In Vivo Maternal-Fetal-Amniotic Fluid Pharmacokinetics of Zidovudine in the Pigtailed Macaque: Comparison of Steady-State and Single-Dose Regimens

TOVE TUNTLAND, ALEKSANDRS ODINECS, CONNIE NOSBISCH and JASHVANT D. UNADKAT

Department of Pharmaceutics, School of Pharmacy, and the Regional Primate Center, University of Washington, Seattle, Washington

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ABSTRACT

The purpose of this study was to characterize the disposition of zidovudine in the maternal-fetal-amniotic fluid unit in vivo. Zidovudine was administered as a constant-rate infusion or a bolus dose to the dam, fetus and amniotic cavity (bolus dose only) of the near-term pigtailed macaque model. A fetal-maternal plasma steady-state concentration ratio of 0.76 ± 0.06 suggested that the drug was transferred extensively to the fetal compartment. Similarly, the mean fetal-maternal plasma area under the curve (AUC) ratio after administration of an i.v. bolus dose to the dam was 0.84 ± 0.09. Both ratios were significantly less than unity (P < .05), which indicates that fetal exposure to zidovudine was lower than maternal exposure. The placental transfer of zidovudine was passive, with a clearance of ~2.0 ml/min/kg, about 35% of the rate of the placental blood flow marker antipyrine. Zidovudine concentration in the amniotic fluid was higher than that in the fetal plasma because the drug is eliminated slowly from the amniotic cavity. The steady-state and i.v. bolus experimental designs resulted in close estimates of the extent of placental transfer of zidovudine (steady-state fetal-maternal plasma concentration ratio or fetal-maternal plasma AUC ratio), which indicates that the extent of transfer of zidovudine is independent of the mode of drug administration. We predict that when zidovudine is administered orally to pregnant women, the average fetal exposure to zidovudine will be approximately three fourths of the maternal exposure. This observation suggests that the dose administered to the pregnant woman need not be changed even if the fetus is the primary target of therapy.

Studies have shown that the rate of HIV transmission from mother to infant is reduced significantly when zidovudine is administered to HIV-infected women during pregnancy and delivery and to the offspring after birth (Connor et al., 1994; Connor and Mofenson, 1995; Simpson et al., 1997). Several studies of mother-to-infant transmission of HIV have demonstrated a correlation between the maternal virus load at delivery and risk of HIV transmission to the child (Fang et al., 1995; Mayaux et al., 1997; Coll et al., 1997; Thea et al., 1997; Dickover et al. 1996). These results suggest that zidovudine treatment reduces the rate of maternal-fetal HIV transmission by limiting the placental transfer of HIV particles and thus the fetal exposure to infectious material. However, other investigators have found that the reduction in vertical transmission after zidovudine treatment is explained only partly by the reduction in plasma levels of viral RNA (Sperling et al., 1996), and may result from the prevention of HIV reverse transcription in the fetus (Melvin et al., 1997). In the latter case, the efficacy of the drug in preventing maternal-fetal HIV transmission depends on whether therapeutic plasma concentrations can be reached in the fetus. If this hypothesis is correct, preventing virus transmission from mother to offspring will depend on the exposure of the fetus and the infant to zidovudine in utero and after birth, respectively.

Despite the current widespread use of zidovudine therapy in pregnancy, information on the disposition of zidovudine in the in vivo maternal-fetal-amniotic fluid unit is limited. Zidovudine studies in pregnant women indicate that the mean fetal:maternal plasma concentration ratio at the time of delivery is 1.1 to 1.3, which suggests that the drug readily crosses the placenta in vivo (Watts et al., 1991; O'Sullivan et al., 1993). However, because these data were obtained under non-steady-state conditions, the observed fetal-maternal plasma concentration ratio cannot be used to discern the exact extent, mechanism or rate of placental transfer.

ABBREVIATIONS: AZT, zidovudine (3'-azido-3'-dideoxythymidine); HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; HPLC, high-performance liquid chromatography; CV, coefficient of variation; CL, clearance; Kᵣ, infusion rate; C₀, steady-state concentration; V, volume of distribution; t½, terminal elimination half-life; AUC, area under the curve.
For several years, our laboratory has been engaged in a series of studies to test the hypothesis that anti-HIV dideoxynucleosides are transported actively across the placenta in vivo. Because these studies are difficult, if not impossible, to conduct in a definitive manner in the human population, we have used the chronically catheterized maternal-fetal pigtailed macaque (*Macaca nemestrina*) as a representative animal model to conduct the studies. To test the hypothesis above, we report the results of our investigation of the maternal-fetal-amniotic fluid pharmacokinetics of zidovudine. In contrast to our experimental design in studies with didanosine (*Pereira et al.*, 1994), zalcitabine (*Yuntland et al.*, 1996), and stavudine (*Odinees et al.*, 1996), in the current study we administered zidovudine to the amniotic fluid. Such a design should allow quantification of the magnitude of the contribution of direct transfer of drug from the amniotic fluid into the maternal circulation to the overall loss of the drug from the amniotic fluid-fetal unit. Although loss of drug from the amniotic fluid to the maternal circulation *via* the fetus is a well-documented phenomenon, the loss of drug directly from the amniotic fluid to the maternal circulation generally is not recognized as a pathway of drug distribution in the maternal-fetal unit.

