

Kinetic analysis of the transport of salicylic acid, an NSAID, across human placenta

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Running Title: Kinetic analysis of transplacental transfer

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the number of

text pages:	25
tables:	1
figures:	9
references:	23

the number of words in the

Abstract:	248
Introduction:	371
Discussion:	1,493

List of non-standard abbreviations

ALP, alkaline phosphatase; BBMVs, placental brush-border membrane vesicles; CYP, cytochrome P450; MCT, monocarboxylate transporter; TPTss, ratio of the rate of amount transferred across the placenta to that infused;

Abstract

The aim of this study was to develop a pharmacokinetic model to describe the transplacental transfer of drugs, based on the human placental perfusion study. The maternal and fetal sides of human placentas were perfused with salicylic acid together with antipyrine, a passive diffusion marker. The drug concentration in the placental tissue was determined at the end of perfusion. A compartment model consisting of maternal space, fetal intravascular space and placental tissue was fitted to the observed concentration profiles of salicylic acid in the maternal and fetal effluents. The developed model could adequately explain the concentration profiles of salicylic acid in the effluents with influx clearances from maternal and fetal perfusates to placental tissue of 0.0407 and 0.0813 mL/min/g cotyledon and efflux rate constants from placental tissue to maternal and fetal perfusates (k_2 and k_3) of 0.0238 and 0.176 min^{-1} , respectively. The kinetics of antipyrine was adequately described by assuming rapid equilibrium between fetal perfusate and placental tissue compartments. The influx plasma clearance from the maternal side (K'_{1}) in humans was estimated by taking into account the protein binding. The K'_{1}/k_2 value of salicylic acid was 1.07 mL/g cotyledon and was larger than that of antipyrine (0.642 mL/g cotyledon). We evaluated the transplacental transfer kinetics of salicylic acid by human placental perfusion study with various perfusion protocols. Based on the data obtained, we developed a pharmacokinetic model, which should enable us to estimate the influx profile of drugs into umbilical arterial blood from the maternal plasma concentration profile.

Introduction

Exchange of materials between mother and fetus occurs across the placenta (Robertson and Karp *et al.*, 1976), where fetal blood perfuses the villi and maternal blood fills the interstitial space. The blood-placental barrier consists of trophoblasts, which face the interstitial space, and fetal capillary endothelium (Stulc, 1989). To estimate the distribution of a drug into the fetus, it is essential to investigate the transplacental transfer process.

Human placental perfusion, first designed by Schneider *et al.* (1972), is a useful technique to investigate drug transfer from the maternal to the fetal circulation in humans, because the influx and efflux profiles of the drug can be directly observed. *In vivo* distribution studies with pregnant animals can not provide such information, and also suffer from the problem of interspecies differences. The placental perfusion technique is also preferable to *in vitro* methods using cultured human cell lines, such as BeWo or Jar, or membrane vesicles prepared from human placenta (Manley *et al.*, 2005, Martel and Keating, 2003), because physiological metabolism is quite well retained (Nanovskaya *et al.*, 2002). However, many studies using human placental perfusion have provided only simple information, *i.e.* the ratio of drug concentration in fetal effluent to that in maternal effluent (Nekhayeva *et al.*, 2005, Heikkinen *et al.*, 2000). No studies have been conducted to analyze the kinetic features of drug transfer among maternal perfusate, placental tissue and fetal perfusate by using the human placental perfusion technique.

Salicylic acid is widely used as an antipyretic and analgesic. However, intake of salicylic acid at pregnancy is reported to be negatively correlated with fertility and body weight of newborns (Turner and Collins, 1975). When given at full-term pregnancy, salicylic acid readily permeates into fetal blood, inhibiting the synthesis of prostaglandins and inducing constriction of the ductus

arteriosus, which leads to pulmonary hypertension in the newborn (Perkin *et al.*, 1980). Therefore, it is worthwhile to investigate in detail the kinetics of the transfer of salicylic acid from mother to fetus.

The aims of this study are to develop a pharmacokinetic model to describe the transfer kinetics of drugs among maternal blood, placental tissue and fetal blood, and to analyze quantitatively the transfer properties of salicylic acid across the blood-placental barriers by applying the developed model.

Materials and Methods

Materials

Human full-term placentas were obtained from gravidae after normal vaginal or Caesarean delivery. The study protocol was approved by the Ethical Committee of the Faculty of Medicine, Kyushu University, and written informed consent was provided by the gravidae. Salicylic acid, antipyrine, phenobarbital, 4-aminoantipyrine, heparin and human serum albumin were purchased from Nacalai Tesque (Kyoto, Japan). All other reagents used were of the highest grade commercially available.

Solutions

The perfusate (Krebs-Ringer-bicarbonate buffer) consisted of 118 mM NaCl, 4.7 mM KCl, 1.3 mM MgSO₄, 24.2 mM NaHCO₃, 2.5 mM CaCl₂, D-glucose (1.0 g/L), heparin (12,500 IU/L), dextran (1.0 g/L) and human serum albumin (2.0 g/L).

