REPORTS

Transplacental Effects of 3′-Azido-2′,3′-Dideoxythymidine (AZT): Tumorigenicity in Mice and Genotoxicity in Mice and Monkeys

*Ofelia A. Olivero, Lucy M. Anderson, Bhalchandra A. Diwan, Diana C. Haines, Steven W. Harbaugh, Thomas J. Moskal, Ann B. Jones, Jerry M. Rice, Charles W. Riggs, Daniel Logsdon, Stuart H. Yuspa, Miriam C. Poirier**

Background: **When given during pregnancy, the drug 3′-azido-2′,3′-dideoxythymidine (AZT) substantially reduces maternal–fetal transmission of human immunodeficiency virus type 1 (HIV-1). However, AZT has been shown to be carcinogenic in adult mice after lifetime oral administration. In this study, we assessed the transplacental tumorigenic and genotoxic effects of AZT in the offspring of CD-1 mice and *Erythrocebus patas* monkeys given AZT orally during pregnancy. *Methods:* Pregnant mice were given daily doses of either 12.5 or 25.0 mg AZT on days 12 through 18 of gestation (last 37% of gestation period). Pregnant monkeys were given a daily dose of 10.0 mg AZT 5 days a week for the last 9.5–10 weeks of gestation (final 41%–43% of gestation period). AZT incorporation into nuclear and mitochondrial DNA and the length of chromosomal end (telomere) DNA were examined in multiple tissues of newborn mice and fetal monkeys. Additional mice were followed from birth and received no further treatment until subjected to necropsy and complete pathologic examination at 1 year of age. An anti-AZT radioimmunoassay was used to monitor AZT incorporation into DNA. *Results:* At 1 year of age, the offspring of AZT-treated mice exhibited statistically significant, dose-dependent increases in tumor incidence and tumor multiplicity in the lungs, liver, and female reproductive organs. AZT incorporation into nuclear and mitochondrial DNA was detected in multiple organs of transplacentally exposed mice and monkeys. Shorter chromosomal telomeres were detected in liver and brain tissues from most AZT-exposed newborn mice but not in tissues from fetal monkeys. *Conclusions:* AZT is genotoxic in fetal mice and monkeys and is a moderately strong transplacental carcinogen in mice examined at 1 year of age. Careful long-term follow-up of AZT-exposed children would seem to be appropriate.** **[J Natl Cancer Inst 1997;89:1602–8]**

Exposure of pregnant females of numerous mammalian species, including nonhuman primates, to various chemical carcinogens results in neoplasms in their offspring *(1).* The human relevance of transplacental carcinogenesis was established with the discovery that diethylstilbestrol (DES) caused vaginal adenocarcinomas in the children of women treated during pregnancy *(2).* Subsequent experimental studies in mice duplicated this effect *(3).* Epidemiologic evidence has implicated transplacental exposures to radiation, certain medications, pesticides, occupational chemicals, and metals *(4–6)* as possible contributors to human cancer risk. Mechanistic studies with rodents indicate that fetuses may be particularly at risk of tumor initiation by chemicals, with high rates of cell division and other fetal characteristics greatly enhancing vulnerability *(7).*

The nucleoside analogue 3′-azido-2′,3′-dideoxythymidine (AZT), widely used to treat human immunodeficiency virus type 1 (HIV-1) infection, has become the standard of care in preventing fetal transmission of the virus in HIV-1-positive pregnant women *(8,9).* Recommended treatment encompasses five daily 100.0-mg doses (approximately 8.3 mg/kg body weight per day) during the second and third trimesters of pregnancy, with additional maternal intravenous dosing at delivery and oral AZT given to the infant after birth *(9,10).* In a recent study from the Pediatric AIDS (i.e., acquired immunodeficiency syndrome) Clinical Trials Group *(9),* this regimen reduced viral transmission to the fetus from 22.6% (n = 204) to 7.6% (n = 198).

In adult mice, AZT is carcinogenic. Ayers et al. *(11)* reported 10% and the National Toxicology Program *(12)* reported 22% incidences of vaginal squamous papillomas and carcinomas after lifetime oral administration of the drug to CD-1 and $B6C3F_1$ mice, respectively. At similar doses in mice exposed to AZT for 28 days, dose-related incorporation of AZT into vaginal DNA and increased vaginal epithelial proliferation were observed *(13).* Because of the projected widespread use of AZT in human pregnancy, we have investigated the genotoxic and carcinogenic effects of AZT in the

**Affiliations of authors:* O. A. Olivero, S. H. Yuspa, M. C. Poirier, Laboratory of Cellular Carcinogenesis and Tumor Promotion, Division of Basic Sciences, National Cancer Institute, Bethesda, MD; L. M. Anderson, A. B. Jones, Laboratory of Comparative Carcinogenesis, Division of Basic Sciences, National Cancer Institute, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD; B. A. Diwan (Intramural Research Supported Program), D. C. Haines (Pathology/Histotechnology Laboratory), D. Logsdon (Laboratory Animal Sciences Program), Science Applications International Corp.–National Cancer Institute-Frederick Cancer Research and Development Center; S. W. Harbaugh, T. J. Moskal, BioQual, Inc., Rockville, MD; J. M. Rice, International Agency for Research on Cancer, Lyon, France; C. W. Riggs, Data Management Services, Inc., National Cancer Institute-Frederick Cancer Research and Development Center.

