ORIGINAL RESEARCH ARTICLE

Prediction of Maternal and Fetal Doravirine Exposure by Integrating Physiologically Based Pharmacokinetic Modeling and Human Placenta Perfusion Experiments

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Abstract

Background and Objective Doravirine is currently not recommended for pregnant women living with human immunodeficiency virus because efficacy and safety data are lacking. This study aimed to predict maternal and fetal doravirine exposure by integrating human placenta perfusion experiments with pregnancy physiologically based pharmacokinetic (PBPK) modeling.

Methods Ex vivo placenta perfusions were performed in a closed–closed configuration, in both maternal-to-fetal and fetal-to-maternal directions ($n = 8$). To derive intrinsic placental transfer parameters from perfusion data, we developed a mechanistic placenta model. Next, we developed a maternal and fetal full-body pregnancy PBPK model for doravirine in Simcyp, which was parameterized with the derived intrinsic placental transfer parameters to predict in vivo maternal and fetal doravirine exposure at 26, 32, and 40 weeks of pregnancy. The predicted total geometric mean (GM) trough plasma concentration (C_{trough}) values were compared with the target (0.23 mg/L) derived from in vivo exposure–response analysis.

Results A decrease of 55% in maternal doravirine area under the plasma concentration–time curve $(AUC)_{0-24h}$ was predicted in pregnant women at 40 weeks of pregnancy compared with nonpregnant women. At 26, 32, and 40 weeks of pregnancy, predicted maternal total doravirine GM C_{trough} values were below the predefined efficacy target of 0.23 mg/L. Perfusion experiments showed that doravirine extensively crossed the placenta, and PBPK modeling predicted considerable fetal doravirine exposure.

Conclusion Substantially reduced maternal doravirine exposure was predicted during pregnancy, possibly resulting in impaired efficacy. Therapeutic drug and viral load monitoring are advised for pregnant women treated with doravirine. Considerable fetal doravirine exposure was predicted, highlighting the need for clinical fetal safety data.

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1 Introduction

Pregnant women living with human immunodeficiency virus (HIV) need adequate antiretroviral treatment for their own health and to prevent mother-to-child transmission of the virus. However, for practical and ethical reasons, pregnant women are often excluded from clinical trials. As a result, a substantial delay to data availability exists, giving rise to uncertainties regarding drug efficacy and safety during pregnancy [1, 2]. Pharmacokinetic data in pregnant women are of special importance because the gradual physiogical changes during pregnancy can significantly impact drug exposure and thus possibly drug efficacy and safety [3].

Key points

This study predicts a substantially reduced maternal doravirine exposure during pregnancy, possibly resulting in impaired efficacy. Therapeutic drug and viral load monitoring are advised for pregnant women treated with doravirine. An increased dose of 100 mg twice daily should be further investigated in pregnant women.

Considerable fetal doravirine exposure was predicted, highlighting the need for clinical fetal safety data.

Intrinsic placental transfer parameters, needed for parameterization of the full-body pregnancy PBPK model, could be derived from placenta perfusion experiments using a mechanistic placenta model. Combining physiologically based pharmacokinetic modeling with human cotyledon perfusion experiments provides a promising framework to predict drug exposure during pregnancy early in drug development.

Doravirine is a first-line drug for patients living with HIV that is commonly prescribed in high-income countries because it has a favorable side effect profile, is not a perpetrator of drug–drug interactions, and has good efficacy [4]. Doravirine is a lipophilic compound (log $P_{o:w}$ 3.0) showing extensive tissue distribution and moderate plasma protein binding (76%) [5, 6]. The major clearance route is mediated by hepatic cytochrome P450 (CYP)3A4, and renal clearance plays a minor role [6]. Doravirine received regulatory approval in 2018 in the United States; however, as no data on doravirine in pregnant women yet exist, this drug is currently not recommended for this population [4, 7].

While clinical data are awaited, alternative approaches, such as physiologically based pharmacokinetic (PBPK) modeling, can be used to predict maternal doravirine exposure in pregnant women. PBPK models combine data on drug properties with physiological data from the human body to predict drug exposure in specified populations. Although pregnancy PBPK models do have limitations, they can help bridge the knowledge gap for drugs with well-characterized properties [8]. Alongside adequate characterization of changes in plasma volume and plasma protein concentrations, various pregnancy PBPK models have been developed and validated for drugs with CYP3A4 clearance, increasing confidence in the equational longitudinal description of these processes [9–12].

To determine fetal drug exposure in a clinical trial, cord blood sampling is the only option from an ethical perspective. However, limitations of this method are the lag time in data collection and the fact that maternal blood/cord blood ratios can vary widely over time after drug intake. Alternatively, the rate and extent of placental passage can be evaluated with ex vivo human placenta experiments. However, this method does not predict in vivo time-varying fetal exposure because overall maternal and fetal pharmacokinetics are not taken into account. Also, maternal drug exposure may yet be unknown. Integrating human placenta perfusion experiments with PBPK models provides a solution for the prediction of in vivo maternal and fetal plasma concentrations over time following maternal dosing.

Several studies have previously worked on integrating ex vivo human placenta perfusion experiments and PBPK models [13–21]. However, a limitation of these studies was that they did not use full-body maternal and fetal PBPK models, and the placental part of the PBPK model was often not described by a permeability-limited model aligned with placental physiology and anatomy. Recently, a permeability-limited placenta model and full-body fetal model was incorporated in the pregnancy model of the PBPK platform Simcyp®, providing opportunities for simultaneous physiologically based prediction of maternal and fetal drug exposure. Therefore, the primary study aim was to predict maternal and fetal doravirine exposure by integrating data from human placenta perfusion experiments in a full-body Simcyp pregnancy PBPK model. To do so, we describe a method for parameterization of the permeability-limited placenta model in Simcyp. In addition, we evaluate the influence of the perfusion experimental setup on predicted maternal and fetal doravirine exposure.

2 Methods

First, we performed human placenta perfusion experiments to study ex vivo transplacental doravirine transfer. Second, we developed a mechanistic placenta model and used it to derive intrinsic placental transfer parameters. Third, we developed a combined maternal–fetal full-body PBPK model for doravirine. We included derived intrinsic placental transfer parameters in the developed pregnancy PBPK model to predict in vivo maternal and fetal doravirine exposure.