Salicylic acid (test drug) and antipyrine (passive diffusion marker) were each dissolved in the perfusate to give concentrations of 8 µg/mL and 50 µg/mL, respectively. The pH was adjusted to 7.4 with 1 M HCl. The maternal and fetal perfusates were aerated with 95% O₂-5% CO₂ and 95% N₂-5% CO₂, respectively, and warmed to 37°C.

Drug transfer from maternal to fetal side (Protocol I)

In vitro human placental perfusion was carried out based on the method reported by Schneider *et al.* (1972). A cotyledon (4 to 6 cm in diameter) was chosen from a placenta obtained after vaginal or Caesarean delivery. The fetal artery of the cotyledon was cannulated, ligated and perfused with drug-free perfusate at a rate of 3 mL/min for 30 minutes by using a peristaltic pump (micro tube pump, EYELA, Tokyo, Japan). Then, while perfusion was continued, the cotyledon was dissected from the placenta and mounted on a plastic chamber with the maternal side up. Three needles (18G) were inserted

from the maternal side to a depth of 7 mm and maternal perfusate was perfused via the needles at a rate of 15 mL/min for 30 minutes to stabilize the preparation. Throughout the experiment, the fetal perfusion pressure was monitored with a personal computer (PC7200/900, Macintosh) equipped with a pressure transducer (Single Transducer Set, Nihon Kohden, Tokyo, Japan), an A/D converter (Power Lab/200, Nihon Kohden) and an amplifier (RMP-6004M, Nihon Kohden). Both salicylic acid and antipyrine were dissolved in maternal perfusate and perfused from the maternal side. Maternal and fetal effluents were sampled for 60 minutes. Before sampling, the effluent in the maternal chamber was stirred to ensure thorough mixing. In all the perfusion protocols, perfused cotyledon was weighed and also sampled just after the last sampling of effluent to determine the tissue concentrations of drugs. All the samples were stored at -20°C until analysis.

Drug efflux from the placenta (Protocol Ia)

After perfusion of the cotyledon with drug-containing perfusate from the maternal side for 62 minutes as in Protocol I, the maternal perfusate was changed to a drug-free perfusate and the effluents from both maternal and fetal sides were further sampled for 3 minutes.

Initial distribution of drugs into placental tissue (Protocol Ib)

After 30-minute stabilization as in Protocol I, the maternal and fetal sides were perfused with drug-containing buffer and drug-free buffer, respectively, for 5 minutes to obtain the placental sample at 5 min.

Drug transfer from fetal to maternal side (Protocol II)

After 30-minute stabilization as in Protocol I, the fetal and maternal sides were perfused with drug-containing buffer and drug-free buffer, respectively, for 52 minutes.

Determination of antipyrine concentration in effluents

Concentrations of antipyrine in effluents were determined by an HPLC-UV method. A sample (500 μ L) was spiked with 500 μ L of internal standard solution (50 μ g/mL 4-aminoantipyrine in ethanol), shaken for 1 minute and centrifuged at 15,500xg for 10 minutes at 4°C. The supernatant was dried under a gentle stream of nitrogen gas and the residue was dissolved in 1 mL of mobile phase (0.02 M phosphate buffer (pH 6.0): methanol = 70:30, v/v). An aliquot of 20 μ L of the solution was subjected to HPLC. The determination limit was 0.1 μ g/mL.

Determination of salicylic acid concentration in effluents

Concentrations of salicylic acid in effluents were determined by an HPLC-UV method. A sample (500 μ L) was spiked with 500 μ L of internal standard solution (50 μ g/mL phenobarbital in water), 5 mL of chloroform and 50 μ L of 1 M HCl, mixed for 1 minute and spun at 740xg for 10 minutes at 4°C. The organic layer (4 mL) was transferred to a glass tube and evaporated into dryness under a gentle stream of nitrogen. The residue was dissolved in 200 μ L of mobile phase (0.04 M KH_2PO_4 (pH 2.0): H_2O :acetonitrile = 40:35:25, v/v) and an 80 μ L aliquot was subjected to HPLC. The determination limit was 0.1 μ g/mL.

Determination of drugs in placental tissue

Placental concentrations of drugs were determined by the HPLC-UV method. A weighed placental section was cut into small pieces with scissors, added to two volumes of water and homogenized with a blender (PHYSCOTRON, Microtech Nichion, Chiba, Japan) and Teflon homogenizer (Mini D.C. stirrer, EYELA). An aliquot of 3 mL of the homogenate was spiked with 50 μ L of 1 M HCl, 5 mL of chloroform and 10 μ L of internal standard solution (50 μ g/mL 4-aminoantipyrine in ethanol for antipyrine and 50 μ g/mL phenobarbital in water for

salicylic acid), mixed for 1 minute and spun at 740xg for 20 minutes at 4°C. The organic layer (3 mL) was transferred to a glass tube, evaporated to dryness under a gentle nitrogen stream and dissolved in 1 mL of the mobile phase. The supernatant (500 µL) was spun at 15,500xg for 10 minutes and a 20 µL aliquot was subjected to HPLC.