Correspondence to: Ofelia A. Olivero, Ph.D., National Institutes of Health, Bldg. 37, Rm. 3B15, MSC-4255, Bethesda, MD 20892–4255. E-mail: olivero@pop.nci.nih.gov

See ''Notes'' following ''References.''

offspring of exposed pregnant CD-1 mice and AZT genotoxicity in the fetuses of exposed pregnant *Erythrocebus patas* monkeys.

Materials and Methods

Source and Purity of AZT

AZT (lot No. 063H7819) was obtained from Sigma Chemical Co., St. Louis, MO. The purity of AZT was assessed by means of elemental analysis, UV spectrum analysis, nuclear magnetic resonance spectrometry, and mass spectrometry, as well as by means of high-performance liquid chromatography with two different mobile phases (data not shown). All of the parameters were consistent with literature values, confirming the AZT structure and assessing the purity of this lot at greater than 99.8%. The determinations were performed by Dr. Haleem J. Issaq of the Chemical Synthesis and Analysis Laboratory, Science Applications International Corp. (SAIC), National Cancer Institute (NCI)-Frederick Cancer Research and Development Center (FCRDC), Frederick, MD.

Tumorigenicity in the Offspring of Pregnant Mice Receiving AZT

Animal care for this experiment was provided in accordance with the procedures outlined in the *Guide for the Care and Use of Laboratory Animals* [National Institutes of Health publication No. (NIH)86–23, 1985] under an animal study proposal approved by the FCRDC Animal Care and Use Committee. Female CD-1 Swiss mice (Charles River, Raleigh, NC) were given food and water *ad libitum.* The mice were mated, and day 1 of pregnancy was ascertained by the presence of a vaginal plug. Forty-five pregnant mice were given 0 (17 litters), 12.5 (13 litters), or 25.0 (15 litters) mg AZT in 0.5 mL sterile distilled water via an intragastric tube once daily on days 12 through 18 of gestation. Preliminary toxicity studies indicated that the maximum fetal-tolerated daily dose was 25.0 mg (approximately 420 mg/kg term body weight), with doses of 30.0–50.0 mg causing fetal or newborn loss. The pups were delivered normally. The average number of pups weaned per litter was 11.7, 12.1, and 10.1 for the control, low-dose, and high-dose groups, respectively. Ten mice per sex from each group of pups were killed on schedule at 13, 26, and 52 weeks after delivery. No tumors were evident at the first two time points. However, at 52 weeks, lung and liver tumors were observed in the AZT-exposed mice, so additional animals (selected at random) were killed to obtain numbers adequate for statistical analysis. In addition, moribund mice from all groups were killed, and the findings from these mice were included with the findings from the mice killed at 52 weeks. Altogether, the numbers of litters represented were five (61 mice) for the control group (i.e., no AZT), six (45 mice) for the 12.5-mg-AZT group, and nine (50 mice) for the 25.0-mg-AZT group. All remaining mice will be killed at 2 years of age.

When moribund or at planned sacrifice, mice underwent a complete necropsy. Lungs, liver, ovary/testis, uterus/cervix/vagina, thymus, spleen, lymph nodes, kidneys, brain, pituitary, mammary gland, femur, cecum, and all grossly noted lesions and masses were examined by means of light microscopy. The pathology findings were peer-reviewed by Dr. Miriam Anver, SAIC, NCI-FCRDC, and by Dr. Jerrold Ward, Office of Laboratory of Animal Sciences, NCI. All lung, liver, and female reproductive tract lesions were reviewed, as well as all questionable and representative definitive hematopoietic lesions. Spiral organisms, thought to be *Helicobacter hepaticus,* were observed in Steiner's stained slides of most ceca, but only a few organisms were observed in one liver, and no hepatitis was observed in any of the livers. The mice were otherwise specific pathogen free. Tumor data are presented as tumor incidence (percent of animals with tumors) and tumor multiplicity (number of tumors per animal).

