Transplacental pharmacokinetics of diclofenac in perfused human placenta

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Running Title: Transplacental transfer of diclofenac

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A list of nonstandard abbreviations:

HPLC, high-performance liquid chromatography; TPT_{ss}, ratio of the rate of amount transferred from maternal to fetal side across the placenta to that infused in the steady state; TPT_{ss,R}; ratio of the rate of amount transferred from fetal to maternal side across the placenta to that infused in the steady state
ABSTRACT:
The aims of this study were to evaluate the transplacental transfer properties of diclofenac and to determine the effect of L-lactic acid on the transplacental transfer of diclofenac. The maternal and fetal vessels of human placenta were perfused in a single pass mode with a solution containing diclofenac and antipyrine. The transplacental pharmacokinetic model was fitted to the time profiles of the drug concentrations in the effluent and placenta to obtain transplacental pharmacokinetic parameters. In addition, chloride ion in the perfusate was partially replaced with L-lactic acid to see the change in the transplacental transfer properties of diclofenac. The TPTss value (ratio of the rate of amount transferred across the placenta to that infused in the steady state) of diclofenac was 2.22%, which was about one-third that of antipyrine, and significantly reduced in the presence of L-lactic acid. The transplacental pharmacokinetic model could adequately explain the transplacental transfer of diclofenac with influx clearances from maternal and fetal perfusates to placental tissue of 0.276 and 0.0345 mL/min/g cotyledon and efflux rate constants from placental tissue to maternal and fetal perfusates of 0.406 and 0.0337 min\(^{-1}\), respectively. By taking into account protein binding, placental tissue-to-plasma concentration ratio in humans for diclofenac was estimated to be 0.108 mL/g cotyledon, and was smaller than that of antipyrine. In conclusion, human placental perfusion and transplacental pharmacokinetic modeling allowed us to determine the transplacental transfer properties of diclofenac quantitatively. Diclofenac may share transplacental transfer system(s) with L-lactic acid.
Introduction

The placenta controls the exchange of a variety of materials between mother and fetus (Robertson and Karp., 1976). Maternal-to-fetal transfer of all materials, including drugs, is primarily regulated by the placenta (Knipp et al., 1999). In the placenta, fetal blood neither mixes with nor contacts to maternal blood. Fetal blood flows in the placental villi, which projects towards the maternal decidua basalis to form the interstitial (intervillous) space. Syncytiotrophoblast cells form the outermost layer of the placental villi. On the other hand, maternal blood is supplied by the spiral arteries and fills the interstitial space.

The use of non-steroidal anti-inflammatory drugs (NSAIDs) in pregnant women is known to lead fetal or neonatal toxicity such as persistent pulmonary hypertension of the newborn (van Marter et al., 1996, Alano et al., 2001) and premature constriction of ductus arteriosus (Menahem et al., 1991). Diclofenac, one of NSAIDs, have been classified as one of the most potent inducers of ductus arteriosus constriction in rats (Momma et al., 1984). When given during human full-term pregnancy, diclofenac readily permeates into fetal blood, inhibiting the synthesis of prostaglandins and inducing constriction of the ductus arteriosus, thereby causing pulmonary hypertension in the newborn (Rein et al., 1999, Siu and Lee, 2004, Auer et al., 2004). Although the evidence for diclofenac-induced fetal adverse reactions remains indefinite, case analyses suggest that diclofenac is one of the common causative drugs of ductus arteriosus and persistent fetal circulation (Luchese et al., 2003, Kuchikura et al., 1984). Therefore, it is worthwhile to investigate in detail the kinetics of the transfer of diclofenac from mother to fetus.

We have shown using BeWo cells, a human placental choriocarcinoma cell line, that diclofenac inhibits the transfer of L-lactic acid, a known substrate for monocarboxylate transporters (MCTs) and decreases the intracellular pH (Emoto et al., 2002). Therefore, it is likely that diclofenac is transported by a proton-coupled transporter, such as MCTs, in
human placenta and this transport is inhibited by L-lactic acid. However, no studies have been conducted to analyze the kinetic interaction between diclofenac and L-lactic acid in human placental tissue. We have reported a method to evaluate quantitatively each process in the transplacental transfer of drugs by using perfused human placenta in combination with pharmacokinetic modeling (Shintaku et al., 2007).

