Fetal Toxicity of Zidovudine (Azidothymidine) in Macaca nemestrina: Preliminary Observations

James C. Ha, Connie Nosbisch, Suzanne H. Conrad, Gerald C. Ruppenthal, Gene P. Sackett, Janis Abkowitz, and Jashvant D. Unadkat

The Regional Primate Research Center, Department of Pharmaceutics, School of Pharmacy, Child Development and Mental Retardation Center, and Division of Hematology, School of Medicine, University of Washington, Seattle, Washington, U.S.A.

Summary: The objective of this study was to determine the dam, fetal, and infant toxicities of zidovudine (AZT) administered to pigtailed macaques during pregnancy. Pregnant macaques were administered AZT (1.5 mg/kg dose every 4 h or water via gastric catheter throughout pregnancy. AZT concentration and hematological changes were monitored in the dam, and fetal growth was monitored via ultrasound. Infant hematocrit was assessed at birth, and the neurological, perceptual, and motor development of the offspring were assessed for 9 to 10 months. Twelve pregnancies were brought to term. Mean plasma concentrations of AZT were comparable to those in human studies. Hemoglobin dropped significantly in pregnant dams and remained low, whereas platelets increased during treatment but returned to normal before the end of the study. There were no significant differences in any ultrasound measure of fetal growth, and AZT-exposed infants exhibited little behavioral delay or impairment. We predict no significant toxic effects of prenatal AZT exposure at this dosage in humans. Key Words: AZT (azidothymidine)—Fetal growth—Infant development—Macaca nemestrina—Pregnancy—Toxicity—ZDV (zidovudine).

Maternal-fetal transmission of the human immunodeficiency virus (HIV) is a leading cause of the increased incidence of AIDS in children (1), and an urgent need exists to develop drugs to prevent or reduce the rate of transmission. This therapeutic goal, however, is complicated by a variable transmission rate of 10–43% (2). Therefore, toxicity to the potentially uninfected fetus due to exposure to anti-HIV drugs must be balanced against the desire to treat the infected mother and potentially infected fetus.

Several lines of investigation support the hypothesis that in utero anti-HIV therapy may be of clinical benefit to the infected fetus (3,4). Although zidovudine (azidothymidine; AZT) appears toxic to mouse fetuses (5), standard teratology studies in both rats and rabbits have found AZT to be remarkably nontoxic to the fetus at doses comparable to those used in the clinic (6–8). In addition, in a pediatric HIV-infected population, a notable neurological improvement was documented when AZT was administered (9). Because of these findings, a nationwide clinical trial sponsored by the National Institutes of Health (ACTG 076) has been initiated in which AZT is administered to pregnant women beginning in their second or third trimester. Before AZT therapy can be extended to the first trimester
of pregnancy, however, the toxicity of AZT to the fetus must be determined in a representative animal model. For this reason, we investigated the fetal toxicity of AZT in the healthy pigtailed macaque (Macaca nemestrina). Specifically, we measured the development of the fetus and the development of motor and neurological milestones of infants exposed to AZT, via the dam, over the entire gestational period. Because data from this study would be essential to planning a human AZT clinical trial during the first trimester, we report here an interim analysis of our study.

The pigtailed macaque is an ideal experimental animal for this purpose. Pharmacokinetic data indicate that, as in humans, AZT crosses the placenta but does not accumulate in the fetus (10,11) and exhibits a similar metabolic profile (12). Second, the placental structure of the macaque is similar to that in humans. Third, the rate of maternal-fetal transmission of the simian immunodeficiency virus (SIV) in pigtailed macaques is similar to that of HIV in humans (13). Thus the macaque could be used to test the efficacy of therapeutic agents on SIV maternal-fetal transmission. An advantage of this experimental approach is that it provides data that are difficult to obtain from human studies. AZT was administered during the entire gestational period to animals that were neither infected nor receiving drugs of abuse. The study environment was well controlled, and concurrent non-AZT-exposed animals underwent testing identical to that of the test animals. The developmental tests used in this study are based on those used to assess the development of human infants; therefore, our results will have direct applications in the clinic.

