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Title Page

Characterization of transplacental transfer of paroxetine in perfused human placenta: development of a pharmacokinetic model to evaluate tapered dosing

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Running Title Page

Running title: Characterization of Transplacental Transfer of Paroxetine

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Abbreviations:

$C_{b,m}$, maternal concentration

$C_{b,f}$, fetal concentration

C_f, drug concentration in fetal effluent or drug concentration in fetal venous compartment

C_{in}, drug concentration into compartment

C_m, drug concentration in maternal effluent or drug concentration in intervillous compartment

$C_{p,f}$, plasma concentration in the fetus

$C_{p,m}$, plasma concentration

CYP, cytochrome P450

$f_{p,f}$: unbound fraction in fetal plasma

$f_{p,m}$: unbound fraction in maternal plasma

f_u: unbound fraction in perfusate

k_a, first-order rate constant

Kp, tissue-to-perfusate concentration ratio

$K_{p,f}$, tissue-to-unbound perfusate concentration ratio

$K_{p,fetal}$, placental tissue-to-fetal effluent partition ratio

K_1, K'_1, K''_1, K_4, K'_4, K''_4: influx clearances into placental tissue

k_2, k_3, k_5, k_6: transfer rate constants

MBI, mechanism-based inactivation

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Q_f, fetal perfusion flow rate

Qh, hepatic blood flow rate

Q_m, maternal perfusion flow rate

SSRI, selective serotonin reuptake inhibitor

TPT_{SS}, transplacental transfer value at the steady state

$TPT_{SS,R}$, TPT_{SS} value in the opposite direction

$V_{b,f}$, fetal distribution volume

V_f, fetal vascular volume in placental tissue

V_m, maternal intervillous volume in placental tissue

V_p: distribution volume of the placental compartment

Xp, drug amount in placental compartment

X_{pa}, drug amount in central placental compartment

X_{pb}, drug amount in deep compartment

Abstract

The aims of this study was to determine whether a tapered dosage regimen of paroxetine in pregnant women might be useful to avoid withdrawal syndrome in neonates after delivery by characterizing transplacental transfer of paroxetine in perfused human placenta, fitting a pharmacokinetic model to the results, and applying the model and parameters to evaluate a tapered dosage regimen. Paroxetine was perfused from the maternal or fetal side of isolated human placental preparation with various perfusion protocols, and paroxetine concentrations in the effluent and placental tissue were determined. Transplacental pharmacokinetic parameters of paroxetine were estimated by simultaneous fitting of a five-compartment transplacental pharmacokinetic model to the set of paroxetine concentration profiles. The developed model and parameters were used to simulate the maternal and fetal concentrations of paroxetine, and the results were compared with reported data. Paroxetine showed a larger distribution volume in placental tissue and a smaller transplacental transfer as compared with antipyrine, a passive diffusion marker. A five-compartment model could well describe the transplacental transfer of paroxetine, and could well simulate the maternal and umbilical venous concentrations of paroxetine at delivery. Transplacental transfer kinetic parameters of paroxetine were estimated by fitting a pharmacokinetic model to perfusion study data. The model and parameters appeared to be suitable for simulation of paroxetine kinetics in fetus. The model was also applicable to design a dosage regimen to avoid an abrupt

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decrease of paroxetine concentration in fetal plasma.

Introduction

Almost one-fifth of pregnant women are considered to suffer from depression or anxiety disorder (O'Keane and Marsh, 2007). To treat psychiatric symptoms during pregnancy, selective serotonin reuptake inhibitors (SSRIs) have been widely used. However, questions have been raised concerning a possible association between the use of paroxetine during pregnancy and an increase in teratogenesis (Källén and Otterblad Olausson, 2006; Louik *et al*., 2007). Moreover, there are several reports of neonates delivered from mothers taking paroxetine showing withdrawal syndrome or paroxetine intoxication, such as respiratory distress, cyanosis, apnea, hyperreflexia, tremor, shivering, irritability, drowsiness and sleeping disorder (Stiskal *et al*., 2001; Dahl *et al*., 1997; Nordeng et al., 2001). From this point of view, paroxetine use in pregnant women should be avoided. However, some authors state that cessation of antidepressant during pregnancy may be physically and mentally unfavorable for mother and may even have a negative influence upon the neonate (Cohen *et al*., 2006; Bonari *et al*., 2004). Substitution of paraxetine with a safer antidepressant is not always feasible, because the clinical response to antidepressants is quite specific among patients, and consequently some pregnant women necessarily continue to use paroxetine. The risk of discontinuation symptoms after cessation of SSRI is considered to be dependent upon the elimination rate of the drug, *i.e.*, a longer half-life results in a lower risk (Judge *et al*., 2002). Therefore, prediction of the concentration profile of paroxetine in fetal plasma after maternal

intake of paroxetine, based on a quantitative evaluation of the transplacental transfer properties of paroxetine, may enable us to design an optimal dosage regimen to avoid fetal withdrawal syndrome after delivery.

It is not feasible to carry out a clinical study to evaluate the placental permeability of drugs in pregnant women for ethical reasons. Although some researchers have attempted to estimate the transplacental permeability by analyzing drug concentrations in umbilical venous plasma and maternal plasma after delivery (Hirt *et al.*, 2007), they could not determine the concentration of drug during pregnancy and could not control the time of sampling after drug intake.

Human placental perfusion (Schneider *et al.*, 1972) can be used to determine the drug concentration-time profile and to observe influx into and efflux from the placental tissue. We have analyzed the transplacental transfer kinetics of salicylic acid, diclofenac and antipyrine in detail by fitting a transplacental pharmacokinetic model to the results of human placental perfusion studies (Shintaku *et al.*, 2007, 2009). This model provides estimates of the kinetic parameters of drug transfer between placental tissue and maternal or fetal perfusate and the results enable us to simulate the drug influx kinetics into fetus across the placenta based on the maternal plasma concentration profile as an input function (Shintaku *et al.*, 2012).

The aim of the present study is to develop a pharmacokinetic model which enables to

predict the pharmacokinetic profiles of paroxetine in neonates after delivery from mothers taking paroxetine. First, we characterized the transplacental transfer of paroxetine in perfused human placenta with various perfusion schedules and fitted the results to a five-compartment pharmacokinetic model. The developed model and estimated parameters were used to design a suitable tapered dosage regimen to achieve a slowly decreasing concentration profile of paroxetine in the fetus.

Materials and Methods

Materials

Human full-term placentae were obtained from gravidae after normal vaginal or cesarean delivery. The study protocol was approved by the ethical committee of the University of Tokyo and written informed consent was provided by the gravidae before delivery. Paroxetine hydrochloride was purchased from Toronto Research Chemicals Inc. (Ontario, Canada). Fluvoxamine malate and diethyl ether were purchased from Sigma Aldrich (St. Louis, MO, USA). Human serum albumin was purchased from Kaketsuken (The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan). All other reagents used were of the highest grade commercially available.

Krebs-Ringer-bicarbonate buffer (118 mM NaCl, 4.7 mM KCl, 1.3 mM $MgSO_4$, 24.2 mM $NaHCO_3$, 2.5 mM $CaCl_2$) containing D-glucose (1.0 g/L), dextran (MW 35,000-50,000, 1.0 g/L), heparin (12,500 IU/L), and human serum albumin (2.0 g/L) was used as the perfusate. The maternal and fetal perfusates were aerated with 95% O_2-5% CO_2 and 95% N_2-5% CO_2, respectively, adjusted to pH 7.3 with HCl, and warmed to 37ºC. Aeration was continued throughout the experiment.

Placental perfusion

Human placental perfusion study was carried out as previously reported (Shintaku *et al.*,

2007) using antipyrine as a passive diffusion marker. Briefly, after cannulation, the cotyledon sample was perfused with drug-free perfusate for 30 minutes to stabilize the preparation and then perfused according to protocol I, Ia, Ib or II as follows. In all the protocols, 1.0 to 1.5 mL aliquots of maternal and fetal effluents were periodically sampled from the maternal chamber and fetal venous cannula, respectively, and perfused cotyledon was also weighed and sampled just after the last sampling of effluent. All the samples were stored at -20ºC until analysis. The concentrations of paroxetine and antipyrine in the perfusate at the point of influx into the cotyledon preparation were also determined just after the completion of the perfusion experiment, because possible adsorption of paroxetine on the tubes was found in our preliminary experiment. In the following text, observed drug concentration refers to the total drug concentration in perfusate. The concentration of paroxetine in the effluent was selected based on the maximum clinical plasma concentration of paroxetine after repetitive dose of 20 mg/day, i.e. 59.5 ng/mL (Murasaki *et al*, 2000).

