fig. 7b). In reality, for lipophilic drugs with limited fetoplacental metabolic capacities, a negligible effect on AUC_f is expected when CL_{f0} or CL_{p0} values are varied. However, even for such drugs, in the presence of significant fetal/placental metabolism and/or placental transport relative to CL_{PD} , fetal drug exposure will be significantly altered (e.g., remifentanyl) (Egan, 1995; Coonen et al., 2010). Several lipophilic HIV protease inhibitors that are P-gp substrates (e.g., nindinavir, ritonavir, and saquinavir) (Unadkat et al., 2004) have UV/MP ratios that are considerably lower than unity (Marzolini and Kim, 2005). However, as pointed out earlier, these non-steady-state_{inf} ratios should be interpreted with caution. Nevertheless, a role of placental P-gp efflux likely contributes to their low fetal exposure as these drugs are excellent substrates of P-gp (van der Sandt et al., 2001; McCormack and Best, 2014).

The Impact of GA on Fetal Drug Exposure. The expression of fetoplacental drug-metabolizing enzymes and placental transporters are known to change with GA (Myllynen et al., 2009). For this reason, fetal drug exposure (with no change in maternal dosage regimen) is likely to

Fig. 6. Impact of changes in fetoplacental metabolism and placental transport on fetal plasma and placental drug X concentration, P/M AUC, F/M AUC, and UV/MP ratios after a single 400-mg oral dose of drug X. Changes in fetoplacental clearance pathways differentially impacted fetal exposure to drug X, P/M plasma AUC ratio (hatched bar), F/M plasma AUC ratio (solid bar), and the UV/MP ratio after a single 400-mg oral dose of drug X at week 40. The CL_{PD} of drug X was held at 1.8 l/h. The clearance pathway variance is indicated at the extreme left of the first panel of each row. The indicated clearance was set at 0, 0.18, 0.9, or 1.8 l/h, respectively (0%, 10%, 50%, or 100% of CL_{PD} ; black, red, green, and blue lines, respectively). Other than CL_{MP} (d1), increasing any of the indicated clearance pathways resulted in lower fetal plasma drug X concentrations (a1–c1 and e1). When CL_{F0} was present, the resultant F/M plasma AUC ratio was lower (b2 and c2) than when only CL_{PD} was present (a2). In all cases, the predicted UV/MP ratio at distributional equilibrium was significantly greater than its steady-state_{inf} value (Supplemental eq. 1). The UV/MP ratio at distributional equilibrium decreased with an increase in CL_{PD} , CL_{F0} , CL_{F0} plus CL_{PD} , or CL_{PM} (a3, b3, c3, and e3, respectively) and increased with an increase in CL_{MP} (d3). See Table 3 for the clearance values used in these simulations.

Fig. 7. Impact of changes in fetoplacental metabolism and placental transport on fetal plasma and placental drug Y concentrations, and P/M AUC, F/M AUC, and UV/MP ratios after a single 15-mg oral dose of drug Y. Changes in fetoplacental clearance pathways differentially impacted fetal exposure to drug Y, P/M plasma unbound AUC ratio (hatched bar), F/M plasma unbound AUC ratio (solid bar), and the UV/MP ratio after a single 15-mg oral dose of drug Y at week 40. The CL_{PD} of drug Y was held at 22.5 l/h. The clearance pathway varied is indicated at the extreme left of the first panel of each row. The indicated clearance was set at 0, 2.25, 11.3, or 22.5 l/h, respectively (0%, 10%, 50%, or 100% of CL_{PD} ; black, red, green, and blue lines, respectively). Other than CL_{MP} (d1), increasing any of the indicated clearance pathways resulted in lower fetal plasma drug X concentrations (a1–c1 and e1). When CL_{F0} was present, the F/M plasma unbound AUC ratio was lower (b2 and c2) than when only CL_{P0} was present (a2). In all cases, the predicted UV/MP ratio at distributional equilibrium was greater than its expected steady-state_{inf} value (Supplemental eq. 1). The UV/MP ratio at distributional equilibrium decreased with an increase in CL_{P0} , CL_{F0} , CL_{F0} plus CL_{P0} , or CL_{PM} (a3, b3, c3, and e3, respectively) and increased with an increase in CL_{MP} (d3). See Table 4 for the clearance values used in these simulations.