Statistical Methods

Dose-dependent linear trends of tumor incidence proportions were evaluated by use of the Cochran–Armitage chi-squared test (two-tailed) *(14),* and high-dose versus control comparisons were made by use of Fisher's exact test (two-tailed) *(15).* Two-tailed chi-squared tests *(16)* were used to analyze the reduction in hematopoietic neoplasms in AZT-exposed males and females in relationship to control animals. Tests of the homogeneity of the proportions of tumor bearers per litter within each of the sex–dose–organ groups were performed by use of the correlated binomial $C(\alpha)$ test statistic as described by Tarone *(17).* In addition, for lungs and liver, comparisons of low-dose treatment with high-dose treatment on the basis of litters involved the analysis of variance of 1) raw proportions of tumor-bearing animals in each litter and 2) Freeman–Tukey arcsine transformations of the proportions *(18),* with each analysis using weights proportional to the litter sizes. Tumor multiplicities were tested for dose-dependent trends by use of the nonparametric Jonckheere test (two-tailed) *(19),* and high-dose versus control comparisons were made by use of the nonparametric Wilcoxon rank-sum test (two-tailed) *(20).*

Incorporation of AZT Into Nuclear and Mitochondrial DNA of Fetal CD-1 Mice Exposed *In Utero* to AZT

Pregnant CD-1 mice were given 0 (n = 3) or 25.0 (n = 3) mg AZT/day by gavage on days 12 through 18 of gestation (final 37% of gestation period), and the pups (14 each for litters 1 and 3 and 11 for litter 2) were born 24 hours or less after the last exposure. For each organ (e.g., kidneys), tissue from all of the pups of one litter was combined. The samples were homogenized, and DNA was isolated by means of the Oncor nonorganic extraction method (Oncor, Inc., Gaithersburg, MD). Mitochondrial DNA was prepared as previously described *(21).*

DNA samples, in distilled water, were sonicated for 30 seconds and then boiled for 5 minutes. Three-microgram aliquots of DNA were subsequently assayed by use of a competitive anti-AZT radioimmunoassay (AZT-RIA) as previously described *(22).* Briefly, a rabbit polyclonal anti-AZT antibody (Sigma Chemical Co.) that also recognizes AZT in DNA was reconstituted in 20 mL 10 m*M* Tris buffer (pH 8.0), representing a 1:5000 dilution of the antibody. A 0.1-mL aliquot of the diluted antibody was incubated in a 12 × 75-mm disposable glass tube with an equal volume of a solution containing either standard AZT plus 3 μg calf thymus carrier DNA (Sigma Chemical Co.) or a 3-μg sample of DNA from AZT-treated or untreated (control) animals for 90 minutes at 37 °C. Approximately 20 000 cpm [^{3}H]AZT tracer (20 Ci/mmol; Moravek Biochemicals, Inc., Mountain View, CA), in a volume of 100 μL, was added per tube together with 100 μL goat anti-rabbit immunoglobulin G secondary antibody (ICN Biomedicals, Inc., Costa Mesa, CA; reconstituted in 12 mL 10 m*M* Tris buffer [pH 8.0]), and the mixture was incubated for 25 minutes at 4 °C. The mixture was centrifuged at 2000*g* for 15 minutes at 4 °C. The resulting supernatant was decanted, and the pellets were dissolved in 100 m*M* NaOH and counted in a liquid scintillation counter.

The AZT-RIA employs a radiolabeled AZT tracer, used in a constant amount in each tube, to compete with the AZT standard, used at different concentrations, or sample DNA for binding to the anti-AZT antibody. In the absence of standard AZT or sample DNA containing AZT, the highest level of radiolabel binding to the antibody will be obtained (cpm with no inhibitor). Because of the competitive nature of this assay, the added presence of nonradiolabeled AZT (i.e., the standard or in the sample DNA) will result in the inhibition of tracer-antibody binding (cpm with inhibitor). The inhibition of tracer-antibody binding observed with a particular amount (''x'') of unlabeled AZT is expressed as a percent according to the following formula:

$$\text{Percent inhibition} = \frac{[(\text{cpm with no inhibitor}) - (\text{cpm with amount ''x'' of inhibitor})]}{(\text{cpm with no inhibitor})} \times 100.$$

The amount of standard AZT added to 3 μg carrier calf thymus DNA that inhibited antibody binding by 50% was 570 ± 420 fmol (mean ± standard deviation [SD]; n = 12) for the mouse assays. The amount of AZT in 3 μg of biologic sample DNA was obtained by comparing DNA from the corresponding tissue of an untreated animal with DNA from a treated animal, calculating the percent inhibition of antibody binding, and reading the amount of AZT from a plotted standard curve. Each sample was assayed in three to five separate radioimmunoassays.

Incorporation of AZT Into Fetal *Erythrocebus patas* Monkey Nuclear and Mitochondrial DNA

Monkeys were maintained and treated under American Association for Accreditation of Laboratory Animal Care-approved conditions at BioQual, Inc. (Rockville, MD), in accordance with humane principles for laboratory animal care. Protocols were reviewed and approved by the Animal Care and Use Committee of BioQual, Inc. Pregnancies were ascertained as described *(23).* Pregnant patas monkeys were given 0 (n = 3) or 10.0 (n = 3) mg of AZT/day (approximately 1.5 mg/kg body weight per day) in a piece of banana, 5 days per week for the last 9.5–10 weeks of gestation (final 41%–43% of gestation period). Dose ingestion was confirmed by an observer. Fetuses were taken by cesarean section, performed on the monkeys under Telazol and isofluorane anesthesia, on days 149 through 152 of gestation (24 hours after the last dose). Tissue processing for DNA preparation and the AZT-RIA were as

described above for newborn mouse tissues, except that the 50% inhibition value for the RIA was 820 ± 290 fmol AZT (mean ± SD; n = 25).