Protocol I

Maternal perfusate was changed to perfusate containing paroxetine (41.5 ng/mL, mean or 149±45 ng/mL, mean±SD) and antipyrine (47.0±3.6 μg/mL, mean±SD) and perfusion was conducted for 60 minutes. Maternal and fetal effluents were sampled periodically for 60 minutes.

Protocol Ia

Maternal perfusate was changed to perfusate containing both paroxetine (128 ng/mL) and antipyrine (48.3 μg/mL) and perfusion was conducted for 10 minutes to determine the concentration of drugs in the placental cotyledon at 10 min.

Protocol Ib

Maternal perfusate was changed to perfusate containing both paroxetine (128±12 ng/mL, mean±SD) and antipyrine (51.4±7.0 μg/mL, mean±SD) and perfusion was conducted for 60 minutes. Then, the perfusate was changed to a drug-free perfusate and perfusion was continued up to 180 minutes. The effluents were sampled periodically for 180 minutes. The concentration profile data until 60 minutes were merged with data from Protocol I and the average profile was used for the model analysis.

Protocol II

Fetal perfusate was changed to perfusate containing both paroxetine (128±4 ng/mL, mean±SD) and antipyrine (55.3±5.2 μg/mL, mean±SD) and perfusion was conducted for 60 minutes. Maternal and fetal effluents were sampled periodically for 60 minutes.

Determination of paroxetine

Total concentration of paroxetine in effluent or perfusate was determined by means of HPLC with UV detection. An aliquot of 500 μL of sample was spiked with 50 μL of internal standard solution (10 μg/mL fluvoxamine), then 30 μL of 1 N NaOH and 5 mL of

organic solvent (a mixture of *n*-hexane and isoamyl alcohol; 99:1, v/v) were added and the mixture was shaken for 10 minutes. The sample was centrifuged at 2,600×*g* at 4ºC for 10 minutes and 4 mL of the organic layer was transferred to a glass tube. Further organic solvent was added to the remaining sample and the mixture was shaken, then the organic layer was separated. The organic layers were combined and evaporated to dryness under a gentle nitrogen stream. The residue was dissolved with 200 µL of mobile phase and subjected to HPLC.

The HPLC system consisted of a pump (LC-20AD, Shimadzu, Kyoto, Japan), a UV-VIS detector (SPD-20AV, Shimadzu), an integrator (CR-8A Chromatopac, Shimadzu), a column oven (CT-20A, Shimadzu) and an auto sampler (SIL-20A, Shimadzu). A reversed-phase column (Cosmosil 5C18; 4.6 mm×150 mm, 5 µm, Nakalai Tesque, Tokyo, Japan) was used for separation. The mobile phase consisted of 0.01 M phosphate buffer (pH 2.8) and acetonitrile (69:31, v/v) and was pumped at a rate of 1.5 mL/min. The detection wavelength was set at 295 nm. . The detection limit of paroxetine was 1 ng/mL.

To determine the concentration of paroxetine in placental tissue, the sample was weighed and cut into small pieces, added to two volumes of water and homogenized with a blender (Physcotron, Microtech Nichion, Chiba, Japan) and a Teflon-glass homogenizer (Mini D.C. Stirrer, Eyela, Tokyo, Japan). An aliquot of 3 mL of homogenate was spiked with 100 µL of internal standard solution (50 µg/mL fluvoxamine), then 5 mL of diethyl ether and 70 µL of 4

M NaOH were added. The mixture was shaken for 10 minutes and centrifuged to separate the organic layer. The organic layer (4 mL) was transferred into a glass tube, to which 2 mL of HCl solution (pH 2.0) was added. The mixture was shaken for 10 minutes and centrifuged again. The aqueous layer (1.8 mL) was transferred to a glass tube and again shaken for 10 minutes with 5 mL of organic solvent (mixture of *n*-hexane and isoamyl alcohol; 99:1, v/v), then centrifuged to separate the organic layer. The organic layer (4 mL) was evaporated to dryness under a gentle nitrogen stream. The residue was dissolved in 200 μL of mobile phase and subjected to HPLC.

Determination of antipyrine

Total concentration of antipyrine in effluent, perfusate or placental tissue was determined by using our previously reported method (Shintaku *et al.*, 2007). The detection limit of antipyrine was 0.1 μg/mL.

Evaluation of permeability across the placenta

The transplacental transfer value at the steady state (TPT_{SS}), an index of permeability across the placenta (Heikkinen *et al.*, 2000), was calculated as the ratio of the amount of drug transferred to fetal effluent across the placenta to that infused at the steady state (60 minutes after the start of perfusion for paroxetine, and 20-60 minutes for antipyrine) and used to

evaluate the permeability in Protocols I, Ia and Ib. The $TPT_{SS,R}$ was defined as a TPT_{SS} value in the opposite direction (Shintaku *et al.*, 2009) and used to evaluate the results of Protocol II.

Pharmacokinetic analysis of transplacental transfer

Antipyrine

The transplacental transfer of antipyrine was characterized by fitting a three-compartment model, consisted of a dead volume compartment, intervillous compartment and placental compartment (Shintaku *et al.*, 2007), without weighing to the set of mean concentration profiles of antipytine in maternal and fetal effluents and placental tissue using a non-linear least-squares program (MLAB; Civilized Software, Bethesda, MD, USA) to estimate transplacental transfer parameters such as K_1 (influx clearance from intervillous compartment to placental compartment; mL/min/g cotyledon) and k_2 (efflux rate from placental compartment to intervillous compartment; min^{-1}). The initial parameters for fitting were arbitrarily determined. The placental compartment represents the union of placental tissue and fetal intravascular space, which are considered to be in rapid equilibrium. The first-order influx rate constant from the dead volume compartment to the intervillous compartment in Protocol I, Ia or Ib was also incorporated to the model to correct the dead volume of the line and set to 1.02 min^{-1} as previously reported (Shintaku *et al.*, 2007).

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Paroxetine

A five-compartment model, which consists of a dead volume compartment, intervillous compartment, placental compartment, fetal venous compartment and deep compartment, was used to analyze the transplacental transfer of paroxetine (Figure 1). The sets of mass-balance equations for Protocols I, Ia, Ib and II based on the five-compartment model are as follows:

[Protocol I and Ib]

$$\frac{dX_a}{dt} = C_{in} \cdot Q_m - k_a \cdot X_a \quad (1)$$

$$\frac{dC_m}{dt} = \frac{k_a \cdot X_a - C_m \cdot (K_1 + Q_m) + k_2 \cdot X_{pa}}{V_m} \quad (2)$$

$$\frac{dX_{pa}}{dt} = C_m \cdot K_1 - (k_2 + k_3 + k_5) \cdot X_{pa} + C_f \cdot K_4 + X_{pb} \cdot k_6 \quad (3)$$

$$\frac{dX_{pb}}{dt} = k_5 \cdot X_{pa} - k_6 \cdot X_{pb} \quad (4)$$

$$X_p = X_{pa} + X_{pb} \quad (5)$$

$$\frac{dC_f}{dt} = \frac{k_3 \cdot X_{pa} - C_f \cdot (K_4 + Q_f)}{V_f} \quad (6)$$

[Protocol Ia] (t>62)

$$\frac{dX_a}{dt} = -k_a \cdot X_a \quad (1')$$

All other equations are same to those for Protocol I.

[Protocol II]

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$$\frac{dC_m}{dt} = \frac{-C_m \cdot (K_1 + Q_m) + k_2 \cdot X_{pa}}{V_m} \quad (7)$$

$$\frac{dX_{pa}}{dt} = C_m \cdot K_1 - (k_2 + k_3 + k_5) \cdot X_{pa} + C_f \cdot K_4 + X_{pb} \cdot k_6 \quad (8)$$

$$\frac{dX_{pb}}{dt} = k_5 \cdot X_{pa} - k_6 \cdot X_{pb} \quad (9)$$

$$X_p = X_{pa} + X_{pb} \quad (10)$$

$$\frac{dC_f}{dt} = \frac{C_{in} \cdot Q_f + k_3 \cdot X_{pa} - C_f \cdot (K_4 + Q_f)}{V_f} \quad (11)$$

where C_m, C_f and X_p represent the concentration of paroxetine in maternal effluent, that in fetal effluent and the amount of paroxetine in placental tissue, respectively. The time t was defined to be zero at the start of infusion. The sets of equations ((1-6), (1', 2-6), and (7-11)) were simultaneously fitted to the set of concentration profiles of paroxetine in maternal effluent, fetal effluent and placental tissue observed in Protocols I/Ib, Ia and II, respectively, to estimate transplacental kinetic parameters such as K_1, k_2, k_3, K_4, k_5 and k_6 by using a non-linear least-squares program as described above. The parameters K_1 and K_4 represent the apparent clearance as given by multiplying the respective unbound clearance by unbound fraction in the effluent.

