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Supplement Article

In Silico Modeling for Ex Vivo Placental Transfer of Morphine

The Journal of Clinical Pharmacology
2022, 62(S1) S140–S146

DOI: 10.1002/jcph.2105

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Abstract

Morphine may be administered in pregnant women as an analgesic agent. The transplacental pharmacokinetics (PK) of morphine varies during pregnancy because of physiological and metabolic changes. In this work, we use a multi-compartment model to simulate ex vivo human placental transfer studies of morphine. The computational model is based on a recently published model for metformin with both passive and active transport kinetics. Modifications were made to incorporate morphine-specific transfer parameters. Parameters for the PK models were determined via the nonlinear regression method. In addition, the Latin hypercube sampling (LHS) method was used for the global parameter analysis of the model. Simulation results show good agreement between the model and observed fetal and maternal morphine concentrations. In addition, the lower efflux of morphine from fetal to maternal plasma reflects reduced P-glycoprotein (P-gp) transport as pregnancy progresses, which leads to slower clearance of morphine in the maternal plasma. The LHS analysis also indicates the more significant roles played by the passive diffusion parameters than the active transport parameter on the fetal/maternal morphine concentrations. In conclusion, we used an in silico model to investigate the transplacental properties of morphine and to predict the in vivo transplacental properties of morphine when PK parameters change.

Keywords

model, morphine, opioid, pharmacokinetics, placenta, pregnant woman

Morphine is an opioid analgesic used to manage moderate to severe pain.[1,2] Specifically, morphine has been used to relieve pain during labor since the early 20th century.[2] However, its clinical use during pregnancy is controversial owing to possible adverse effects on fetuses, such as neonatal opioid withdrawal syndrome.[3] One study claims that morphine does not significantly reduce labor pain intensity, and that systematically giving morphine seems inappropriate.[4] Nevertheless, opioids, including morphine, may be used and abused during pregnancy. For example, it was found that 6.6% of women self-reported opioid use during pregnancy in the USA, and 21.2% of them disclosed opioid misuse.[5] Hence, it is important to investigate the absorption, distribution, metabolism, and excretion (ADME) of morphine in pregnant women and the potentially detrimental effects of morphine on fetuses and neonates.

In vivo pharmacokinetic studies of morphine in pregnant women are scarce. One such study was published more than 3 decades ago.[2] The authors found faster clearance and shorter half-time of morphine in 13 pregnant women than in 6 nonpregnant women. The same research group also found that morphine crossed the placenta quickly. Still, the placental transfer of its metabolite morphine-3-glucuronide (M3G) was retarded in 5 cases of suspected Rhesus (Rh) isoimmunization.[6] With the ethical constraints on in vivo investigations, evaluations of fetotoxicity from maternal exposure to chemicals and drugs are usually performed in vitro, for example using an ex vivo perfusion system of the placenta.[7] In such a system, the placenta was collected from pregnant women at delivery and a single unit of cotyledon was cannulated and perfused in the perfusate.[7] Depending on whether the perfusate is recirculated, an ex vivo perfusion system can be closed (with recirculation) or open (without recirculation). Specific to morphine, an ex vivo human cotyledon perfusion study was reported by Kopecky et al.[8] The authors found that changes in vascular resistance did not alter the transfer of morphine across the placental barrier.

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Submitted for publication 31 January 2022; accepted 23 May 2022.

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However, it should be stressed that with cotyledon harvested postpartum, the transplacental properties at term could be measured from an ex vivo perfusion experiment. Yet great physiological and metabolic changes occur throughout pregnancy, which affects the pharmacokinetics and transplacental properties of morphine. Anatomically, the human placental barrier at term thins out to about one-tenth of its thickness in the first trimester, that is, from 50 μm at the end of the 2nd month to about 5 μm by the end of week 37.[9] With the physicochemical properties of lipophilicity (pKa 7.9) and low molecular weight (285 Da), morphine readily crosses the placenta from the maternal side to the fetal side.[8] Furthermore, morphine is bound to albumin in plasma, yet albumin decreases as pregnancy progresses.[10] Another factor to consider is membrane transporters that actively transport morphine across the placenta. Morphine is a substrate of P-glycoprotein (P-gp), expressed at the apical side (maternal facing) of the syncytiotrophoblast,[11] and facilitates morphine transfer from the fetal side to the maternal side. However, the placental expression of P-gp decreases as gestational age advances.[12] The phenomenon may result in reduced morphine transport from fetal to maternal circulations.[10]