2.1 Ex Vivo Human Placenta Perfusion Experiments

To study the transplacental transfer of doravirine, we performed ex vivo dual-side placenta perfusions in a closed–closed configuration, in both maternal-to-fetal (MTF) and fetal-to-maternal (FTM) directions ($n = 4$ each), as described previously [22] (see electronic supplementary material [ESM]-1). In short, the fetal (6 mL/min) and

maternal (12 mL/min) circulation of an intact cotyledon were re-established upon delivery in the laboratory. After flushing the placenta with perfusion buffer for 30 minutes in an open–open setting, both circulations were closed and buffers were switched to experimental perfusion buffers containing human albumin (29 g/L maternal buffer and 32 g/L fetal buffer) and doravirine. Doravirine was added to either the maternal or the fetal circulation at a concentration of 0.96 mg/L to mimic the observed peak plasma concentration (C_{max}) in nonpregnant adults living with HIV [23]. Antipyrine 100 mg/L was added to the closed circulation as a control marker to determine the overlap between the cannulated maternal and fetal circulation. The experiments were considered successful if the antipyrine FTM or MTF concentration ratio, depending on the transport direction, at the end of the perfusion was >0.75. To determine total and unbound doravirine concentrations, maternal and fetal buffer samples were taken at fixed timepoints over 180 minutes. Three tissue samples of the perfused cotelydon were collected directly after the perfusion experiment. A control experiment was performed without placental tissue to test for adhesion of doravirine to the perfusion system and doravirine stability. The bioanalytical assays are described in ESM 2.

2.2 Estimation of Intrinsic Placental Transfer Parameters

To estimate intrinsic placental transfer parameters based on the ex vivo placenta perfusion data, we developed a mechanistic placenta model using NONMEM 7.4 (ICON Development Solutions, Hanover, MD, USA) using the first-order conditional estimation method with interaction. We used a placenta model representing a single functional cotyledon split into three compartments corresponding to the structure of the permeability-limited placenta model in Simcyp. Additional compartments for the maternal and fetal reservoir were added in line with the ex vivo perfusion setup (Fig. 1). The compartments were considered to be well stirred.

Based on placenta physiology and previous experiments with doravirine, we tested two different transfer models: (1) a model representing simple passive diffusion and (2) a model representing simple passive diffusion + P-glycoprotein (P-gp) transport over the maternal-facing barrier (Fig. 1) [6, 24, 25]. Equations 1–7 estimated placental transfer represented by passive diffusion over the maternal-facing (CL_{pdm}) and fetal-facing (CL_{pdf}) barrier and active transport (CL_{p-gp}):

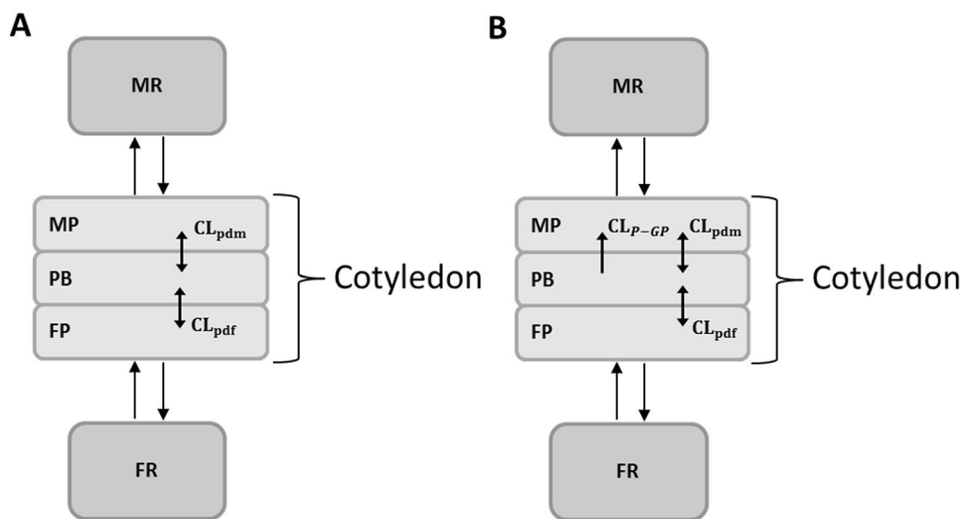$$\frac{dN_{MR}}{dt} = \frac{Q_M \times N_{MP} - Q_M \times N_{MR}}{V_{MR}}, \tag{1}$$

Fig. 1 Schematic overview of the tested mechanistic placenta model structures to estimate intrinsic placental transfer parameters of doravirine based on ex vivo perfusion data in closed–closed configuration. **A** simple diffusion transfer model; (**B**) diffusion model combined with p-glycoprotein-mediated active transport over the maternal-facing barrier. CL_{pdf} clearance between fetal part of the placenta and the placental barrier, CL_{pdm} clearance between maternal part of the placenta and the placental barrier, CL_{P-GP} p-glycoprotein-mediated active transport, *FR* fetal reservoir, *FP* fetal part of the placenta, *PB* barrier of the placenta, *MP* maternal part of the placenta, *MR* maternal reservoir

$$\frac{dN_{\text{MP}}}{dt} = \frac{Q_{\text{M}} \times N_{\text{MR}} + \text{CL}_{\text{pdm}} \times N_{\text{PB}} \times \text{FU}_{\text{P}} - Q_{\text{M}} \times N_{\text{MP}} - \text{CL}_{\text{pdm}} \times N_{\text{MP}} \times \text{FU}}{V_{\text{MP}}}, \tag{2}$$

$$\frac{dN_{\text{MP}}}{dt} = \frac{Q_{\text{M}} \times N_{\text{MR}} + \text{CL}_{\text{pdm}} \times N_{\text{PB}} \times \text{FU}_{\text{P}} - Q_{\text{M}} \times N_{\text{MP}} - \text{CL}_{\text{pdm}} \times N_{\text{MP}} \times \text{FU} + \text{CL}_{\text{p-gp}} \times N_{\text{PB}} \times \text{FU}_{\text{P}}}{V_{MP}}, \tag{3}$$

