Transplacental Pharmacokinetics of Diclofenac in Perfused Human Placenta

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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 approximately one-third that of antipyrine and was 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 0.0346 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, the placental tissue/plasma concentration ratio in humans for diclofenac was estimated to be 0.108 ml/g of 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.

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 maternal blood. Fetal blood flows in the placental villi, which projects toward 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 nonsteroidal anti-inflammatory drugs in pregnant women is known to lead to 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, 1991). Diclofenac, a nonsteroidal anti-inflammatory drug, has been classified as one of the most potent inducers of ductus arteriosus constriction in rats (Momma et al., 1984). When 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; Auer et al., 2004; Siu and Lee, 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 (Kuchikura et al., 1984; Luchese et al., 2003). 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 an MCT, 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

ABBREVIATIONS: MCT, monocarboxylate transporter; HPLC, high-performance liquid chromatography; TPTss, ratio of the rate of amount transferred from maternal to fetal side across the placenta to that infused in the steady state; TPTss, Ip, ratio of the rate of amount transferred from fetal to maternal side across the placenta to that infused in the steady state.
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 ethics committees of the Faculty of Medicine, Kyushu University and The University of Tokyo, and written informed consent was provided by the gravidae before the delivery.

Diclofenac was provided by Novartis Pharma Co., Ltd. (Basel, Switzerland). 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 reported previously (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 was used for the inhibition study (35 mM L-lactic acid, 83 mM NaCl, 4.7 mM stabilization by perfusing drug-free perfusate for 30 min, placenta 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 7 mm and maternal perfusate was perfused via the needles at a rate of 15 ml/min. Were inserted from the maternal side to the cotyledon to a depth of 14.7 mm, 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 of the samples were stored frozen at -20°C until analysis.

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

The 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 800g 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)-acetoniitrile, 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 reported previously (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 800g 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 15,500g 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, Kyoto, Japan). A reverse-phase column (Cosmosil 5C18 column, 4.6 mm × 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)-acetoniitrile, 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 TPTₐₐ 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 eq. 1 (Heikkinen et al., 2000):

\[
\text{TPT}_{\text{ss}, \text{R}}(\%) = \frac{C_{\text{m,ss}} \cdot Q_{\text{f}} \cdot 100}{C_{\text{in}} \cdot Q_{\text{n}}} = \frac{Q_{\text{f}}}{Q_{\text{n}}}
\]

where \(C_{\text{m,ss}}\), \(C_{\text{in}}\), \(Q_{\text{f}}\), and \(Q_{\text{n}}\) represent the mean drug concentration in the maternal effluent (calculated value based on the preparation), and the fetal and maternal flow rates (3 and 15 ml/min), respectively. The \(TPT_{\text{ss}}\) value in the opposite direction (\(TPT_{\text{ss}, \text{R}}\)) was calculated in the same manner by using eq. 2:

\[
\text{TPT}_{\text{ss}, \text{R}}(\%) = \frac{C_{\text{m,ss}} \cdot Q_{\text{n}} \cdot 100}{C_{\text{in}} \cdot Q_{\text{f}}}
\]

where \(C_{\text{m,ss}}\) and \(C_{\text{in}}\) 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 transplacental transfer of antipyri ne (Fig. 1A). 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 \(K_{\text{e}}\) (influx clearance from interstitial space to placental tissue, milliliters per minute per gram of cotyledon), \(k_{\text{f}}\) (efflux rate constant from placental tissue to interstitial space, minute⁻¹), and \(V_{\text{p}}\).
**A Antipyrine**

maternal-to-fetal perfusion

![Diagram of pharmacokinetic model for antipyrine transfer across the placenta](image)

Statistical Analysis. The significance of differences between the TPT\textsubscript{sa} values in the presence or 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.