Examination of Telomere Length in Mouse and Monkey Tissues

The length of telomeric (chromosomal end) DNA *(24)* was examined in DNA from the tissues of 10 newborn mouse litters, either unexposed (n = 5) or exposed (n = 5) *in utero* to 25.0 mg AZT/day on days 12 through 18 of gestation. For each litter, tissues from different organs were combined and homogenized with a Dounce homogenizer, and high-molecular-weight DNA was prepared by use of the nonorganic extraction method of Oncor, Inc. The DNA was digested with *Alu* I, *Rsa* I, and *Sau* 3A I restriction endonucleases (New England Biolabs, Inc., Beverly, MA) and resolved in 1% agarose gels along with biotinylated molecular markers (Oncor, Inc.). The DNA was transferred to nylon support membranes overnight by means of capillary action *(25)*, and it was cross-linked to the membranes by use of UV light. The membranes were blocked for 30 minutes at 45 °C with blocking solution (Oncor, Inc.) and hybridized with a biotinylated human telomeric repeat sequence probe (Oncor, Inc.) overnight in a sealed bag at 45 °C. Posthybridization washes were performed in a solution of 0.16× standard saline citrate and 0.1% sodium dodecyl sulfate for 1 hour at 60 °C. The membranes were subsequently blocked with a 5% low-fat milk solution at room temperature for 30 minutes, and an amplified alkaline phosphatase immunoblot assay kit (Biorad Laboratories, Hercules, CA) was used in membrane staining. The sizes of the telomere repeats were determined by comparison with the biotinylated molecular markers. A similar approach was used to examine telomere length in DNA from the tissues of six fetal monkeys exposed to either 0 (n = 3) or 10.0 mg AZT/day for the last 9.5–10 weeks of gestation (n = 3).

Results

Evaluation of AZT as a Transplacental Carcinogen

To test for transplacental tumorigenicity, pregnant CD-1 Swiss dams were exposed once daily to 0, 12.5, or 25.0 mg AZT via intragastric intubation on days 12 through 18 of gestation. In the carcinogenicity study, litter sizes did not differ substantially between AZT-exposed and control mice, and no further exposures were given postnatally. Ten offspring of each sex from each treatment group were necropsied at 3 and 6 months, and no neoplasms were found. However, the interim sacrifice at approximately 1 year revealed the presence of tumors, prompting the sacrifice of additional animals of each sex in each group for further analysis. Complete necropsy and histopathologic analysis of dead, moribund, or sacrificed animals at 190–400 days of age (average ages of 350–382 days) revealed a severalfold, dose-dependent increase in the incidence (Fig. 1) and the multiplicity (Table 1) of tumors in the AZT-exposed groups.

The spontaneous liver and lung adenomas in the control animals are typical for this strain of mice *(26)*. At the same sites, incidences of these types of tumors were increased twofold to eightfold in AZT-treated animals (Fig. 1, A–C). For both sexes combined, the incidence of lung carcinomas increased significantly with AZT dose (3%, 7%, and 14% for the 0-, 12.5-, and 25.0-mg AZT groups, respectively; $P = .037$, two-tailed chi-squared test). Neoplasms of the female organs (ovary, uterus, and vagina) were absent from the control animals but were detected in 14% and 17% of the AZT-exposed female offspring at the low and high doses, respectively (Fig. 1, D).

Neoplasms of the hematopoietic system, including lymphomas, myelogenous leukemia, and histiocytic sarcoma, which occur spontaneously in this mouse strain, were reduced from 33% and 16% in unexposed females and males, respectively, to 8% in the mouse pups of both sexes (Fig. 1, E and F). This reduction suggests that an endogenous oncogenic retrovirus may be inhibited by the AZT treatment and/or that alterations in the immune system may occur in the offspring exposed to AZT *in utero.*

Statistical Analyses for the Tumor Study

Probability values for the tumor incidences presented in Fig. 1 are shown in the legend, and those for the tumor mul-