We conducted this study in the near-term pregnant pigtailed macaque because this species, similar to humans, has a hemochorial placenta in which fetal trophoblast cells are in direct contact with the circulating maternal blood (*King, 1986*). Studies in our laboratory have demonstrated that the systemic disposition of zidovudine in the pigtailed macaque is similar to that in humans (*Lopez-Anaya et al.*, 1990b). In addition, the macaque is an excellent model because both HIV-2 and SIV are transmitted from the mother to the fetus (*Ho et al.*, 1996; *Ochs et al.*, 1991) in this species. Therefore, future studies may be conducted in this species to determine whether dideoxynucleoside therapy can reduce the maternal-fetal viral transmission.

**Materials and Methods**

**Surgical Preparation of the Animals**

Four pregnant macaques (*M. nemestrina*; mean body weight, 7.0 ± 1.0 kg; weight range, 5.7–8.1 kg; mean age, 9.1 ± 3.2 years; age range, 6.5–13.6 years) with a history of viable pregnancy outcomes were acclimated to a tether jacket beginning at about 105 days of gestation. The protocol was approved by the Institutional Animal Care and Use Committee at the University of Washington. The animals were housed individually in stainless steel cages at the Washington Regional Primate Research Center. There they received food, water, care and environmental enrichment in accordance with the standards of the American Association of Laboratory Animal Care and the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council.

At about 122 days of gestation (range, 117–124 days) the macaques were chronically catheterized as follows. With the animals under isoflurane anesthesia, PV-6 polyvinyl catheters were inserted 17 cm into the femoral artery and vein. The arterial line was used for blood pressure and acid/base balance monitoring during surgery, and a temporary I.V. line was used for administration of lactated Ringer's solution. A paramedian laparotomy was performed followed by a transverse supracervical incision in the uterus. Amniotic fluid was collected *via* a 20-gauge needle syringe to decompress the uterine pressure. The amniotic fluid was saved so it could be returned to the dam at the conclusion of the surgery. The fetal head was exteriorized and an incision was made in the fetal neck to enable insertion of catheters. PV-3 and PV-4 catheters were inserted 2 to 3 cm into the fetal carotid artery and internal jugular vein, respectively. The catheters were anchored to the fetal skin closure, and the fetus was then returned to the uterus. Two amniotic “bird-cage” catheters were placed in the amniotic cavity and the uterus was closed tightly in three layers (amnion-chorion, myometrium and serosa). Each catheter was anchored to the uterus adjacent to the uterine incision, and the abdomen was closed in layers. All catheters were tunneled subcutaneously, exteriorized between the dam's shoulder blades, pulled through a tether and placed in a swivel that enabled access to the catheters from outside the cage (*Morton et al.*, 1987). The swivel arrangement allowed the animal to move freely within the cage while receiving the drug infusions and during sampling. Each animal was housed individually and given access to food and water *ad libitum*.

Patency of the maternal and fetal catheters was maintained by continuous perfusion with 0.9% heparinized (3 U/ml) sterile saline solution *via* a Minipuls 3 peristaltic pump (Gilson Medical Electronic Inc., Middleton, WI). Flow rates were 6.7 ml/h for the maternal catheters and 0.3 ml/h for the fetal catheters. In the 3 to 7 days immediately following catheterization, the animals were given analgesics (ketamine hydrochloride, 60–100 mg daily i.v.; ketoprofen, 1.0 mg daily i.m.), antibiotics (cefazolin, 1.0 g daily i.v.) and tocolytics (terbutaline, 2.0 mg daily i.v.; indomethacin, 50 mg daily orally) to prevent postsurgical complications. Antibiotics and tocolytics were not administered during the zidovudine transplacental studies, but were occasionally administered during the 5 to 7 days that separated experiments. The well-being of the mother and the fetus was determined by daily observation of dam, and weekly (under ketamine sedation; 10 mg/kg i.m.) weight measurement, blood and chemistry panels on the dam and weekly fetal position, palpation and fetal heart rate measurement of the fetus. At these weekly sedations, tether jackets were changed and cages were cleaned. The placental transfer studies began at 129 ± 5 days of gestation (range, 124–135 days) and ended approximately a month later at 153 ± 10 days of gestation (range, 140–164 days). Cesarean section was performed at 165 ± 8 days of gestation (range, 153–172 days). All catheters were removed from the dams and infants shortly after delivery.

**Drug Administration and Sample Collection Protocol**

Zidovudine was a gift from Burroughs Wellcome Corp. (Research Triangle Park, NC) and antipyrine was purchased from Sigma Chemical Corp. (St. Louis, MO). Drug solutions were freshly prepared in sterile normal saline (pH 7.4) and resterilized by filtration.