$$\frac{dN_{\text{PB}}}{dt} = \frac{\text{CL}_{\text{pdm}} \times N_{\text{MP}} \times \text{FU} + \text{CL}_{\text{pdf}} \times N_{\text{FP}} \times \text{FU} - \text{CL}_{\text{pdm}} \times N_{\text{PB}} \times \text{FU}_{\text{P}} - \text{CL}_{\text{pdf}} \times N_{\text{PB}} \times \text{FU}_{\text{P}}}{V_{\text{PB}}}, \tag{4}$$

$$\frac{dN_{\text{PB}}}{dt} = \frac{\text{CL}_{\text{pdm}} \times N_{\text{MP}} \times \text{FU} + \text{CL}_{\text{pdf}} \times N_{\text{FP}} \times \text{FU} - \text{CL}_{\text{pdm}} \times N_{\text{PB}} \times \text{FU}_{\text{P}} - \text{CL}_{\text{pdf}} \times N_{\text{PB}} \times \text{FU}_{\text{P}} - \text{CL}_{\text{p-gp}} \times N_{\text{PB}} \times \text{FU}_{\text{P}}}{V_{\text{PB}}}, \tag{5}$$

$$\frac{dN_{\text{FP}}}{dt} = \frac{Q_{\text{F}} \times N_{\text{FR}} + \text{CL}_{\text{pdf}} \times N_{\text{PB}} \times \text{FU}_{\text{P}} - Q_{\text{F}} \times N_{\text{FP}} - \text{CL}_{\text{pdf}} \times N_{\text{FP}} \times \text{FU}}{V_{FP}}, \tag{6}$$

$$\frac{dN_{\text{FR}}}{dt} = \frac{Q_{\text{F}} \times N_{\text{FP}} - Q_{\text{F}} \times N_{\text{FR}}}{V_{\text{FR}}}, \tag{7}$$

where N denotes amount (µg), Q denotes flow rate (mL/min), V denotes volume (mL), and FU denotes fraction unbound. Subscripted M, F, P, MR, MP, PB, FP, and FR, denote mother, fetus, placental, maternal reservoir, maternal placenta, placental barrier, fetal placenta, and fetal reservoir, respectively. Equations 3 and 5 were used instead of 2 and 4 in the second transfer model including transport. Model selection was based on maximum likelihood statistics (quantified by the objective function value [OFV]), with a 5% significance level (differences in OFV [dOFV] −3.84), physiological plausibility, precision in parameters estimates, standard goodness-of-fits plots, and visual predictive checks.

Input parameters were based on experimental conditions and placental physiology and are depicted in Table 1. Absolute volumes of MP, PB, and FP were scaled with the individual cotyledon weight of each perfusion experiment and were standarized to a typical cotyledon volume of 44.02 mL [26, 27]. We tested log-normal and box-cox transformed distributions for the interindividual variability (IIV) on transfer parameters and the correlation between IIVs [28]. Normally distributed additive, proportional, and combined error models were tested as one error model for the whole model and as separate error models for the different compartments or for the experimental directions.

2.3 Pregnancy Physiogically Based Pharmacokinetic Model

All simulations were performed using the Simcyp® PBPK Simulator, version 20 (Simcyp®, Certara company, Sheffield, UK). Doravirine parameters described in an existing three-compartment PBPK model, which was validated in nonpregnant individuals and verified with drug–drug interaction studies, were used as starting points [5, 6]. The model was optimized in terms of volume of distribution to allow use of the permeability-limited placenta model in Simcyp. This full-body PBPK model was then revalidated using independent data from intravenous and oral plasma concentrations in nonpregnant individuals with matched populations [6, 29–31]. We defined that predicted/observed ratios for area under the plasma concentration–time curve $(\text{AUC})_{0\text{–}24\text{h}}$, C_{max}, and trough plasma concentration (C_{trough}) had to be between 0.7 and 1.3, following the strict criteria for drugs with a small therapeutic window to increase confidence in model predictions [32].

After validation of the optimized compound file for nonpregnant subjects, the virtual population was switched to the pregnant population present in Simcyp (Fig. 2). This virtual pregnancy population has been validated for different compounds, including several drugs metabolized by CYP3A4 [9]. Additionally, the permeability-limited placenta model present in Simcyp was used, which was parameterized using the derived intrinsic placental transfer values from the mechanistic placenta model. Simulations were performed for 26, 32, and 40 weeks of pregnancy ($n = 100$ virtual subjects per group) and compared with a matched nonpregnant

Table 1 Parameters of the mechanistic placenta model

Parameter	Input	Reference
V_{MR}, mL	200	Experimental condition
V_{MP}, mL	5.08	11.55% of total placental volume [48]. Standardized for placenta of 42 g = 44.02 mL [26]
V_{PB}, mL	4.86	11.05% of total placental volume [48]. Standardized for placenta of 42 g = 44.02 mL [26]
V_{FP}, mL	3.63	8.25% of total placental volume [48]. Standardized for placenta of 42 g = 44.02 mL [26]
V_{FR}, mL	200	Experimental condition
Q_M, mL/min	12	Experimental condition
Q_F, mL/min	6	Experimental condition
FU	0.529	Measured
FU_p	0.01	Estimated with Simcyp® PBPK simulator version 20, based on the physiochemical properties
CL_{pdm}, mL/min	To be estimated	
CL_{pdf}, mL/min	To be estimated	
$CL_{P\text{-}gp}$, mL/min	To be estimated	