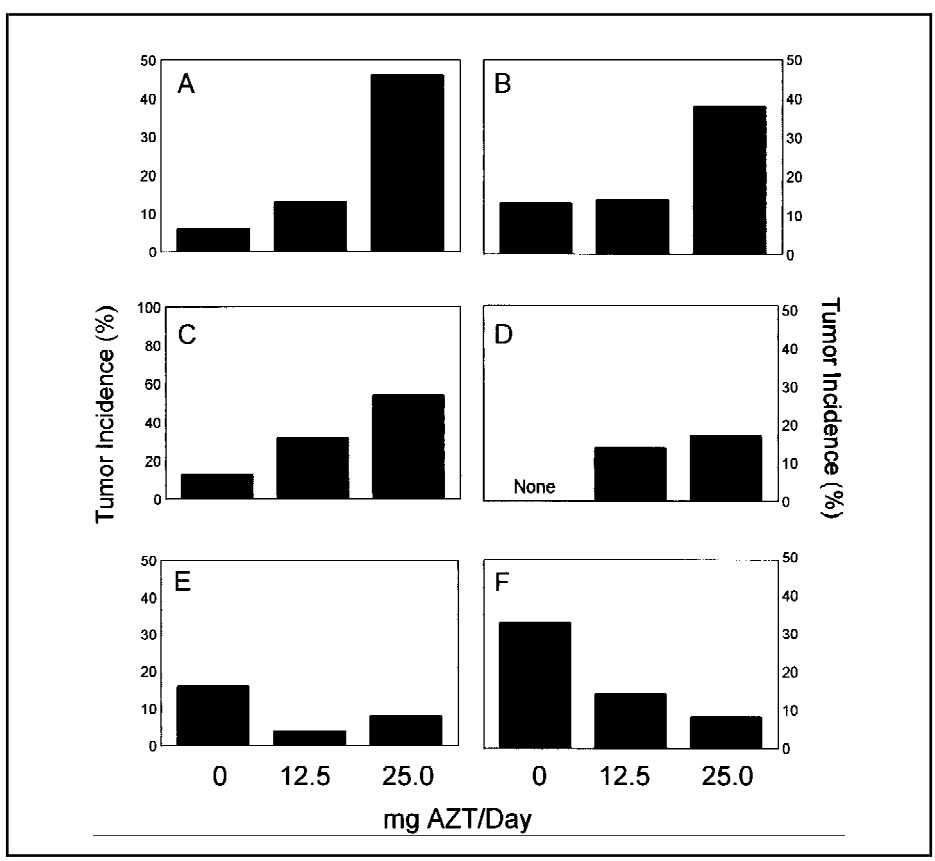

Fig. 1. Tumor incidences in internal organs of offspring of pregnant mice 190–400 days after transplacental 3′-azido-2′,3′-dideoxythymidine (AZT) exposure. Pregnant CD-1 mice were given 0, 12.5, or 25.0 mg AZT in sterile distilled water once daily on days 12 through 18 of gestation. Pups were delivered normally and given no further treatment. When moribund or at sacrifice (190–400 days), the mice underwent complete necropsy (*see* ''Materials and Methods'' section). The figure shows the following tumor incidences: lung tumors (from bronchoalveolar cells) in males (**A**) and in females (**B**); hepatocellular adenomas, including two carcinomas, in males (**C**); female reproductive organ tumors (**D**); and hematopoietic tumors in males (**E**) and females (**F**). Tumor incidences were analyzed for dose-related linear trend by use of the Cochran–Armitage chi-squared test (two-sided). High-dose AZT (25.0 mg AZT) versus control (no AZT) comparisons employed the Fisher's exact test (two-sided). Probability values for these two tests are, respectively, .0004 and .00066 for male lung tumors (**A**); .037 and .056 for female lung tumors (**B**); .0011 and .0014 for male liver tumors (**C**); and .033 and .034 for female reproductive organ tumors (**D**).

tiplicities in Table 1 are shown in a table footnote. The presented statistical relationships are based on pooled groups, since preliminary tests indicated that litter proportions within each test group were generally homogeneous. A possible exception was noted for the male–lung–high-dose group, where near-significant heterogeneity was found ($P = .058$), which was due exclusively to one litter with lung tumors in all five male offspring. Reanalysis of the data excluding this litter resulted in somewhat lower significance probabilities but no overall change in conclusions. When analyzed on a litter basis, comparisons of low- with high-dose AZT for the lungs and the liver produced results that generally reaffirmed the conclusions based on pooled groups.

Transplacental AZT Exposure and Genotoxicity in Newborn Mice and Fetal Monkeys

Using a sensitive anti-AZT radioimmunoassay (AZT-RIA) *(22)* and comparing DNA from exposed and unexposed animals (*see* ''Materials and Methods'' section), we detected AZT incorporation in the nuclear and mitochondrial DNA from multiple pooled organs of newborn CD-1 mouse pups (three litters) after transplacental exposure to 25.0 mg AZT/day on days 12 through 18 of gestation (Table 2, A). Incorporation was widely variable among litters, organs, and DNA compartments, suggesting that undefined pharmacokinetic and specific tissue factors influence persistent AZT incorporation. Litter size appeared to influence measurable incorporation levels, since litters 1 and 3 each consisted of 14 pups and had the lowest incorporation levels, and litter 2, with only 11 pups, had higher incorporation levels.

Telomerase is active in fetal tissues and tumor cells *(27)*, and previous studies have demonstrated that AZT can be preferentially incorporated into the telomeric DNA of cells containing telomerase *(24)*. *In vitro* long-term exposure to 800 μM AZT was shown to produce an irreversible shortening of telomeres *(28)*. In this study, telomere length was examined in five litters of newborn mice exposed to 25.0 mg AZT/day as described in Table 2, A. Fig. 2 shows the results for liver, lungs, and brain from one litter. In comparison with the DNA from control animals, smaller sized telomeric DNA was detected in the livers from five litters, the brains from three of five litters, the lungs from two of five litters, and the kidneys from one of five litters of AZT-treated animals. The variability between litters observed for telomere length is similar to that seen for AZT incorporation into DNA.