**Bolus dose studies.** For the sequential bolus studies, zidovudine and antipyrine were delivered as a bolus dose to the dam *via* the femoral artery, and to the fetus *via* the carotid artery and into the amniotic cavity on separate occasions. The bolus studies were held a week apart to minimize the possibility of carry-over effects. The study order generally was determined by the patency of the fetal and maternal catheters on the first study day. Drugs were administered to the fetus in the first week and to the dam in the next week in two animals, whereas the order was reversed in two animals. Administration to the amniotic fluid occurred in the third week in the three animals to which such administration was possible. Zidovudine was administered at doses of 10 mg/kg to the dam, 2.5 mg/kg to the fetus and 20 mg/kg to the amniotic cavity. Antipyrine was coadministered at doses of 20 mg/kg, 20 mg/kg and 40 mg/kg, respectively. When the dose was given to dam and fetus, blood samples were drawn from the dam (1.0–2.0 ml) *via* the femoral artery and from the fetus (0.1–0.6 ml) *via* the jugular vein at 0, 5, 10, 15, 30, 60, 90, 120 and 180 min after dosing. Amniotic fluid samples (0.2–2.0 ml) were drawn at these times and also at 4, 6, 8, 24 and 30 h after administration of the dose. When the dose was delivered to the amniotic fluid, blood samples were collected from the dam and the fetus at regular intervals for 8 h, and amniotic fluid was sampled for 30 h after dosing. All samples were centrifuged immediately and frozen at −20°C until analysis.
Constant infusion studies. After completion of the i.v. bolus studies, infusion studies were begun in three of the four macaques. The fourth macaque could not be used in the infusion study because a fetal catheter had become permanently occluded. Zidovudine and antipyrine were administered as a constant infusion to the dam via the femoral vein (zidovudine, 66 \( \mu \text{g/min/kg} \); antipyrine, 41.7 \( \mu \text{g/min/kg} \)) or to the fetus via the carotid artery (zidovudine, 33 \( \mu \text{g/min/kg} \); antipyrine, 41.7 \( \mu \text{g/min/kg} \)). Assuming that zidovudine clearances in the pregnant macaque would be similar to those previously observed in our laboratory (Lopez-Anaya et al., 1990a, b), we selected zidovudine infusion rates that would produce therapeutic plasma concentrations (1–2 \( \mu \text{g/ml} \)) in both the dam and the fetus. The infusions continued for 30 h and were separated by about 7 days. The order of infusions was determined by the patency of the fetal and maternal catheters on the first study day. Drugs were infused first to the fetus and then to the dam in two cases, whereas the order was reversed in one case. Drugs were administered via a WalkMed 420 Infusion Pump (Medfusion Inc., Broomfield, CO). Maternal blood samples (2.0 ml) were collected via the femoral artery at 0.25, 0.5, 1, 3, 6, 24, 25.5, 27, 28.5 and 30 h after the start of the infusion. Fetal blood samples (0.3–0.5 ml) were collected via the jugular vein before and at 0.25, 0.5, 1, 3, 6, 27, 28.5 and 30 h after the start of the infusion. Amniotic fluid samples (2.0 ml) were collected at the same times as the maternal blood samples. All samples were centrifuged immediately and frozen at \(-20^\circ\text{C}\) until analysis. After centrifugation of the fetal blood samples, the red blood cell fraction was resuspended in sterile saline and returned to the fetus.

**Analytical Procedures**

The concentrations of drugs in the biological samples were analyzed by a HPLC method developed in our laboratory (Lopez-Anaya et al., 1990b) and modified so that zidovudine and antipyrine could be analyzed simultaneously. Aliquots (100 \( \mu \text{l} \)) of plasma and 100 \( \mu \text{l} \) of internal standard solution (3-acetamidophenol, 5.0 \( \mu \text{g/ml} \) in water) were loaded onto solid-phase extraction columns (Bond Elut C18, 3 ml, Varian, Harbour City, CA) which were preconditioned with 2 ml of methanol and 2 ml of water. After two washes with 2 ml water, the analytes were eluted with 2 ml methanol. The methanol fraction was evaporated to dryness in a vacuum evaporator (model RC1010, Jouan, Winchester, VA) and reconstituted in 100 \( \mu \text{l} \) of 6% acetonitrile solution in 0.05 M ammonium phosphate buffer (pH 4.5). Then, 50 \( \mu \text{l} \) of this mixture was injected into the reverse-phase HPLC system. The HPLC system included two programmable pumps (LC-6A, Shimadzu Corp., Kyoto, Japan), a C18 column (Ultrasphere ODS 5 \( \mu \text{m} \), 4.6 mm \( \times \) 25 cm, Beckman Instruments Inc., Fullerton, CA), an ultraviolet absorbance detector (SPD-6A, Shimadzu Corp., Kyoto, Japan) with the wavelength set at 280 nm and an autoinjector (Waters 712 WISP, Waters Assoc., Millford, MA). The analytes were eluted with a 25-min linear gradient at a flow rate of 1.0 ml/min with the following mobile phases: acetonitrile, 0.05 M ammonium phosphate buffer (pH 4.5), 6:94 (mobile phase A) and 25:75 (mobile phase B). At 0 min, mobile phase A was 100%; from 0 to 10 min, mobile phase B increased linearly to 100%; from 10 to 17 min, mobile phase B was 100%; from 17 to 20 min, mobile phase A increased linearly to 100%; from 20 to 25 min, mobile phase A was 100%. The samples were integrated by the Maxima 820 Chromatographic Workstation (Waters, The Millipore Inc., Millford, MA).

The elution times for 3-acetamidophenol, zidovudine and antipyrine were approximately 9, 13 and 15 min, respectively. The calibration curve for zidovudine was linear over the concentration range 0.4 to 4.0 \( \mu \text{g/ml} \) (\( r = 0.999 \)). Intraday coefficients of variation of the zidovudine control samples at 0.5 \( \mu \text{g/ml} \) and 2.0 \( \mu \text{g/ml} \) were 5.0% and 4.5%, respectively (\( n = 6 \)). The assay was accurate to within 0.48 \( \pm \) 6.6% and \(-4.2 \pm 8.7% \), respectively, as determined with the use of the control samples.