CL_{pdf} clearance between fetal part of the placenta and the placental barrier, CL_{pdm} clearance between maternal part of the placenta and the placental barrier, $CL_{P\text{-}gp}$ P-glycoprotein-mediated active transport (or other efflux transporter at the syncytiotrophoblast–blood interface), FU ex vivo fraction unbound, FU_P fraction unbound in placental barrier, Q_F fetal blood flow, Q_M maternal blood flow, V_{FP} volume fetal part of the placenta, V_{FR} volume fetal reservoir, V_{MP} volume maternal part of the placenta, V_{MR} volume maternal reservoir, V_{PB} volume placental barrier
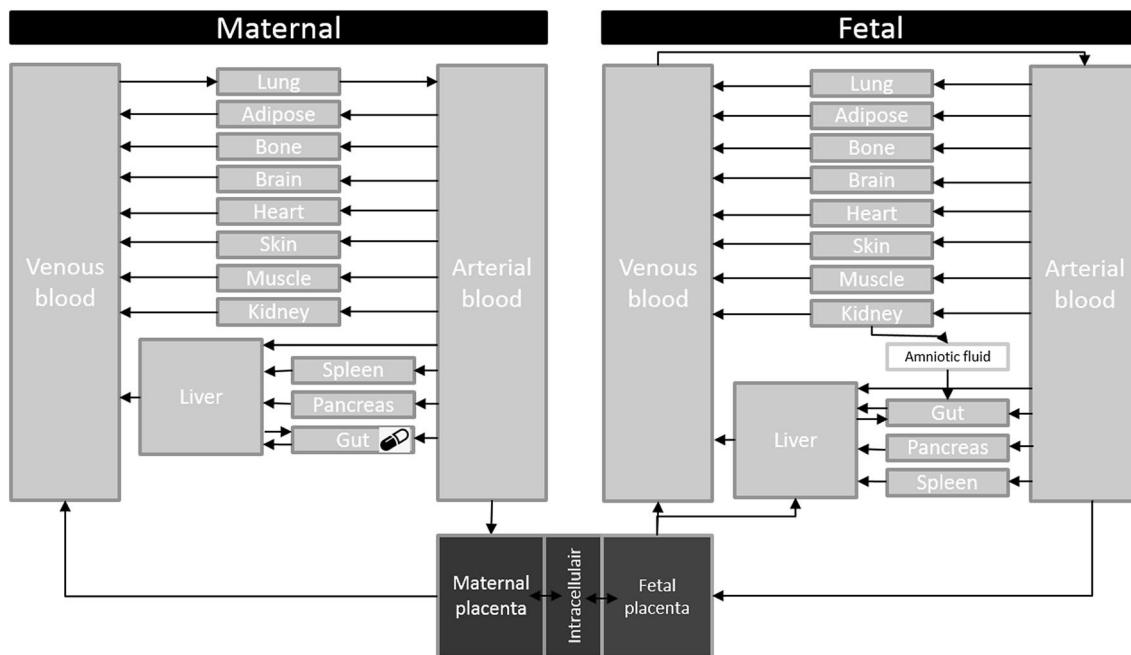

Fig. 2 Structure of the pregnancy physiologically based pharmacokinetic (PBPK) model in the PBPK platform Simcyp

simulation ($n=100$). Also, predicted maternal and fetal total C_{trough} was compared with the in vivo target of 0.23 mg/L; this target was the 10th percentile C_{trough} in a population pharmacokinetic study of phase IIb and III data that was significantly associated with lower clinical efficacy [33]. A twice-daily (BID) 100 mg regimen was evaluated in case of a predicted maternal geometric mean (GM) total C_{trough} <0.23 mg/L during pregnancy. Considering fetal safety, no target could be predefined, but the safety margins seem to be large. In embryo–fetal development toxicity studies in rats and rabbits, no embryo–fetal effects were observed up to doses of 450 mg/kg/day, corresponding to a maternal animal exposure (AUC_{0-24} 147 ng*h/mL) approximately nine-fold above the clinical exposure [34].

2.4 Sensitivity Analysis

The sensitivity of the estimated parameters included in the permeability-limited placenta model, namely the intrinsic placental transfer parameters (CL_{pdf} and CL_{pdm}) and the fraction unbound in the placental barrier (FU_p), on maternal and fetal plasma concentration were studied using the pregnancy PBPK model of women at 40 weeks of pregnancy. CL_{pdf} and CL_{pdm} were changed to the 2.5th and 97.5th percentile of the typical parameter estimates obtained from the sampling importance resampling procedure (Table 2) and pragmatically changed to 0.1 and 10-fold of the original value [35]. The FU_p was esimated with Simcyp based on the physicochemical properties of doravirine, and the uncertainty in parameter estimation was unknown. Therefore, FU_p was changed to 0.1, 0.5, 2, and 10-fold of the original value, taking a pragmatic approach. The sensitivity analysis was performed in Simcyp using 100 subjects.

2.5 Impact of Perfusion Experiment Setup

Ex vivo human placenta perfusion experiments in closed–open configuration, representative of sink conditions to measure clearance, have been used to estimate placental transfer for integration in PBPK models [15, 20, 36]. To investigate the impact of perfusion experiment configuration, we also performed perfusion experiments in a closed–open configuration in MTF ($n=3$) and FTM ($n=3$) direction, as previously described [15, 22]. We subsequently estimated the intrinsic placental transfer parameters with an adjusted mechanistic placenta model. The estimated intrinsic placental transfer parameters were imputed in the pregnancy PBPK model, and the results were presented together with the general sensitivity analysis. The detailed workflow for closed–open perfusion experiments is described in a separate ESM file: 13-19.

3 Results

3.1 Ex vivo Human Placenta Perfusion Experiments

Four successful closed–closed ex vivo placenta perfusion experiments were performed in each direction. Antipyrine FTM or MTF concentration ratios were all above 0.75, indicating adequate overlap between the maternal and fetal circulation (ESM 3). In addition, fetal vasculature remained intact during all these perfusions, as indicated by negligible volume loss of the closed circulation. Seven of eight placentas were obtained via caesarean section (ESM 4).

Figure 3 shows that doravirine crossed the placenta extensively. Steady state was reached at the end of the experimental period in the MTF direction and almost reached steady-state at the end of the experiments in the FTM direction. After addition of doravirine to the maternal circulation, median total concentrations in the maternal and fetal compartments at 180 minutes were 0.34 (range 0.30–0.39) and 0.28 (range 0.24–0.32) mg/L, respectively, corresponding to a mean FTM concentration ratio of 0.82. After addition of doravirine to the fetal circulation, final median total concentrations were, respectively, 0.28 (range 0.24–0.29) and 0.42 (range 0.40–0.56) mg/L, corresponding to a mean MTF concentration ratio of 0.61. The observed mean ex vivo free fraction was 52.9%.

The recovery of doravirine was 46% of the dose added in the MTF direction and 53% in the FTM direction, indicating placental tissue accumulation of doravirine. A control experiment without placental tissue did not show any adhesion of doravirine to components of the perfusion system (<5%) and showed doravirine stability. The median observed placental tissue concentration at 180 minutes was 0.76 (range 0.53–1.07) µg/g placenta, which was about 15% of the dose added. This explained approximately 25% of the doravirine loss as depicted in the mass–balance calculation (ESM 5 and 6).