Correction of clearance values by unbound fraction

The unbound fraction (f_u) of paroxetine and antipyrine in the perfusate was determined by ultrafiltration. The perfusate (2 mL) containing 148 ng/mL paroxetine and 50.2 μg/mL

antipyrine was transferred to a Centricon Ultracel YM-30 (Millipore, Bedford, MA, USA) and spun at 1,000 g for two minutes. The f_u value was calculated from the concentration in the filtrate (C_2) and that in solution remaining in the upper cell (C_1) by means of equation (12).

$$f_u = \frac{C_2}{C_1} \quad (12)$$

The unbound influx clearance, K'_1 and K'_4, was calculated by dividing K_1 and K_4 by f_u. The estimated influx clearance from the maternal plasma, K''_1, was calculated by multiplying K'_1 by the unbound fraction in maternal plasma (0.884 for antipyrine, Ohkawa *et al.*, 2001; 0.05 for paroxetine in healthy adults, Sakamoto *et al.*, 2000). Similarly, the estimated influx clearance from the fetal plasma, K''_4, was calculated by multiplying K'_4 by the unbound fraction in fetal plasma (0.869 for antipyrine, 0.05 for paroxetine in healthy adults).

Estimation of paroxetine output from placental compartment to fetal umbilical vein *in utero*

To simulate the pharmacokinetics of paroxetine in the maternal circulation, we used a pharmacokinetic model incorporating mechanism-based inactivation (MBI) previously developed by us (Mikami *et al*, 2013). The influx profile of paroxetine into fetal plasma and the plasma concentration profile in the fetal plasma after repetitive oral administration of paroxetine to the mother at a dose of 40 mg once a day were simulated with a hybrid model (Figure 2) using the maternal concentration profile of paroxetine ($C_{b,m}$) as an input function based on the parameters previously reported by us (Mikami *et al*, 2013). The mass-balance

equations that describe the hybrid model are as follows.

$$\frac{dC_m}{dt} = \frac{C_{b,m} \cdot Q_m - C_m \cdot (K_1" + Q_m) + k_2 \cdot X_{pa}}{V_m} \quad (13)$$

$$\frac{dX_{pa}}{dt} = C_m \cdot K_1" - (k_2 + k_3 + k_5) \cdot X_{pa} + C_f \cdot K_4" + X_{pb} \cdot k_6 \quad (14)$$

$$\frac{dX_{pb}}{dt} = k_5 \cdot X_{pa} - k_6 \cdot X_{pb} \quad (15)$$

$$X_p = X_{pa} + X_{pb} \quad (16)$$

$$\frac{dC_f}{dt} = \frac{k_3 \cdot X_{pa} + C_{b,f} \cdot Q_f - C_f \cdot (K_4" + Q_f)}{V_f} \quad (17)$$

$$\frac{dC_{b,f}}{dt} = \frac{(C_f - C_{b,f}) \cdot Q_f}{V_{b,f}} \quad (18)$$

where $C_{b,f}$ and $V_{b,f}$ represent the plasma concentration and distribution volume of proxetine in the fetus, respectively. The $V_{b,f}$ value was fixed to 20.3 L based on the assumption that $V_{b,f}$ per body weight is the same as $V_{b,m}$ per body weight ($V_{b,m}$ =405.1 L/body, Mikami *et al.*, 2013; body weight of mother = 60 kg; body weight of fetus = 3 kg) and that paroxetine is not metabolized in the fetus.

Validation of the model

To validate the model and estimated parameters, the observed concentrations of paroxetine in maternal plasma and umbilical venous plasma at delivery for five cases were collected

from the literature and compared with the values estimated by the model, using the dosage regimen in each case as an input function. Simulation was carried out under the steady-state condition because, in all five cases, the mother had been taking paroxetine for more than 60 days.

Simulation study

The concentration profiles of paroxetine in fetal plasma and placental tissue at a dose of 40 mg once daily to a pregnant mother, as well as under a tapered dosage regimen from 40 mg/day, were simulated by using the developed model.

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Results

Permeability of antipyrine across the placenta

In protocols I, Ia and Ib, the concentration of antipyrine in the fetal and maternal effluents reached the steady-state at about 10 minutes after the start of perfusion (Figure 3) and the TPT_{SS} value at the steady state (21 to 60 minutes) was 9.16±2.67% (mean±SD).

Protocol Ib revealed that the efflux of antipyrine from placental tissue to maternal and fetal effluents was rapid, with half-lives of 3 and 1 minutes, respectively (Figure 3). The concentration of antipyrine in the placental tissue at 180 minutes was 0.44±0.51 μg/ g tissue. The tissue-to-perfusate concentration ratios (Kp) of antipyrine are summarized in Table 1, together with those of paroxetine.

In Protocol II, the antipyrine concentration in the maternal and fetal effluents also reached the steady-state at about 10 minutes after the start of perfusion.

Transplacental kinetic parameters obtained by fitting equations (1-11) to the data are shown in Table 2.

Permeability of paroxetine across the placenta

In protocols I and Ib, the concentration of paroxetine in the maternal and fetal effluents did not reach the steady-state within 60 minutes (Figure 4). In protocol I with maternal perfusate containing 41.5 ng/mL paroxetine, the concentration of paroxetine in the fetal

effluent remained below the quantification limit until 19 minutes after the start of infusion and reached 3.35 ng/mL at 60 minutes (Figure 4(E)). The paroxetine concentrations in the maternal effluent and placental tissue at 60 minutes were 24.4 ng/mL and 1,670 ng/g tissue, respectively (Figure 4(E), (F)).

When the concentration of paroxetine in the maternal perfusate was as high as 138±21 ng/mL, the concentration of paroxetine in the fetal effluent reached the quantification limit at 7 minutes and was 20.2±15.2 ng/mL at 60 minutes (Figure 4(A)). The concentration in the maternal effluent was 80.6±20.5 ng/mL at 60 minutes, while that in the placental tissue was as high as 742 ng/g tissue at 10 minutes and 2,840±2,510 ng/g tissue at 60 minutes (Figure 4(A), (B), Table 1). The placental tissue-to-fetal effluent partition ratio ($K_{p,\ fetal}$) was 232 at 60 minutes. The unbound fraction (f_u) of paroxetine in the effluent was 0.263. Thus, the $K_{p,f,fetal}$ value was calculated to be 882 (Table 1). The TPT_{60} value for paroxetine was 3.64±2.26%, which is 40% of TPT_{SS} for antipyrine. The total recovery rates of paroxetine in Protocol I and Protocol Ib were 103±9% and 121±18%, respectively.

In protocol Ib, paroxetine was detected in maternal and fetal effluents at concentrations of 12.1±7.3 and 2.22±1.93 ng/mL, respectively, even after washout with drug-free perfusate for 118 minutes. The decay of the concentration in maternal effluent was bi-exponential with half-lives of 18.2 and 108 minutes. The placental concentration of paroxetine remained as high as 1,750±20 ng/g tissue even after washout for 118 minutes (Table 1).

In protocol II, the concentration in the fetal effluent was 10.3 ng/mL at 60 minutes, while that in the maternal effluent reached the quantification limit after 30 minutes and did not reach a steady-state within 60 minutes (Figure 4(C)).

Equations (1) to (11) were simultaneously fitted to all the results in Protocols I, Ia, Ib and II to estimate the transplacental pharmacokinetic parameters (Table 2). The influx clearances from maternal and fetal effluents were corrected by the f_u value of 0.263 to yield the respective unbound influx clearances (K_1' and K_4') in Table 2 as well as K_1" and K_4", which are the influx clearances in human plasma calculated by multiplying by the plasma unbound fraction of paroxetine in human plasma (0.05).

The model and parameters could explain well the observed profile of the concentration of paroxetine in the effluents and placental tissue (Figure 4).

Validation of the model

Table 3 shows the results of model validation by using the observed paroxetine concentrations in the maternal and umbilical venous plasma at delivery. There are considerable discrepancies between observed and model-predicted concentrations.

Simulation study

Figure 5 shows the simulated profiles of the paroxetine concentration in fetal and maternal

plasma and placenta after maternal administration of paroxetine (40 mg/day, p.o.), using the developed model and parameters. During repeated administration, the fetal concentration gradually increased with the maternal profile reaching the steady state. The maternal and fetal plasma concentrations were simulated to reach steady states within one week. After the mother stops taking paroxetine, the maternal and fetal plasma concentrations are simulated to decrease with half-lives of 8 and 10 hours, respectively. The fetal plasma concentration was considered to decrease more slowly than the maternal plasma concentration because the placental tissue acts as a reservoir, supplying paroxetine to the fetal plasma. However, abrupt cessation of paroxetine may cause withdrawal syndrome in the mother, so we next simulated the case that the daily dose of paroxetine was gradually decreased by 10 mg a week (Figure 6). With this regimen (row B at the top of Figure 6), the fetal and maternal plasma concentrations were predicted to decrease quite slowly as compared with the case of abrupt cessation of dosing (row A at the top of Figure 6; dashed lines).

