General Supplementary Information for

Prediction of maternal and fetal doravirine exposure by integrating physiologically-based pharmacokinetic modelling and human cotyledon perfusion experiments

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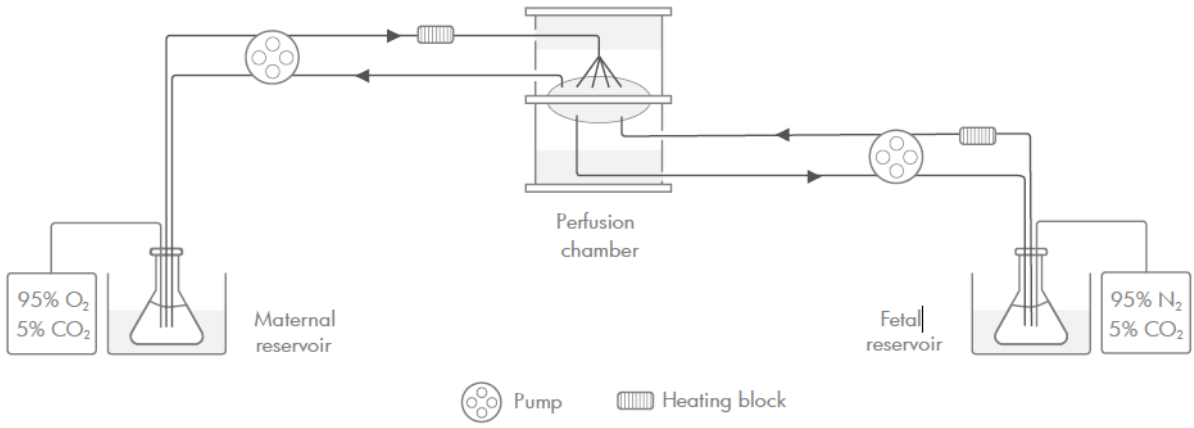


Online Resource 1: Schematic overview of the *ex vivo* dual-side placenta perfusion experiment in closed-closed configuration. to study placental transfer of doravirine from the maternal-to-fetal circulation and from the fetal-to-maternal circulation Both maternal and fetal circulations were recirculated (closed).

Online Resource 2: Bioanalytical assays

Bioanalytical assay antipyrine

The collected samples were analyzed at the laboratory of the Department of Pharmacology and Toxicology, Radboud university medical center. Antipyrine measurements were performed using an LC-MS/MS method with an Acquity UPLC (Waters, Milford, MA, USA) coupled to a Xevo TQ-S micro (Waters) triple quadrupole mass spectrometer. Protein precipitation was achieved by the addition of acetonitrile to the samples in a ratio 3:1. After vortexing, samples were centrifuged at 13000 rpm for 3 minutes. Samples were injected on an Acquity UPLC HSST3 column and separation was accomplished via gradient elution (flowrate 0.300 mL/min). Gradient conditions were as follows: 0-1.0 min, 100% solvent A and 0% solvent B; 1-3 min, 100% solvent B and 0% solvent A. Solvent A consisted of 100% water and 0.1% formic acid, solvent B of 100% acetonitrile and 0.1% formic acid. The effluent from the UPLC was passed directly into the electrospray ion source. Positive electrospray ionization was achieved using nitrogen as a desolvation gas with ionization voltage at 0.3 kV. Furthermore, argon was used as a collision gas and the source temperature was set at 500 °C. Detection of the compounds was based on isolation of the protonated molecular ion and subsequent MS/MS fragmentations and a multi reaction monitoring (MRM) was carried out. The following MRM transitions were used: for antipyrine, m/z 188.98 (parent ion) to m/z 55.83 and 76.96 (both product ions), and for d3-antipyrine, m/z 192.04 (parent ion) to m/z 58.95 and 104.14 (both product ions). The internal standard d3-antipyrine (Toronto Research Chemicals) was used.