3.2 Estimation of Intrinsic Placental Transfer Parameters

A diffusion-only transfer model adequately described the doravirine placenta perfusion data (ESM 7). Inclusion of
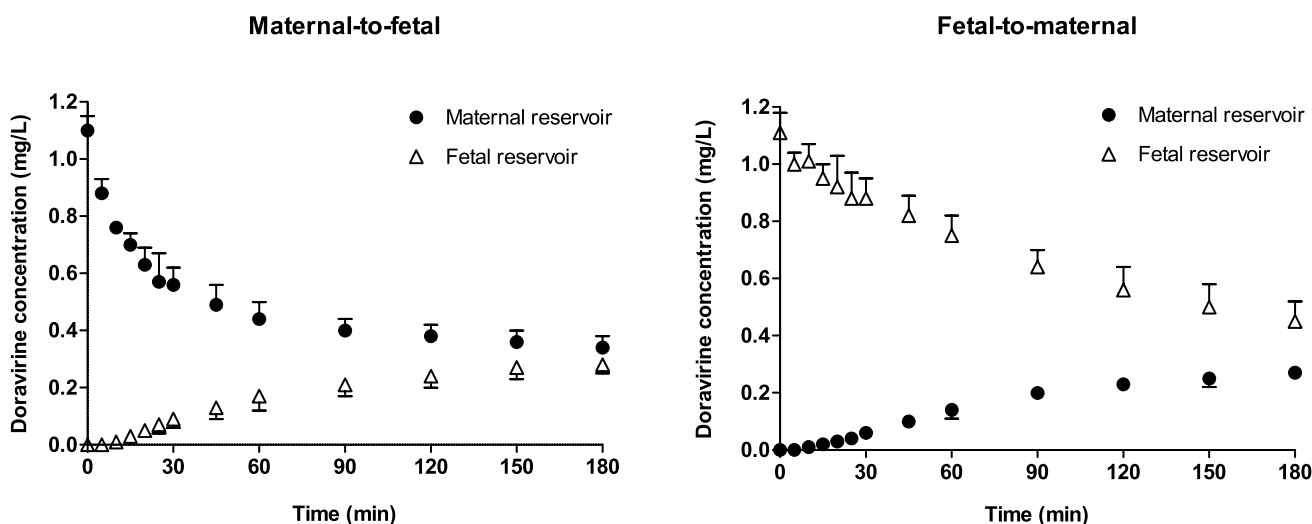
Table 2 Final estimates of intrinsic placental transfer parameters using the mechanistic placenta model

Parameter	Parameter estimate	95% CI from SIR
CL_{pdm}, mL/min[a]	37.2	19.8–73.7
CL_{pdf}, mL/min[a]	5.5	3.1–9.8
IIV CL_{pdm}, %	Fixed to 100[b]	
IIV CL_{pdf}, %	Fixed to 100[b]	
Additive residual error, µg/mL	0.000007	0.000004–0.00001
Proportional residual error after dosing in maternal compartment, %	7.5[b]	6.4–8.8 [c]
Proportional residual error after dosing in fetal compartment, %	12.6[b]	10.7–15.2 [c]

CI confidence interval, CL_{pdf} clearance between the fetal part of the placenta and the placental barrier, CL_{pdm} clearance between maternal part of the placenta and the placental barrier, *IIV* interindividual variability, *SIR* sampling importance resampling

[a]For the typical cotyledon weighing 42 g, assumed to be equal to 44.02 mL

[b]Transformed from log normal variance to % coefficient of variation with $\sqrt{(\exp(variance)-1)}$

[c]Transformed individual SIR results from log normal variance to % coefficient of variation with $\sqrt{(\exp(variance)-1)}$ for calculation of the 95% CI

Maternal-to-fetal **Fetal-to-maternal**

Fig. 3 Placental transfer of doravirine determined with ex vivo human cotyledon perfusion experiments in closed–closed configuration ($n = 4$ each). Data are shown as mean $\pm$ standard deviation

separate active transport had no significant effect (dOFV 0.007), and the typical parameter estimate of active transport over the maternal-facing barrier was very small, with high imprecision. The final parameter estimates are depicted in Table 2. For the typical cotyledon of 44.02 mL, we estimated that doravirine CL_{pdm} was 37.2 mL/min (95% confidence interval [CI] 19.8–73.7) and CL_{pdf} was 5.5 mL/min (95% CI 3.1–9.8). Interplacental variability was high, but IIV could not be estimated with a relative standard error <100% and was, therefore, pragmatically fixed at 100%.

3.3 Pregnancy Physiogically Based Pharmacokinetic Model

The final full-body PBPK model adequately described the mean data observed in healthy nonpregnant individuals (ESM 8). The predefined verification criterion was met because the predicted/observed ratios of AUC_{0-24h}, C_{max}, and C_{trough} were between 0.7 and 1.3 for intravenous, single-dose oral, and steady-state oral doravirine study comparisons in nonpregnant patients (ESM 9) [6, 29–31]. The final input parameters of the pregnancy PBPK model are depicted in ESM 10. The permeability-limited placenta model was parameterized with the intrinsic transfer estimates from the closed–closed placenta perfusion. After unit conversion, the imputed estimates were 0.0507 and 0.0075 L/h/mL placenta for CL_{pdm} and CL_{pdf}, respectively.

The PBPK model predicted a significant doravirine exposure decrease during pregnancy up to a GM steady state AUC_{0-24} decrease of 55% at 40 weeks of pregnancy compared with nonpregnant women (Fig. 4). The predicted GM

total C_{trough} was 0.10, 0.07, and 0.05 mg/L at 26, 32, and 40 weeks of pregnancy, respectively, corresponding to a C_{trough} decrease of 65%, 75%, and 84% compared with nonpregnant women. All GM C_{trough} values during pregnancy were below the predefined target of 0.23 mg/L. Maternal unbound concentrations decreased almost similarly to total concentrations; the fraction unbound was 31% at 40 weeks of pregnancy compared with 27% in nonpregnant women. An extensive overview of all predicted pharmacokinetic parameters is shown in ESM 11.