**Pharmacokinetic Analysis**

**Constant infusion studies.** The transplacental and irreversible clearances of zidovudine and antipyrine were estimated by nonparametric analysis of their steady-state maternal and fetal plasma concentrations (Szeto et al., 1982). Equations 1 through 4 were used on the basis of first-order kinetics and the assumption that the net rate of drug transfer between the fetus and amniotic fluid at steady-state is zero. This assumption is reasonable because steady-state conditions in the fetus and the amniotic fluid will exist only under these conditions provided there is no loss of drug from the amniotic fluid compartment by other routes.

\[
\text{CL}_{\text{eff}} = \frac{K_{\text{ssd}}C_{\text{ssd}}^\infty}{C_{\text{ssd}}^\infty - C_{\text{ssd}}} (1)
\]

\[
\text{CL}_{\text{eff}} = \frac{K_{\text{ssd}}}{C_{\text{ssd}}^\infty - C_{\text{ssd}}} (2)
\]

\[
\text{CL}_{\text{eff}} = \frac{K_{\text{ssd}}C_{\text{ssd}}^\infty}{C_{\text{ssd}}^\infty - C_{\text{ssd}} - \text{CL}_{\text{del}}} (3)
\]

\[
\text{CL}_{\text{eff}} = \frac{K_{\text{ssd}}^\infty}{C_{\text{ssd}}^\infty - C_{\text{ssd}}} - \text{CL}_{\text{del}} (4)
\]

where \( K_{\text{ssd}} \) is the influx rate; \( \text{CL}_{\text{eff}} \) and \( \text{CL}_{\text{del}} \) are the transplacental drug clearances from the dam to the fetus and from the fetus to the dam, respectively; \( \text{CL}_{\text{eff}} \) and \( \text{CL}_{\text{del}} \) are the irreversible drug clearances from the dam and the fetus, respectively; \( C_{\text{ssd}}^\infty \) and \( C_{\text{ssd}} \) are the steady-state plasma drug concentrations in the dam and fetus, respectively. Variables without asterisks indicate infusion of drug to the dam; variables with asterisks indicate infusion of drug to the fetus. The fetal:maternal concentration ratios and clearances were calculated for each animal with data obtained solely from that animal. For each animal, the antipyrine-normalized transplacental clearances were computed as the ratio of the respective transplacental clearance of AZT and antipyrine.

To confirm the estimates obtained by the nonparametric approach, we fit a three-compartment model to the zidovudine and antipyrine concentrations in maternal and fetal plasma and amniotic fluid by nonlinear least squares regression (PCnonlin 4.0, Scientific Consulting Inc., Apex, NC). The three-compartment model was chosen because it is physiologically realistic and results in the best fit to the data when compared with several simpler configurations. The differential equations in the model were the same as described previously (equations 5–10 of Tuntland et al., 1996), except that the clearance of drug from the amniotic fluid to the maternal compartment (CL_{\text{amn}}) was determined independent of the clearance in the opposite direction (CL_{\text{fm}}). Weights equal to the reciprocal predicted concentration gave the best fit of the model to the data.

**Bolus studies.** The transplacental and irreversible clearances of zidovudine and antipyrine were estimated by nonparametric analysis of their area under the concentration-vs.-time profiles (AUC) in maternal and fetal plasma. The AUC values were estimated by the trapezoidal rule with the use of the Lagrang method (Rocchi and Jusko, 1983), and then were analyzed with equations 1 to 4, where the steady-state concentration values were replaced with the corresponding AUC values and the infusion rates were replaced by the bolus doses. Furthermore, the terminal half-life of elimination (\( t_{1/2} \)) and the volume of distribution at steady-state (\( V_{ss} \)) in the maternal compartment, fetal compartment and the amniotic fluid were determined as described by Gibaldi and Perrier (1982).

In addition to the analyses above, a five-compartment model (fig. 1) was fit simultaneously to the maternal plasma, fetal plasma and amniotic fluid drug concentration-vs.-time data obtained from each
animal by nonlinear least squares regression. We assumed that zidovudine follows first-order pharmacokinetics and that the bidirectional clearances between central and peripheral compartments were the same (i.e., passive diffusion) in both dam and fetus. The regression analysis was accomplished with the use of WinNonlin 1.0 (Scientific Consulting Inc.) combined with Microsoft FORTRAN 5.1 (Microsoft Corp., Redmond, WA). The five-compartment model was chosen over several simpler configurations on the basis of the outcome of the F-ratio test and residual plot analysis. Weights equal to the reciprocal of variance concentration gave the best fit of the model to the data. The compartmental approach involved the simultaneous fit of the following five differential equations per route of drug administration (i.e., a total of 15 equations) to the concentration-time data from each animal:

\[
\frac{dC_d}{dt} = \frac{CL_{a_d}C_a}{V_a} + \frac{CL_{df}C_d}{V_{df}} - \frac{CL_{ad}C_f}{V_{ad}} + \frac{(CL_{a_d} + CL_{df} + CL_{fd} + CL_{dp} + CL_{dp})C_d}{V_d} \tag{5}
\]

\[
\frac{dC_f}{dt} = \frac{CL_{f_d}C_d}{V_{fd}} + \frac{CL_{a_f}C_a}{V_{fa}} + \frac{CL_{fd}C_f}{V_{fd}} - \frac{(CL_{fa} + CL_{fd} + CL_{fd} + CL_{fp} + CL_{fp})C_f}{V_f} \tag{6}
\]

\[
\frac{dC_a}{dt} = \frac{CL_{a_p}C_p}{V_p} + \frac{CL_{dp}C_d}{V_p} + \frac{(CL_{a_d} + CL_{dp} + CL_{da})C_a}{V_{ap}} \tag{7}
\]

\[
\frac{dC_{fp}}{dt} = \frac{CL_{fp}C_f}{V_{fp}} \tag{8}
\]

\[
\frac{dC_{dp}}{dt} = \frac{CL_{dp}C_d}{V_{dp}} \tag{9}
\]

where \(C_d\) and \(C_{dp}\) are the drug concentrations in the central and peripheral maternal compartments, respectively; \(C_f\) and \(C_{fp}\) are the drug concentrations in the central and peripheral fetal compartments; \(C_a\) is the drug concentration in the amniotic fluid compartment; \(V_{a}\) and \(V_{dp}\) are the volumes of distribution of the central and peripheral maternal compartments; \(V_{f}\) and \(V_{dp}\) are the volumes of distribution of the central and peripheral fetal compartments; \(V_{a}\) and \(V_{dp}\) represent the intercompartmental clearances.