change with GA (Figs. 8 and 9). But these changes are not intuitive. When drug X passively diffused across the placenta without being transported or metabolized in the fetoplacental unit (Fig. 8, a and b), the progression of gestation did not affect AUC_f because the latter was solely driven by AUC_m , which remained virtually the same from week 20 to week 40. In scenario 2, where only fetal metabolic clearance was

present, AUC_f was significantly reduced and appeared nearly equal between week 20 and week 40 (Fig. 8d). This is because the CL_{F0}/CL_{PD} ratio remained similar from week 20 to week 40 (0.53 and 0.50, respectively) due to the fact that fetal body volume (and therefore fetal metabolic clearance) and the placental surface area (and therefore CL_{PD}) increased with GA in a nearly parallel fashion (Table 1). Placental P-gp

Fig. 8. Impact of GA on maternal and fetal drug X plasma concentration and F/M AUC ratio after a single 400-mg oral dose of drug X at week 20 (red) or week 40 (blue). The effect of GA on fetal exposure to drug X varies with fetoplacental clearance mechanisms involved. Maternal (solid) and fetal (dashed) C-T profiles as well as F/M plasma AUC ratios were simulated after a single 400 mg oral dose of drug X at week 20 (red) and week 40 (blue) under different scenarios. In scenario 1, where no irreversible loss of drug X occurred in the fetoplacental unit, the advancement of GA did not significantly affect fetal-maternal drug disposition (a) or the F/M plasma AUC ratio of unity (b). In scenario 2, the addition of fetal metabolism (increased proportionally with the fetal body volume as GA increased) significantly reduced fetal plasma drug concentrations (c versus a) and resulted in an ~50% decrease in F/M AUC plasma ratio at both GAs (d). In scenario 3, placental P-gp efflux clearance decreased with GA (based on reported P-gp expression and changes in placental volume with GA). Consequently, fetal exposure to drug X increased with GA, reflected by higher fetal plasma drug concentrations (e) and increased F/M plasma AUC ratios (f). Finally, in scenario 4, where both fetal metabolism and placental efflux were present, a further reduction in fetal exposure was observed at both GAs (g and h). See Table 3 and Table 5 for the clearance values used in these simulations.

Fig. 9. Impact of GA on maternal and fetal drug Y plasma concentration and F/M AUC ratio after a single 15-mg oral dose of drug Y at week 20 (red) and week 40 (blue). The effect of GA on fetal exposure to drug Y varies with the fetoplacental clearance mechanisms involved. Maternal (solid) and fetal (dashed) C-T profiles, maternal (solid) and fetal (hatched) plasma AUCs, as well as F/M [total (solid) and unbound (hatched)] AUC ratios were simulated after a single 15-mg oral dose of drug Y at week 20 (red) and week 40 (blue) under different scenarios. In scenario 1, where no irreversible loss of drug Y occurred in the fetoplacental unit under a constant maternal hepatic unbound intrinsic clearance ($CL_{int,u,hep}$), the advancement of GA resulted in an increased fetal plasma AUC despite the decrease in MP AUC (a and b) but did not affect the F/M plasma AUC unbound ratio of unity (c). In scenario 2, the assumed 100% higher $CL_{int,u,hep}$ at week 40 compared with week 20 significantly decreased maternal-fetal plasma drug Y concentrations (hence, AUCs) at term (d versus a; e versus b), whereas F/M AUC plasma ratios at both GAs were identical to those in scenario 1 (f versus c). In scenario 3, placental P-gp-mediated efflux clearance decreased with GA. Consequently, fetal exposure to drug Y increased with GA, which is reflected by higher fetal plasma drug concentrations (g) and increased F/M plasma AUC ratios (i). Finally, in scenario 4, the increase in fetal exposure from week 20 to week 40 persisted in the presence of both a decrease in placental efflux and an increase in CL_{m0} with GA, but to a smaller extent compared with scenario 3 (k versus h). Note that F/M AUC plasma ratios at both GAs were identical to those in scenario 3 (l versus i). See Table 4 and Table 5 for the clearance values used in these simulations.