Discussion

In the present study, we evaluated the transplacental transfer kinetics of paroxetine by analyzing the results of a human placental perfusion study with several perfusion protocols using a pharmacokinetic model, and we successfully used the developed model and estimated parameters to predict the concentration of paroxetine in fetal plasma.

We employed the transplacental pharmacokinetic model previously reported by us (Shintaku *et al.*, 2007) with a minor modification, *i.e.*, introduction of a deep compartment in the placental tissue (Figure 1), because biphasic decay of the paroxetine concentration in effluents was observed in the washout phase of protocol Ia. Most human placental perfusion studies employ a single protocol such as protocol I. However, we clearly detected the existence of a deep compartment in the placental tissue by using protocol Ia, suggesting that the use of a set of various perfusion protocols is preferable to characterize the transplacental transfer kinetics of drugs. The modified model could successfully explain the concentration profiles of paroxetine in the maternal and fetal effluents. Although the present study did not provide any information with regard to the physiological entity of the deep compartment, it is noteworthy that paroxetine shows high affinity for a serotonin transporter expressed on the basal membranes of human trophoblast cells (Cool *et al*., 1990).

As for the recovery rate of paroxetine, it was estimated to exceed 100%. To calculate the total amount of drug recovered, we integrated the drug concentrations in maternal and fetal

effluents to estimate the amount of drug flowed out, and added the amount of drug remaining in the placental tissue at the end of perfusion. This amount recovered was then divided by the total amount of drug flowed into the preparation. Most of paroxetine was recovered from the placental tissue so that increase in the flow rate of perfusate leads to the overestimation of recovery rate. Another feasible cause is the overestimation of the placental concentration by some reasons such as heterogeneous distribution of drug in the placental preparation, although we did not detect any significant differences in the drug concentration among various parts of placental preparation in our preliminary experiments.

We have already carried out a series of studies to analyze the transplacental transfer kinetics of various drugs, such as diclofenac and salicylic acid, using a similar experimental approach (Shintaku *et al.*, 2007, 2009). When those results are compared with the present findings, the unbound influx clearance (K_1') for paroxetine (12.4 mL/min/g cotyledon) was found to be higher to those for antipyrine (0.28 mL/g cotyledon; Table 2), salicylic acid (0.0451 mL/min/g cotyledon; Shintaku *et al.*, 2007) and diclofenac (6.27 mL/g cotyledon; Shintaku *et al.*, 2009). The membrane permeability of a drug which is not transported by a specific transport system(s) is known to be correlated with the ratio of the octanol-water partition coefficient at pH 7.4 to the square root of molecular weight. The permeability values of the four drugs estimated according to this assumption show a good correlation with the K'_1 values (data not shown), suggesting that the high permeability of paroxetine into

placental tissue may be largely explained by its physicochemical properties.

However, recent *in vitro* studies have demonstrated that paroxetine is a substrate, as well as an inhibitor, of P-glycoprotein (Maines *et al.*, 2005; Yasui-Furukori *et al.*, 2007). P-Glycoprotein is reported to be expressed in placental tissue, and its substrates, such as saquinavir, methadone, paclitaxel and quentapine, were shown to be unidirectionally transported in *in vitro* placental preparations (Nagashige *et al.*, 2003; Ushigome *et al.*, 2003; Mölsä *et al.*, 2005; Nanovskaya *et al.*,2005; Rahi *et al.*, 2007). Therefore, it is possible that P-glycoprotein affects the transplacental transfer of paroxetine. A perfusion study using both paroxetine and a P-glycoprotein inhibitor may clarify the influence of P-glycoprotein.

The paroxetine concentrations in umbilical and maternal plasma have been reported and can be used to estimate the permeation of paroxetine into fetus. We compared the observed values reported by Hendrick *et al.* (2003) and found that the estimated fetal plasma concentrations were within a fivefold range of the observed values (Table 3), except for No.5. A fivefold difference is considered not to be unreasonable, because paroxetine is primarily metabolized by cytochrome P450 (CYP) 2D6, which is highly polymorphic. Moreover, the estimated maternal-to-umbilical concentration ratios were also comparable to the observed values, except for No.5. We assumed that paroxetine is not metabolized in the fetus, because the enzymatic activity of CYP2D6 in fetal liver is reported to be absent or to be only about 4% of that in adults in some (30%) fetal liver samples (Treluyer *et al.*, 1991; Jacqz-Aigrain

and Cresteil 1992; Hakkola *et al.*, 1996). Taken together, the results suggest that the model has potential to predict the fetal plasma level of paroxetine from the concentration profile in the maternal plasma, although no definite criteria exists to validate the prediction.

After the validation of the model and parameters, we attempted to estimate the concentration profile of fetal plasma paroxetine by using the maternal plasma concentration as an input function. The developed model predicted that during the repetitive oral administration of paroxetine at a dose of 40 mg daily, fetal plasma concentration would increase day by day along with the increase in the maternal plasma concentration and would reach a steady state within one week. After the cessation of paroxetine before delivery, the fetal plasma level is predicted to decrease rather abruptly, though with a half-life longer than that in maternal plasma. However, the model simulation indicated that tapering of the paroxetine dosage prior to delivery would be effective to produce a slow and prolonged decrease of paroxetine in fetal plasma. Therefore, we suggest that a strategy of tapering the dosage of paroxetine before delivery might be effective to reduce the incidence of withdrawal syndrome of neonates, as well as mothers.

In conclusion, we characterized the transplacental transfer kinetics of paroxetine by means of perfusion studies with human placenta, using various perfusion protocols. The developed model and estimated parameters enable us to predict the fetal plasma concentration profile from the maternal one. The model and parameters determined in this study are expected to

be useful to design an optimal dosage regimen to reduce adverse reactions such as withdrawal syndrome and to investigate the relationship between the fetal concentration profile and the nature of adverse reactions in fetus.

Authorship Contributions

Participated in research design: Ohtani, Satoh, Hori and Sawada

Conducted experiments: Nagai, Ohtani, Satoh and Hori

Contributed analytic tools: Fujii and Taketani

Performed data analysis: Nagai, Ohtani, Satoh and Matsuoka

Wrote or contributed to the writing of the manuscript: Nagai, Ohtani, Hori and Sawada

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Footnotes

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Legends for Figures

Figure 1 Pharmacokinetic models of paroxetine transfer across the placenta

C_{in}, drug concentration into compartment; C_m, drug concentration in intervillous compartment (μg/mL); C_f, drug concentration in fetal venous compartment (μg/mL); X_{pa}, drug amount in central placental compartment (μg/g cotyledon); X_{pb}, drug amount in deep compartment (μg/g cotyledon); k_a, first-order rate constant (0.635 min^{-1}); K_1, K_4, influx clearance (mL/min/g cotyledon); k_2, k_3, k_5, k_6, first-order rate constant (min^{-1}); Q_m, maternal perfusion flow rate (mL/min/g cotyledon); Q_f, fetal perfusion flow rate (mL/min/g cotyledon); V_m, maternal volume in perfusion chamber (mL/g cotyledon); V_f, fetal vascular volume in placental tissue (0.06 mL/g cotyledon).

Figure 2 Pharmacokinetic model of paroxetine transfer across the placenta in vivo

$C_{b,m}$, maternal concentration (ng/mL); C_m, drug concentration in maternal intervillous compartment (ng/mL); C_f, drug concentration in fetal venous compartment (ng/mL); Xp, drug amount in placental compartment (ng); K_1' and K_4', intrinsic influx clearance obtained by dividing K_1 and K_4 by the unbound fraction of the drug (f_u) (mL/hr); $f_b \cdot K_1$' (=K_1"), $f_b \cdot K_4$' (=K_4"), plasma influx clearance in vivo (mL/hr); k_2, k_3, k_5, k_6, first-order rate constants (hr^{-1}); Q_m, maternal perfusion flow rate (18 L/hr); Q_f, fetal perfusion flow rate (3.6 L/hr); V_m, maternal intervillous volume in placental tissue (113 mL); V_f, fetal vascular volume in placental tissue (24 mL); $V_{b,f}$, fetal distribution volume (L); $C_{b,f}$, fetal concentration

(μg/L).

Figure 3 Time courses of total antipyrine concentration and their model analysis (A) for perfusates in protocols I and Ib (t≤60, n=9) or protocol Ib (t>60, n=3), (B) for placental tissue during protocols I (n=4), Ia (n=1) and Ib (n=3), (C) for perfusates in protocol II (n=3), (D) for placental tissue during protocol II (n=3)

Open triangles, closed triangles and gray diamonds represent maternal outflow, fetal outflow and placental tissue concentrations, respectively. Each point represents the mean±SD. The lines were calculated as described in the methods (solid line, maternal perfusate; dotted line, fetal perfusate; gray line, placental tissue concentrations).