**Statistical analysis**

The values of the transplacental clearances were compared with the use of the paired Student’s \(t\) test. The unpaired \(t\) test was used to determine whether the fetal-maternal plasma drug concentration ratios or AUC ratios differed from unity. The values were considered significantly different when \(P < .05\).

**Results**

**Zidovudine.** Both the mean (± S.D.) steady-state fetal:maternal plasma concentration ratio of zidovudine after infusion to the dam (0.76 ± 0.06, \(n = 3\)) and the mean fetal:maternal plasma AUC ratio after administration of an i.v. bolus dose to the dam (0.84 ± 0.09, \(n = 4\)) were significantly different from unity (\(P < .05\), table 1). The two ratios were not significantly different from each other (\(P > .05\)). Irrespective of dosing regimen or analysis approach, the mean maternal-fetal transplacental clearance of zidovudine was not significantly different from the fetal-maternal clearance (\(P > .05\), table 2). Similarly, when CL_{ad} and CL_{dp} were normalized to the corresponding antipyrine clearance, no significant differences were observed (\(P > .05\)). Overall, there was good agreement in the parameter estimates obtained in the infusion study with those obtained in the bolus study. Representative zidovudine concentration-time profiles and their model predictions from the infusion study and the bolus study are shown in figures 2 and 3. The mean terminal half-lives of zidovudine elimination from the maternal (\(t_{1/2le} \sim 42\) min) and fetal plasma (\(t_{1/2le} < 29\) min) after maternal drug administration were 3- to 4-fold lower than that from the amniotic fluid (\(t_{1/2le} \sim 137\) min).

**Antipyrine.** The mean (± S.D.) steady-state fetal:maternal plasma concentration ratio of antipyrine after infusion to the dam (0.83 ± 0.13, \(n = 3\)) was not significantly different from unity (\(P > .05\); table 3). In contrast, the mean fetal:maternal plasma AUC ratio after administration of an i.v. bolus dose to the dam (0.83 ± 0.06, \(n = 4\)) was significantly different from unity (\(P < .05\), table 3). The two ratios were

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Zidovudine steady-state concentrations and ratios in plasma and amniotic fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infusion Study</strong></td>
<td><strong>Bolus Study</strong></td>
</tr>
<tr>
<td>Variable</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td></td>
<td>((n = 3))</td>
</tr>
<tr>
<td>(C_{a_d})</td>
<td>2.02 ± 0.52</td>
</tr>
<tr>
<td>(C_{a_f})</td>
<td>1.54 ± 0.46</td>
</tr>
<tr>
<td>(C_{a_p})</td>
<td>3.96 ± 0.96</td>
</tr>
<tr>
<td>(C_{a_{ap}})</td>
<td>1.03 ± 0.20</td>
</tr>
<tr>
<td>(C_{a_{ap}}^*)</td>
<td>14.88 ± 1.75</td>
</tr>
<tr>
<td>(C_{a_{ap}}^*)</td>
<td>19.08 ± 0.53</td>
</tr>
<tr>
<td>(C_{a_{ap}}^{2.72})</td>
<td>2.72 ± 0.86</td>
</tr>
</tbody>
</table>

**Abbreviations and symbols:** S.D., standard deviation; \(C_{a_d}\), steady-state concentration; AUC, area under the concentration-time curve; \(t_{1/2le}\), terminal half-life; \(C_{a_d}\) bolus dose to the fetus; \(d\), dam; \(f\), fetus; \(a\), amniotic fluid.

*Significantly less than unity (\(P < .05\)).
Irrespective of dosing regimen or analysis approach, the mean maternal-fetal transplacental clearance of antipyrine was not significantly different from each other (P > .05). Irrespective of dosing regimen or analysis approach, the mean maternal-fetal transplacental clearance of antipyrine was not significantly different from the fetal-maternal clearance (P > .05).

**Table 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infusion Study</th>
<th>Bolus Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonparametric</td>
<td>Three-compartment</td>
</tr>
<tr>
<td></td>
<td>model*</td>
<td>model</td>
</tr>
<tr>
<td>CL_{df}</td>
<td>1.79 ± 0.24</td>
<td>1.65 ± 0.16</td>
</tr>
<tr>
<td>CL_{fd}</td>
<td>2.46 ± 0.50</td>
<td>2.36 ± 0.27</td>
</tr>
<tr>
<td>CL_{df} (norm)</td>
<td>0.37 ± 0.06</td>
<td>0.29 ± 0.02</td>
</tr>
<tr>
<td>CL_{fd} (norm)</td>
<td>0.39 ± 0.06^b</td>
<td>0.35 ± 0.05^a</td>
</tr>
<tr>
<td>CL_{da}</td>
<td>32.65 ± 7.34</td>
<td>32.21 ± 6.62</td>
</tr>
<tr>
<td>CL_{in}</td>
<td>0.00 ± 0.02</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>CL_{tn}</td>
<td>0.04 ± 0.05</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>CL_{td}</td>
<td>0.03 ± 0.02</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>CL_{ao}</td>
<td>314.40 ± 17.61</td>
<td>63.59 ± 2.77</td>
</tr>
<tr>
<td>V_{dc}</td>
<td>314.40 ± 17.61</td>
<td>63.59 ± 2.77</td>
</tr>
<tr>
<td>V_{fa}</td>
<td>10.00 ± 5.81</td>
<td>10.00 ± 5.81</td>
</tr>
</tbody>
</table>

Abbreviations and symbols: S.D., standard deviation; * calculated with steady-state concentrations and infusion rates; † calculated with AUCs and bolus doses; CL, clearance, ml/min/kg; V, volume of distribution, ml/kg; d, dam; f, fetus; a, amniotic fluid; o, irreversible clearance; norm, normalized to the corresponding antipyrine clearance; c, central compartment.