The aims of this study were to analyze quantitatively the transfer properties of diclofenac across the placenta by means of a combination of human placental perfusion experiments and transplacental pharmacokinetic modeling, and to examine the effect of L-lactic acid on the kinetics of diclofenac.
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 Committees of the Faculty of Medicine, Kyushu University and The University of Tokyo, and written informed consent was provided by the gravidae prior to the delivery.

Diclofenac was provided by Novartis Pharma Co., Ltd. Antipyrine, ketoprofen, 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

Krebs-Ringer-bicarbonate buffer (pH 7.4), prepared by the method previously reported (Shintaku et al., 2007), was used as the perfusate. High-lactate Krebs-Ringer-bicarbonate buffer was prepared by replacing 35 mM NaCl in Krebs-Ringer-bicarbonate buffer with 35 mM L-lactic acid and used for the inhibition study (35 mM L-lactic acid, 83 mM NaCl, 4.7 mM KCl, 1.3 mM MgSO4, 24.2 mM NaHCO3, 2.5 mM CaCl2, pH adjusted to 7.4 by NaOH). The buffers also contained D-glucose (1.0 g/L), heparin (12,500 IU/L), dextran (1.0 g/L) and human serum albumin (2.0 g/L). The maternal and fetal perfusates were aerated with 95% O2/5% CO2 and 95% N2/5% CO2, respectively, and warmed to 37°C.

Placental perfusion technique

In vitro human placental perfusion was carried out as previously reported (Shintaku et al., 2007, Schneider et al., 1972). 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/h. One cotyledon was chosen from each placenta and was set up on a plastic chamber with the maternal side up. Three needles (18 gauge), connected
to a trifurcation (branching glass pipe) with a dead volume of 14.7 mL, were inserted from the maternal side to the cotyledon to a depth of 7 mm and maternal perfusate was perfused via the needles at a rate of 15 mL/min. The fetal side was cannulated and perfused at a rate of 3 mL/min. After stabilization by perfusing drug-free perfusate for 30 min, placenta was perfused according to the respective protocols (Table 1). In protocol I, the maternal side was perfused with a diclofenac (100 ng/mL) and antipyrine (50 µg/mL)-containing perfusate, and the maternal and fetal effluents were sampled for 60 min. Antipyrine was used as a passive diffusion marker. In protocol Ia, the efflux kinetics of drugs from the placental tissue was investigated by changing the drug-containing perfusate to a drug-free perfusate at 62 min. In protocol Ib, the tissue sample at the early phase of perfusion was investigated. Protocol II was designed to investigate the transfer of drugs from fetal to maternal side. In protocol I-LA, the effect of lactic acid on the transfer of drugs was investigated. In all the perfusion protocols, maternal and fetal effluents were sampled periodically and the perfused cotyledon was weighed and sampled just after the last sampling of effluents to determine the tissue concentrations of drugs. All the samples were stored frozen at -20°C until analysis.

**Determination of drugs in effluents**

Antipyrine concentration in effluents was determined by means of the HPLC-UV method previously reported (Shintaku et al., 2007). The detection and quantification limits were <0.05 and 0.1 µg/mL, respectively.

Diclofenac concentration in effluents was also determined by means of an HPLC-UV method. An aliquot of sample (500 µL) was spiked with 500 µL of internal standard solution (1 µg/mL ketoprofen in water), 5 mL of chloroform, and 50 µL of 1 M HCl, mixed for 1 min, and spun at 800xg for 10 min at 4°C. The organic layer (4 mL) was transferred to another glass tube and evaporated to dryness under a gentle nitrogen stream. The residue
was dissolved in 200 µL of mobile phase (0.05 M KH₂PO₄ (pH 5.0) : acetonitrile = 55 : 45 v/v) and an aliquot of 80 µL was subjected to HPLC. The detection and quantification limits were <0.5 and 1 ng/mL, respectively.

**Determination of drugs in placental tissue**

Placental concentrations of antipyrine were determined by the HPLC-UV method previously reported (Shintaku et al., 2007).

Placental concentrations of diclofenac were determined by an HPLC-UV method. An aliquot of 3 mL of the homogenate was spiked with 50 µL of 1 M HCl, 5 mL of chloroform, and 10 µL of internal standard solution (1 µg/mL ketoprofen in water), mixed for 1 min, and spun at 800xg for 10 min 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 min at 4°C and a 20-µL aliquot was subjected to HPLC.