The study aims to develop an in silico model of morphine to simulate the ex vivo perfusion of the human placental cotyledon described in a previous study.[8] Similar models have been developed for other drugs, for example, metformin and acetaminophen (paracetamol).[13,14] With such a model, one may simulate some intricate transplacental mechanisms during pregnancy, such as protein binding and transporter-assisted active perfusion, which are otherwise difficult or costly to measure from ex vivo experiments.

Methods

Ex Vivo Cotyledon Perfusion Data for Morphine

In the ex vivo placenta perfusion experiment described by Kopecky et al,[8] the cotyledon was at first perfused without morphine for 2 hours as a pre-experiment control period. A 50 ng/mL morphine dose was then added to the perfusing system, and the protein-free perfusate was circulated between maternal and fetal circulations for 4 hours. Perfusate samples were collected from maternal and fetal reservoirs every 5 minutes for 30 minutes, every 15 minutes for 30 minutes, and every 30 minutes for the rest of the experiment. The temporal profiles of morphine in the maternal and fetal reservoirs were digitized from the paper by using a WebPlotDigitizer tool.

In Silico Model

We adopted a recently published in silico model by Kurosawa et al,[13] which was originally developed to investigate the transplacental kinetics of metformin. We also refer to the work of Mian et al,[14] which simulates the transplacental properties of paracetamol. As morphine readily crosses the placenta, we hypothesize that morphine transfer from the maternal to the fetal side is dominated by passive diffusion, yet the transfer from fetal to maternal plasma depends on both passive diffusion and active transport, which is aided by the P-gp transporter. Compartments imitating the ex vivo perfusion system are shown in Figure 1. Three compartments, that is, a maternal placenta (MP), a trophoblast and a fetal capillary compartment, were used to represent a single cotyledon unit. Drug transfer across the microvillous plasma membrane (MVM) and the basal plasma membrane (BM) were modeled separately for the trophoblast compartment. This arrangement facilitates the modeling for passive diffusion and active transport mechanisms individually at the apical (maternal facing) and basal (fetal facing) membranes. The model consists of 7 equations:

$$\frac{dC_{mr}}{dt} = \frac{Q_m \times (C_{mp} - C_{mr})}{V_{mr}}, \tag{1}$$

$$\frac{dC_{ma}}{dt} = \frac{Q_m \times (C_{mr} - C_{ma})}{V_{ma}}, \tag{2}$$

$$\frac{dC_{mp}}{dt} = \frac{Q_m \times (C_{ma} - C_{mp}) + (PS_{MVM,act,eff} + PS_{MVM,diff}) \times C_t - PS_{MVM,diff} \times C_{mp}}{V_{mp}}, \tag{3}$$

$$\frac{dC_t}{dt} = \frac{PS_{MVM,diff} \times C_{mp} + PS_{BM,diff} \times C_{fc} - (PS_{MVM,act,eff} + PS_{MVM,diff} + PS_{BM,diff}) \times C_t}{V_t}, \tag{4}$$

