Effect of pregnancy, mode of administration and neonatal age on the pharmacokinetics of zalcitabine (2′,3′-dideoxycytidine) in the pigtailed macaque (Macaca nemestrina)

Tove Tuntland, Connie Nosbisch and J ashvant D. Unadkat*

Our objective was to determine the effect of pregnancy, mode of administration and neonatal age on the pharmacokinetics of the anti-HIV drug zalcitabine (2′,3′-dideoxycytidine; ddC) in the pigtailed macaque (Macaca nemestrina). Zalcitabine was administered as an iv bolus dose to pregnant dams (n = 3) at term and at 6 weeks post-partum. No significant differences were found between the pre- and post-partum systemic plasma clearance, steady-state volume of distribution or terminal plasma half-life of zalcitabine, indicating that pregnancy does not affect the pharmacokinetics of the drug in the macaque. The observed maternal plasma, fetal plasma and amniotic fluid concentration–time profiles were compared with profiles that were simulated using pharmacokinetic parameter estimates obtained in an earlier constant iv infusion study in pregnant macaques. The fetal:maternal ratio of the area under the simulated zalcitabine plasma concentration–time profile after an iv bolus dose (0.58) was close to the earlier observed fetal:maternal steady-state plasma concentration ratio after iv infusion of the drug (0.58 ± 0.05). The excellent agreement between observed and simulated fetal:maternal ratio of zalcitabine demonstrates that the steady-state infusion experimental design can be used to estimate the drug exposure to the fetus after a single dose. To determine the influence of age on the pharmacokinetics of zalcitabine, the drug was administered as a single iv bolus dose to four infant macaques serially at the ages of 1–2 weeks, 1 month and 4 months. The systemic plasma clearance of zalcitabine was significantly smaller and the terminal plasma half-life significantly longer at age 1–2 weeks than at 1 and 4 months of age. If replicated in humans, these substantial age-dependent changes in the pharmacokinetics of zalcitabine would warrant smaller and less frequent dosing with zalcitabine in HIV-infected neonates than in older children and adults.

Introduction

Women of childbearing age represent an increasing proportion of the population infected with human immuno-deficiency virus (HIV). The transmission of HIV from mother to fetus is the single most important contributor to the increase of AIDS in children. Zidovudine treatment of women during pregnancy and offspring after birth significantly reduced the rate of transmission of HIV from the mother to the infant. However, zidovudine treatment may not be the best therapeutic strategy for patients intolerant to zidovudine, or for patients experiencing disease progression resulting from emerging zidovudine-resistant strains of HIV. Other dideoxynucleosides, including zalcitabine, may prove effective alternatives to zidovudine in the treatment of HIV-infected pregnant women and their offspring. The most important adverse effects of zalcitabine are dose-related sensorimotor neuropathy, rash and oral ulceration. Studies of the in-vitro embryotoxicity of anti-HIV dideoxynucleosides have demonstrated that zalcitabine is significantly less cytotoxic than zidovudine at equivalent concentrations. When zalcitabine was administered to paediatric HIV-infected patients either alone or in an alternating schedule with zidovudine, it appeared to be well tolerated, with signs of clinical improvement in the majority of the treated children. However, several...
important issues need to be resolved before phase I/II clinical trials of zalcitabine, alone or in combination with other anti-HIV drugs, can be carried out in pregnant women or in neonates. Specific questions include the effect of pregnancy on the pharmacokinetics of the drug, the extent of maternal-fetal transfer of the drug, the optimal study design for determining the extent of placental transfer, and the age-dependent changes in the pharmacokinetics of the drug in neonates. As part of a series of studies, we have determined the influence of pregnancy and neonatal age on the pharmacokinetics of zalcitabine in a representative non-human primate model, the pigtailed macaque (Macaca nemestrina).

Materials and methods

Animals

Pre- and post-partum study. Three near-term pregnant pigtailed macaques (M. nemestrina, body weight 9.0 ± 1.5 kg, age 9.1 ± 3.5 years) were chronically catheterized at 128 ± 8 days of gestation. While keeping the animal under general anaesthesia, polyvinyl catheters were placed in the femoral artery and vein of the dam, in the carotid artery and jugular vein of the fetus, and in the amniotic cavity. The catheters were exteriorized between the shoulder blades after subcutaneous tunnelling, pulled through a tether and placed in a swivel that enabled access to the catheters from outside the cage. Each animal was housed individually and given access to food and water ad libitum.