**HPLC apparatus**

The HPLC system consisted of a pump, a UV-visible detector and an integrator (LC-10AD, SPD-10AV and CR-6A Chromatopac; Shimadzu Co., Kyoto, Japan). A reverse-phase column (Cosmosil 5C18 column, 4.6 mm x 150 mm, 5 µm; Nacalai Tesque) was used for separation at ambient temperature. For determination of antipyrine, the mobile phase (0.02 M phosphate buffer (pH 6.0) : methanol = 70 : 30 (v/v)) was pumped at a rate of 1.0 mL/min and the detection wavelength was set at 250 nm to detect antipyrine and internal standard (4-aminoantipyrine). For determination of diclofenac, the mobile phase (0.05 M KH₂PO₄ (pH 5.0) : acetonitrile = 55 : 45 (v/v)) was pumped at a rate of 1.2 mL/min and the detection wavelength was set at 280 nm to detect diclofenac and the internal standard.

**Evaluation of transplacental permeability**
The TPTss value 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)

\[
TPT_{ss} (\%) = \frac{C_{f,ss} \cdot Q_f \cdot 100}{C_{in} \cdot Q_m}
\]  

where \( C_{f,ss} \), \( C_{in} \), \( Q_f \) and \( Q_m \) represent the mean drug concentration in the fetal effluent, that in the maternal inflow (calculated value based on the preparation), and the fetal and maternal flow rates (3 mL/min and 15 mL/min), respectively.

The TPTss value in the opposite direction (TPTss,R) was calculated in the same manner by using equation (2).

\[
TPT_{ss,R} (\%) = \frac{C_{m,ss} \cdot Q_m \cdot 100}{C_{in,f} \cdot Q_f}
\]  

where \( C_{m,ss} \) and \( C_{in,f} \) represent the mean drug concentration in the maternal effluent and that in the fetal inflow (calculated value based on the preparation), respectively.

**Pharmacokinetic analysis for transplacental transfer**

A pharmacokinetic model consisting of two placental compartments (Shintaku et al., 2007) was used to evaluate the placental transfer of antipyrine (Figure 1-A). The transplacental pharmacokinetic modeling was simultaneously fitted to the sets of time profiles of maternal and fetal effluents and the amount of drug in the perfused placental tissues in protocols I, Ia, Ib and II, by using a nonlinear least-squares program (MLAB; Civilized Software, Bethesda, MD) to obtain transplacental pharmacokinetic parameters, such as \( K_1 \) (influx clearance from intestinal space to placental tissue, mL/min/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).
A pharmacokinetic model consisting of three placental compartments (Shintaku et al., 2007) was used to evaluate the placental transfer of diclofenac (Figure 1-B). Transplacental pharmacokinetic parameters, such as K1 and K4 (influx clearance from maternal and fetal perfusates to placental tissue, mL/min/g cotyledon), k2 and k3 (efflux rate constants from placental tissue to maternal and fetal perfusates, min⁻¹) and kₖ (elimination rate constant from the placenta, min⁻¹) were obtained in the same manner as described above.

In both models, we incorporated a dead volume compartment into the maternal circulation to explain the fluid volume in the trifurcated glass pipe.

Correction of influx clearances with protein binding

Since the perfusate contains 2.0 g/L of human serum albumin, the protein-binding rates of diclofenac and antipyrine in the perfusate were determined by equilibrium dialysis using the method previously reported (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 (fₖ) of each drug in the perfusates. Furthermore, the influx plasma clearances into placental tissue (K''₁ and K''₄) were estimated by multiplying by the unbound fraction of each drug in the human maternal plasma (diclofenac: 0.007, Chan et al., 1987, antipyrine: 0.884, Ohkawa et al., 2001) and human fetal plasma (diclofenac: 0.006, Chan et al., 1987, antipyrine: 0.869, Ohkawa et al., 2001).