Figure 4 Time courses of total paroxetine concentration and their model analysis (A) for perfusates in protocols I and Ib (t≤60, n=6) or protocol Ib (t>60, n=3), (B) for placental tissue during protocols I (n=3), Ia (n=1) and Ib (n=3), (C) for perfusates in protocol II (n=2), (D) for placental tissue during protocol II (n=3), (E) for perfusates in protocol I at low doses of paroxetine (n=2), (F) for placental tissue during protocol I at low doses of paroxetine (n=2).

Open circles, closed circles and gray diamonds represent maternal outflow, fetal outflow and placental tissue concentrations, respectively. Each point represents the mean±SD. The

lines were calculated as described in the methods (solid line, maternal perfusate; dotted line, fetal perfusate; gray line, placental tissue concentrations).

Figure 5 Simulated profiles of paroxetine concentration in maternal and fetal plasma and placental tissue during and after repeated oral administration of paroxetine (40 mg) to a pregnant woman

Dashed, black and gray lines represent maternal plasma, fetal plasma and placental paroxetine concentrations, respectively. After the cessation of paroxetine at day 0, the paroxetine concentration in fetal plasma decreases with a half-life of 10 hours, whereas that in maternal plasma decreases with a half-life of 8 hours.

Figure 6 Simulated profiles of paroxetine concentration in maternal and fetal plasma during and after repeated tapered oral administration of paroxetine to a pregnant woman

Black and gray lines represent paroxetine concentrations in maternal plasma and fetal plasma, respectively. Dashed lines show the simulated plasma concentrations when the dose of 40 mg/day paroxetine is abruptly stopped (dosing schedule in row A above the plot). Solid lines show the simulated plasma concentrations when the dose of 40 mg/day paroxetine was tapered to 10 mg/day at the rate of 10 mg per week and then stopped (dosing schedule in row B). The effect of subsequent doses of 10 mg/day at 2-day intervals

is also shown on the right.

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Table 1 Tissue-to-perfusate concentration ratio (Kp) of paroxetine and antipyrine (mean±SD, n=3)

Paroxetine

Time (min)	Placental Concentration (ng/g tissue)	K_p		$K_{p,f}$	
		Fetal	Maternal	Fetal	Maternal
10	742	210	27.7	799	105
60	2840 ± 2510	232 ± 212	28.3 ± 19.8	882 ± 805	108 ± 75
180	1750 ± 20	395 ± 232	179 ± 85	1500 ± 880	679 ± 324

Antipyrine

Time (min)	Placental Concentration (μg/g tissue)	K_p		$K_{p,f}$	
		Fetal	Maternal	Fetal	Maternal
10	25.2	0.937	0.672	1.01	0.726
60	26.0 ± 4.2	1.66 ± 1.29	0.582 ± 0.108	1.79 ± 1.40	0.629 ± 0.116
180	0.441 ± 0.512	-	-	-	-

The unbound fractions of paroxetine and antipyrine in perfusate were 0.263 and 0.926, respectively. $K_{p,f}$: tissue-to-unbound perfusate concentration ratio

Table 2 Transplacental pharmacokinetic parameters of paroxetine and antipyrine

Drugs		Parameters obtained by model-fitting						
		K_1	k_2	k_3	K_4	k_5	V_p	k_6
		(mL/min/g cotyledon)	(min^{-1})	(min^{-1})	(mL/min/g cotyledon)	(min^{-1})	(mL/g cotyledon)	(min^{-1})
Paroxetine	estimate	3.25	0.0717	0.0779	9.67	0.00490	-	0.0241
	(SD)	(0.42)	(0.0217)	(0.0572)	(6.90)	(0.01442)	-	(0.0695)
Antipyrine	estimate	0.259	0.353	-	-	-	0.739	-
	(SD)	(0.016)	(0.044)	-	-	-	(0.080)	-

Drugs	Parameters from literatures or derived from the above										
	f_u	K_1'	$f_{p,m}$	K_1"	K_1'/k_2	$K_1"/k_2$	K_4'	$f_{p,f}$	K_4"	K_4'/k_3	$K_4"/k_3$
	-	(mL/min/g cotyledon)	-	(mL/min/g cotyledon)	(mL/g cotyledon)	(mL/g cotyledon)	(mL/min/g cotyledon)	-	(mL/min/g cotyledon)	(mL/g cotyledon)	(mL/g cotyledon)
Paroxetine	0.263	12.4	0.050 [a]	0.618	172	8.61	36.8	0.050 [a]	1.84	472	23.6
Antipyrine	0.926	0.280	0.884 [b]	0.247	0.792	0.700	-	0.869 [b]	-	-	-

-: not applicable; K_1, K'_1, $K"_1$, K_4, K'_4, $K"_4$: influx clearances into placental tissue; k_2, k_3, k_5, k_6: transfer rate constants; f_u: unbound fraction in perfusate; $f_{p,m}$: unbound fraction in human maternal plasma; $f_{p,f}$: unbound fraction in human fetal plasma; V_p: distribution volume of the placental compartment

(Healthy subject data used for $f_{p,m}$ and $f_{p,f}$ for paroxetine, as maternal and fetal data are unavailable.)

a The values in fetal and maternal plasma are not available so the unbound fraction in healthy subjects was used.

b Ohkawa *et al*., 2001

Table 3 Comparison between observed and predicted concentrations of paroxetine in maternal and umbilical venous plasma at delivery

No	Daily Dose	Duration of paroxetine treatment	Time of delivery after the last dose of paroxetine	Observed values at delivery*			Model-predicted values		
				Maternal concentration	Umbilical venous concentration	Umbilical venous-to-maternal ratio	Maternal concentration	Umbilical venous concentration	Umbilical venous-to-maternal ratio
	(mg)		(hr)	(ng/mL)	(ng/mL)		(ng/mL)	(ng/mL)	
1	10	wk 12-	6	59	24	0.407	16.1	4.31	0.267
2	20	wk 15-	21	34	31	0.912	24.2	11.8	0.486
3	25	wk 10-	13	10	6	0.600	47.9	19.0	0.397
4	40	throughout pregnancy	13	38	24	0.632	85.8	33.8	0.394
5	15	wk 1-5, 16-	19	22	<1	<0.05	16.9	8.14	0.483

* Hendrick *et al.*, 2003

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Appendix

Model equations for antipyrine (Shintaku *et al.*, 2007)

(Protocol I, Ia, Ib (t≤60))

$$\frac{dX_a}{dt} = C_{in} \cdot Q_m - k_a \cdot X_a$$

$$\frac{dC_m}{dt} = \frac{k_a \cdot X_a - C_m \cdot (K_1 + Q_m) + k_2 \cdot C \cdot (V_p + V_f)}{V_m}$$

$$X_p = C_f \cdot (V_p + V_f)$$

$$\frac{dC_f}{dt} = \frac{C_m \cdot K_1 - (k_2 + k_s) \cdot C_f \cdot (V_p + V_f) - Q_f \cdot C_f}{V_p + V_f}$$

(Protocol Ib (t>60))

$$\frac{dX_a}{dt} = -k_a \cdot X_a$$

(Protocol II)