Bioanalytical assay doravirine

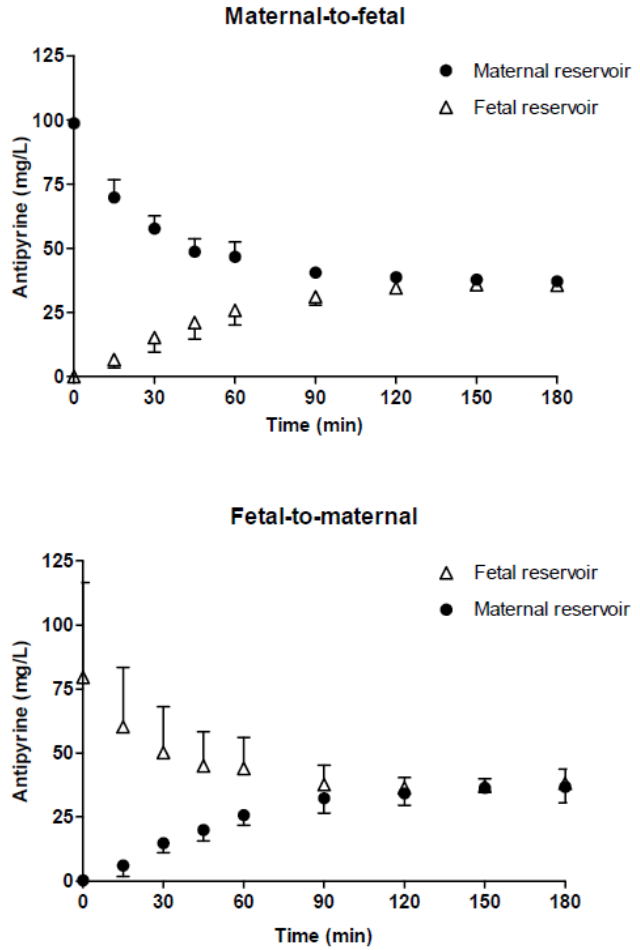
The collected samples were analyzed at the laboratory of the Department of Pharmacy, Radboud university medical center. The laboratory participates in external quality assurance programs for antiretroviral drug quantification (1, 2). The LC-MS/MS measurements of doravirine were performed using an Acquity UPLC (Waters, Milford, MA, USA) coupled to a Xevo TQ-S micro (Waters) triple

quadrupole mass spectrometer. Protein precipitation was achieved by the addition of methanol (ratio sample:methanol 1:25). Next, samples were vortexed for 2 minutes at speed 2500 rpm and subsequently centrifuged at 14000 rpm (18620 g) for 5 minutes. Samples were injected on an Acquity UPLC BEH C18 1,7 µm 2,1 x 50 mm column and separation was accomplished via gradient elution (flowrate 0.475 mL/min). Gradient conditions were as follows: 0-5.0 min, 80% solvent A and 20% solvent B; 5.0-5.1, change to 10% solvent A and 90% solvent C; 5.1-5.9 min, 10% solvent A and 90% solvent C; 5.9-6.0 min, change to 80% solvent A and 20% solvent B; 6.0-10.0 min, 80% solvent A and 20% solvent B. Solvent A consisted of 100% water and 0.1% formic acid, solvent B of 100% acetonitrile and 0.1% formic acid, and solvent C was composed of 100% acetonitrile and 1% formic acid. The UPLC transmitted the effluent directly into the electrospray ion source which was established using nitrogen as a desolvation gas with an ionization voltage of 2.5 kV. Furthermore, argon was used as a collision gas and the source temperature of the MS was set at 150 °C. The following MRM transitions were used for doravirine, m/z 426.2 (parent ion) to m/z 112.0 and 315.0 (both product ions), and for [$_{13}C_6$]-doravirine, m/z 432.2 (parent ion) to m/z 112.0 (product ion).The internal standard [$^{13}C_6$]-Doravirine (Merck Sharp & Dohme (MSD)) was used.

Unbound doravirine concentrations were determined using ultrafiltration. Samples were filtered by an ultrafiltration device (Centrifree -30K, Amicon) and centrifuged for 20 minutes at 1650 g at 37 °C to separate bound from unbound molecules, followed by subsequent LC-MS/MS detection of the fraction containing unbound doravirine as previously described.