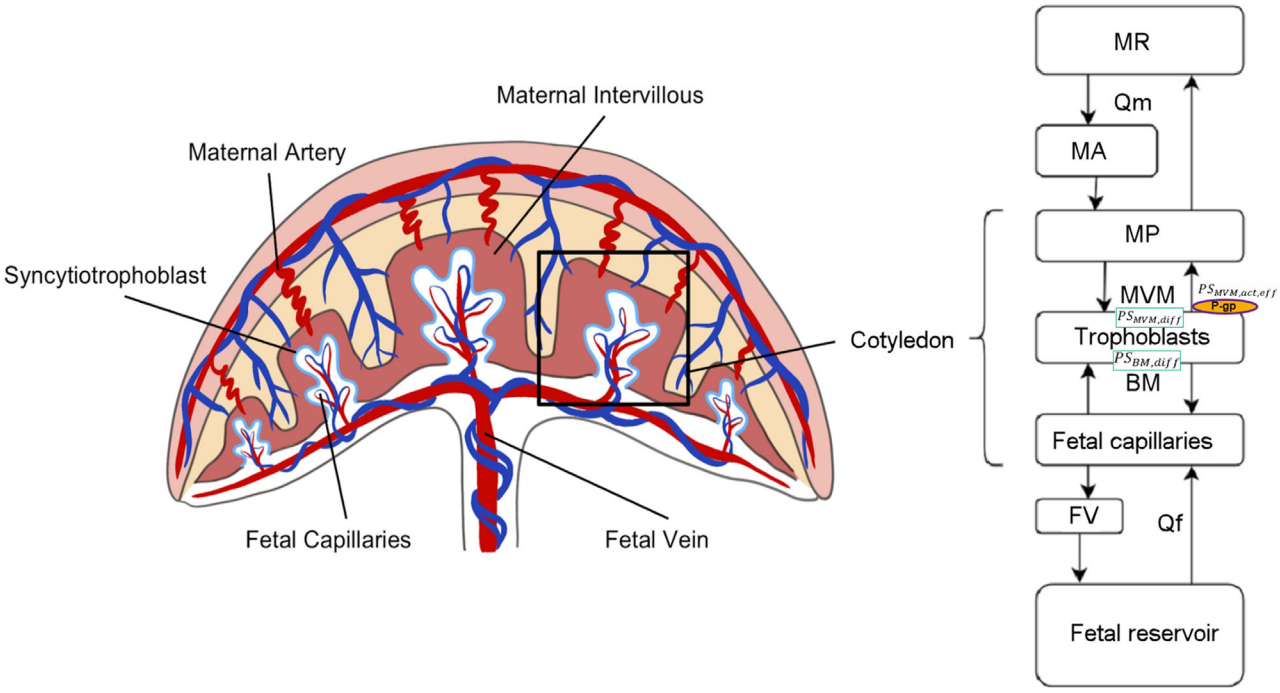

Figure 1. A single cotyledon of placenta is dual-perfused from the maternal and fetal sides in an ex vivo transplacental experiment, which can be simulated by a multi-compartment in silico model. Note that the efflux transporter P-gp is located at the apical membrane on the maternal side. The passive diffusion ($PS_{MVM,diff}$ and $PS_{BM,diff}$) and efflux transport ($PS_{MVM,act,eff}$) parameters are also denoted in the model. BM, basal plasma membrane; FV, fetal vein; FR, fetal reservoir; MA, maternal artery; MP, maternal placenta; MR, maternal reservoir; MVM, microvillous plasma membrane; P-gp, P-glycoprotein.

$$\frac{dC_{fc}}{dt} = \frac{Q_f \times (C_{fr} - C_{fc}) + PS_{BM,diff} \times C_t - PS_{BM,diff} \times C_{fc}}{V_{fc}}, \quad (5)$$

$$\frac{dC_{fv}}{dt} = \frac{Q_f \times (C_{fc} - C_{fv})}{V_{fv}} \text{ and,} \quad (6)$$

$$\frac{dC_{fr}}{dt} = \frac{Q_f \times (C_{fv} - C_{fr})}{V_{fr}}. \quad (7)$$

Each equation represents the concentration changes of morphine in one compartment. C_i are the concentrations and V_i are the volumes of the corresponding i compartments, where i is the maternal reservoir (mr), maternal artery (ma), MP (mp), trophoblast (t), fetal capillaries (fc), fetal vein (fv), or fetal reservoir (fr). Q_m and Q_f are the flow rate of the perfusate in maternal (m) and in fetal (f) circulation, respectively. The placental transfer of morphine consists of passive diffusion and active transport. Passive diffusion is considered in both MVM and BM membranes and is represented by $PS_{MVM,diff}$ and $PS_{BM,diff}$. $PS_{MVM,act,eff}$ stands for the active efflux transport on the MVM side of the trophoblast, aided by the transporter P-gp.

The diagram in Figure 1 shows the main sites where morphine travels from the maternal reservoir to the fetal reservoir via the trophoblasts of a cotyledon. Detailed explanations of individual parameters, nominal values, and units are listed in Table 1.