HPLC apparatus

The HPLC system consisted of a pump (LC-10AD, Shimadzu Co., Kyoto, Japan), a UV-VIS detector (SPD-10AV, Shimadzu) and an integrator (CR-6A Chromatopac, Shimadzu). A reversed-phase column (Cosmosil 5C18, 4.6 mm x 150 mm, 5 µm, Nacalai Tesque) was used for separation at ambient temperature. The mobile phases described above were pumped at a rate of 1.0 mL/min. The detection wavelength was set at 250 nm or 235 nm for the determination of antipyrine or salicylic acid, respectively.

Evaluation of transplacental permeability

The value of TPT_{ss} (transplacental transfer in the steady state) was calculated as the ratio of the amount transferred across the placenta to that infused at the steady state in Protocol I or Ia by using equation (1) (Heikkinen et al., 2000).

$$TPT_{ss}(\%) = \frac{C_{f,ss} \cdot Q_f \cdot 100}{C_{in} \cdot Q_m} \quad (1)$$

where $C_{f,ss}$, C_{in} , Q_f and Q_m represent the mean drug concentration in the fetal effluent samples at the steady state, the drug concentration in the maternal perfusate, and the fetal and maternal flow rates (3 mL/min and 15 mL/min), respectively.

Outline of Pharmacokinetic Analysis (Figure 1)

The experimental design was as follows; one of the above protocols was applied to each placenta to obtain one set of concentration-time profiles of antipyrine and salicylic acid in

maternal and fetal effluents. Then, all the time profiles for each compound were pooled, and the appropriate pharmacokinetic model described below was simultaneously fitted to the collective data to obtain pharmacokinetic parameters.

Model analysis for antipyrine (Figure 2)

A pharmacokinetic model consisting of two placental compartments (*i.e.*, maternal compartment and placental tissue compartment) and a dead volume compartment for the maternal reservoir, where the three needles were attached, was used to evaluate the transplacental transfer kinetics of antipyrine. The maternal compartment consists of the interstitial space and the maternal chamber that receives the maternal effluent. The volume of the maternal compartment, V_m , was assumed to be 23 mL. The influx rate constant from the dead volume compartment into the maternal compartment, k_a , was determined to be 1.02 min^{-1} by analyzing the time profile of antipyrine in the effluent from the three needles after changing the influx solution from drug-free to drug-containing perfusate.

Equations (2)-(5), equations (2)-(6) and equations (7)-(9) were simultaneously fitted to the sets of time profiles of maternal and fetal effluents (C_m and C) and the amounts of drug in the perfused tissues (X_p , $\mu\text{g/g}$ cotyledon) in Protocol I and Ib, Protocol Ia and Protocol II, respectively, by using a nonlinear least-squares program (MLAB, Civilized Software, Bethesda, MD) to obtain transplacental pharmacokinetic parameters, *i.e.*, K_1 (influx clearance from interstitial space to placental tissue, $\text{mL}/\text{min}/\text{g}$ cotyledon), k_2 (efflux rate constant from placental tissue to interstitial space, min^{-1}), k_s (elimination rate constant from the placenta, min^{-1}) and V_p (apparent volume of distribution from fetal blood, mL/g cotyledon). X_a represents the amount of drug in the dead volume compartment. V_m and V_f represent the volumes of maternal compartment and fetal intravascular space ($0.06 \text{ mL}/\text{g}$ cotyledon,

Drury *et al.*, 1981).

(Protocol I, Ia ($t \leq 62$), Ib)

$$\frac{dX_a}{dt} = C_{in} \cdot Q_m - k_a \cdot X_a \quad (2)$$

$$\frac{dC_m}{dt} = \frac{k_a \cdot X_a - C_m \cdot (K_1 + Q_m) + k_2 \cdot C \cdot (V_p + V_f)}{V_m} \quad (3)$$

$$X_p = C \cdot (V_p + V_f) \quad (4)$$

$$\frac{dC}{dt} = \frac{C_m \cdot K_1 - (k_2 + k_s) \cdot C \cdot (V_p + V_f) - Q_f \cdot C}{V_p + V_f} \quad (5)$$

(Protocol Ia ($62 < t \leq 65$))

$$\frac{dX_a}{dt} = -k_a \cdot X_a \quad (6)$$

(Protocol II)

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

$$X_p = C \cdot (V_p + V_f) \quad (8)$$

$$\frac{dC}{dt} = \frac{C_{in} \cdot Q_f + C_m \cdot K_1 - (k_2 + k_s) \cdot C \cdot (V_p + V_f) - Q_f \cdot C}{V_p + V_f} \quad (9)$$

Model analysis for salicylic acid (Figure 3)

A pharmacokinetic model consisting of three placental compartments (*i.e.*, maternal compartment, placental tissue compartment and fetal intravascular compartment) and a dead volume compartment was used to evaluate the transplacental transfer kinetics of salicylic acid.