Infant study. Infant pigtailed macaques, one male and three females, were studied serially at ages 1–2 weeks (8.8 ± 2.9 days), 1 month (29.2 ± 1.1 days) and 4 months (124.8 ± 14.1 days). The mean (±s.d.) body weights at these ages were 0.50 ± 0.08 kg, 0.64 ± 0.09 kg and 1.12 ± 0.16 kg, respectively.

Drug administration and sampling of biological fluids

Pre-partum study. Zalcitabine was a gift from Hoffmann-La Roche, Nutley, NJ, USA; antipyrine was purchased from Sigma Chemical Corporation, St Louis, MO, USA. Drug solutions were freshly prepared in sterile normal saline, adjusted to pH 7.4 and resterilized by filtration. An iv bolus dose of zalcitabine (0.25 mg/kg) and antipyrine (20 mg/kg) was administered to the dam via the femoral vein at 150 ± 12 days of gestation (full term is approximately 170 days). A ntipyrine was included because it is a marker of total body water. Blood samples (1–3 mL) were drawn from the dam via the femoral artery 0, 5, 15, 30, 45, 60, 90 and 180 min after dosing. Fetal blood samples (0.5–1 mL) were collected via the jugular vein 15, 30 and 90 min after dosing. A mniotic fluid samples (2 mL) were collected to coincide with each of the maternal blood samples. Total urine was collected over a period of 24 h. The blood, amniotic fluid and urine samples were immediately centrifuged and frozen at –80°C until analysis.

Post-partum study. Parturition occurred at 158 ± 11 days of gestation, via either caesarean section (n = 2) or vaginal delivery (n = 1). The mean (±s.d.) post-partum body weight was 8.4 ± 1.5 kg. A second iv bolus dose of zalcitabine (0.25 mg/kg) and antipyrine (20 mg/kg) was administered to the dams via the femoral vein 46 ± 4 days post-partum, and blood samples were drawn from the femoral artery of the dam. The blood and urine sampling schemes were identical to those used during the pre-partum study.

Infant studies. Infants were administered a single iv bolus dose of zalcitabine (0.25 mg/kg) at age 1–2 weeks, 1 month and 4 months through a catheter placed in the cephalic vein while under sedation with ketamine (5 mg/kg im). Blood samples (0.2–0.8 mL) were drawn through the femoral vein by venipuncture pre-dose and 5, 10, 15, 30, 60, 90 and 180 min after drug administration. The total volume of blood drawn did not exceed 3.5 mL in any study.

Determination of zalcitabine and antipyrine concentrations

The concentration of zalcitabine in the plasma, amniotic fluid and urine was determined using a radioimmunoassay kit purchased from Sigma. The concentration of antipyrine was determined by an HPLC method described previously.

Data analysis

The pharmacokinetic parameters systemic plasma clearance (Cl), steady-state volume of distribution (Vss), terminal plasma half-life (t1/2) and mean body residence time (MBRT) of zalcitabine and antipyrine were determined by the non-compartmental approach. The area under the plasma concentration–time curve (AUC) and area under the moment curve (AUMC) were estimated by the Lagrang method. Renal clearance (Clr) was calculated as the product of Cl and the fraction of dose excreted unchanged in the urine. The parameter estimates from the various studies were compared using Student’s paired t-test with the significance level set at 0.05.

Data simulations

The goal of these simulations was to predict the concentrations of zalcitabine and antipyrine in maternal plasma, fetal plasma and amniotic fluid after administration of an iv bolus dose from kinetic parameters acquired during an earlier constant infusion steady-state experiment. We simulated the concentration–time profiles of dam 89127, an
animal which had not participated in the constant infusion study. The mean pharmacokinetic parameters obtained for zalcitabine and antipyrine from dams ($n = 4$) and their fetuses ($n = 3$) were used in the simulation. Based on these estimates and a four-compartment model (Figure 1), the concentration-time profiles of zalcitabine in maternal plasma, fetal plasma and amniotic fluid after administration of an iv bolus dose to dam 89127 were simulated using PCNONLIN (Scientific Consulting Inc., Apex, NC, USA).

**Results**

**Pre- and post-partum study**

No statistical differences ($P > 0.05$) were observed in the pre- and post-partum $Cl$, $V_{ss}$, $t_{1/2}$, MBRT and fraction excreted in urine or $Cl_r$ of zalcitabine or antipyrine when normalized to body weight (Table I). Likewise, no significant changes were observed when comparing non-normalized pre- and post-partum pharmacokinetic parameter estimates (data not shown). The simulated zalcitabine concentration-time profiles in maternal plasma, fetal plasma and amniotic fluid agreed closely with those obtained experimentally (Figure 2). The ratios of the areas under the simulated fetal and maternal plasma concentration-time curves ($AUC_f/AUC_m$) for zalcitabine and antipyrine were 0.58 and 0.99, respectively.