In the in vitro cotyledon experiment of morphine,[13] the flow rates in the maternal and fetal circuits were 13–15 mL/min and 4–5 mL/min, respectively. The maternal and fetal reservoir volumes in the closed system were 250 mL and 150 mL, respectively. The placenta was perfused for 4 hours with 50 ng/mL of morphine in a closed-circuit system.

Parameter Sensitivity Analysis

We perform 2 kinds of parameter sensitivity analysis to assess the influence of each parameter on the whole ordinary differential equation (ODE) system of the model. The first method is local parameter sensitivity

Table 1. Physiological and Pharmacokinetic Parameters

Parameters	Notes	Value	References
Q_m	Flow rate of the perfusate in maternal circulation	13 mL/min	Kopecky et al[8]
Q_f	Flow rate of the perfusate in fetal circulation	4 mL/min	Kopecky et al[8]
V_m	Maternal reservoir volume	250 mL	Kopecky et al[8]
V_f	Fetal reservoir volume	150 mL	Kopecky et al[8]
V_{mp}	Maternal placenta volume	74.7 mL	Kurosawa et al[13]
V_{ma}	Maternal artery vein	0.15 mL	Kurosawa et al[13]
V_{fc}	Fetal capillary volume	1.34 mL	Kurosawa et al[13]
V_t	Trophoblast volume	2.68 mL	Kurosawa et al[13]
V_{fv}	Fetal vein volume	0.15 mL	Kurosawa et al[13]
$PS_{MVM,diff}$	Passive diffusion rate on microvillus membrane side of the cotyledon	5.92 mL/min	Fitted
$PS_{MVM,act,eff}$	Active efflux rate via P-gp	1.25 mL/min	Fitted
$PS_{BM,diff}$	Passive diffusion rate on base membrane side of the cotyledon	2.3	Fitted

BM, basal plasma membrane; MVM, microvillous plasma membrane; P-gp, P-glycoprotein.

analysis, achieved by varying a parameter while fixing the other parameters. The second method is a global parameter sensitivity analysis, where all parameters vary simultaneously. Latin hypercube sampling (LHS) is a global parameter analysis method where random values for parameters of interest are generated synchronously within a specific range [1/N, N] of their nominal parameter values.[15] In the current study, we applied this method to examine the effect of parameters related to the transport of morphine across the placenta barrier, that is, $S_{MVM,act,eff}$, $PS_{MVM,diff}$, and $PS_{BM,diff}$, in our ODE system. We take the value of N as 3. With each set of the parameters, which represents a virtual ex vivo experiment, we simulated a time–concentration profile of morphine in maternal circulation. We configured 1000 simulations in total and observed the statistical pattern of the morphine concentration. The analysis was performed using "lhsdesign" in Matlab (Mathworks Inc., Natick, Massachusetts), and the ODE system was solved with the "ode45" function.

Results

In the maternal reservoir, the observed morphine concentration at time 0 was 60 ng/mL, but the reported dose was 50 ng/mL.[8] To bridge the discrepancy, we used 55 ng/mL as the initial condition. With the parameters provided in Table 1, the simulation results are shown in Figure 2. Note that the simulation results match the observation results well. The concentration in the maternal reservoir decreases sharply in the first 10 minutes, and then the decrease slows down, whereas the morphine concentration in the fetal reservoir increases. Figure 3 shows the result of local sensitivity analysis by varying the parameter $PS_{MVM,act,eff}$, which corresponds to the active transport of morphine from fetal to maternal circulations. As gestational age increases, the P-gp expression at the apical membrane reduces,[12] implying weaker active transport from fetal to maternal plasma. When $PS_{MVM,act,eff}$ is smaller than the reference value (with denominators 1/1.3, 1/2, 1/5), Figure 3a shows increased clearance of morphine at the maternal reservoir. In contrast, the morphine concentration in the fetal reservoir increases (Figure 3b) owing to a smaller $PS_{MVM,act,eff}$ or weaker efflux from the fetal to the maternal plasma.