Equations (10)-(13), equations (10)-(14) and equation (15)-(17) were simultaneously fitted to the sets of time profiles of maternal and fetal effluents and the amounts of drug in the perfused tissues in Protocol I and Ib, Protocol Ia and Protocol II,

respectively, by using a least-squares program (MLAB) to obtain transplacental pharmacokinetic parameters, *i.e.*, K_1 , k_2 , k_3 , k_4 and k_s . K_4 and k_3 represent the influx clearance from fetal intravascular space to placental tissue (mL/min/g cotyledon) and efflux rate constant from placental tissue to fetal intravascular space (min^{-1}), respectively.

(Protocol I, Ia ($t \leq 62$), Ib)

$$\frac{dX_a}{dt} = C_{in} \cdot Q_m - k_a \cdot X_a \quad (10)$$

$$\frac{dC_m}{dt} = \frac{k_a \cdot X_a - C_m \cdot (K_1 + Q_m) + k_2 \cdot X_p}{V_m} \quad (11)$$

$$\frac{dX_p}{dt} = C_m \cdot K_1 - (k_2 + k_3 + k_s) \cdot X_p + C \cdot K_4 \quad (12)$$

$$\frac{dC}{dt} = \frac{k_3 \cdot X_p - C \cdot (K_4 + Q_f)}{V_f} \quad (13)$$

(Protocol Ia ($62 < t \leq 65$))

$$\frac{dX_a}{dt} = -k_a \cdot X_a \quad (14)$$

(Protocol II)

$$\frac{dC_m}{dt} = \frac{-C_m \cdot (K_1 + Q_m) + k_2 \cdot X_p}{V_m} \quad (15)$$

$$\frac{dX_p}{dt} = C_m \cdot K_1 - (k_2 + k_3 + k_s) \cdot X_p + C \cdot K_4 \quad (16)$$

$$\frac{dC}{dt} = \frac{C_{in} \cdot Q_f + k_3 \cdot X_p - C \cdot (K_4 + Q_f)}{V_f} \quad (17)$$

Correction of kinetic parameters for protein binding in the perfusate

Protein binding rates of salicylic acid and antipyrine in the perfusate were determined by equilibrium dialysis (Takedomi *et al.*, 1998). The unbound influx clearances from the maternal and fetal

sides to the placental tissue ($K'1$ and $K'4$) were obtained by dividing the $K1$ and $K4$ values by the observed free fraction (f_p) of each drug in the respective perfusates. Furthermore, the influx plasma clearances into placental tissue ($K''1$ and $K''4$) were estimated by multiplying the unbound rate of drugs in the maternal plasma ($f_{p,m}$, salicylic acid: 0.568, Hill and Abramson *et al.*, 1975, antipyrine: 0.884, Ohkawa *et al.*, 2001) and fetal plasma ($f_{p,f}$, salicylic acid : 0.457, antipyrine: 0.869).

Results

Placental perfusion technique

Fetal perfusion pressure was monitored throughout the experiment and confirmed not to exceed 40 mmHg. Leakage of perfusate from the fetal side was less than 3.0 mL/hour.

Protocols I and Ia

Antipyrine and salicylic acid appeared rapidly in the fetal effluent. Their concentrations in the maternal and fetal perfusates reached a steady state within 10 minutes after the start of drug perfusion (Figures 4 and 5). The TPTss values of antipyrine and salicylic acid were 7.62% and 4.13%, respectively, showing that the transfer of salicylic acid was 54.2% of that of antipyrine. In Protocol Ia, drug concentrations in the effluents gradually decreased after the perfusate was changed (Figures 4 and 5).

Protocol II

After the start of drug perfusion, the concentration of antipyrine in the fetal effluent rapidly reached a steady state, and the fetal-to-maternal transfer was low (Figure 6). Fetal-to-maternal transfer of salicylic acid was lower than that of antipyrine (Figure 7).

Model analysis

Concentration profiles of antipyrine in the maternal and fetal effluents under Protocols I, Ia, Ib and II were adequately explained by the developed model (Figure 8). The values of the pharmacokinetic parameters K_1 , k_2 and V_p obtained were 0.0791 mL/min/g cotyledon, 0.118 min⁻¹ and 0.195 mL/g, respectively (Table 1). The concentration profiles of salicylic acid in the maternal and fetal effluents in Protocols I, Ia, Ib and II were also adequately explained by the developed model (Figure 9). The values of the

pharmacokinetic parameters K_1 , k_2 , k_3 and K_4 obtained were 0.0407 mL/min/g cotyledon, 0.0238 min^{-1} , 0.176 min^{-1} and 0.0813 mL/min/g cotyledon, respectively (Table 1).

Correction for protein binding

The unbound fractions (fb) of salicylic acid and antipyrine in the perfusate were 0.902 and 0.932, respectively. The unbound influx clearances of salicylic acid, K'_1 and K'_4 , were estimated to be 0.0451 and 0.0901 mL/min/g cotyledon, so that the plasma influx clearances, K''_1 and K''_4 , were estimated to be 0.00256 and 0.0412 mL/min/g cotyledon, respectively. Placental tissue-to-plasma concentration ratio, K''_1/k_2 , for salicylic acid was estimated to be 1.07 mL/g cotyledon (Table 1).