Tissue samples were weighted and subsequently diluted in drug-free perfusion medium (tissue:perfusion medium, 1:4). Next, samples were homogenized in the perfusion medium using a T10 basic Ultra-Turrax® disperser (IKA, Germany) for 20 seconds and two subsequent times per sample, while keeping the samples on ice to obtain a 20% tissue homogenate. Protein precipitation was achieved using methanol (sample:methanol 1:25). Samples were vortexed and then centrifuged for

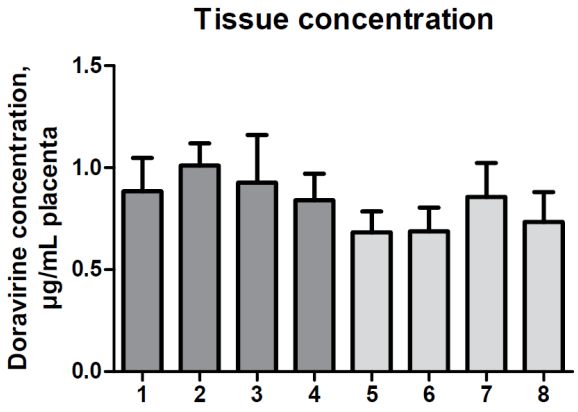
5 minutes at 13000 rpm. Finally, the supernatant was transferred to vials and LC-MS/MS analysis was performed as previously described to determine doravirine concentrations in placental tissue.



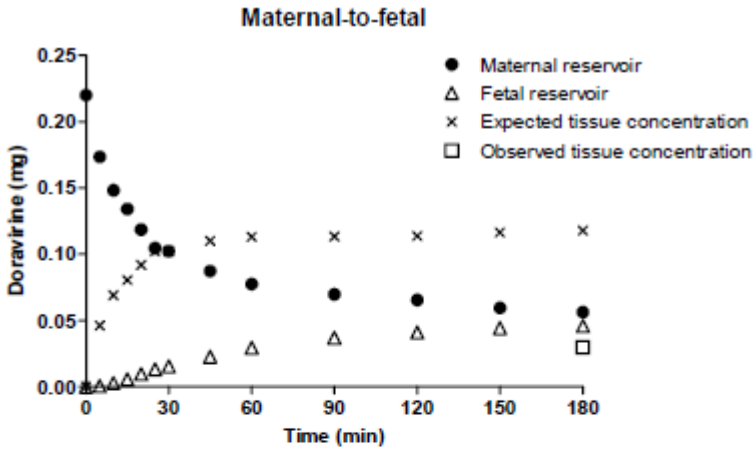
Online Resource 3: Antipyrine control data of the *ex vivo* human cotyledon perfusion experiments in closed-closed configuration (n=4 each). Data are shown as mean ± SD

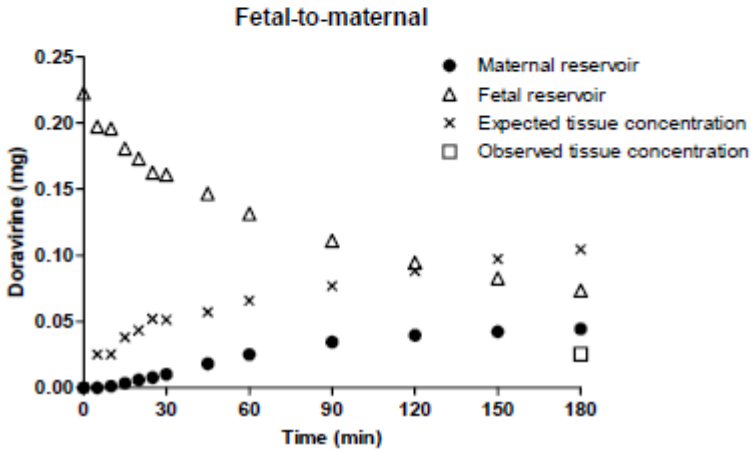
Online Resource 4: Overview of the main characteristics of the placentas used for the *ex vivo* dual-side placenta perfusion experiments in closed-closed configuration