^1 Not significantly different from corresponding CL_{df} (P > .05).
^2 Not significantly different from corresponding CL_{df}(norm) (P > .05).
we anticipated that the loss of drug from the maternal and fetal compartments would be larger than that from the amniotic fluid, we administered a relatively high dose of zidovudine to the amniotic fluid to get detectable concentrations in the maternal and fetal plasma. Infusion of drug directly to the amniotic fluid would result in high and long-lasting drug exposure, which might be harmful to the fetus. Thus, we conducted the bolus study to estimate the clearance and the route(s) of loss of zidovudine from the amniotic fluid, and the infusion study to obtain independent estimates of the transplacental clearances at steady state.

In both the infusion and i.v. bolus studies, the mean maternal-fetal transplacental clearance of zidovudine (CLdf) was not significantly different from the corresponding fetal-maternal transplacental clearance (CLfd). Because placental perfusion may vary from day to day and the estimates of the transplacental clearances were obtained by studies conducted several days apart, it is particularly helpful to compare the transplacental clearances after changes in placental blood flow have been taken into account. Hence, our experimental design included antipyrine, a marker of placental blood flow, to account for differences in placental blood flow between different in vivo experiments. The absence of significant differences in the two transplacental clearances, including the antipyrine-normalized clearances, indicated that the placental transfer of zidovudine is not active within the concentration range studied and most likely occurs by passive diffusion. This conclusion is supported by the finding that the transfer of zidovudine in the in vitro perfused human placenta model is passive (Liebes et al., 1990; Schenker et al., 1990; Bawdon et al., 1992).

Transplacental or systemic clearance interactions between zidovudine and antipyrine in the pregnant macaque model were not likely, given that antipyrine diffuses passively across membranes, that protein binding of zidovudine is less than 20% in the macaque (Lopez-Anaya et al., 1990a) and that the two drugs follow different routes of systemic elimination (Danhof et al., 1982; Lopez-Anaya et al., 1990b; Tuntland et al., 1997).

The disposition of zidovudine during infusions resulted in mean fetal:maternal concentration ratios at a steady-state (Cmat/Cfet) of 0.76 ± 0.06, significantly less than unity (P < .05), which indicates that fetal exposure to the drug was

### TABLE 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infusion Study</th>
<th>Bolus Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonparametric model</td>
<td>Three-compartment model</td>
</tr>
<tr>
<td></td>
<td>(n = 3)</td>
<td></td>
</tr>
<tr>
<td>CLad</td>
<td>5.62 ± 0.78</td>
<td></td>
</tr>
<tr>
<td>CLid</td>
<td>13.33 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>CLdo</td>
<td>1.18 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>CLfo</td>
<td>6.00 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Vd</td>
<td>19.98 ± 1.46</td>
<td></td>
</tr>
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</table>

### TABLE 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± S.D.</th>
<th>Variable</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
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<tr>
<td>CL &amp; C</td>
<td>µg/ml</td>
<td>µg/ml</td>
<td></td>
</tr>
<tr>
<td>Cmat</td>
<td>6.63 ± 0.49</td>
<td>AUC</td>
<td>3,114.6 ± 517.5</td>
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<tr>
<td>Cdf</td>
<td>5.51 ± 1.20</td>
<td>C</td>
<td>2,594.7 ± 607.4</td>
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<tr>
<td>Cfo</td>
<td>8.98 ± 0.93</td>
<td>C</td>
<td>9,028.7 ± 1,123.7</td>
</tr>
<tr>
<td>Cfo</td>
<td>6.78 ± 0.74</td>
<td>AUC</td>
<td>2,817.0 ± 553.0</td>
</tr>
<tr>
<td>Cfo</td>
<td>13.20 ± 2.14</td>
<td>AUC</td>
<td>5,493.0 ± 888.0</td>
</tr>
<tr>
<td>Cfo</td>
<td>10.94 ± 1.04</td>
<td>AUC</td>
<td>4,626.1 ± 1,253.9</td>
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<tr>
<td>Cfo</td>
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<td>AUC</td>
<td>2,982.6 ± 512.2</td>
</tr>
<tr>
<td>Cfo</td>
<td>1.66 ± 0.18</td>
<td>AUC</td>
<td>9,053.9 ± 840.5</td>
</tr>
<tr>
<td>Cfo</td>
<td>0.93 ± 0.12</td>
<td>AUC</td>
<td>5,493.0 ± 888.0</td>
</tr>
<tr>
<td>Cfo</td>
<td>2.14 ± 0.13</td>
<td>AUC</td>
<td>1,048.0 ± 182.0</td>
</tr>
<tr>
<td>Cfo</td>
<td>0.49 ± 0.06</td>
<td>AUC</td>
<td>898.0 ± 152.0</td>
</tr>
<tr>
<td>Cfo</td>
<td>17.71 ± 0.74</td>
<td>AUC</td>
<td>898.0 ± 152.0</td>
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<tr>
<td>Cfo</td>
<td>1.20 ± 0.13</td>
<td>AUC</td>
<td>1,253.9 ± 205.0</td>
</tr>
<tr>
<td>Cfo</td>
<td>1.04 ± 0.12</td>
<td>AUC</td>
<td>5,493.0 ± 888.0</td>
</tr>
<tr>
<td>Cfo</td>
<td>1.03‡ ± 0.1</td>
<td>AUC</td>
<td>1,253.9 ± 205.0</td>
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</tbody>
</table>