On the other hand, unbound and plasma influx clearances (K'_1 and K''_1) were estimated to be 0.0849 and 0.0751 mL/min/g cotyledon, respectively. Placental tissue-to-plasma concentration ratio, K''_1/k_2 , for antipyrine was estimated to be 0.642 mL/g cotyledon (Table 1).

Discussion

In the present study, we developed a pharmacokinetic model based on the results of a human placental perfusion study with various perfusion protocols (Protocols II, Ia and Ib), in addition to the conventional protocol, Protocol I (Figures 8 and 9, Table 1). Each fitting line fell largely within the range of observed values \pm S.D., and the S.D. value of each parameter did not greatly exceed the parameter estimate, indicating that the model fit can be considered adequate. Distinct models were used to describe the kinetics of antipyrine and salicylic acid in the present study. The model for antipyrine is a variation of that for salicylic acid based on the assumption that placental tissue and fetal intravascular space are in rapid equilibrium (Figure 2). Although we had hoped to apply the model for salicylic acid (Figure 3) directly to antipyrine, the calculated k_3 and K_4 values were extremely large and failed to converge (data not shown). Therefore, we considered that rapid equilibrium can be assumed between placental tissue and fetal intravascular space within the present sampling schedule. As antipyrine is rapidly distributed into tissues, more frequent sampling may be required to quantitatively evaluate the kinetics between placental tissue and fetal intravascular space and also to estimate k_3 and K_4 individually. This may also be the case for other drugs that are rapidly distributed into tissues.

We assumed a well-stirred maternal compartment with the volume (V_m) of 23 mL. Taking into account the maternal flow rate (Q_m) of 15 mL/min, the replacement of fluid in the maternal compartment is likely to be rapid within the present sampling schedule, and therefore we assumed the compartment to be well-stirred. Although a model involving a concentration gradient (e.g. tube model, dispersion model, etc.) might be applicable for each compartment, we consider this to be unnecessary for the same reason as above, unless we wish to analyze the kinetics in the subminute range. On

the other hand, the concentration of maternal effluent reached the steady-state more slowly than the model describes, suggesting that an additional distribution compartment, which is adjacent to the maternal compartment, but unconnected to the fetal compartment, may be involved. However, more complicated models with many parameters failed to converge. Reducing the dead volume (X_a , Figures 2 and 3), increasing the sampling frequency, and/or using other perfusion protocol(s) may be necessary if more complicated models are to be examined.

The calculated transplacental kinetic parameters of salicylic acid and antipyrine obtained with this model are summarized in Table 1. The unbound influx clearance ($K'1$) of salicylic acid was 0.0451 mL/min/g cotyledon, which is about half of that of antipyrine (0.0849 mL/min/g cotyledon). On the other hand, the efflux rate constant of salicylic acid from the placental tissue to interstitial space (k_2) was 0.0238 min^{-1} , which is about one-fifth of that of antipyrine. This difference may be explained by the fact that salicylic acid at physiological pH (7.4) is almost entirely ionized (membrane-impermeable form). The membrane permeability of a drug that does not undergo transporter-mediated transport is proportional to the product of the octanol/water partition coefficient and (molecular weight)^{1/2} (Camenisch *et al.*, 1996). Although this value for salicylic acid at the present experimental pH is 0.000562 and far smaller than that of antipyrine (0.136), the observed $K'1$ value of salicylic acid was comparable with that of antipyrine, suggesting that the $K'1$ value of salicylic acid is larger than would be predicted from its physicochemical properties. Therefore, transplacental transfer of salicylic acid may be mediated by a specific system, at least in part. Indeed, we have shown that BeWo cells, a human placental choriocarcinoma cell line, take up salicylic acid together with protons via a specific transport system (Emoto *et al.*, 2002). A known proton-dependent transporter, monocarboxylate transporter (MCT) 4, is highly expressed on the maternal side of human placental

trophoblasts (Settle *et al.*, 2003). Thus, proton-dependent transporter(s) such as MCTs may contribute to the transplacental transport of salicylic acid.

In the present model analysis, the value of the parameter, k_s , which represents the elimination of drug from the placenta, was derived from the mean recovery of antipyrine (82.6%). Antipyrine has been employed as a typical passive diffusion marker in human placental perfusion studies. However, we also retrospectively calculated the recovery from the antipyrine concentrations in the influx perfusate and effluents in other studies, and found that it was generally less than 100%, i.e., 80% in the case of Schenker *et al.* (1992), 70% according to Lampela *et al.* (1999), and 75% in the study by Nanovskaya *et al.* (2002). A likely explanation is metabolism of antipyrine in the placenta. Although there is no report concerning metabolism of antipyrine in the placenta, antipyrine is metabolized by a variety of cytochrome P450 isoforms such as CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4 (Engel *et al.*, 1996; Carcillo *et al.*, 2003), and the placenta is known to express CYP3A4 protein and mRNAs of CYP1A2 and CYP2C family members (Hakkola *et al.*, 1996; Pasanen, 1999). Thus, the rate constant, k_s , may include a contribution from metabolism of antipyrine in the placenta. On the other hand, the k_s value for salicylic acid was quite small ($1.81 \times 10^{-4} \text{ min}^{-1}$), suggesting that it may not be metabolized in the placenta.