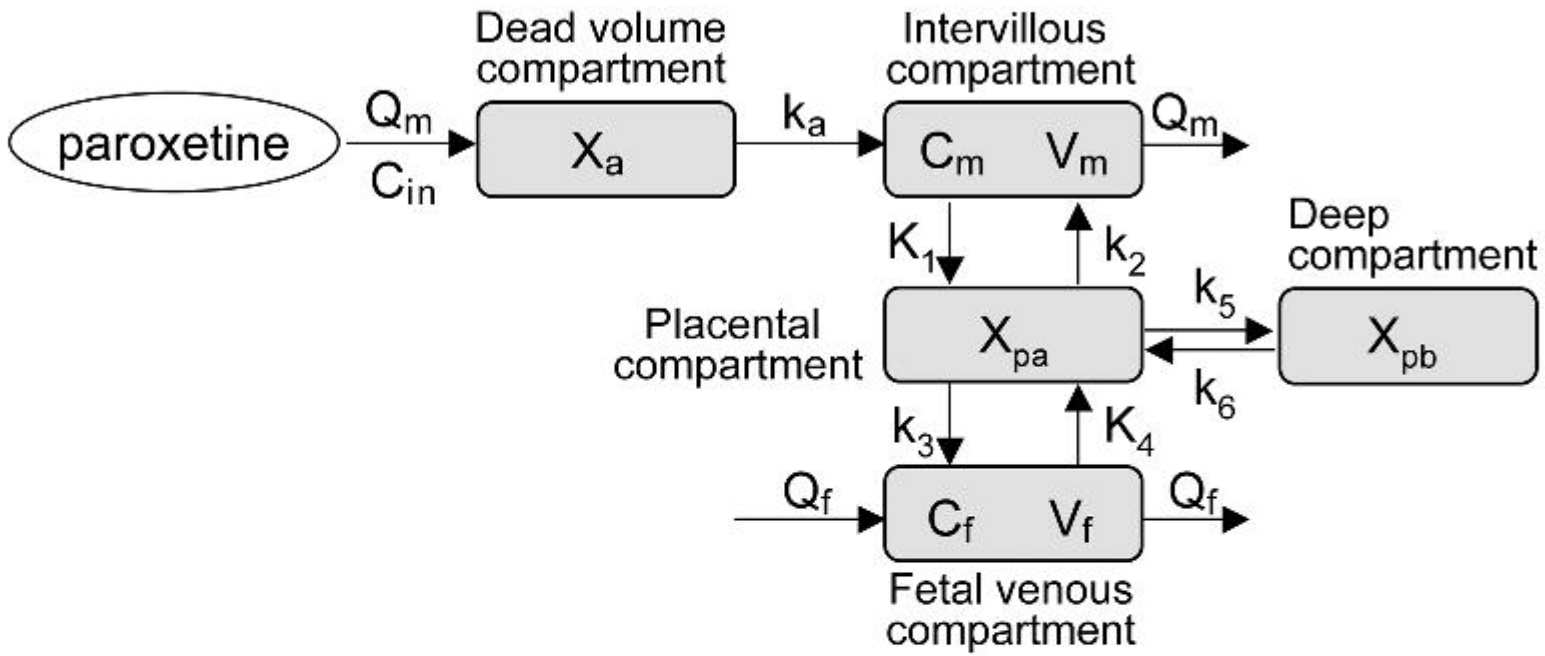
$$\frac{dC_m}{dt} = \frac{-C_m \cdot (K_1 + Q_m) + k_2 \cdot C_f \cdot (V_p + V_f)}{V_m}$$

$$X_p = C_f \cdot (V_p + V_f)$$

$$\frac{dC_f}{dt} = \frac{C_{in} \cdot Q_f + C_m \cdot K_1 - (k_2 + k_s) \cdot C_f \cdot (V_p + V_f) - Q_f \cdot C_f}{V_p + V_f}$$

Fig 1

Maternal-to-fetal perfusion



Fetal-to-maternal perfusion

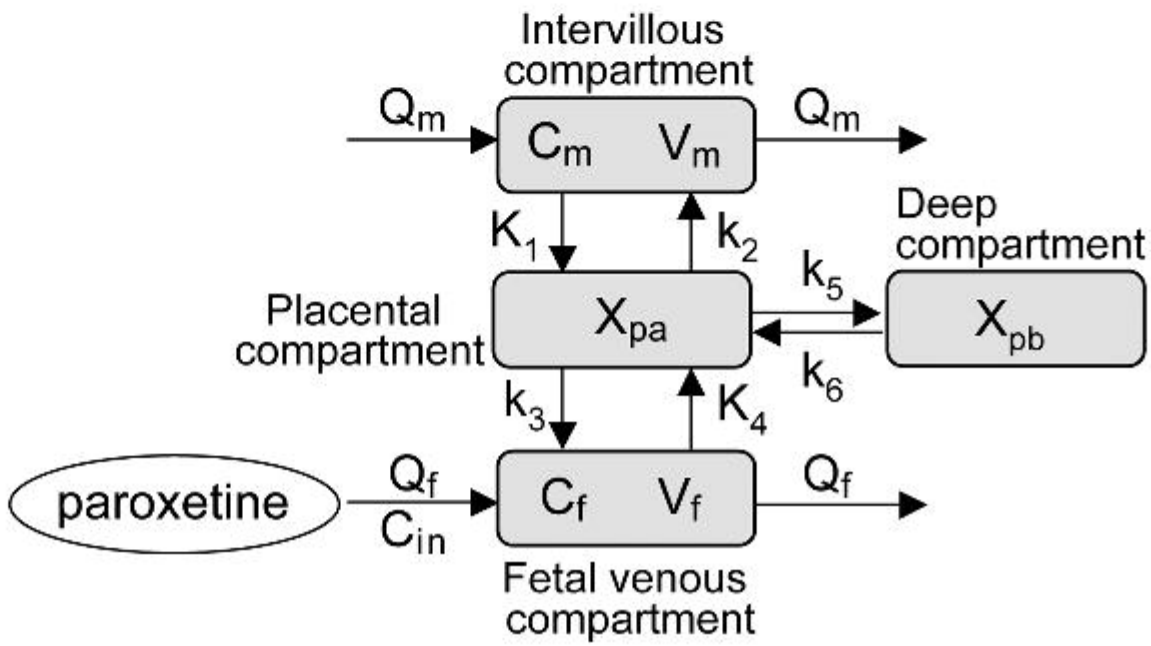


Fig 2

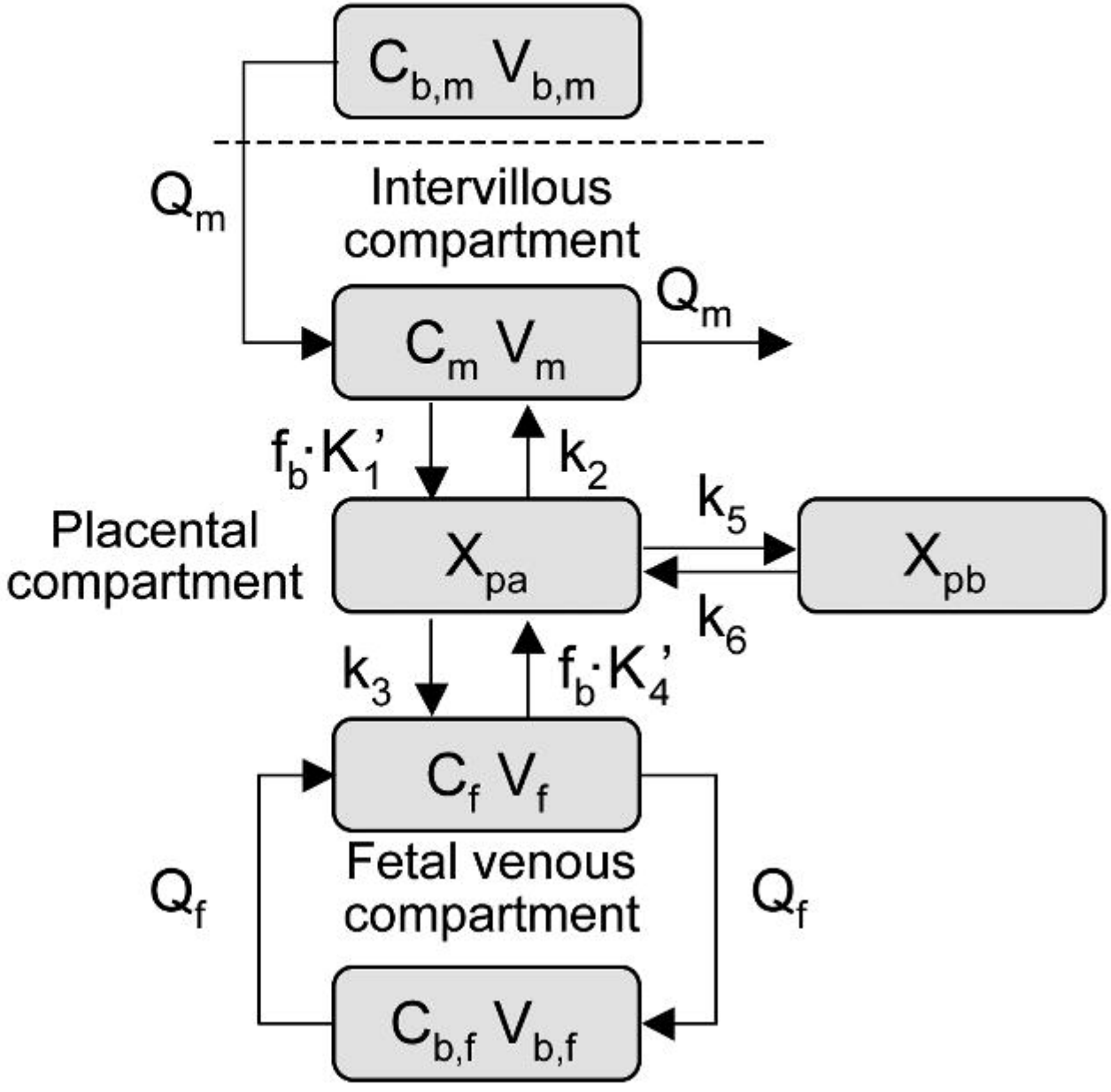

$C_{b,m}$ $V_{b,m}$
Q_m
Intervillous compartment
Q_m
C_m V_m
$f_b \cdot K_1'$
k_2
Placental compartment
X_{pa}
k_5
k_6
X_{pb}
k_3
$f_b \cdot K_4'$
C_f V_f
Q_f
Fetal venous compartment
Q_f
$C_{b,f}$ $V_{b,f}$