Closed-closed perfusion						
Placenta nr.	**Maternal age, years**	**Medication**	**Gestational age at delivery, weeks**	**Birth weight neonate, g**	**Mode of delivery**	**Cotyledon weight, g**
1	31	None	39	4580	Caesarean section	18.9
2	30	Omeprazole	38	3135	Caesarean section	31.4
3	37	Omeprazole & acetaminophen	37	3090	Caesarean section	43.5
4	37	None	40	3978	Vaginal delivery	43.6
5	34	None	39	3815	Caesarean section	64.4
6	41	Vitamin D3	38	3985	Caesarean section	20.4
7	36	None	40	2785	Caesarean section	33.7
8	30	Vitamin D3	39	Unkown	Caesarean section	27.4

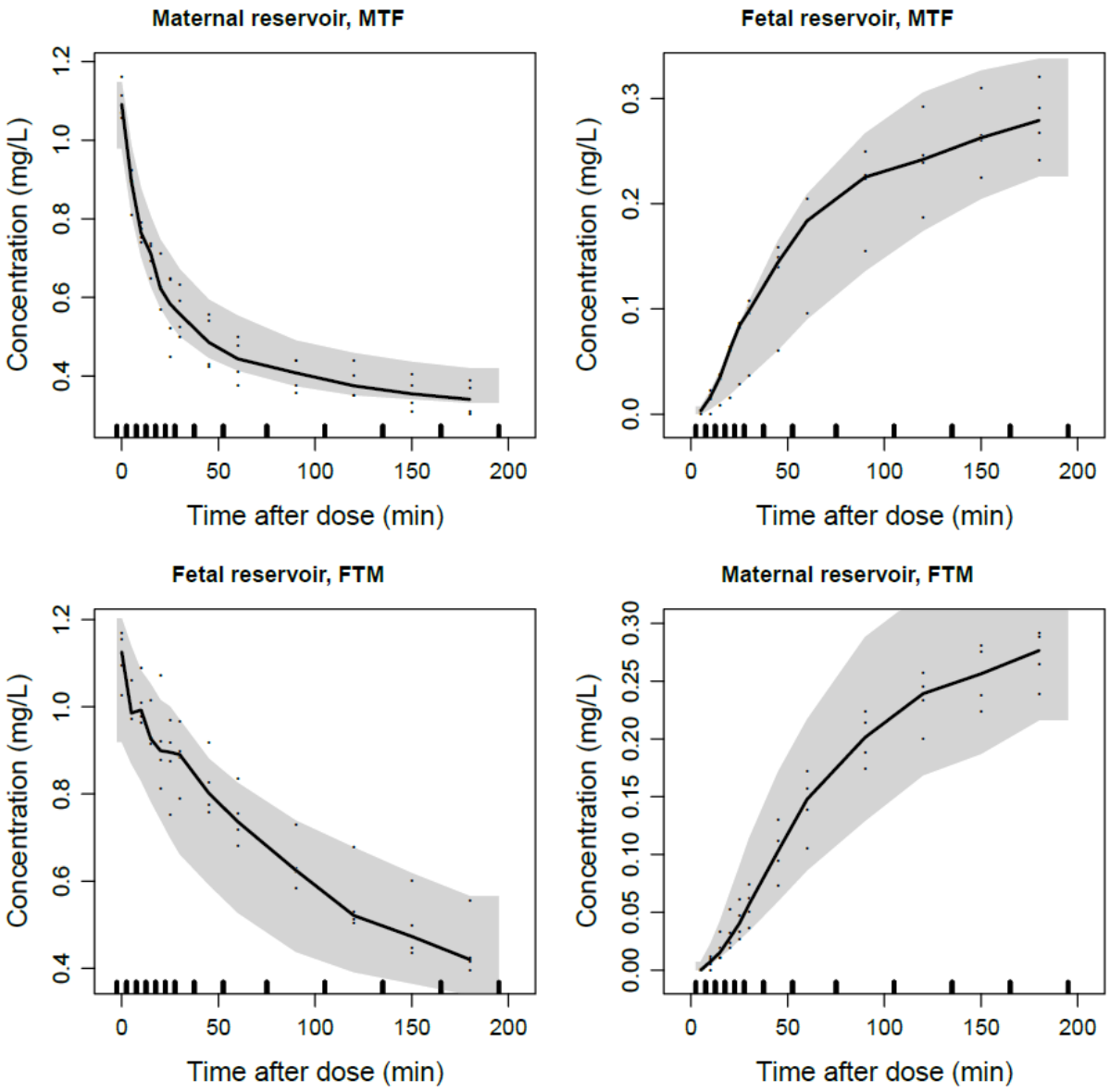


Online Resource 5: Doravirine concentration in the placental tissue at the end of the experiment in closed-closed configuration. Dark grey represents perfusion in maternal-to-fetal direction, and light grey represents perfusions in fetal-to-maternal direction. Data are reported for n= 8 placentas and 3 tissue samples per placenta, data is shown as mean ± SD.

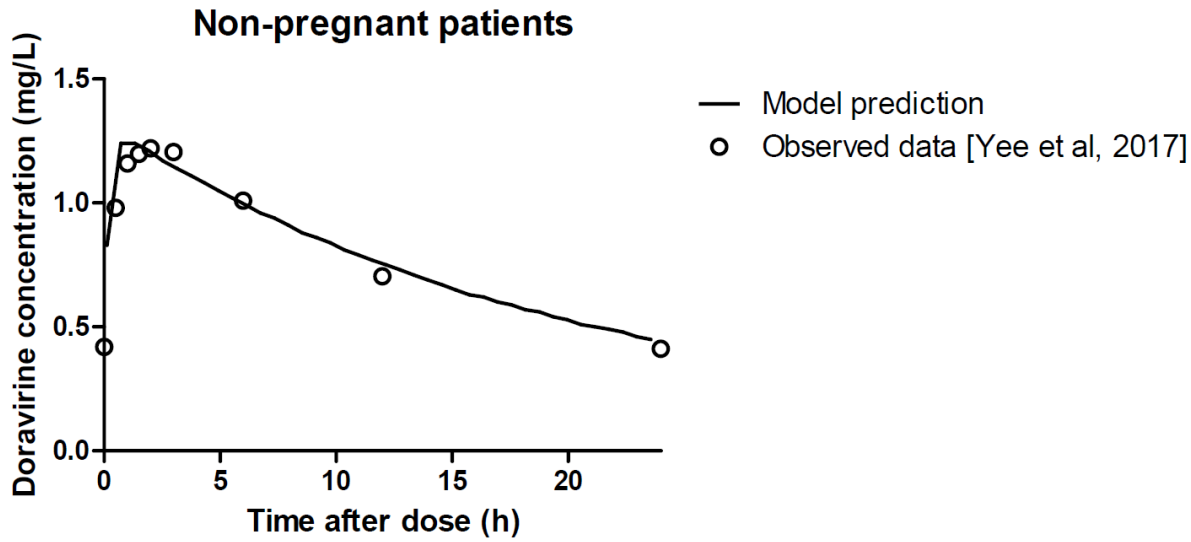




Online Resource 6: Doravirine mass balance of the *ex vivo* placenta perfusion experiments in closed-closed configuration (n=4 each). Calculations are based on the measured concentration of doravirine at each timepoint in the maternal and fetal buffer. Corrections have been made for volume loss of the perfusion medium due to sampling.



Online Resource 7: Visual predictive check of the final physiologically-based placenta model (simulations n=1000) with the observed data of the *ex vivo* human cotyledon perfusion experiments in closed-closed configuration. The observations of the perfusion experiments are indicate by the black dots. The median of the observations is indicated by the continuous black line. The grey shaded area indicate the 95% confidence interval around the median of the simulated data. Vertical markers indicate the bins around the sampling points. MTF; maternal-to-fetal perfusion experiment, FTM; fetal-to-maternal perfusion experiment.



Online Resource 8: Predicted doravirine plasma concentration-time profile at steady-state in healthy, non-pregnant patients using the PBPK model compared with the observed data of Yee et al, 2017. (3)

Online Resource 9: predicted/observed ratios of primary pharmacokinetic parameters for intravenous, single dose oral and steady state oral doravirine study comparisons in non-pregnant patients using the non-pregnant physiogically-based pharmacokinetic model.