We employed four protocols (Protocol I, II, Ia and Ib) of human placental perfusion. While most human placental perfusion studies have been done at the steady state, a non-steady state model was applied to the time-profiles of the drug concentrations in the effluent. Our model with frequent sampling after the start of drug perfusion could provide kinetic parameters such as K_1 . Protocol II is considered to have been helpful to precisely calculate the parameters k_3 and K_4 , because in the absence of the results of Protocol II, these parameters could not be accurately estimated (data

not shown). In other words, observation of transfer kinetics of a drug from maternal perfusate to fetal effluent is not sufficient to allow evaluation of the kinetics of drug transfer among interstitial space, placental tissue and fetal intravascular space. Protocol Ia was helpful to evaluate the efflux of the drug from placental tissue to the effluents, i.e., k_2 and k_3 , more accurately. Although we did not evaluate the acceptability of the precision by using any index, the relative S.D. value of k_2 , especially for salicylic acid, was larger than those of other parameters. This may reflect the existence of a maternal dead volume compartment (X_a , Figures 2 and 3), which may weaken the concentration clamp of the maternal perfusate. Protocol Ib was also helpful to determine the initial drug uptake rate from maternal perfusate to placental tissue. Although the drug amount in the placental tissue can theoretically be obtained at the steady-state from a mass-balance equation, if the drug is not eliminated in the placenta, the tissue sampling indicated that antipyrine may be eliminated in the placenta. In protocol II, the model underestimated the concentration-time profiles of the fetal effluents (Figures 8 and 9). This seems to be consistent with the observation that the recovery rates of drugs ($[C_m \cdot Q_m + C \cdot Q_f] / C_{in} \cdot Q_{in}$) in protocol II were higher than those in protocol I. Although the reason for this difference remains to be elucidated, a possible explanation is that some factor(s), such as a heterogeneous enzyme distribution in the placental tissue, leads to differences in the metabolic capacity for the drug between protocols. In any case, simultaneous fitting of the model to the sets of concentration profiles and tissue concentrations enabled us to determine the transplacental parameters with sufficient accuracy.

Our model allowed us to evaluate the elemental processes of transplacental transfer of salicylic acid. This in turn makes it possible to predict the influx profile of the drug into fetal umbilical veins by using the plasma concentration-time profile of the drug in gravidae. The present model analysis may therefore

provide clinically significant information about fetal exposure to drugs given to the mother during full-term gestation, and may be applicable to the prediction of possible fetal toxicity of diclofenac, digoxin, paroxetine and so on. It may also be possible to evaluate the physiological contributions of certain transporter(s) to the transplacental transfer of drugs by the present technique, if a specific inhibitor of the transporter is perfused simultaneously with the substrate. Moreover, if the placental transfer is altered by some factor (e.g. pathology, genotype, concomitant drug administration, etc.), the present model-based analysis may be useful to reveal what process was affected. We may also simulate the effects of change in an individual process (e.g. change in drug transporter function) on the overall transfer function of the placenta.

In conclusion, the time profiles of the concentration of salicylic acid in the effluents under various protocols of placental perfusion can be adequately explained by our newly developed pharmacokinetic model. The model analysis provided the transplacental transfer kinetics of salicylic acid and enabled us to estimate the influx profile of the drug into the fetal umbilical vein from the plasma concentration profile of the drug in the gravidae.

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Footnotes

This study was supported in part by grants from Japan Human Sciences Foundation and Japan Society for the Promotion of Science (JSPS).

Legends for Figures

Figure 1 Schematic representation of the experimental design. A set of concentration-time profiles of antipyrine and salicylic acid in maternal and fetal effluents was obtained from one placenta. Then, all the time profiles of each compound in the four experimental protocols were pooled. Finally, the appropriate pharmacokinetic model was simultaneously fitted to the collective data to obtain pharmacokinetic parameters. See the text for details of the pharmacokinetic models.