Figure 4 shows the results of global parameter sensitivity analysis, where $PS_{MVM,act,eff}$, $PS_{MVM,diff}$, and $PS_{BM,diff}$ vary simultaneously. Clearly variation in $PS_{MVM,act,eff}$ does not form a definite statistical pattern, as seen for $PS_{MVM,diff}$ and $PS_{BM,diff}$ in this system. In other words, the system is less sensitive to the active transport parameter than the passive diffusion parameters. Changes in the unbound fraction of morphine during pregnancy affect the membrane permeability, that is, $PS_{MVM,diff}$ and$PS_{BM,diff}$. Higher values of $PS_{MVM,diff}$ and $PS_{BM,diff}$ than the reference values lead to a reduced concentration of morphine in the maternal reservoir but an increased concentration of morphine in the fetal reservoir.

Discussion

The situation of opioid abuse has deteriorated in recent years. In 2019, 49 860 people died from opioid overdoses in the USA, which was a 6-fold increase since 2000.[16] Of particular concern is opioid abuse, including morphine abuse, by pregnant women. Prenatal exposure to morphine is associated with detrimental effects on neonates, including diminishing fetal breathing efforts, delaying fetal lung maturation, and eliciting neurological symptoms.[8] To treat neonatal diseases induced by opioid abuse, an in-depth understanding of the transplacental properties of morphine and other opioids are required. The work aimed to develop the first in silico model for ex vivo placenta perfusion with morphine. It was hoped that the findings

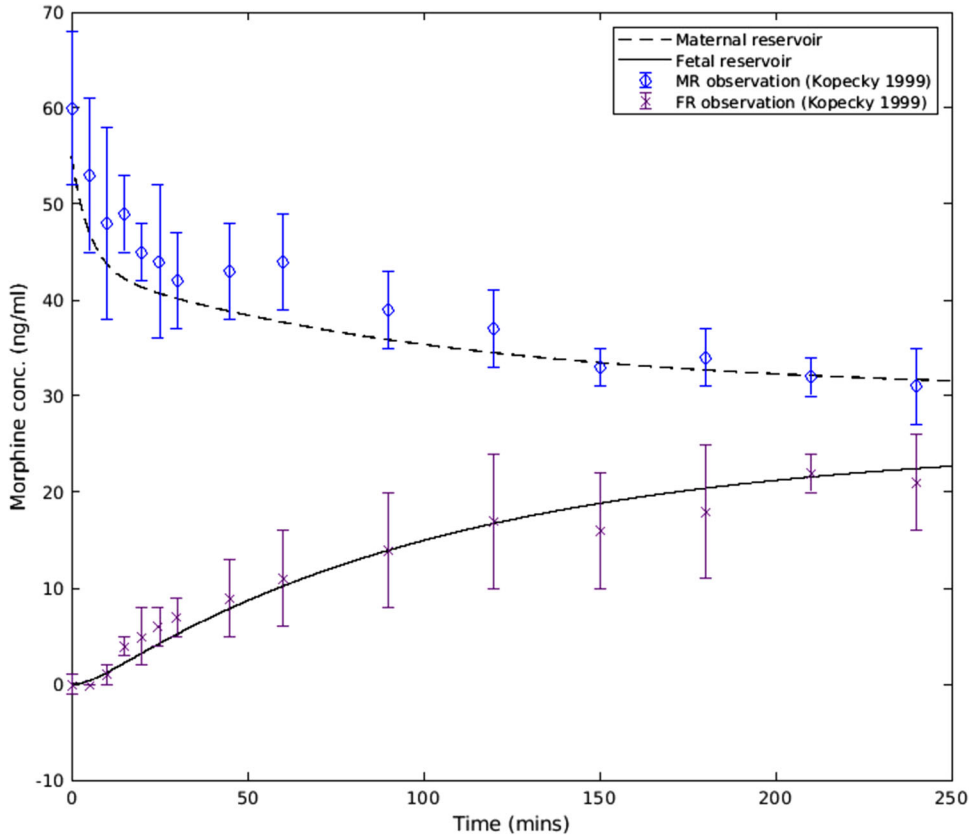

Figure 2. Simulated and observed morphine concentrations in the maternal and fetal reservoirs.