**Infant studies**

All pharmacokinetic parameters except $V_{ss}$ changed significantly from the first weeks of life to 1 month of age in the neonatal pigtailed macaques (Table II). In contrast, the parameters did not change significantly from 1 month to 4 months of age ($P > 0.05$). The change in zalcitabine $Cl$ with age was similar to that observed by us in previous studies with zidovudine, didanosine and stavudine (Figure 3).

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**Table I.** Pharmacokinetic parameters (mean $\pm$ s.d.) of zalcitabine and antipyrine at term and 6 weeks post-partum in the pigtailed macaque

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-partum zalcitabine</th>
<th>Pre-partum antipyrine</th>
<th>Post-partum zalcitabine</th>
<th>Post-partum antipyrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Cl$ (mL/min/kg)</td>
<td>14.81 $\pm$ 0.71</td>
<td>6.97 $\pm$ 0.67</td>
<td>16.58 $\pm$ 1.73</td>
<td>6.70 $\pm$ 0.09</td>
</tr>
<tr>
<td>$V_{ss}$ (L/kg)</td>
<td>1.32 $\pm$ 0.24</td>
<td>0.84 $\pm$ 0.07</td>
<td>1.26 $\pm$ 0.43</td>
<td>0.78 $\pm$ 0.03</td>
</tr>
<tr>
<td>$t_{1/2}$ (min)</td>
<td>86.9 $\pm$ 13.6</td>
<td>87.7 $\pm$ 12.5</td>
<td>73.1 $\pm$ 10.9</td>
<td>89.5 $\pm$ 9.0</td>
</tr>
<tr>
<td>MBRT (min)</td>
<td>89.1 $\pm$ 15.3</td>
<td>108.8 $\pm$ 17.1</td>
<td>74.4 $\pm$ 17.8</td>
<td>116.8 $\pm$ 3.36</td>
</tr>
<tr>
<td>$f_e$</td>
<td>0.77 $\pm$ 0.10</td>
<td>0.04 $\pm$ 0.01</td>
<td>0.72 $\pm$ 0.08</td>
<td>0.03 $\pm$ 0.01</td>
</tr>
<tr>
<td>$Cl_r$ (mL/min/kg)</td>
<td>11.32 $\pm$ 0.99</td>
<td>0.30 $\pm$ 0.07</td>
<td>11.99 $\pm$ 1.36</td>
<td>0.18 $\pm$ 0.05</td>
</tr>
</tbody>
</table>

Abbreviations: $Cl$, systemic plasma clearance; $V_{ss}$, steady-state volume of distribution; $t_{1/2}$, terminal plasma half-life; MBRT, mean body residence time; $f_e$, fraction of dose excreted in urine; $Cl_r$, renal clearance.

**Table II.** Pharmacokinetic parameters (mean $\pm$ s.d.) of zalcitabine in the infant pigtailed macaque at ages 1–2 weeks, 1 month and 4 months

<table>
<thead>
<tr>
<th>Age</th>
<th>$Cl$ (mL/min/kg)</th>
<th>$V_{ss}$ (L/kg)</th>
<th>$t_{1/2}$ (min)</th>
<th>MBRT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–2 weeks</td>
<td>13.4 $\pm$ 4.5</td>
<td>0.87 $\pm$ 0.21</td>
<td>46.8 $\pm$ 9.8</td>
<td>67.5 $\pm$ 14.2</td>
</tr>
<tr>
<td>1 month</td>
<td>19.8 $\pm$ 4.6a</td>
<td>0.89 $\pm$ 0.20</td>
<td>31.8 $\pm$ 5.2a</td>
<td>45.9 $\pm$ 7.6a</td>
</tr>
<tr>
<td>4 months</td>
<td>21.9 $\pm$ 7.1a</td>
<td>0.76 $\pm$ 0.15</td>
<td>24.8 $\pm$ 3.3a</td>
<td>35.8 $\pm$ 4.8a</td>
</tr>
</tbody>
</table>

Abbreviations as in Table I.