Fig 3

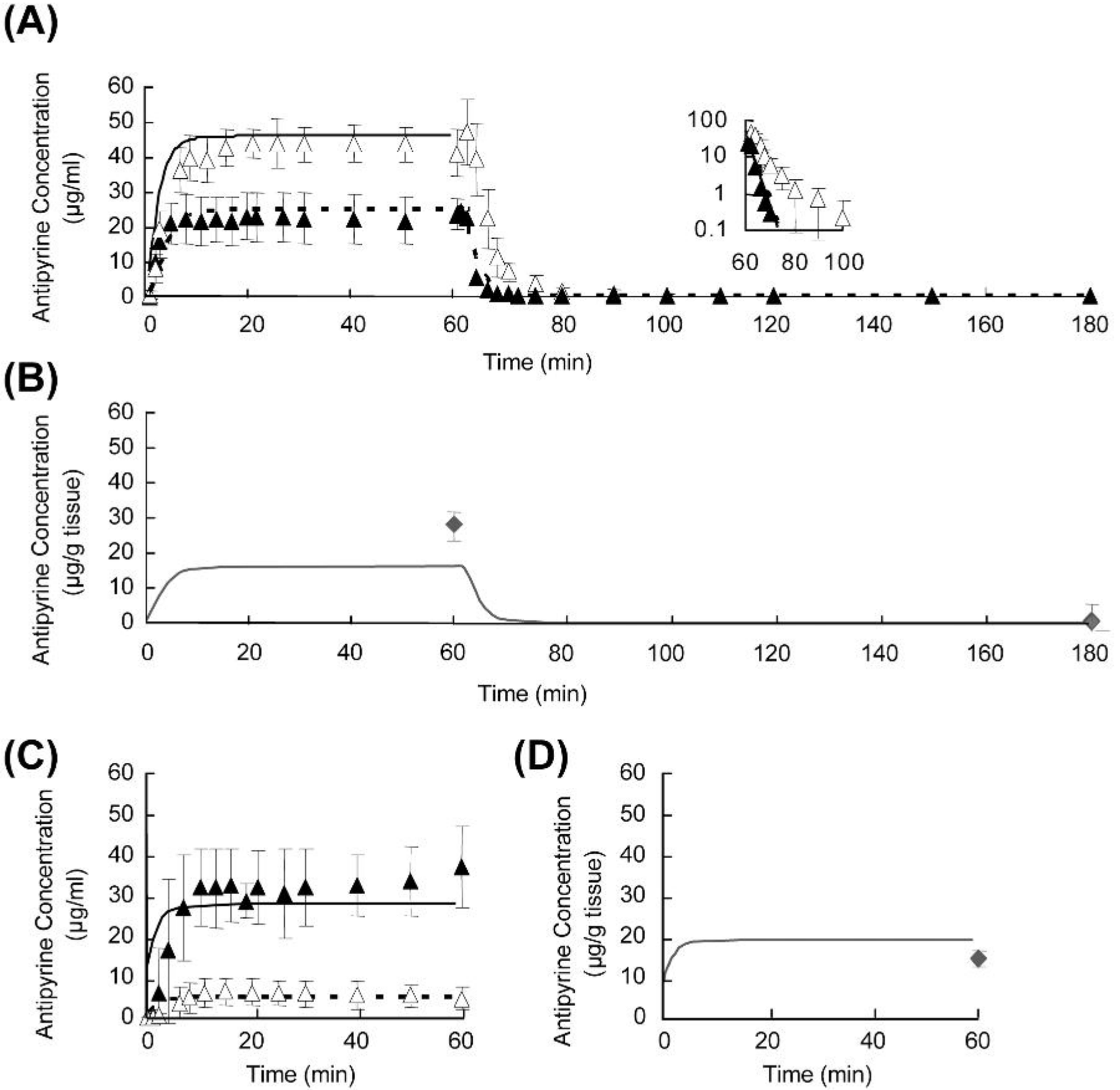

(A)
Antipyrine Concentration (μg/ml)
Time (min)
(B)
Antipyrine Concentration (μg/g tissue)
Time (min)
(C)
Antipyrine Concentration (μg/ml)
Time (min)
(D)
Antipyrine Concentration (μg/g tissue)
Time (min)

Fig 4

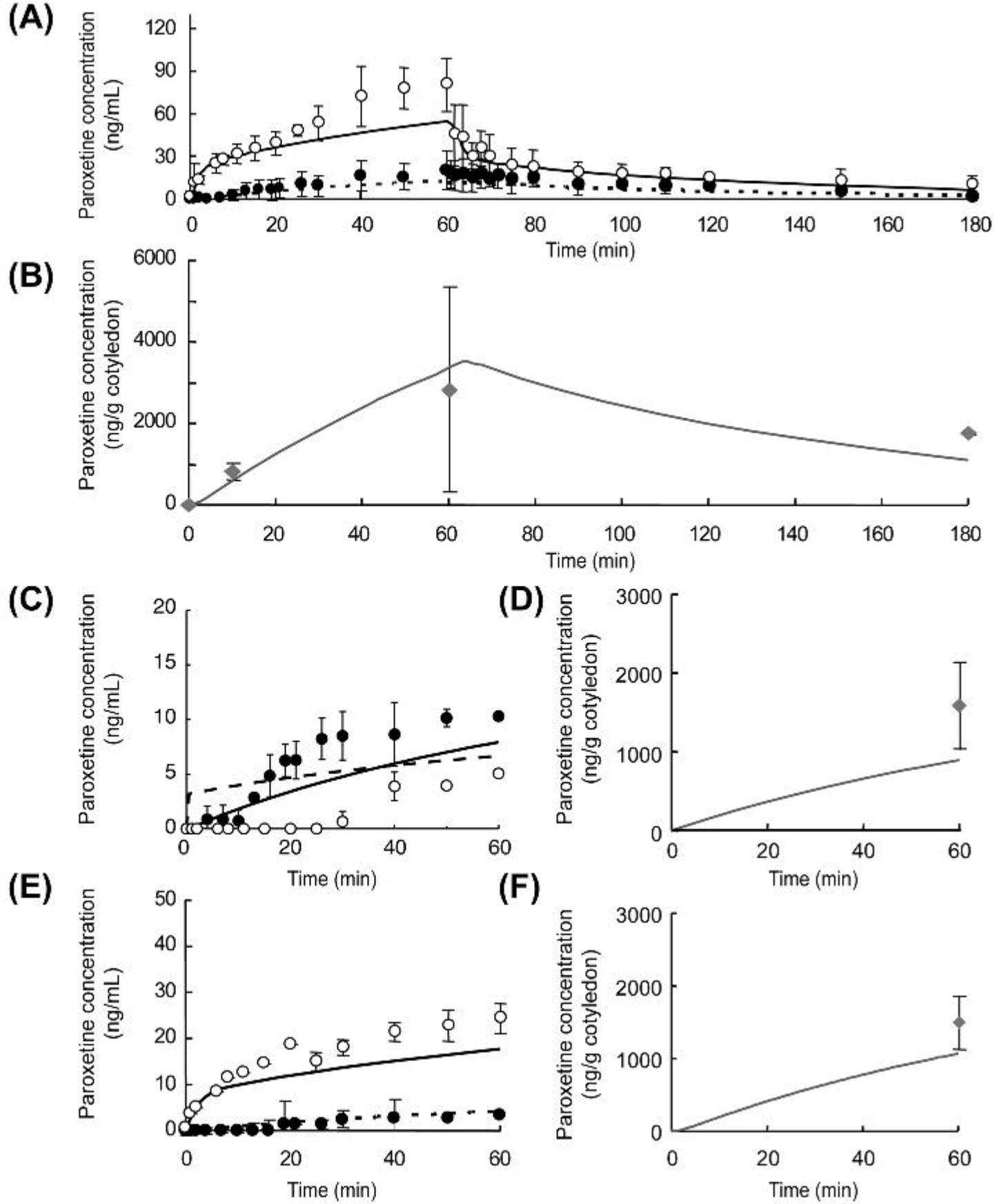

(A)
Paroxetine concentration (ng/mL)
120
90
60
30
0
0 20 40 60 80 100 120 140 160 180
Time (min)
(B)
Paroxetine concentration (ng/g cotyledon)
6000
4000
2000
0
0 20 40 60 80 100 120 140 160 180
Time (min)
(C)
Paroxetine concentration (ng/mL)
20
15
10
5
0
0 20 40 60
Time (min)
(D)
Paroxetine concentration (ng/g cotyledon)
3000
2000
1000
0
0 20 40 60
Time (min)
(E)
Paroxetine concentration (ng/mL)
50
40
30
20
10
0
0 20 40 60
Time (min)
(F)
Paroxetine concentration (ng/g cotyledon)
3000
2000
1000
0
0 20 40 60
Time (min)