**B Diclofenac**

maternal-to-fetal perfusion

![Diagram of pharmacokinetic model for diclofenac transfer across the placenta](image)

**Results**

**Drug Transfer from Maternal to Fetal Side (Protocols 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 (Fig. 2, A and B). The TPT\textsubscript{sa} 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 (Fig. 2, A and 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 (Fig. 2C). Fetal-to-maternal transfer of diclofenac was much lower than that of antipyrine (Fig. 2D). The TPT\textsubscript{sa,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 (Fig. 2, E and 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 (Fig. 3A). The values obtained for the pharmacokinetic parameters $K_1$, $k_2$, and $V_p$ were 0.0685 ml/min/g cotyledon, 0.0657 min$^{-1}$, and 0.284 ml/g, respectively (Table 2).

The concentration profiles for diclofenac in the maternal and fetal effluents in protocols I, Ia, Ib, and II were adequately explained by a three-compartment transplacental pharmacokinetic model (Fig. 3B). The values of the pharmacokinetic parameters $K_1$, $k_2$, $k_3$, and $K_4$ obtained were 0.276 ml/min/g of cotyledon, 0.406 min$^{-1}$, 0.0337 min$^{-1}$, and 0.0345 ml/min/g of cotyledon, respectively (Table 2).

Correction of Influx Clearances for Protein Binding. The unbound fractions ($f_u$) 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 of cotyledon, so the plasma influx clearances, $K'_{1}$ and $K'_{4}$, were estimated to be 0.0439 and 0.00470 ml/min/g cotyledon, respectively. The placental tissue-to-plasma concentration ratio, $K'_{1}/k_{2}$, for diclofenac was estimated to be 0.108 ml/g of cotyledon (Table 2).

Conversely, the unbound and plasma influx clearances ($K'/K_{2}$ and $K'/K_{1}$) of antipyrine were estimated to be 0.0735 and 0.0650 ml/min/g cotyledon, respectively. The placental tissue-to-plasma concentration ratio, $K'_{1}/k_{2}$, for antipyrine was estimated to be 0.989 ml/g of cotyledon (Table 2).

Discussion

The present model analysis provided the transplacental transfer kinetic parameters of diclofenac (Fig. 3B; Table 2). Although the 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 a 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 protocols I, Ia, and Ib and that of diclofenac in protocol II. Although the cause of this discrepancy remains uncertain, the smaller number of placental samples may have undervalued 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 a 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 newborns may possibly be predicted by combining the present model with a pharmacodynamic model explaining the above adverse reactions.

In the present model analysis, the value of the parameter, $k_{c}$, 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 CYP3A4) 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 et al., 2002). However, there are no reports directly showing the metabolism of diclofenac in the placenta, and we did not find any evidence, such as metabolites in the effluent. Although the model required the rate constant, $k_{c}$, 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.

Maternal and fetal transport properties are 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
The fetal side. They have also shown that the uptake into maternal plasma/membrane has been reported that MCT4 is expressed on the maternal side of the placenta (Emoto et al., 2002). To clarify the transplacental transport mechanisms 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/maternal concentration ratio, $K_{p}^{s}$/ $k_{s}$, for diclofenac was estimated to be 15.4 ml/g of cotyledon and was approximately 10-fold larger than that for salicylic acid (1.89 ml/g cotyledon) (Shintaku et al., 2007). In addition, the unbound placental tissue/fetal concentration ratio, $K_{p}^{t}$$^{s}$/ $k_{s}$, for diclofenac was estimated to be 23.3 ml/g of cotyledon and was approximately 45-fold larger than that for salicylic acid (0.512 ml/g cotyledon) (Shintaku et al., 2007). Therefore, these results 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/maternal plasma concentration ratio, $K_{p}^{s}$/ $k_{s}$, for diclofenac was estimated to be 0.108 ml/g of cotyledon and was approximately one-tenth that of salicylic acid (1.07 ml/g cotyledon). The placental tissue/fetal plasma concentration ratio, $K_{p}^{t}$/ $k_{s}$, for diclofenac was also estimated to be as low as 0.139 ml/g of cotyledon and was approximately half that for salicylic acid (0.234 ml/g cotyledon). In other words, the placental tissue/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.

**References**


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