Substantial fetal doravirine exposure was predicted using the pregnancy PBPK model (Fig. 4). Although total concentrations were > 0.23 mg/L until approximately 8 h after drug intake, all predicted fetal mean C_{trough} were < 0.23 mg/L. The predicted placental tissue doravirine concentrations are shown in ESM 12. As observed from the ex vivo placenta perfusion experiments, substantial placental tissue concentrations were predicted. Predicted C_{max} concentrations in the placental tissue were almost two-fold higher than the maternal plasma C_{max}.

Because the predicted GM C_{trough} was < 0.23 mg/L during pregnancy, simulations were performed with a higher dose of doravirine (up to 100 mg BID) (Fig. 4). We predicted that maternal GM total C_{trough} would be 0.46, 0.37, and 0.28 mg/L at 26, 32, and 40 weeks of pregnancy, respectively. Also, predicted GM AUC_{0-24h} during pregnancy was similar to that in nonpregnant women receiving doravirine 100 mg once daily (QD). Predicted fetal mean total C_{trough} during pregnancy was around 0.23 mg/L. The maternal exposure after treatment with doravirine 100 mg BID fell within the exposure limits tested in animal embryo–fetal development toxicity studies (AUC_{0-24h} 147 ng*h/mL).
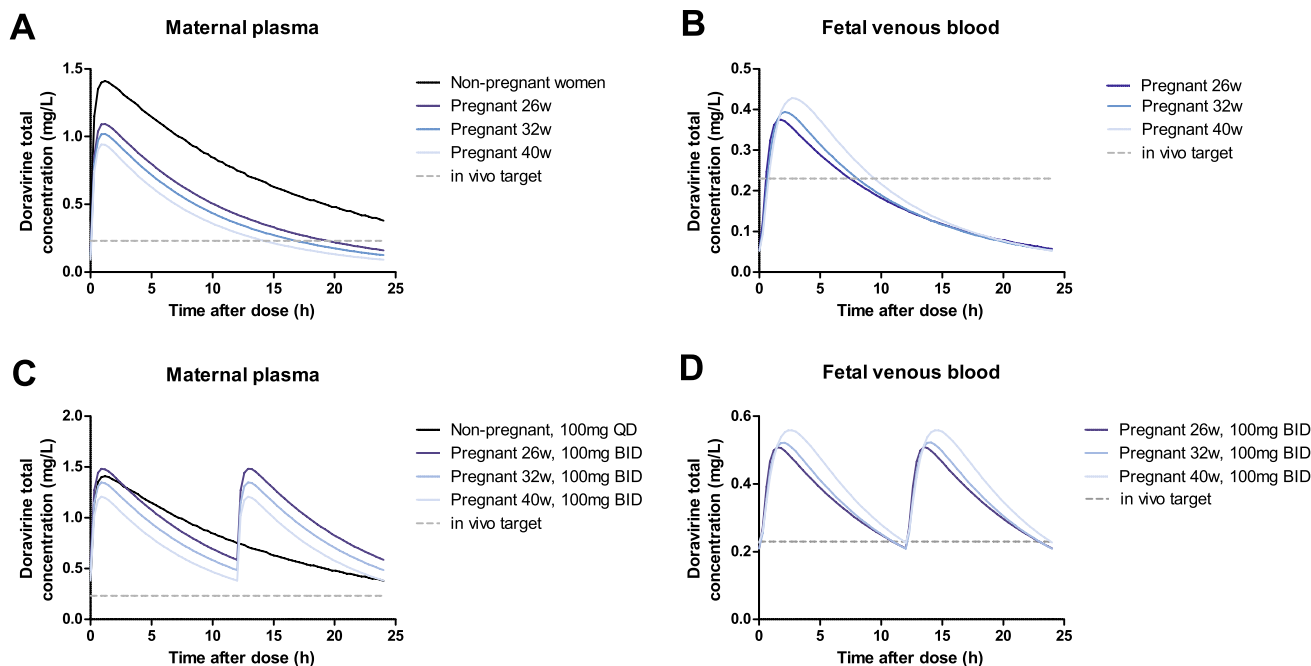
Fig. 4 Predicted mean doravirine total plasma concentration at steady state after treatment with **A**, **B** doravirine 100 mg QD or **C**, **D** 100 mg BID using the pregnancy physiologically based pharmacokinetic model ($n = 100$ subjects). The in vivo target of 0.23 mg/L was derived from in vivo exposure–response analysis [33]. *BID* twice daily, *QD* once daily, *w* weeks

3.4 Sensitivity Analysis

The observed uncertainty in the estimations of CL_{pdm}, CL_{pdf}, and FU_p had no impact on the predicted maternal plasma concentrations (Fig. 5). When looking at the impact on fetal plasma concentrations, only ten-fold lower estimations of CL_{pdf}, CL_{pdm}, and FU_p delayed and decreased fetal C_{max} by approximately 20%, whereas no difference could be observed when increasing these parameter values.

3.5 Impact of Perfusion Experiment Configuration

The results of the ex vivo human placenta perfusion experiments in a closed–open configuration are shown in ESM 15. With an adjusted mechanistic placenta model, we estimated that doravirine CL_{pdm} was 11.0 mL/min (95% CI 5.6–22.7) and CL_{pdf} was 4.3 mL/min (95% CI 2.3–8.3) (ESM 19). CL_{pdm} and CL_{pdf} derived from closed–open perfusion experiments were, respectively, 70% and 22% lower than the parameters from closed–closed experiments, but the 95% CIs overlapped. The observed differences did not influence maternal plasma concentration predictions and only slightly decreased fetal C_{max}, by 8% (Fig. 5).

4 Discussion

In vivo maternal and fetal doravirine exposure was predicted by integrating preclinical placenta perfusion experiments and PBPK modelling. In the absence of clinical human data, this combination of ex vivo and in silico approaches can help predict and understand the pregnancy effect on maternal doravirine exposure and predict fetal exposure. Our model predicted substantially reduced maternal doravirine exposure during pregnancy, possibly resulting in impaired doravirine efficacy.