The data in mice demonstrated AZT-induced genotoxic and carcinogenic effects at a high dose. To approximate the human exposure of about 8.3 mg/kg body weight per day, we gave three pregnant *Erythrocebus patas* monkeys 10.0 mg AZT/day (approximately 1.5 mg/kg body weight per day) 5 days a week during the last 9.5–10 weeks of a 23-week gestation. Multiple fetal tissues were obtained after cesarean section. AZT incorporation was observed in the nuclear and mitochondrial DNA of transplacentally exposed monkeys but not in the DNA of unexposed control animals (Table 2, B). Even though the monkey dose was much lower than the dose received by the mouse, incorporation levels were generally several-fold higher than those observed in the mouse.

Table 1. Tumor multiplicities at 190–400 days of age in offspring of mice given 0, 12.5, or 25.0 mg 3′-azido-2′,3′-dideoxythymidine (AZT) per day by gavage on days 12 through 18 of gestation*

		Organ of tumorigenesis			
AZT exposure, mg	Offspring evaluated	Lungs, mean No. of tumors ± SE†	Liver, mean No. of tumors ± SE‡	Female organs, No. of tumors	Incidental, No. of tumors
0	31 males	0.10 ± 0.07 (1 CA)	0.23 ± 0.14	na	2 liver hemangiosarcomas
	30 females	0.13 ± 0.06 (1 CA)	0	0	1 skin basal cell tumor
12.5	23 males	0.13 ± 0.07 (1 CA)	0.48 ± 0.18 (1 CA)	na	1 skin papilloma; 1 skin hemangiosarcoma
	22 females	0.14 ± 0.07 (2 CA)	0	2 uterine endometrial stromal polyps; 1 vaginal leiomyosarcoma	None
25.0	26 males	0.50 ± 0.11 (4 CA)	0.79 ± 0.26 (1 CA)	na	None
	24 females	0.38 ± 0.10 (3 CA)	0	1 Sertoli cell tumor, ovary; 1 histiocytic sarcoma, uterus; 1 hemangiosarcoma, uterus; 1 endometrial stromal polyp, uterus	1 skin papilloma; 1 malignant tumor§

*Tumor multiplicity refers to the number of tumors per animal. Results include findings from moribund and dead animals and those killed at 12–13 months. SE = standard error; CA = carcinoma; na = not applicable. *P* values (two-tailed) for trends and comparisons were as follows: Male lung tumors—trend test, $P = .014$; control (0 mg AZT) versus high-dose AZT (25.0 mg), $P = .001$; low-dose AZT (12.5 mg) versus high-dose AZT, $P = .012$. Female lung tumors—trend test, $P = .15$; control versus high-dose AZT, $P = .042$; low-dose AZT versus high-dose AZT, $P = .071$. Male liver tumors—trend test, $P = .013$; control versus high-dose AZT, $P = .0016$; control versus low-dose AZT, $P = .097$. Trend tests were performed with the Jonckheere test, and pairwise treatment comparisons were made by use of the Wilcoxon rank-sum test (both are nonparametric methods).

†Lung alveolar cell adenomas and carcinomas.

‡Liver hepatocellular adenomas.

§Autolyzed neoplasm involving pancreas and spleen.

Table 2, A. Incorporation of 3′-azido-2′,3′-dideoxythymidine (AZT) into nuclear and mitochondrial DNA of fetal CD-1 mice exposed *in utero* to AZT*

	fmol AZT/μg DNA†					
	Litter 1		Litter 2		Litter 3	
Organ	Nuclear	Mt	Nuclear	Mt	Nuclear	Mt
Brain	nd	nd	nd	49.5 ± 3.9‡	nd	nd
Lungs	nd	nd	226.7 ± 128.4	nd	40.3 ± 9.4	51.4 ± 15.0
Liver	nd	nd	121.1 ± 78.9‡	42.9 ± 14.9‡	nd	nd
Kidneys	23.2 ± 2.5	28.4 ± 7.5	41.2 ± 1.2‡	nd	nd	33.3 ± 13.3‡
Skin	nd	160.8 ± 43.6	87.5 ± 23.5‡	302.7 ± 64.0‡	ns	227.0 ± 195.0‡

*Pregnant CD-1 mice were given 0 (n = 3) or 25.0 (n = 3) mg AZT/day, and the pups (14 each for litters 1 and 3 and 11 for litter 2) were born 24 hours or less after the last exposure. For each organ (e.g., kidneys), tissue from all of the pups of one litter was combined, nuclear and mitochondrial DNA was prepared, and 3-μg aliquots of DNA were assayed by use of an anti-AZT radioimmunoassay (*see* ''Materials and Methods'' section). Incorporation is expressed as fmol AZT/μg DNA in relation to results obtained with tissue DNA from unexposed mice. Except where noted, values are means ± standard error for three to five assays.