Statistical analysis

The significance of differences between the TPTₜₜ values in the presence or the absence of L-lactic acid (35 mM) was determined by using Student’s t-test, and a value of p < 0.05 was considered statistically significant.
Results

Drug transfer from maternal to fetal side (Protocol I, Ia and Ib)

Both antipyrine and diclofenac appeared rapidly in the fetal effluent after the start of perfusion. Their concentrations in the maternal and fetal perfusates reached the steady state within 10 min (Figure 2-A, 2-B). The TPTss values of antipyrine and diclofenac were 6.94% and 2.22%, respectively, showing that the transfer of diclofenac was 32.0% of that of antipyrine. In Protocol Ia, drug concentrations in the effluents gradually decreased after the perfusate was changed to a drug-free perfusate (Figure 2-A, 2-B).

Drug transfer from fetal to maternal side (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 2-C). Fetal-to-maternal transfer of diclofenac was much lower than that of antipyrine (Figure 2-D). The TPTss,R values of antipyrine and diclofenac were 34.6% and 19.5%, respectively.

Effect of L-lactic acid on the transplacental transfer of diclofenac (Protocol I-LA)

After the start of drug perfusion, the antipyrine and diclofenac concentrations in the maternal and fetal perfusates reached a steady state within 10 min (Figure 2-E, 2-F). In the presence of L-lactic acid (Protocol I-LA), the mean concentration profile of diclofenac in the fetal effluent was lower than that in the absence of L-lactic acid (Protocols I and Ia).

The TPTss value of diclofenac was significantly reduced by 30.6% (from 2.22% to 1.54%, p<0.05) by L-lactic acid, whereas that of antipyrine remained unchanged (from 6.94% to 6.16%).

Model analysis

Concentration profiles of antipyrine in the maternal and fetal effluents under Protocols I, Ia, Ib and II were adequately explained by a two-compartment transplacental pharmacokinetic model (Figure 3-A). The values of the pharmacokinetic parameters $K_1$, $k_2$ and $V_p$ obtained were 0.0685 mL/min/g.
cotyledon, 0.0657 min⁻¹ and 0.284 mL/g, respectively (Table 2).

The concentration profiles of diclofenac in the maternal and fetal effluents in Protocols I, Ia, Ib and II were adequately explained by a three-compartment transplacental pharmacokinetic model (Figure 3-B). The values of the pharmacokinetic parameters K₁, k₂, k₃ and K₄ obtained were 0.0276 mL/min/g cotyledon, 0.406 min⁻¹, 0.0337 min⁻¹ and 0.0345 mL/min/g cotyledon, respectively (Table 2).

**Correction of influx clearances for protein binding**

The unbound fractions (f₀) of diclofenac and antipyrine in the perfusate were 0.044 and 0.932, respectively. The unbound influx clearances of diclofenac, K’₁ and K’₄, were estimated to be 6.27 and 0.784 mL/min/g cotyledon, so that the plasma influx clearances, K''₁ and K''₄, were estimated to be 0.0439 and 0.0650 mL/min/g cotyledon, respectively. Placental tissue-to-plasma concentration ratio, K''₁/k₂, for diclofenac was estimated to be 0.108 mL/g cotyledon (Table 2).

On the other hand, the unbound and plasma influx clearances (K’₁ and K’’₁) of antipyrine were estimated to be 0.0735 and 0.0650 mL/min/g cotyledon, respectively. Placental tissue-to-plasma concentration ratio, K’’₁/k₂, for antipyrine was estimated to be 0.989 mL/g cotyledon (Table 2).
Discussion

The present model analysis provided the transplacental transfer kinetic parameters of diclofenac (Figure 3-B, Table 2). Although three compartment model seems to be physiologically more proper, the calculation for antipyrine did not converge as in our previous report (Shintaku et al., 2007). Therefore, to analyze the kinetics of antipyrine, we again chose two compartment model with the assumption that rapid equilibrium is attained between placental tissue and fetal perfusate. The model underestimated the placental concentration of antipyrine in protocol I, Ia and Ib and that of diclofenac in protocol II. Although the cause of this discrepancy remains uncertain, the smaller number of placental sample may have made light of the tissue concentration profiles. It should also be noted that any attempts to increase the number of compartments in the model resulted in failure to converge or to give enough accuracy in parameter estimates.