METHODS AND MATERIALS

Ten multiparous Macaca nemestrina were surgically implanted with a gastric catheter and fitted with a jacket and tether system (14) before AZT administration and mating. AZT at 1.5 mg/kg dose every 4 h adjusted monthly for body weight was administered via the gastric catheter for at least 30 days before conception occurred, and treatment continued throughout pregnancy. Control females were administered water placebo. All females were mated with the same sire and examined by ultrasound to determine conception. If a female failed to conceive, AZT or placebo was continued, and the animal was mated during her next menstrual cycle.

Plasma AZT concentrations were monitored regularly by high-performance liquid chromatography (HPLC) (15) or by radiomunnoassay (Sigma). Hematology measures hemoglobin, mean corpuscular volume, platelets, and white blood cell counts were obtained biweekly for the first month of treatment and then monthly until delivery. Infant hematocrits were determined within 24 h of birth.

To assess fetal viability and growth, ultrasound exams were conducted at frequent intervals between 30 and 160 days of gestation. After delivery of the infant, all standard housing, feeding, and behavioral test challenges used in our Infant Primate Research Laboratory protocol were employed (16). Infants were separated from the dams after birth and weighed daily for the first 4 months and weekly for the remaining 7 months. All infants were provided with similar visual, auditory, olfactory, and tactile stimulation in their home cages, as well as with a daily, 30-min suckling period with specifics.

All data were compared between the test and control groups. Hematological and growth data were compared using t-tests and analysis of variance (ANOVA); they are reported as means ± SD. Behavioral test data were compared using nonparametric Mann-Whitney U tests and are reported as medians. A statistical significance level of 0.05 was used throughout the analysis.

RESULTS AND DISCUSSION

Some females underwent both a control and an AZT-treated pregnancy. Therefore, 12 pregnancies were brought to term (six AZT, six control); however, one of the control fetuses died during delivery. Two additional fetuses were lost during the first trimester (one from each treatment group; cause unknown). Four of 11 infants were delivered by cesarean section. It took significantly more matings to achieve the six AZT pregnancies than the six control pregnancies (17 vs. 9; binomial test, p = 0.007). Studies in both mice and rats have reported similar findings (5,7). We speculate that this observation may be related to the inhibitory effect of AZT on the production of progesterone by placental trophoblasts (18).

Although the mean peak AZT plasma concentration (334.4 ± 151.1 ng/ml) was lower than that obtained in humans after a 100-mg oral dose (500 mg/day), the area under the plasma concentration-time curve (550.6 ± 216.3 ng·h/ml) was comparable (19,20).

Baseline hematological values for the two groups (AZT and control) were not significantly different (Table 1). Hemoglobin dropped significantly in the AZT-treated animals after treatment began and remained low until the end of the study. Platelet counts increased significantly in AZT-treated animals during the treatment period but returned to control levels before the end of the study. Mean corpuscular volume and white blood cell counts were not significantly different from those of controls throughout the treatment period. Hemoglobin and other blood cell indices in AZT-treated animals returned to baseline when AZT was discontinued. The hematological toxicities reported here are consistent with those seen in 500 mg/day AZT-treated
TABLE 1. Mean values for hematological measures at baseline, at each subject's extreme value during therapy, and immediately before the end of treatment

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Extreme</th>
<th>End of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>AZT</td>
<td>Control</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>11.5</td>
<td>11.2</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>(0.9)</td>
<td>(0.8)</td>
<td>(0.4)</td>
</tr>
<tr>
<td>Mean corpuscular volume (fl)</td>
<td>64.8</td>
<td>64.4</td>
<td>66.6</td>
</tr>
<tr>
<td></td>
<td>(0.8)</td>
<td>(0.2)</td>
<td>(1.4)</td>
</tr>
<tr>
<td>Platelets (10^3 /mm^3)</td>
<td>469.5</td>
<td>442.2</td>
<td>564.7</td>
</tr>
<tr>
<td></td>
<td>(78.1)</td>
<td>(157.7)</td>
<td>(120.9)</td>
</tr>
<tr>
<td>White blood cells (10^3 /mm^3)</td>
<td>9.0</td>
<td>7.0</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>(2.5)</td>
<td>(1.6)</td>
<td>(2.6)</td>
</tr>
</tbody>
</table>

No. of subjects 6 6 6 6 6 6

*a* Significant differences between control and AZT subjects at the p < 0.05 level.