Recirculating placental perfusion experiments showed that doravirine extensively crosses the placenta, which is consistent with previous open-circuit ex vivo placenta perfusion experiments [37]. A doravirine FTM ratio of 0.82 was observed, which is similar to the observed FTM ratio of 0.89 of antipyrine, a commonly used passive diffusion marker. About 15% of the added doravirine was observed in the placental tissue, in accordance with the lipophilic nature of doravirine. However, the observed doravirine placental tissue concentrations could not fully explain the doravirine loss, likely because of tissue measurement uncertainty. Placental elimination is unlikely to be the cause of the doravirine loss because placental CYP3A4 activity is expected to be negligible in term placentas [38]. It cannot be excluded that other placental enzymes alternatively metabolize doravirine, but how to quantify this remains unknown.
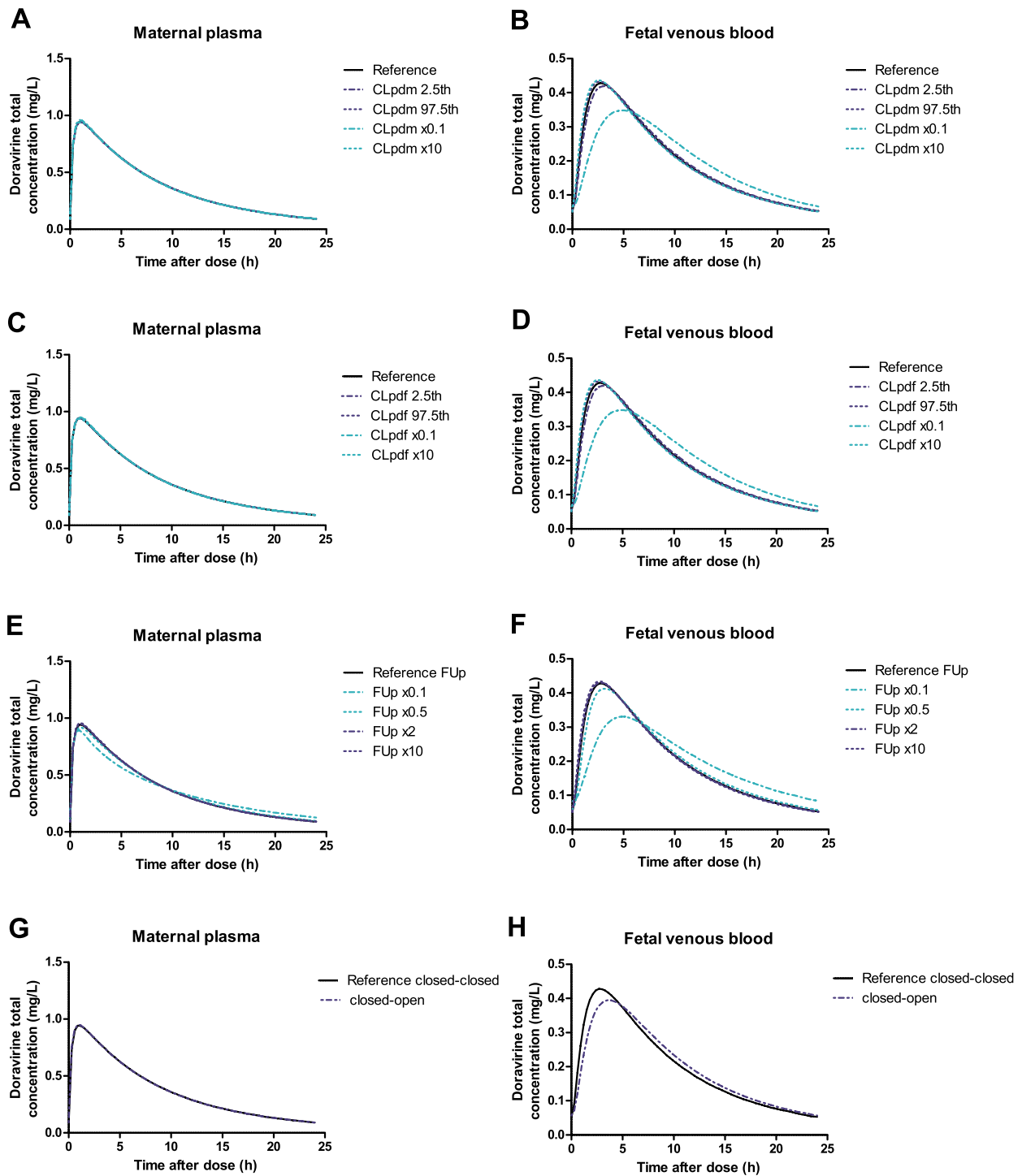
Fig. 5 Sensitivity analysis of the estimated placental parameters using the pregnancy physiologically based pharmacokinetic model ($n=100$ subjects). Sensitivity of CL_{pdm} input on the doravirine **A** maternal plasma concentration and **B** fetal venous blood concentration. Sensitivity of CL_{pdf} input on the doravirine, **C** maternal plasma concentration and **D** fetal venous blood concentration. The 2.5th and 97.5th percentile of the typical CL_{pdf} and CL_{pdm} are estimated with the mechanistic placenta model. Sensitivity of the FUp impute on the doravirine, **E** maternal plasma concentration and **F** fetal venous blood concentration. Sensitivity of the experimental placenta perfusion configuration on the doravirine, **G** maternal plasma concentration and **H** fetal venous blood concentration. CL_{pdf} clearance between fetal part of the placenta and the placental barrier, CL_{pdm} clearance between maternal part of the placenta and the placental barrier, FU_P fraction unbound in the placental barrier

Intrinsic placental transfer parameters, needed for parameterization of the pregnancy PBPK model in Simcyp, could be derived from both closed–closed and closed–open placenta perfusion experiments using a mechanistic placenta model. Marginal impact of the placental perfusion configuration was observed for a passive diffusion compound such as doravirine. The mechanistic placenta model was based on previous mechanistic models, matched with the placental model in Simcyp and optimized by standardization of individual cotyledon weight and by including doravirine ex vivo free fraction [13, 14, 17–19, 21]. Including the free fraction of the drug enabled us to correct for differences in free fraction observed in the experimental setup compared with in vivo. A limitation was that differentiating between active P-gp transport and passive diffusion was challenging with our placenta perfusion data. We advise that, in futher experiments, if the literature for the drug of interest indicates transporter affinity, perfusion experiments should be performed with different drug concentrations and transporter-specific inhibitors so that active transport over the placental barrier may be accurately assessed [39]. Also, further development of methods to quantify drug concentrations in the different placental cells is needed to better differentiate between the transfer processes, such as transfer over the maternal- versus fetal-facing placental barrier. In line with previous perfusion experiments with fatty acids and corticosteroids, we estimated a greater placental permeability over the maternal-facing barrier than over the fetal-facing barrier [40].