Maternal and fetal physiologically based pharmacokinetic models can be connected by the present transplacental pharmacokinetic model and parameters to provide prediction of the fetal plasma concentration-time profile of diclofenac after administration of diclofenac to the mother, although it remains to be investigated how to estimate quantitatively the distribution properties and clearance of diclofenac in the fetus during full-term gestation. The risk of pulmonary hypertension or premature constriction of ductus arteriosus in newborn may be possibly predicted by combining the present model with pharmacodynamic model explaining the above adverse reactions.

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 in the steady-state (89.4%). Although this was less than 100%, it was similar to values obtained in our previous report (82.6%, Shintaku et al., 2007) and other reports (70-80%, Schenker et al., 1992, Lampela et al., 1999, Nanovskaya et al., 2002). On the other hand, the mean recovery of diclofenac (92.9%) was also slightly less than 100%. Diclofenac is known to be metabolized by cytochrome P450 isoforms (CYP2C9 and 3A4) and glucuronyl transferase (UGT2B7) in the liver (Kenny et al., 2004).
and the placenta is known to express CYP3A4 and UGT2B family proteins (Hakkola et al., 1996; Collier, 2002). However, there are no reports directly showing the metabolism of diclofenac in the placenta, and we did not find any evidence, such as one of metabolites in the effluent. While the model required the rate constant, $k_s$, to explain the incomplete recovery of drug in the placental perfusion study, it remains uncertain whether it reflects the metabolism of drugs in the placenta.

The membrane permeability of a drug that is not the substrate of a specific transport system is often predicted from the partition coefficient (P, quantified as logP), the distribution coefficient (D, quantified as logD) that takes into consideration the degree of ionization is a more appropriate index to estimate the permeability at physiological pH (7.4) (Camenisch et al., 1996). The permeability-surface area product value (PS) is a quantitative index to explain the membrane permeability. Its logarithm (logPS) was shown to correlate well with log $(D \times MW^{0.5})$, i.e., logPS = $-2.32 + 0.322 \times \log (D \times MW^{0.5})$ (Liu et al., 2004). Although the estimated PS value of diclofenac $(4.41 \times 10^{-3})$ is similar to that of antipyrine $(2.51 \times 10^{-3})$, the observed $K_1'$ value of diclofenac is more than 80-times larger than that of antipyrine $(6.27 \text{ vs } 0.0735)$ (Table 3). This discrepancy was observed in our previous report for salicylic acid with lesser extent (Table 3). These results indicate that physicochemical properties cannot adequately explain the extensive influx clearance of diclofenac from maternal side to placental tissue, suggesting that influx of diclofenac is, at least in part, mediated by a specific system. MCTs are proton-coupled active transporters. Settle et al. (2004) have reported that MCT4 is expressed on the maternal side of syncytiotrophoblast, while MCT1 is predominantly expressed on the fetal side. They have also shown that the uptake into maternal membrane vesicles was higher than that into fetal membrane vesicles. We have previously shown that diclofenac inhibits the uptake of L-lactic acid, a typical substrate of MCTs, and is co-transported with proton in BeWo cells, a human placental choriocarcinoma cell line (Emoto et al., 2002). In order to clarify the
transplacental transport mechanism of diclofenac and its relation to L-lactic acid transport, the inhibitory effect of L-lactic acid on diclofenac transport was determined in the present study. The TPTss value of diclofenac was significantly reduced by L-lactic acid. This result suggests that transplacental transfer of diclofenac is mediated partially via transport system(s) inhibited by L-lactic acid. Further perfusion study incorporating other protocols in the presence of L-lactic acid would be necessary to estimate accurately the transplacental pharmacokinetic parameters and to predict the inhibitory effect of L-lactic acid on the transplacental transfer of diclofenac.

The unbound placental tissue-to-maternal concentration ratio, $K'_{1/k2}$, for diclofenac was estimated to be 15.4 mL/g cotyledon and was about 10-fold larger than that for salicylic acid (1.89 mL/g cotyledon) (Shintaku et al., 2007). In addition, the unbound placental tissue-to-fetal concentration ratio, $K'_{4/k3}$ for diclofenac was estimated to be 23.3 mL/g cotyledon and was about 45-fold larger than that for salicylic acid (0.512 mL/g cotyledon) (Shintaku et al., 2007). Therefore, these result show that the placental distribution of diclofenac is larger than that of salicylic acid, and is consistent with the values of distribution coefficient, D (diclofenac, 18.2, salicylic acid, 0.00676) under physiological conditions (pH 7.4). However, diclofenac is highly bound to plasma protein in vivo. Therefore, the placental tissue-to-maternal plasma concentration ratio, $K''_{1/k2}$, for diclofenac was estimated to be 0.108 mL/g cotyledon and was about one-tenth that of salicylic acid (1.07 mL/g cotyledon). The placental tissue-to-fetal plasma concentration ratio, $K''_{4/k3}$, for diclofenac was also estimated to be as low as 0.139 mL/g cotyledon and was about half that for salicylic acid (0.234 mL/g cotyledon). In other words, the placental tissue-to-plasma concentration ratio for diclofenac was considered to be smaller than that for salicylic acid because diclofenac is highly bound to plasma protein in humans in vivo.