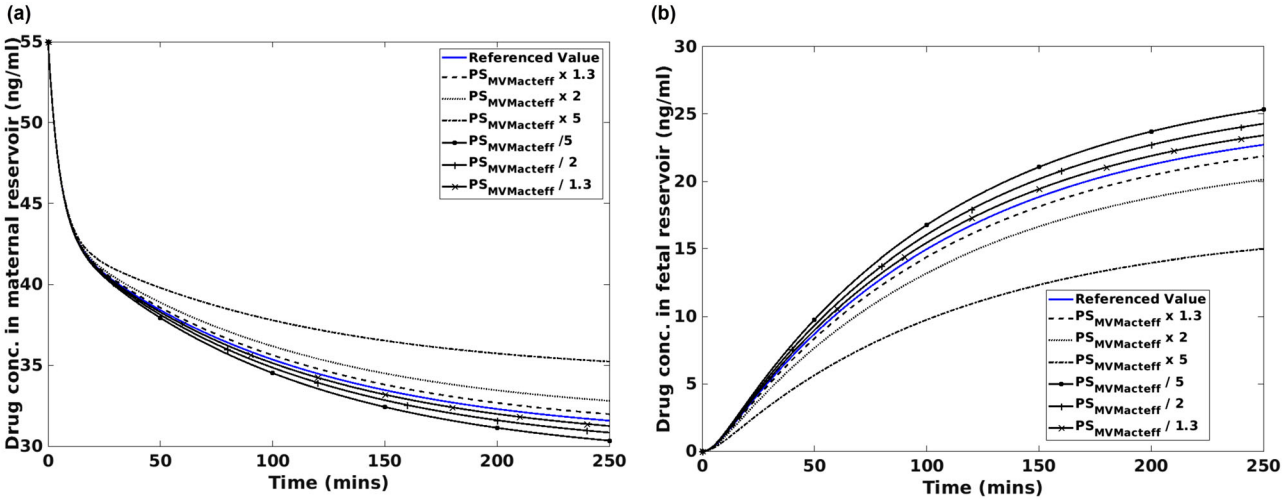

Figure 3. Results of local parameter analysis by varying the efflux transport parameter $PS_{MVM,act,eff}$ while keeping the other parameters intact. The plots are morphine concentrations in the maternal (a) and fetal (b) reservoirs. The reference value was used to produce results in Figure 1. MVM, microvillous plasma membrane.

might also be extrapolated to predict in utero exposure to other opioids.[10]

In this study, we reproduced the concentration profiles of morphine in the maternal and fetal reservoirs reported by Kopecky et al.[8] The model allowed us to perform simulations for scenarios that could affect the transplacental properties of morphine during pregnancy. First, we considered changes in the unbound fraction of morphine during pregnancy that could affect the membrane permeability. In this case, $PS_{MVM,diff}$ at the apical membrane and $PS_{BM,diff}$ at the base membrane will be affected (Figure 4). As the