	Predicted/observed ratio			
	$AUC_{0\text{-}24h}$ [a]	**C_{max}**	**C_{trough}**	**CL**
Single dose intrevenous, healthy subjects (4)	1.05			0.98
Single dose oral, fasted, healthy females (5)	0.99	1.26	1.01	
Single dose oral, fed state, healthy subjects (6)	1.03	1.11	0.90	
Steady state oral, healthy subjects (3)	0.97	0.97	0.92	

a AUCinf for intravenous study comparison

Online Resource 10*:* Summary of physiochemical and pharmacokinetic parameters used in the pregnancy PBPK model for doravirine

Parameter	Input	Reference
Molecular weight (g/mol)	425.75	Yee et al, 2021 (7)
LogP	3.0	Yee et al, 2021 (7)
Compound type	Monoprotic base	Yee et al, 2021 (7)
pKa	9.47	Yee et al, 2021 (7)
Plasma free fraction	0.24	Yee et al, 2021 (7)
Blood/plasma ratio	1.0	Yee et al, 2021 (7)
Intestinal free fraction	1.0	Assumed, sensitivity analysis showed parameter that it is not a sensitive parameter
Protein binding to	AGP	Assumed, because basic compound (8)
AGP K_D (µM)	5.81	Predicted with Simcyp
AGP reference concentration (g/L)	0.81	Simcyp
Absorption model (first order)		
K_a (h^{-1})	1.4	PopPK analysis (4)
F_a	0.664	PopPK analysis, calculated from F (4)
P_{eff} (x 10^{-4} cm/s)	2.44	Calculated in Simcyp, based on doravirine P_{app} of $25*10^{-6}$ cm/s and verapamil P_{app} of $40.1*10^{-6}$ cm/s in LLC-PK1 cells (4)
Distribution model		
V_{ss} (L/kg)	0.94	Calculated in Simcyp with prediction method 2 and Kp scalar of 0.211 to simulate V_{ss} as reported in PopPK analysis (4)
Elimination model (enzyme kinetics)		
Total CYP3A4 CL_{int} (µL/min/pmol CYP)	0.026	Yee et al, 2021 (4, 7)
CL_{renal}	0.556	Yee et al, 2021 (7, 9)
Permeability placenta model		
CL_{pdm} (L/h/mL placenta)	0.0507	Estimated with the mechanistic placenta model
CL_{pdf} (L/h/mL placenta)	0.0075	Estimated with the mechanistic placenta model
FUp	0.013	Predicted with Simcyp
Fetal model		
FUf	0.544	Predicted with Simcyp