In conclusion, our human placental perfusion study and the pharmacokinetic model
analysis have established the transplacental transfer properties of diclofenac. Our findings also indicate that diclofenac may, at least in part, share transplacental transport system(s) with L-lactic acid.
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Footnotes

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Legends for Figures

Figure 1 Pharmacokinetic models of antipyrine (A) and diclofenac (B) transfer across the placenta, observed in protocol I, Ia and Ib.

C_{in}, drug concentration in perfusate (antipyrine: 50 µg/mL, diclofenac: 100 ng/mL); C_{m}, drug concentration in maternal compartment (antipyrine: µg/mL, diclofenac: ng/mL); C, drug concentration in fetal intravascular compartment (antipyrine: µg/mL, diclofenac: ng/mL); X_{p}, amount of drug in placental compartment (antipyrine: µg/g cotyledon, diclofenac: ng/mL); 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_{e}, elimination rate constant (min^{-1}); V_{p}, apparent distribution volume of placental compartment (mL/g cotyledon); 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_{f}, intravascular volume of placental tissue (0.06 mL/g cotyledon)

Figure 2 A, B; Time courses of antipyrine (A) and diclofenac (B) concentration in effluents during maternal-to-fetal and washout perfusion (Protocol I, Ia, and Ib),
C, D; Time courses of antipyrine (C) and diclofenac (D) concentration in effluents during fetal-to-maternal perfusion (Protocol II).
E, F; Time courses of antipyrine (E) and diclofenac (F) concentration in effluents during maternal-to-fetal perfusion with L-lactic acid (Protocol I-LA).
Closed circles, open circles and gray diamonds represent the total drug concentrations in maternal effluent, fetal effluent and placental tissue, respectively. The values in each panel show the drug concentrations in maternal and fetal effluent at 60 min. The TPT_{ss} or TPT_{ss,R} value was also shown in each panel. Each point represents the mean ± S.D. (n = 3 for each protocol).
**Figure 3** Model analysis of transplacental transfer of antipyrine (A) and diclofenac (B)

Closed circles, open circles and gray diamonds represent total 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 Summary of the experimental protocols

<table>
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<tr>
<th>Protocol</th>
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<th>Drug-free perfusate</th>
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Drug-containing perfusate also contains a passive diffusion marker, antipyrine.

† containing 35 mM lactic acid
Table 2 Pharmacokinetic parameters of transplacental transfer of diclofenac and antipyrine