### Discussion

We used both steady-state and i.v. bolus experimental designs to determine the transplacental clearances of zidovudine. An advantage of the steady-state approach is that it allows determination of clearance parameters with a limited number of blood samples. A disadvantage is the need to have two patent catheters, one for drug administration and one for blood sampling. Earlier studies with dideoxynucleosides in our laboratory demonstrated that the concentration of dideoxynucleoside drugs at steady-state is higher in the amniotic fluid than in the maternal or fetal plasma. To obtain a better estimate of the clearance and the route(s) of irreversible loss of zidovudine from the amniotic fluid, we administered zidovudine directly into the amniotic cavity. Because
reduced relative to maternal exposure. This result is at odds with the nonparametric analysis of the infusion data because the irreversible loss (CL_{aolf}) of both zidovudine and antipyrine estimated by this approach is negligible. The differences between the transplacental clearances were larger in the infusion study (range, 5–37%) than in the i.v. bolus study (range, 0–9%; tables 2 and 4). We hypothesize that this difference in clearances is caused by a combination of the simpler model used for the infusion studies and the experimental design. In the simpler model used, no loss of drug from the amniotic fluid to the maternal compartment was allowed, nor was an irreversible loss of drug from the amniotic fluid, CL_{aolf}. These model misspecifications in the simpler model most likely were offset by the higher value of CL_{aolf}. In addition, the different study design in the i.v. bolus experiment did not allow the model misspecification to influence the parametric estimates of CL_{aolf} because of the truncated sampling schedule and the relatively slow rate of equilibration of the amniotic fluid compartment. The resulting differences in transplacental clearances between the two models highlight the problem of model identifiability. Without administration of the drug into the amniotic cavity, the disposition of the drug from the amniotic cavity in the infusion study could not be elucidated. Even when the loss of the drug from the amniotic cavity to the maternal circulation is relatively small compared with the transplacental clearance, exclusion of such loss can lead to different estimates of transplacental clearances.

The amniotic fluid:fetal plasma concentration ratio at steady state during zidovudine infusion to the dam (C_{amn;of} = 2.72 ± 0.86) was greater than unity. The amniotic fluid: fetal plasma concentration ratio at steady-state during infusion to the fetus (C_{amn;of} = 1.29 ± 0.16) was lower than that during infusion to the dam, which suggests that zidovudine is cleared from the amniotic fluid in part directly via the amniochorion and the uterine wall (i.e., the paraplacental route) or by diffusion across the placental surface. The higher concentration of zidovudine in the amniotic fluid was evident in the ratio of the amniotic fluid and fetal AUCs after the i.v. bolus dose to the dam (AUC_{amn} / AUC_{f} = 1.87 ± 0.63). Similarly, higher concentrations of zidovudine in the amniotic fluid after administration of single doses have been observed in pregnant baboons (Hankins et al., 1990) and in humans at the time of delivery (Sperling et al., 1992).

Unlike the other protocols for transplacental studies of dideoxynucleosides in our laboratory, zidovudine was administered as a bolus dose directly into the amniotic fluid. Not surprisingly, this route of administration resulted in a ratio of the fetal:maternal AUC that was considerably higher than that during infusion to the fetus (AUC_{f} / AUC_{amn} = 3.63 ± 2.95). If the only route of elimination from the amniotic fluid were viva the fetus, the two ratios theoretically should be equal. Thus, zidovudine must be eliminated from the amniotic fluid viva routes other than through the fetus. Furthermore, the elevated amniotic fluid:fetal plasma AUC ratio after the drug was administered to the amniotic fluid (AUC_{amn} / AUC_{f} = 180.08 ± 98.37) suggested that the clearance of zidovudine from the amniotic fluid is small compared with clearance from the fetal compartment. When the drug was administered to the amniotic fluid, the concentration-vs.-time profiles in maternal and fetal plasma were almost parallel to that in the amniotic fluid. The elimination of zidovudine from plasma apparently was rate-limited by the transfer of drug from the amniotic fluid. Consequently, the mean terminal half-lives of zidovudine elimination from the maternal and fetal plasma after maternal drug administration were 3- to 4-fold lower than that from the amniotic fluid.

As indicated earlier, a pharmacokinetic model consisting of five compartments (fig. 1) was fitted simultaneously to the observed zidovudine i.v. bolus data from the three dosing regimens for each animal. This compartmental analysis resulted in estimates of the transplacental and irreversible clearances that were close to those obtained in the nonparametric analysis. The model-predicted zidovudine concentration-vs.-time profiles in maternal and fetal plasma and amniotic fluid closely followed the observed data in most cases (fig. 3), despite the complexity of the model. Because of the limited volume of fetal blood and assay sensitivity, the fetal blood sampling schemes were limited to 3 and 8 h postdose during fetal and amniotic fluid administration, respectively. Deviations between predicted and observed data were apparent in the maternal and fetal plasma at lower concentrations in these dosing regimens. It is possible that the maternal and fetal plasma concentrations were just reaching the terminal elimination phase at the end of sampling; however, the overall closeness of the pharmacokinetic parameters in the i.v. bolus study and the infusion study indicate that the early sampling termination did not significantly affect the parameter estimates. For animals that received zidovudine in the amniotic fluid, the data were best fitted to a model that included irreversible loss from the amniotic fluid, CL_{aolf}. Including this clearance term in the amniotic fluid compartment resulted in improved fit of the model to the maternal and fetal plasma concentration-vs.-time profiles. Extremely high zidovudine concentrations (C_{max} > 1000 μg/ml) were observed in the amniotic fluid when the drug was administered directly into this compartment. At these high concentrations, even a minor route of clearance will contribute significantly to the loss of mass of the drug. It is unlikely that such loss can be explained by the metabolism of the amniotic fluid. We hypothesize that a portion of the drug was lost by partitioning into surrounding tissues and was excreted directly without reaching the maternal circulation.