Figure 2 Pharmacokinetic models of antipyrine transfer across the placenta observed with a human placental perfusion method. C_{in} , drug concentration in perfusate (50 $\mu\text{g/mL}$); C_m , drug concentration in maternal compartment ($\mu\text{g/mL}$); C , drug concentration in fetal intravascular compartment ($\mu\text{g/mL}$); X_p , amount of drug in placental compartment ($\mu\text{g/g cotyledon}$); k_a , first-order influx rate constant (1.02 min^{-1}); K_1 , influx clearance ($\text{mL/min/g cotyledon}$); k_2 , first-order efflux rate constant (min^{-1}); k_s , elimination rate constant (min^{-1}); V_p , apparent distribution volume of placental compartment (mL/g cotyledon); Q_m , maternal flow rate ($\text{mL/min/g cotyledon}$); Q_f , fetal flow rate ($\text{mL/min/g cotyledon}$); V_m , volume of maternal compartment (23 mL); V_f , intravascular volume of placental tissue ($0.06 \text{ mL/g cotyledon}$)

Figure 3 Pharmacokinetic model of salicylic acid transfer across the placenta observed with a human placental perfusion method. C_{in} , drug concentration in perfusate (8 $\mu\text{g/mL}$); C_m , drug concentration in maternal compartment ($\mu\text{g/mL}$); C , drug concentration in fetal intravascular compartment ($\mu\text{g/mL}$); X_p , amount of drug in placental compartment ($\mu\text{g/g cotyledon}$); k_a , first-order influx rate constant

(1.02 min^{-1}); K_1 , K_4 , influx clearances (mL/min/g cotyledon); k_2 , k_3 , first-order efflux rate constant (min^{-1}); k_5 , elimination rate constant (min^{-1}); Q_m , maternal flow rate (mL/min/g cotyledon); Q_f , fetal flow rate (mL/min/g cotyledon); V_m , volume of maternal compartment (23 mL); V_e , intravascular volume in placental tissue (0.06 mL/g cotyledon)

Figure 4 Time courses of antipyrine concentration in effluents during maternal-to-fetal and washout perfusion (Protocol I, Ia and Ib).

Closed circles, open circles and gray diamonds represent the drug concentrations in maternal effluent, fetal effluent and placental tissue, respectively. Each point represents the mean \pm S. D. (n = 3 - 9).

Figure 5 Time courses of salicylic acid concentration in effluents during maternal-to-fetal and washout perfusion (Protocols I, Ia and Ib).

Closed circles, open circles and gray diamonds represent drug concentrations in maternal effluent, fetal effluent and placental tissue, respectively. Each point represents the mean \pm S. D. (n = 3 - 9).

Figure 6 Time courses of antipyrine concentration in effluents during fetal-to-maternal perfusion (Protocol II).

Closed circles, open circles and gray diamonds represent drug concentrations in maternal effluent, fetal effluent and placental tissue, respectively. Each point represents the mean \pm S. D. (n = 3).

Figure 7 Time courses of salicylic acid concentration in effluents during fetal-to-maternal perfusion (Protocol II).

Closed circles, open circles and gray diamonds represent drug concentrations in maternal effluent, fetal effluent and placental

tissue, respectively. Each point represents the mean \pm S. D. (n = 3).

Figure 8 Model analysis of transplacental transfer of antipyrine. Closed circles, open circles and gray diamonds represent maternal concentration, fetal concentration and placental tissue concentration, respectively. The lines represent the model fit (solid line, maternal effluent; dotted line, fetal effluent; dashed line, placental tissue).

Figure 9 Model analysis of transplacental transfer of salicylic acid. Closed circles, open circles and gray diamonds represent maternal concentration, fetal concentration and placental tissue concentration, respectively. The lines represent the model fit (solid line, maternal effluent; dotted line, fetal effluent; dashed line, placental tissue).

Table 1. Transplacental pharmacokinetic parameters of salicylic acid and antipyrine

	K_1 (mL/min/g cotyledon)	f_b	K'_1 (mL/min/g cotyledon)	$f_{p,m}$	K''_1 (mL/min/g cotyledon)	k_2 (min ⁻¹)	K'_1/k_2 (mL/g cotyledon)	K''_1/k_2 (mL/g cotyledon)
salicylic acid	0.0407±0.0062	0.902	0.0451	0.568 ^a	0.0256	0.0238±0.0344	1.89	1.07
antipyrine	0.0791±0.0059	0.932	0.0849	0.884 ^b	0.0751	0.118±0.093	0.726	0.642

	k_3 (min ⁻¹)	K_4 (mL/min/g cotyledon)	K'_4 (mL/min/g cotyledon)	$f_{p,f}$	K''_4 (mL/min/g cotyledon)	V_p	K''_4/k_3 (mL/g cotyledon)	K'_4/k_3 (mL/g cotyledon)	k_s (min ⁻¹)
salicylic acid	0.176±0.054	0.0813±0.0284	0.0901	0.457 ^a	0.0412	- ^c	0.512	0.234	1.81×10 ⁻⁴
antipyrine	- ^c	- ^c	- ^c	0.869 ^b	- ^c	0.195±0.050	- ^c	- ^c	0.108±0.091

$K_1, K'_1, K''_1, K_4, K'_4, K''_4$, clearance; k_2, k_3, k_s , rate constants; f_b , 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 placental compartment. See text for details.

Each value represents the estimate ± S.D. (68.3% confidence interval)

^a Hill and Abramson et al (1988).

^b Ohkawa et al (2001).