†Mt = mitochondrial; nd = not detectable; ns = no sample.

‡Mean ± range for samples assayed twice.

Table 2, B. Incorporation of AZT into fetal *Erythrocebus patas* monkey nuclear and mitochondrial DNA*

	fmol AZT/μg DNA†					
	Monkey No. R105		Monkey No. R200		Monkey No. R226	
Organ	Nuclear	Mt	Nuclear	Mt	Nuclear	Mt
Brain	nd	120.0 ± 40.0‡	150.0 ± 70.0‡	nd	nd	242.0 ± 89.4
Cerebellum	227.7 ± 94.7	nd	81.5 ± 11.5‡	ns	252.7 ± 84.0	282.0 ± 54.7
Lungs	739.0 ± 221.1	253.7 ± 135.9	nd	nd	166.7 ± 13.3	203.0 ± 8.8
Liver	566.0 ± 274.0	nd	230.0 ± 70.0‡	55.0 ± 15.0‡	146.0 ± 69.1	nd
Kidneys	703.0 ± 262.6	nd	170.0 ± 30.0‡	55.0 ± 5.0‡	316.6 ± 101.1	nd
Heart	541.6 ± 84.9	155.0 ± 63.7	60.0 ± 10.0‡	45.0 ± 5.0‡	316.0 ± 152.8	324.3 ± 42.4
Placenta	nd	22.0 ± 6.0‡	nd	60.0 ± 0‡	341.7 ± 123.8	nd

*Pregnant patas monkeys were given 0 (n = 3) or 10.0 (n = 3) mg of AZT/day (approximately 1.5 mg/kg body weight per day) in a piece of banana 5 days per week for the last 9.5–10 weeks of gestation. Fetuses were taken by cesarean section on days 149 through 152 of gestation (24 hours after the last dose). Tissue processing and AZT-DNA radioimmunoassay were as described in the ''Materials and Methods'' section, with comparison of DNAs from unexposed and exposed monkeys. Except where noted, values are means ± standard error for three to five assays.

†Mt = mitochondrial; nd = not detectable; ns = no sample.

‡Mean ± range of two assays.

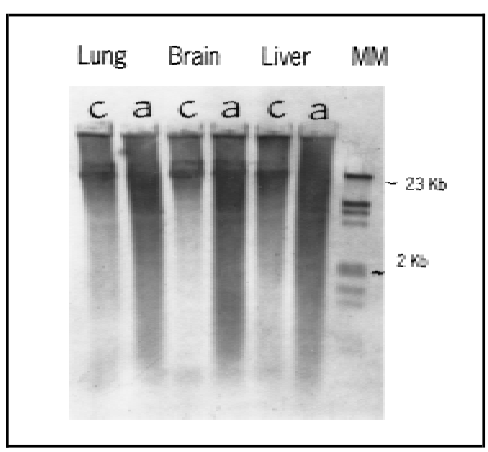

Fig. 2. Telomere length in nuclear DNA from lung, brain, and liver tissue of newborn mice either unexposed (c, control) or exposed (a) to 3′-azido-2′,3′-dideoxythymidine (AZT) *in utero* at a dose of 25.0 mg/day on days 12 through 18 of gestation. DNA was isolated, digested with restriction endonucleases, resolved in a 1% agarose gel, transferred to a nylon support membrane, and hybridized with a biotinylated probe specific for human telomeric DNA repeat sequences. The sizes of the telomeric repeats were determined by comparison with the biotinylated molecular marker (MM) DNAs (note the 2- and 23-kilobase reference regions). *See* ''Materials and Methods'' section for more details.

Again, there was a large interanimal variability in AZT-DNA levels among the monkey fetuses and in the DNA compartments. The size of telomeric DNA was not measurably altered in the DNA from liver, brain, cerebellum, heart, lungs, and placenta obtained from AZT-exposed fetal monkeys (data not shown).

Discussion

At the doses tested here, AZT is unequivocally a transplacental genotoxin and carcinogen in CD-1 mice. The liver and lungs, targets for tumor formation in this study, are typical organ sites for genotoxic transplacental carcinogens in mice *(7)*. The incidence, latency, multiplicity, and histopathology of the AZT-induced tumors indicate that AZT is intermediate in potency as a mouse transplacental carcinogen. On a toxic-equivalent dose basis, this drug is less potent than *N*-nitrosoethylurea *(29)*, 7,12-dimethylbenz[*a*]anthracene *(30)*, and 3-methylcholanthrene *(31)*, but it is more potent than *N*-nitrosodimethylamine *(29)* and the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone *(32)*. When given transplacentally to mice, benzo[*a*]pyrene produced lung and liver tumor multiplicities similar to those observed here *(30)*. The female reproductive organ tumors, absent in untreated animals, are similar in type and incidence to those resulting from mouse transplacental exposure to DES *(3)*.