Pregnancy PBPK models are a promising tool for early pharmacokinetic predictions because these models mechanistically assess the influence of several physiological changes during pregnancy simultaneously. Although the pharmacokinetic processes relevant for doravirine are longitudinally described and validated in the pregnancy Simcyp model, we believe some caution in data interpretation is still needed. Variation in assumptions and longitudinal equations of the pregnant PBPK model exists across the various PBPK platforms, such as for CYP3A4 activity [12]. Simcyp assumes increased hepatic CYP3A4 activity solely, whereas other models assume uniform hepatic and intestinal CYP3A4 induction [12, 41]. Our pregnancy PBPK model of doravirine showed little impact from intestinal CYP3A4 induction on the predictions (data not shown), but further standarization and validation of the longitudinal CYP3A4 activity equation is desired. Another limitation of the PBPK model is that the variability in all mechanistic input parameters was not well defined and pragmatically set to 30% coefficient of variation, as common in PBPK modelling. Therefore, only mean pharmacokinetic parameters were presented.

Our developed pregnancy PBPK model predicted that maternal total doravirine C_{trough} was below 0.23 mg/L at 26, 32, and 40 weeks of pregnancy, suggesting impaired efficacy of doravirine during pregnancy. This target of 0.23 mg/L was significantly associated with virologic efficacy in an exposure–response analysis, although the observed relationship was relatively flat and possibly driven by nonadherence [33]. When simulating with the regimen of doravirine 100 mg BID, total GM C_{trough} in pregnant women remained above 0.23 mg/L. Possibly, BID dosing of doravirine is needed during pregnancy, which is in line with recommendations for rifabutin coadministration (C_{trough} decrease by 68%) [42].

The predicted decrease of doravirine exposure during pregnancy was similar to that observed in vivo for other drugs with a wide distribution volume, primarily CYP3A4 clearance and moderate plasma protein binding. A pharmacokinetic study observed a 74% lower indinavir minimum plasma drug concentration during pregnancy compared with postpartum, and another study observed a 76% decrease in quetiapine drug concentrations 8–30 h after drug intake in therapeutic drug monitoring samples of women in the third trimester [43, 44].

Using a permeability-limited placenta model and a full-body fetal PBPK model, considerable fetal doravirine plasma concentrations were predicted with concentrations >0.23 mg/L until around 8 h after drug intake. The permeability-limited placenta model was parameterized with intrinsic transfer parameters derived from placenta perfusion experiments with term placentas. Although the pregnancy model corrects for some altered physiologic processes in the placenta during the course of pregnancy, such as blood flow and placenta volume, we had no data on intrinsic placental transfer at 26 and 32 weeks of pregnancy. This resulted in some uncertainty in the predictions of fetal exposure at 26 and 32 weeks of pregnancy. Another limitation is that fetal clearance may not be optimally defined in the pregnancy PBPK model. Fetal CYP3A4 clearance was not included because fetal CYP3A4 activity is expected to be negligible [45, 46]. However, the involvement of other CYP450 enzymes in the fetal biotransformation of doravirine in utero cannot be excluded.

Sensitivity analysis showed that, for doravirine, increasing the intrinsic placental transfer parameters had little impact on fetal exposure because estimated doravirine intrinsic placental transfer parameters were similar to the placental blood flow, resulting in a blood flow-limited placenta model [47]. Lower estimations of intrinsic placental transfer parameters are expected to decrease fetal exposure as flow will no longer be limiting.

5 Conclusion

The developed pregnancy PBPK model predicted substantially reduced maternal doravirine exposure during pregnancy, possibly resulting in impaired doravirine efficacy. Therefore, therapeutic drug monitoring (if available) and

viral load monitoring are advised for pregnant women treated with doravirine, and the use of this drug should preferentially be restricted to clinical trial settings, which are ongoing [48–50]. In addition, perfusion experiments showed that doravirine crosses the placenta extensively, and considerable fetal doravirine exposure was predicted. Although fetal exposure can have a prophylactic effect and the maternal exposure at doses of 100 mg QD and 100 mg BID stayed within the safety limits for embryo–fetal effects observed in animal reproduction studies, clinical safety data are needed. Based on the predicted maternal and fetal exposure in this study, we advise further investigation of the 100 mg BID dose in clinical pharmacokinetic studies to show whether this overcomes the decreased exposure and to collect maternal and fetal clinical safety data. The approach of integrating a placenta perfusion experiment and PBPK modeling is promising for the investigation of drug pharmacokinetics during pregnancy early in drug development, but further standardization and extension is needed.

Declarations

Conflicts of interest AC has received honoraria from Merck Sharp & Dohme Corp 2021, paid to their institution. A Colbers and D Burger have received study grants from Merck Sharp & Dohme Corp, Gilead, and ViiV Healthcare, paid to their institution. VE Bukkems, H van Hove, D Roelofsen, JJM Freriksen, EWJ van Ewijk-Beneken Kolmer, J van Drongelen, EM Svensson, and R Greupink have no conflicts of interest that are directly relevant to the content of this article.

Availability of data and material Data are stored at the Deparment of Pharmacy and the Deparment of Pharmaclogy-Toxicology of the Radboud University Medical Center. Data may be available upon request to the authors.

Ethics approval For performing the placenta perfusion experiments, the regional institutional medical ethical committee provided a waiver for formal approval based on the Dutch Law for Human research (CMO Arnhem/Nijmegen File 2014-1397).

Informed consent Informed consent was obtained from the women who donated their placentas for the placenta perfusing experiments.

Author contributions V E Bukkems, H van Hove, D Roelofsen, J J M Freriksen, D Burger, J van Drongelen, R Greupink, and A Colbers designed the research. V E Bukkems, H van Hove, and D Roelofsen performed the research. V E Bukkems, H van Hove, D Roelofsen, J J M Freriksen, E W J van Ewijk-Beneken Kolmer, E Svensson, R Greupink, and A Colbers analyzed the data. V E Bukkems, H van Hove, and D Roelofsen wrote the manuscript.

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