^c Not applicable to antipyrine

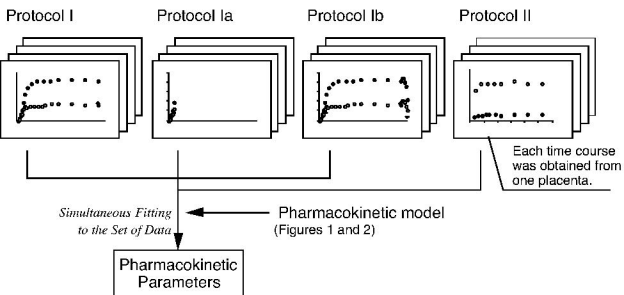
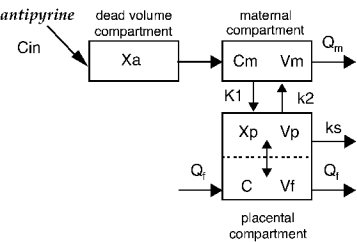


Figure 1

maternal-to-fetal perfusion
(Protocol I, Ia and Ib)



fetal-to-maternal perfusion
(Protocol II)

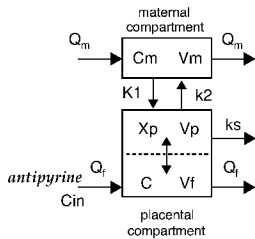
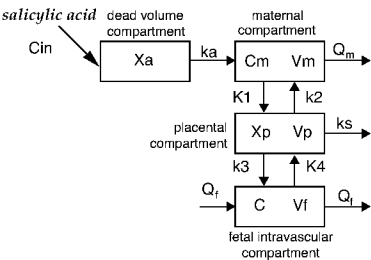


Figure 2

maternal-to-fetal perfusion
(Protocol I, Ia and Ib)



fetal-to-maternal perfusion
(Protocol II)

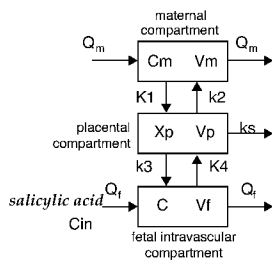


Figure 3

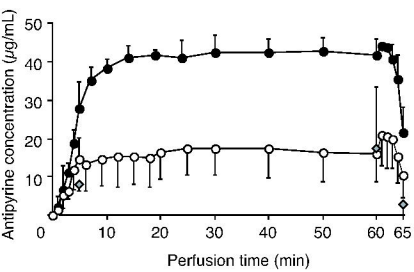


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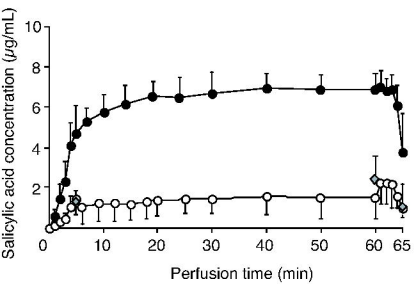


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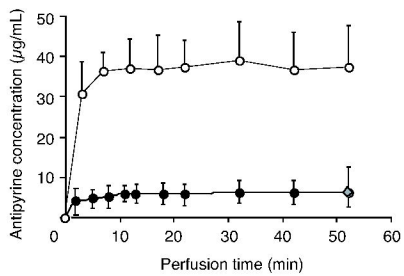


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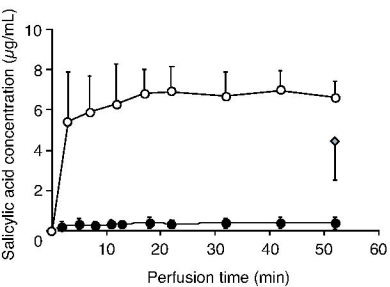


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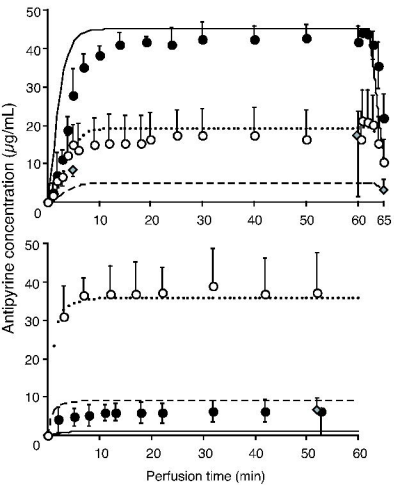


Figure 8

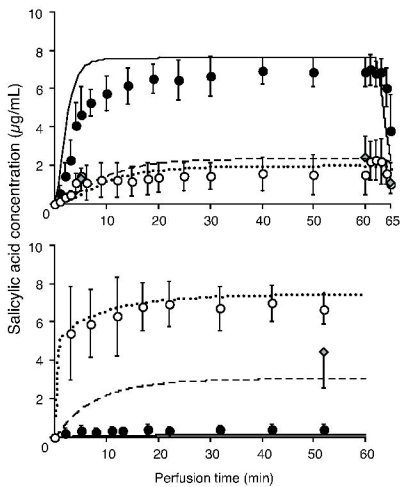


Figure 9