In these experiments, AZT was given to mice for the last 37% of gestation at a daily dose that was approximately fivefold higher than the equivalent daily dose received by pregnant women [with the use of the mouse–human scaling factor of 1:12 described by Freireich et al. *(33)*]. With no scaling factors applied, an HIV-1-positive pregnant woman receiving AZT during the last two trimesters will have a total dose of about 1.4 g/kg body weight. The mice receiving 25.0 mg AZT/day were given a total dose of about 3.5 g/kg body weight, and the monkeys in this study received about 0.08 g AZT/kg body weight. Nevertheless, fetal *Erythrocebus patas* monkeys, given AZT at a much lower daily dose than that received by the CD-1 mice, had more AZT-DNA incorporation than that detected in the newborn mice.

Human–mouse dose comparisons are further complicated by a number of factors. Phosphorylation of AZT is more efficient in mice than in humans *(34)*. The plasma half-life of AZT in mice is 20 minutes *(35,36)*, whereas it is 1–2 hours in humans *(10)*. Because CD-1 mice typically carry 10–14 pups, the ratio of fetal weight to total maternal weight at deliv-

ery is 0.4 or more in the mouse, compared with 0.1 or less in humans. Together with tissue-specific differences in target organ responses and differences in litter size (11–14 pups for these experiments), the variability in AZT-DNA incorporation and telomere length might be expected. However, despite this variability, litter differences in tumorigenesis were not significant (Fig. 1). Pregnant monkeys, which carry only one fetus, may therefore be a more appropriate model for the human.

Two transplacental studies of AZT in mice, in which much lower doses than our doses of 12.5 and 25.0 mg/day were used, failed to find a carcinogenic effect. Ayers et al. *(37)* gave pregnant CD-1 mice 20.0 and 40.0 mg AZT/kg body weight per day (approximately 0.5 and 1.0 mg AZT/day) from the 10th day of gestation through weaning. Pups were then treated with 0, 20.0, or 40.0 mg AZT/kg body weight per day for 24 months. In animals given lifetime exposure to 40.0 mg/kg body weight per day, the males were unaffected, and vaginal tumors were observed only in the adult females; however, the tumor yield was not increased in animals given no drug exposure past weaning. Bilello et al. *(35)* gave 4.5 mg AZT/day to pregnant mice on days 16 through 21 of gestation and postnatally to nursing dams; these investigators did not detect tumors in the offspring by gross examination of tissues at 18 months. Taken together with our study, the data suggest that cumulative dose effects are critically important in determining the prenatal carcinogenicity of AZT.

Two novel genotoxicity assays were employed in this study. The AZT-RIA, which has previously been validated by comparison with incorporation of radiolabeled AZT into nuclear DNA *(22)*, was applied here for the first time to mitochondrial DNA. Unlike the situation with many chemical carcinogens that preferentially modify mitochondrial DNA by covalent binding, the incorporation of AZT into mitochondrial DNA was highly variable and did not parallel the incorporation of the drug into nuclear DNA. Because there is no literature for the comparison of nuclear and mitochondrial DNA incorporation of AZT, further validation must await confirmation and the development of alternative methods. Although it is unclear whether mitochondrial genotoxicity is related to tumorigenicity, it may be significant that mice developed tumors in organs where nuclear AZT DNA was not always detectable. In addition, the monkeys had higher levels of AZT incorporation into DNA than the mice in spite of receiving a much lower dose. The telomere length assays also provide novel information but no clear context for evaluation of the biologic consequences. The observation that telomeres were shortened in the mice and not in the monkeys would suggest that dose effects are important; however, the fetal mice appeared to function normally, and any possible relationship with the process of tumorigenesis remains obscure. Both of these biomarkers will be examined in great detail in future studies to assess their relationships with the observable biologic consequences of AZT exposure.

The relevance of the mouse studies to human exposure must be considered in the context of dose-equivalency, an especially difficult extrapolation for transplacental exposures. Available literature does not allow an accurate estimation of human risk implied by these data. However, our results suggest that the current practice of treating HIV-1-positive women and their infants with high doses of AZT could increase cancer risk in the drug-exposed children when they reach young adulthood or middle age. The remarkable effectiveness of AZT in preventing fetal HIV infection *(8,9)* indicates that the immediate need for treatment of a potentially fatal disease should outweigh the potential cancer risk. However, given the relatively high tumor incidences observed here at only half of the lifetime of the mouse, it would seem appropriate both to include additional notification in informed consent documents and to plan extensive follow-up of AZT-exposed children. In addition, since human carcinogenesis is multifactorial and takes many years to develop *(38)*, protective modulation may be possible *(39)*.

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Notes

We extend our appreciation to Drs. Jerrold Ward and Miriam Anver for review of the pathology slides and to Dr. Robert Tarone for verifying the statistics. For review of the data and the manuscript, we thank Drs. Richard D. Klausner, George Vande Woude, Alan S. Rabson, Henry C. Pitot, Lorenzo Tomatis, and Paul Kleihues. For editorial assistance, we thank Ms. Margaret Taylor.

Manuscript received March 7, 1997; revised August 20, 1997; accepted August 28, 1997.