The coefficients of variation of the other parameter estimates involving the amniotic fluid, i.e., CL_{da}, CL_{af}, CL_{da}, CL_{aolf} and V_{aolf} were considerably less in the bolus study (CV < 35%) than in the infusion study (CV < 80%). Similar trends were observed in the parameter estimates of antipyrine, which implicated, as anticipated, increased confidence of the CL_{da}, CL_{af}, CL_{da}, CL_{aolf} and V_{aolf} estimates when the drug was administered into the amniotic fluid. This improved compartmental analysis enabled us to discern the quantitative importance of various clearance pathways to and from the amniotic fluid with confidence. In addition, the volume of distribution of the amniotic fluid compartment could be estimated with confidence for the first time. The analyses showed that the clearances of zidovudine between the maternal compartment and the amniotic fluid (CL_{da} and CL_{aolf}) were approximately equal to the clearances between the maternal compartment and the amniotic fluid (CL_{da} and CL_{aolf}). Drug transfer directly between the amniotic fluid and the maternal blood may occur viva the paraplacental route or by diffusion across the placental surface. Our findings suggest that
diffusion across the uterine wall or across the placental surface plays a major role in the transfer of zidovudine to and from the amniotic fluid. As expected, the overall clearance of zidovudine from the amniotic fluid (–0.11 ml/min/kg) was very low compared with that from the maternal (–30.34 ml/min/kg) or fetal (–2.26 ml/min/kg) compartments.

The disposition of antipyrine after the bolus dose to the amniotic fluid was similar to that of zidovudine, but consistent with higher lipophilicity of antipyrine, the concentration of antipyrine in both the fetal and the amniotic fluid compartments relative to the maternal compartment were not as high as that of zidovudine. The concentration-vs.-time profiles in maternal plasma, fetal plasma and the amniotic fluid were parallel irrespective of route of administration. No major differences were noted in the mean terminal half-lives of antipyrine elimination in the maternal plasma, fetal plasma and amniotic fluid.

Based on both the i.v. bolus and i.v. infusion studies, antipyrine diffused passively across the placenta in the pigtailed macaques, with a transplacental clearance (~6 ml/min/kg) close to the placent al blood flow in rhesus macaques (6–9 ml/min/kg) (Novy et al., 1975). The antipyrine-normalized transplacental clearance of zidovudine estimated in our *in vitro* model (CLₐ,d,norm), 0.34 ± 0.05) was similar to that estimated in the *in vitro* perfused human placenta model (0.40 ± 0.16) when results from several studies were averaged (Liebes et al., 1990; Schenker et al., 1990; Bawdon et al., 1992). This observation indicates that the antipyrine-normalized rate of zidovudine transfer across the placenta in the *in vitro* human placenta model agrees with the rate observed in the *in vivo* pregnant macaque model. That is, the rate of zidovudine transfer across the placenta is approximately 35% of the transfer rate of the lipophilic placental blood flow marker antipyrine.

The results from this and other studies we have conducted with the anti-HIV dideoxynucleosides zidovudine, didanosine, zalcitabine and stavudine in the chronically catheterized maternal-fetal macaque model (Pereira et al., 1994, Odinecs et al., 1996, Tunland et al., 1996) collectively should provide us with the data necessary to test the hypothesis that the mechanism, rate and extent of placent al transfer of dideoxynucleosides *in vivo* can be predicted from the *in vitro* perfused human placenta model. Indeed, we have observed an excellent agreement between the two models with respect to the mechanism and the rate of antipyrine-normalized placental transfer for these four dideoxynucleosides: zidovudine, didanosine, stavudine and zalcitabine (manuscript in preparation).

In summary, we conclude that the placental transfer of zidovudine in the pregnant pigtailed macaque is passive, with a clearance of ~2.0 ml/min/kg, a rate that is roughly 35% that of the placent al blood flow marker antipyrine. The fetal:maternal plasma concentration ratio of zidovudine at steady-state, 0.76 ± 0.06, suggests that the drug is transferred extensively to the fetal compartment. Similarly, the mean fetal:maternal plasma AUC ratio after administration of an i.v. bolus dose to the dam was 0.84 ± 0.09. Both ratios were significantly less than unity (P < 0.05), which indicates that the fetal exposure to zidovudine was reduced relative to that in the dam. Administration of drug into the amniotic cavity allowed greater confidence in the estimation of the contribution of the drug transfer in and out of the amniotic fluid to the overall disposition of zidovudine in the maternal-fetal unit. Slow elimination of drug from the amniotic cavity, which partly occurs via the paraplacental route and by diffusion across the placental surface, explains the high concentration of zidovudine observed in the amniotic fluid after drug administration to the dam. The excellent agreement between the maternal-fetal plasma concentration ratio and AUC ratio from the infusion and i.v. bolus studies, respectively, indicates, as per theory, that the extent of maternal-fetal transfer of zidovudine is independent of the mode of drug administration. We predict that when zidovudine is administered to pregnant women in an oral dosing regimen, the average fetal exposure to zidovudine will be approximately three fourths of maternal exposure. This observation agrees with data obtained in the clinic and suggests that the dose administered to the pregnant woman need not be changed even if the fetus is the primary target of therapy.

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