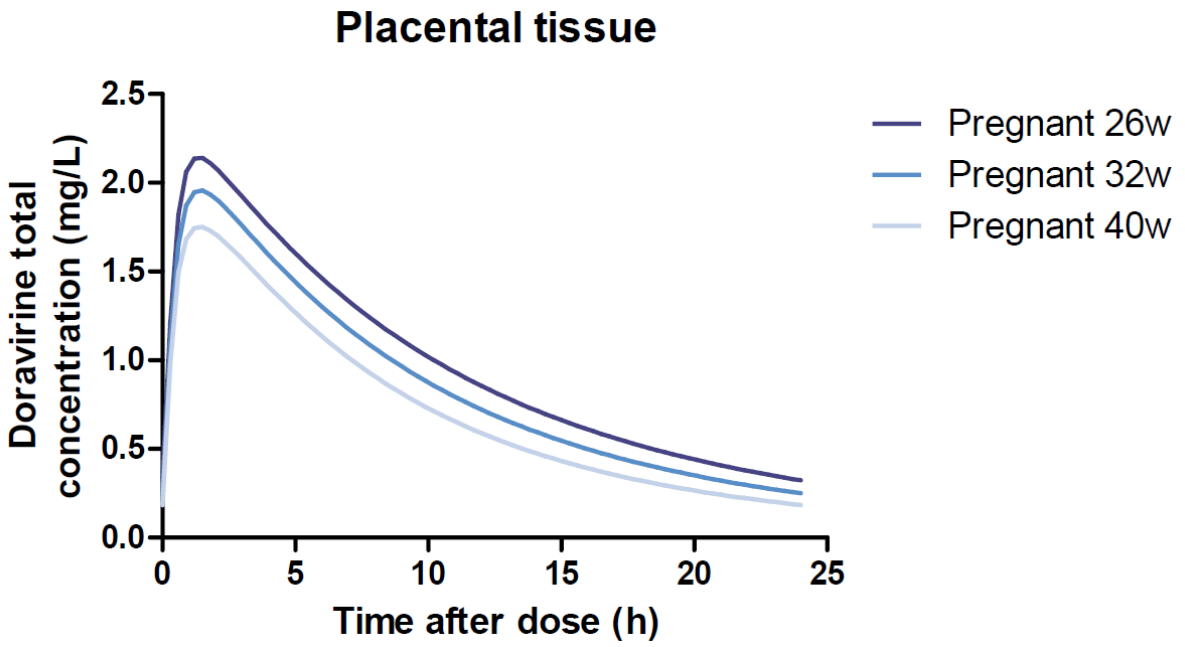
Abbreviations: Log $P_{o:w}$; octanol-water partition coefficient, pKa; Acid dissociation constant, AGP; Alpha-1-acid glycoprotein, KD; dissociation constant of the drug-protein complex, Papp; apparent permeability coefficient, Peff; intestinal permeability, Ka; absorption rate constant, Fa; fraction of dose absorbed, Vss; volume of distribution, CLint; intrinsic clearance, CLrenal; renal clearance, PopPK; population pharmacokinetic, FUp; fraction unbound in the placental barrier, FUf; fraction unbound in fetal plasma.

Online Resource 11: Predicted pharmacokinetic parameters of doravirine in pregnant and non-pregnant women using the pregnancy PBPK-model.

PK parameter	26w of pregnancy, GM [a]	32w of pregnancy, GM [a]	40w of pregnancy, GM [a]	Non-pregnant women, GMR	26w vs. non-pregnant, GMR	32w vs. non-pregnant, GMR	40w vs. non-pregnant, GMR
Maternal plasma, total concentration, 100mg QD							
$AUC_{0\text{-}24h}$, mg*h/L	10.70	9.33	7.88	17.32	0.62	0.54	0.45
C_{max}, mg/L	1.06	0.99	0.92	1.35	0.79	0.73	0.68
C_{trough}, mg/L	0.10	0.07	0.05	0.28	0.35	0.25	0.16
Maternal plasma, unbound concentration, 100mg QD							
$AUC_{0\text{-}24h}$, mg*h/L	3.16	2.82	2.42	4.66	0.68	0.61	0.52
C_{max}, mg/L	0.31	0.30	0.28	0.36	0.86	0.82	0.77
C_{trough}, mg/L	0.03	0.02	0.01	0.08	0.39	0.28	0.19
Fraction unbound, %	30	30	31	27			
Maternal plasma, total concentration, 100mg BID							
$AUC_{0\text{-}24h}$, mg*h/L	21.43	18.69	15.78	-	-	-	-
C_{max}, mg/L	1.40	1.27	1.14	-	-	-	-
C_{trough}, mg/L	0.46	0.37	0.28	-	-	-	-
Fetal plasma, total concentration, 100mg QD							
$AUC_{0\text{-}24h}$, mg*h/L	4.22	4.34	4.79	-	-	-	-
C_{max}, mg/L	0.38	0.39	0.43	-	-	-	-
C_{trough}, mg/L	0.06	0.05	0.05	-	-	-	-
Fetal plasma, total concentration, 100mg BID							
$AUC_{0\text{-}24h}$, mg*h/L	8.54	8.78	9.70				
C_{max}, mg/L	0.51	0.52	0.56				
C_{trough}, mg/L	0.21	0.21	0.23				

Abbreviations: w; weeks, GM; geometric mean, GMR; geometric mean ratio, QD; once-daily, BID; twice-daily

[a] mean for fetal plasma pharmacokinetic parameters



Online Resource 12: Predicted doravirine concentration in the placental tissue using the pregnancy PBPK model. w; weeks.

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