*Significantly different from the parameter estimate at 1–2 weeks of age ($P < 0.05$).
Discussion

Throughout pregnancy, physiological changes occur that can affect the distribution and elimination of drugs. The growth of the uterus, placenta, fetus and amniotic fluid results in increased total body water and fat tissue, thereby changing the volume of distribution of many drugs. Increased extracellular water in the mother, including a 50% increase in plasma volume, is also an important contributor to increased total body water in pregnancy. Increases in cardiac output and renal function are often observed, both of which may accelerate the elimination of drugs. There are several examples in the literature of drugs whose pharmacokinetics are altered sufficiently in pregnancy to warrant a change in the dosing regimen of the drug.\cite{12,13,14}

We observed no significant differences in any of the pre- and post-partum pharmacokinetic parameter estimates of zalcitabine or antipyrine, regardless of whether the parameters were normalized to body weight (Table I). Hence, the pharmacokinetics of the two compounds are unaffected by pregnancy in M. nemestrina, which suggests that a zalcitabine dosing regimen in HIV-infected women need not be altered during pregnancy. The lack of change in zalcitabine and antipyrine pharmacokinetics in our animal model indicates that the amount of drug in the fetal or amniotic fluid compartments is negligible compared with that in the maternal compartment. Indeed, assuming that the distribution of the compounds is proportional to the weight of the individual compartments, this hypothesis appears to be correct. The average maternal body weight at term (8.4 kg) was significantly greater than the infant body weight at birth (250–350 g) or the volume of the amniotic fluid (50–100 mL). Since antipyrine is a marker of total body water, the lack of change in the $V_{ss}$ of this compound indicates that total body water is not affected by pregnancy.

Interesting differences were observed in the plasma and amniotic fluid concentration–time profiles of zalcitabine

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**Figure 2.** The observed and simulated concentration–time profiles of (a) zalcitabine and (b) antipyrine in the maternal (●) and fetal (■) plasma and amniotic fluid (○) after administration of an iv bolus dose to dam 89127. The solid lines represent the simulated profiles.

**Figure 3.** Age-related changes in systemic plasma clearance, expressed as percentage of the 4 month value, of several dideoxynucleosides (●, zalcitabine; ○, zidovudine; □, stavudine; ▲, didanosine) in the neonatal pigtailed macaque.
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and antipyrine. The zalcitabine concentrations in the fetal plasma 15 and 30 min after dosing were lower than those in the maternal plasma, while the situation was reversed at 90 min (Figure 2). This pattern of distribution in the maternal–fetal unit is typical of an intermediate polar drug (zalcitabine: octanol–water partition coefficient, \( P_{ow} \approx 0.06 \)) whose maternal–fetal transplacental clearance is slow relative to the irreversible loss of the drug from the mother.\(^{11}\) Likewise, the amniotic fluid compartment demonstrated an even slower pseudo-equilibration with the maternal and fetal plasma compartments with concentrations of drugs exceeding that in the maternal and fetal plasma at the longer time points. Estimates of the terminal half-life from the amniotic fluid (approximately 6 h) indicate that the elimination from this compartment is three- to four-fold slower than that from the maternal or fetal compartments. Such accumulation in the amniotic fluid has been observed previously with a variety of drugs, including the dideoxynucleosides didanosine, zidovudine and stavudine.\(^{15–17}\)

In contrast to zalcitabine, the antipyrine concentration–time profiles in maternal plasma, fetal plasma and amniotic fluid demonstrated a parallel decline (Figure 2). Consistent with the somewhat lipophilic nature of the agent (\( P_{ow} \approx 1.9 \)), antipyrine distributed rapidly in the fetal compartment and reached pseudo-equilibrium within 30 min after dosing. This pattern of distribution in the maternal–fetal unit is typical of non-polar drugs whose maternal–fetal transplacental clearance is of the same magnitude as the irreversible loss of drug from the dam.\(^{11}\) The lipophilic nature of antipyrine also allows rapid diffusion of drug from the amniotic fluid to the maternal and fetal compartments, thus preventing accumulation of the drug in the amniotic fluid.

When an anti-HIV drug is tested in pregnant women to reduce the incidence of maternal–fetal HIV transmission, a question of paramount importance is the extent to which the drug crosses the placenta. The only experimental clinical paradigm available to answer this question is to obtain a cord (i.e. fetal) and a maternal blood sample simultaneously at the time of delivery. From these samples, the fetal:maternal plasma concentration ratio of the drug can be determined. Unfortunately, such fetal:maternal plasma concentration ratios are usually obtained under non-steady-state conditions. Depending upon the time elapsed between the last administered dose and parturition, blood samples are obtained at varying times from different individuals. Under these experimental conditions, the maternal and fetal blood drug concentrations are constantly changing in a non-parallel fashion. When the terminal plasma half-life of the drug is short, as is the case with anti-HIV dideoxynucleosides, the fetal:maternal plasma concentration ratio can vary more than ten-fold depending on the time of sampling relative to dosing.\(^{18}\)