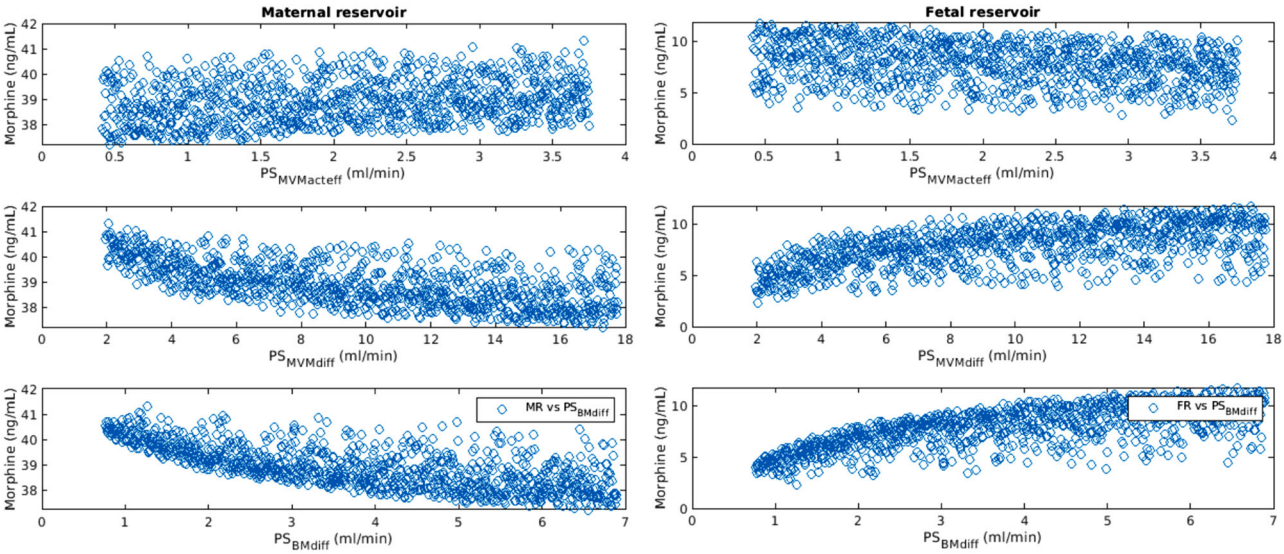

Figure 4. Parameter analysis using the Latin hypercube sampling method, where the 3 diffusion and transport parameters are changed simultaneously within a given range. The results show that the passive diffusion parameters $PS_{MVM,diff}$ and $PS_{BM,diff}$ have more pronounced effects on the pattern of maternal and fetal morphine concentration than the active transport parameter $PS_{MVM,act,eff}$. BM, basal plasma membrane; MVM, microvillous plasma membrane.

unbound morphine is already high in maternal plasma (approx. 65% in the adult),[8] the difference induced by changes in the unbound fraction of morphine may not be significant. Second, the decreased P-gp expression in the apical membrane could affect the efflux of morphine from fetal to maternal plasma, leading to the accumulation of morphine in the fetal plasma.

Concerning the limitations of the current study, we have dropped the terms for active transport from maternal to fetal circulations in the original model of Kurosawa et al,[13] as passive perfusion plays a dominant role. This treatment is justified by the global parameter analysis (Figure 4), where the diffusion parameters have much more influence on the fetal/maternal morphine concentrations than the transport parameter. However, being more hydrophilic ($\log D_{7.4} = -0.15$) than some other opioids (eg, $\log D_{7.4} = 1.81$ for fentanyl),[10] active transport may still play a minor role in the placental transfer of morphine. Second, while we could predict the time course of morphine concentration caused by changes in the protein binding, as well as the effects of the variations of P-gp on the model, data for validating our prediction are still scant. It has been shown that P-gp expression is significantly higher in early preterm placentas than in term placentas, and decreases throughout pregnancy.[17] This implies increased maternal morphine transport from the maternal to the fetal side as a result of reduced $PS_{MVM,act,eff}$, as shown in Figure 3b.

Although the in silico model presented in the paper was developed for morphine, it may also be applied to other drugs administered to pregnant women. This is because ex vivo cotyledon perfusion experiments, first described by Panigel et al in 1967,[18] and with various modifications by different research groups over the years, have similar experimental protocols. For example, an ex vivo placenta perfusion study was reported for methadone, another opiate.[17] In addition, lists of drugs that have therapeutic interest were tested in the perfused cotyledon model from 1972 to 1994,[19] and from 1995 to 2006.[20] The noninvasive collection of information regarding human placental transfer and clearance of drugs from ex vivo experiments, further assisted by in silico analysis, will be a powerful research tool for obstetric, fetal, and neonatal medicine.

Conclusion

In this work, we used an in silico model to simulate an ex vivo placenta perfusion experiment for morphine. We were able to simulate several challenging scenarios to observe in vivo, such as the efflux effects of transporter P-gp.

Acknowledgments

Open access publishing facilitated by The University of Auckland, as part of the Wiley - The University of Auckland agreement via the Council of Australian University Librarians.

Conflicts of Interest

The authors declare that they have no conflicts of interest associated with this work.

Data Sharing

The Matlab source code is available upon reasonable requests.

Funding

H.H. acknowledges financial support from the Li Ka Shing Foundation.

Author Contributions

H.H. drafted the article, S.Z. performed the MATLAB coding and parameter analysis, and K.K. and K.C. advised on transplacental model construction.

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