Fig 5

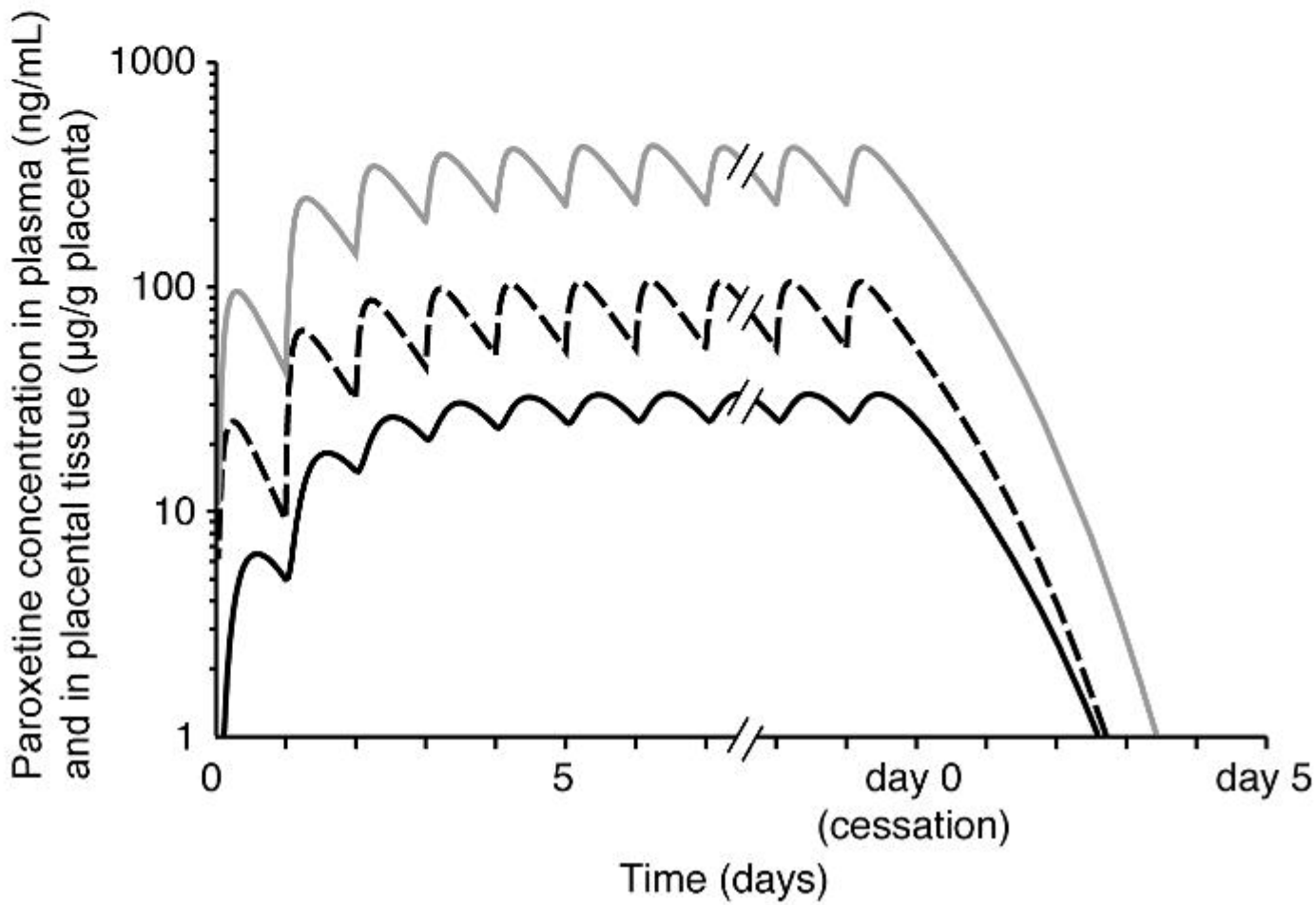
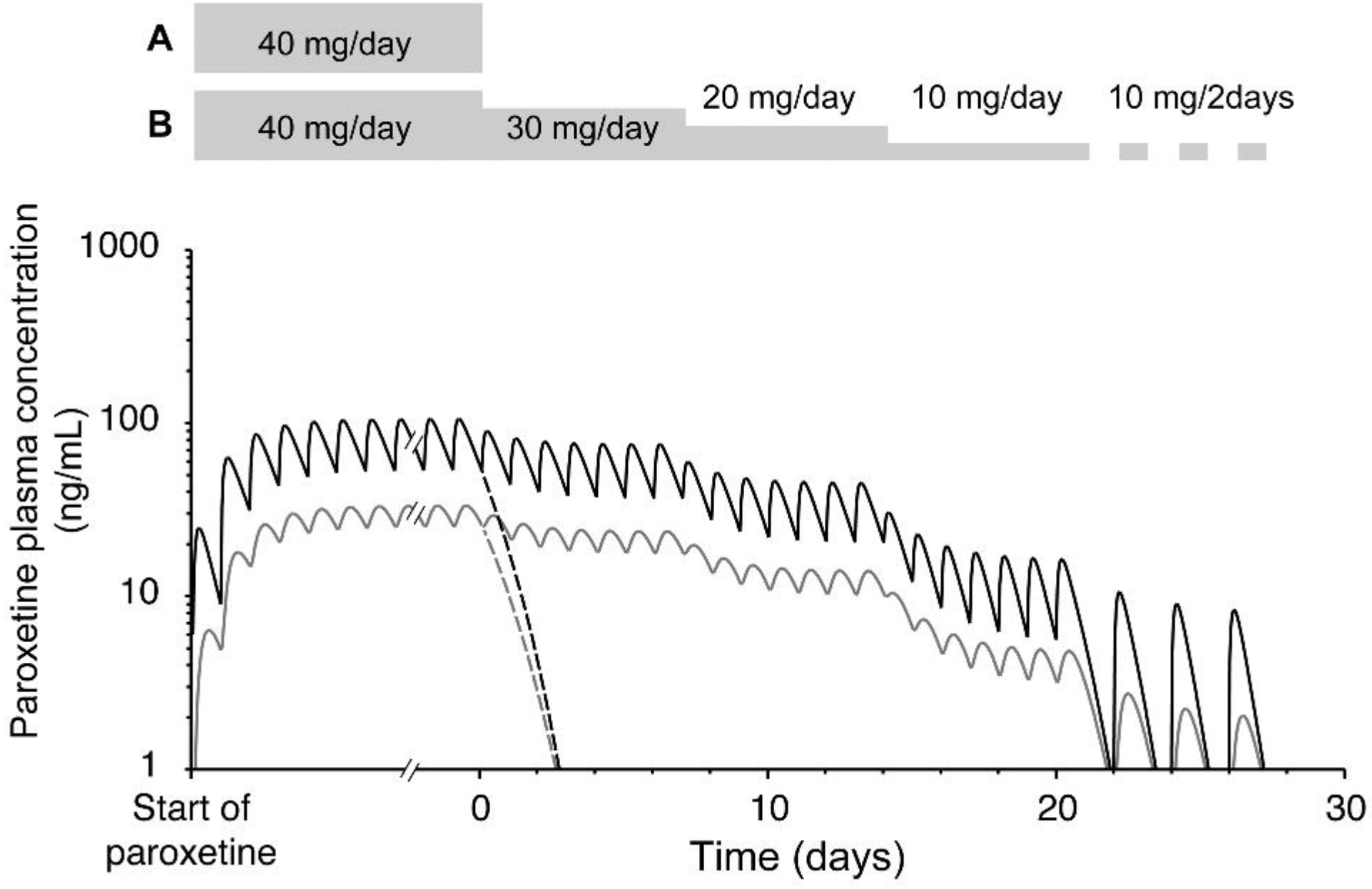

Paroxetine concentration in plasma (ng/mL)
and in placental tissue (µg/g placenta)
1000
100
10
1
0
5
day 0
(cessation)
day 5
Time (days)

Fig 6


A
40 mg/day
B
40 mg/day
30 mg/day
20 mg/day
10 mg/day
10 mg/2days
Paroxetine plasma concentration (ng/mL)
1000
100
10
1
Start of paroxetine
0
10
20
30
Time (days)