The optimal way of determining the fetal:maternal plasma concentration ratio accurately is to obtain maternal and fetal blood samples at steady-state after a constant drug infusion. However, the relevance of the steady-state plasma concentration ratio compared with that obtained after single or multiple oral dose administration needs to be addressed. Provided the kinetics of the drug are linear, the fetal:maternal plasma concentration ratio at steady-state \((\text{C}_{\text{ssf}}/\text{C}_{\text{sm}})\) must be the same as the \(\text{AUC}_{\text{f}}/\text{AUC}_{\text{m}}\) ratio after a single dose or multiple dose administration. That is, \(\text{C}_{\text{ssf}}/\text{C}_{\text{sm}}\) should be predictive of the kinetics of the drug after both single and multiple oral dose administration irrespective of the route or mode of administration. To demonstrate this principle, we have determined whether the pharmacokinetic parameters of zalcitabine obtained after constant rate iv infusion to the dam and her fetus\(^{8}\) are predictive of the pharmacokinetics of the drug in the maternal–fetal unit after a single iv bolus dose to the dam.

Simulation of the zalcitabine and antipyrine concentration–time profiles in the maternal plasma, fetal plasma and amniotic fluid resulted in predictions that closely agreed with the observed data, even though mean parameter estimates from other animals were used to simulate the concentration–time profiles in dam 89127 (Figure 2). The closeness between observed and predicted data confirms the pharmacokinetic principle that provided the pharmacokinetics of drugs are linear, the disposition of a drug in the maternal–fetal–amniotic fluid unit is independent of the mode of administration. This principle is further demonstrated by the excellent agreement of the predicted \(\text{AUC}_{\text{f}}/\text{AUC}_{\text{m}}\) ratio of zalcitabine (0.58) and the observed \(\text{C}_{\text{ssf}}/\text{C}_{\text{sm}}\) (0.58 ± 0.06).

In contrast to the 7–10 year incubation period observed in adults, HIV infection in children is characterized by a very brief latency period. By 1 year of age, AIDS is present in 26% of all children infected by vertical transmission.\(^{19}\) Early treatment may be required to prevent or alter the course of the disease in neonates.\(^{20}\) Age-related changes in pharmacokinetics of a drugs are known to be especially large during the neonatal period.\(^{21}\) These age-related changes often require progressive adjustment of dosing regimens from the neonatal population to that of older children and adults. As demonstrated for zidovudine, knowledge of the pharmacokinetic profile of the drug during the first weeks and months of life is necessary to devise appropriate dosing regimens for the treatment of neonates.\(^{22}\)

Renal function in humans is immature at birth, but approaches the value in adults by 5–12 months of age.\(^{23}\) Age-dependent factors of renal function include renal blood flow, glomerular filtration rate and tubular secretion.\(^{24}\) In adult human HIV patients, a majority of zalcitabine (75%) is excreted unchanged in urine. The renal clearance is more than double the glomerular filtration rate, suggesting active renal secretion of zalcitabine.\(^{25}\) As in humans, a large fraction of a zalcitabine dose (72 ± 8%) is excreted unchanged in the urine in the adult pigtailed macaque. Lack of maturity of the renal function
in the newborn macaque is the most likely explanation for the low zalcitabine CI observed in the young animals (Table II). A t 1 month of age, CI is increased while $f_{ss}$ and MBRT are decreased significantly relative to those in the first weeks of life (P < 0.05). In contrast, no significant differences were observed between the parameter estimates at 1 month and 4 months of age (P > 0.05). Collectively, the results indicate that the pharmacokinetics of zalcitabine change significantly from the time of birth to 1 month of age. This finding is consistent with earlier results obtained for zidovudine, didanosine and stavudine when studied in infant M. nemestrina. As depicted in Figure 3, the profiles of the age-related increase in CI were similar for the dideoxynucleosides studied.

We conclude that the pharmacokinetics of zalcitabine is unaffected by pregnancy in M. nemestrina. Based on the findings in this non-human primate model, we predict that the pharmacokinetics of zalcitabine in women will be unaffected by pregnancy and so it should not be necessary to change the dosing regimen of this drug when treating pregnant HIV-infected women. In addition, we suggest that the optimal clinical experimental design for determining the extent of placental transfer of a drug is to measure the drug's $C_{ssf}/C_{ssm}$ ratio at the time of delivery. This ratio is determined by administering a drug as a constant iv infusion to women through labour and up to the time of sampling of maternal and cord blood. Our study has also established that zalcitabine CI is age-dependent in the newborn M. nemestrina due to immaturity of the renal function at birth. If replicated in humans, the substantial age-dependent changes in the pharmacokinetics of zalcitabine suggest that treatment of human HIV-infected neonates will require smaller and less frequent dosing of zalcitabine than commonly used in older children and adults.

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References


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