*b* Standard deviations in parentheses.

humans (21). The mechanism for the elevation in platelet count in AZT-treated animals is unknown.

Infant hematocrits taken at time of birth were lower in the AZT-exposed group but not statistically different [AZT, 0.51 ± 0.10 (n = 5); control, 0.57 ± 0.08 (n = 5)]. This is similar to human studies that report no serious hematologic toxicity or other adverse outcomes of pregnancy attributed to AZT exposure (22–24). Most of these patients, however, did not receive AZT throughout pregnancy.

No significant differences were found between control and AZT subjects in any ultrasound measurement of fetal growth (head area, biparietal distance, or femur length). The ratios of head circumference to abdominal circumference (a measure of intrauterine growth retardation) were not significantly different between treatment groups. Birth weights were identical in the two treatment groups. Postnatal weight increase was significantly lower in AZT-exposed infants (Fig. 1; p < 0.001; AZT, n = 3; control, n = 4). In our experience, this difference is probably not biologically significant.

Of the behavioral tests conducted, the results of three are reported here.

1. The recognition memory test measures the response of infants to novel versus familiar stimuli. Normal infants (human and macaque) exhibit significant preference for novel stimuli; infants at high risk for developmental problems do not (25). No significant differences were observed between AZT-exposed and control infants in recognition memory scores.

2. The development of object permanence ability is a series of milestones in central nervous system development of the infant. We found no significant differences in age of achieving any object concept milestone between infants exposed to AZT and controls.

3. The Wisconsin General Testing Apparatus series challenges the infant with an increasingly difficult series of learning tasks. Some statistical differences were seen in learning ability in this series of tests. AZT-exposed infants took three times as many sessions as controls to meet criterion on Black–White Learning, a simple discrimination task [AZT, 6.0 sessions (n = 3); control, 2.0 sessions (n = 3; p = 0.046)]. There were no differences, however, in the number of sessions needed to meet criterion on Black–White Reversal, a test of ability

---

**FIG. 1.** Postnatal weight data for AZT (open triangles) (n = 3) and control (pluses) (n = 4) subjects. Lines represent quadratic regressions for each group. There is a significant difference in weights between treatment groups (p < 0.001, analysis of variance). There is no significant difference in birthweight.
to reverse the conditioning that was learned during Black-White Learning. No significant differences in abilities were demonstrated on the last day of more complex learning tasks involving search for a hidden food reward among four possible locations. Hamilton Search (HSI) and Hamilton Search Set-Breaking (HSSB). However, AZT-exposed infants exhibited significantly faster latencies to locate the reward in both tasks [HS AZT, 4.1 s (3); control, 7.2 s (3), p < 0.05; HSSB AZT, 2.9 s (3); control, 5.9 s (3), p < 0.05].

In conclusion, AZT therapy of pregnant pigtailed macaques caused a decrease in hemoglobin, similar to that seen in AZT-treated humans. Exposure of the fetus to AZT resulted in an increase in the number of primates required to achieve pregnancy, but no increased risk of premature births or intrauterine growth retardation. AZT-exposed infants were slower to gain weight but showed little behavioral delay or impairment. Collectively, the data obtained to date suggest that, at the dose administered, AZT exposure poses no significant toxicities during gestation in *M. nemestrina*. Based on these findings, we predict that there would be no significant toxic effects of prenatal AZT exposure (100 mg dose; 500 mg/d) in humans.

Acknowledgment: This research was supported by grants from the National Institutes of Health (HD 25391, RR00160). AZT was supplied by the Burroughs-Wellcome Company. We thank Lisa Bexfield and Colin Walker-Gellat for assistance in conducting this study.

REFERENCES