expression is significantly higher during early gestation versus term (Mathias et al., 2005). This gestational effect in P-gp expression resulted in lower fetal exposure to drug X at week 20, whereas at week 40, due to lower placental P-gp efflux clearance, fetal exposure to drug X increased by 1.9-fold (Fig. 8, e and f). When both clearance pathways were present, the change in placental P-gp expression was a major determinant of the GA effect on drug X AUC_f (Fig. 8, g and h).

Challenges for the Next Generation of Fetal PBPK Models. Like other fetal PBPK models, our m-f-PBPK model also has some limitations.

The use of our model to predict fetal exposure to drugs prior to week 20 may be limited for the following reasons. First, data on many fetal physiologic parameters prior to week 20 (Table 1) are not available. Second, fetal skin is not completely keratinized and is highly permeable during the first half of gestation (Polin et al., 2004). Therefore, drugs that extensively partition into the skin/subcutaneous layer may readily cross into the amniotic fluid. Such movement of drugs could be incorporated into a future iteration of this model. In order for the model to be useful for drugs that are extensively metabolized by the fetus or transported/metabolized

in the placenta, the model will need to be populated with GA-dependent changes in the expression of these enzymes and transporters. Such studies, using quantitative targeted proteomics, are in progress in our laboratory.

Currently, the lack of critical data hinders further development of fetal PBPK models. First, fetoplacental physiologic data across developmental stages are much needed, including the substantial changes in placental and fetal circulation and fetal organ sizes and composition. Obtaining such information will rely on the advancements in non-invasive physiologic measurement techniques and devices. Before such information becomes available, cross-species extrapolation through PBPK modeling and simulation can be conducted (Poet et al., 2010; Yoon et al., 2011). However, this approach will not work when there are significant interspecies differences in fetoplacental metabolism and placental transport. Another challenge lies in obtaining data for PBPK model validation. As detailed above, the UV/MP ratio can change dramatically with time. Therefore, we propose that the maternal dosing regimen, the time after the last maternal dose when maternal and UV drug plasma concentrations are obtained, be recorded and the unbound drug concentration in these samples be measured.

In summary, through simulations we have shown that even when fetoplacental metabolic or placental transport clearance is small, it can significantly determine fetal drug exposure provided that the magnitude of these clearances is comparable to the CL_{PD} of the drug (likely for hydrophilic drugs). In addition, we have shown that the single time point UV/MP ratio (except at steady state after an intravenous infusion or when maternal concentrations do not fluctuate much after multiple oral administration), routinely reported in the literature, cannot be used as an indicator of F/M plasma drug exposure ratio even at distributional equilibrium (after single or multiple doses). Therefore, one promising alternative is to dynamically estimate fetal drug exposure in humans at term and earlier in gestation through PBPK models such as the one presented here. However, prior to using the proposed model, it needs to be verified with fetal exposure data. In our companion article (Zhang and Unadkat, 2017), we describe such a verification using midazolam, zidovudine, and theophylline.

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Authorship Contributions

Participated in research design: Zhang and Unadkat.

Conducted experiments: Zhang and Imperial.

Contributed new reagents or analytic tool: Patilea-Vrana, Wedagedera, and Gaohua.

Performed data analysis: Zhang.

Wrote or contributed to the writing of the manuscript: Zhang and Unadkat

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