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<thead>
<tr>
<th></th>
<th>$K_1$ (mL/min/g cotyledon)</th>
<th>$f_b$</th>
<th>$K'_1$ (mL/min/g cotyledon)</th>
<th>$f_{p,m}$</th>
<th>$K''_1$ (mL/min/g cotyledon)</th>
<th>$k_2$ (min$^{-1}$)</th>
<th>$K'_1/k_2$ (mL/g cotyledon)</th>
<th>$K''_1/k_2$ (mL/g cotyledon)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diclofenac</strong></td>
<td>0.276±0.0098</td>
<td>0.044</td>
<td>6.27</td>
<td>0.007$^a$</td>
<td>0.0439</td>
<td>0.406±0.186</td>
<td>15.4</td>
<td>0.108</td>
</tr>
<tr>
<td><strong>Antipyrine</strong></td>
<td>0.0685±0.0044</td>
<td>0.932</td>
<td>0.0735</td>
<td>0.884$^b$</td>
<td>0.0650</td>
<td>0.0657±0.0519</td>
<td>1.12</td>
<td>0.989</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>$k_3$ (min$^{-1}$)</th>
<th>$K_4$ (mL/min/g cotyledon)</th>
<th>$K'_4$ (mL/min/g cotyledon)</th>
<th>$f_{p,t}$</th>
<th>$K''_4$ (mL/min/g cotyledon)</th>
<th>$V_p$ (mL/g cotyledon)</th>
<th>$K'_4/k_3$ (mL/g cotyledon)</th>
<th>$K''_4/k_3$ (mL/g cotyledon)</th>
<th>$k_s$ (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diclofenac</strong></td>
<td>0.0337±0.0060</td>
<td>0.0345±0.0049</td>
<td>0.784</td>
<td>0.006$^a$</td>
<td>0.00470</td>
<td>23.3</td>
<td>0.139</td>
<td>0.0811±0.0241</td>
<td>0.0495±0.0417</td>
</tr>
<tr>
<td><strong>Antipyrine</strong></td>
<td>0.139</td>
<td>0.0811±0.0241</td>
<td>0.869$^b$</td>
<td>0.869$^b$</td>
<td>0.284±0.043</td>
<td>0.284±0.043</td>
<td>0.284±0.043</td>
<td>0.284±0.043</td>
<td>0.284±0.043</td>
</tr>
</tbody>
</table>

$K_1$, $K'_1$, $K''_1$, $K_4$, $K'_4$, $K''_4$, influx clearance; $k_2$, $k_3$, $k_s$, elimination rate constants from the placental tissue; $f_b$, 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. (63% confidence interval)


$^c$Not applicable to antipyrine.
Table 3. A comparison of the physicochemical properties and transplacental PK parameters

<table>
<thead>
<tr>
<th></th>
<th>diclofenac</th>
<th>salicylic acid</th>
<th>antipyrine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>class</strong></td>
<td>acid</td>
<td>acid</td>
<td>base</td>
</tr>
<tr>
<td><strong>MW</strong></td>
<td>296.15</td>
<td>138.12</td>
<td>188.23</td>
</tr>
<tr>
<td><strong>pKa</strong></td>
<td>4.0&lt;sup&gt;a)&lt;/sup&gt;</td>
<td>2.90&lt;sup&gt;b)&lt;/sup&gt;</td>
<td>1.4&lt;sup&gt;c)&lt;/sup&gt;</td>
</tr>
<tr>
<td>log D (pH 7.4)</td>
<td>1.13&lt;sup&gt;a)&lt;/sup&gt;</td>
<td>-1.86&lt;sup&gt;d)&lt;/sup&gt;</td>
<td>0.27&lt;sup&gt;d)&lt;/sup&gt;</td>
</tr>
<tr>
<td>log{D/(MW&lt;sup&gt;1/2&lt;/sup&gt;)}</td>
<td>-0.11</td>
<td>-2.93</td>
<td>-0.87</td>
</tr>
<tr>
<td><strong>estimated permeability (PS)</strong>&lt;sup&gt;d)&lt;/sup&gt;</td>
<td>44.1×10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>5.45×10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>25.1×10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>K&lt;sub&gt;1'&lt;/sub&gt; (mL/min/g cotyledon)</strong></td>
<td>6.27</td>
<td>0.0451&lt;sup&gt;e)&lt;/sup&gt;</td>
<td>0.0735</td>
</tr>
<tr>
<td><strong>k&lt;sub&gt;2&lt;/sub&gt; (/min)</strong></td>
<td>0.406</td>
<td>0.0238&lt;sup&gt;e)&lt;/sup&gt;</td>
<td>0.0657</td>
</tr>
<tr>
<td><strong>K&lt;sub&gt;1'&lt;/sub&gt;/k&lt;sub&gt;2&lt;/sub&gt; (mL/g cotyledon)</strong></td>
<td>15.4</td>
<td>1.89&lt;sup&gt;e)&lt;/sup&gt;</td>
<td>1.12</td>
</tr>
</tbody>
</table>

MW: molecular weight, a) Novartis Pharma 2005, b) Hänninen et al., 2003, c) Ghafourian T et al., 2004, d) Liu et al., 2004, e) Shintaku et al., 2007
Figure 1

A. Antipyrine

maternal-to-fetal perfusion

B. Diclofenac

maternal-to-fetal perfusion

fetal-to-maternal perfusion

fetal-to-maternal perfusion