Tissue Disposition of Zidovudine and Its Phosphorylated Metabolites in Zidovudine-treated Healthy and Retrovirus Infected Mice

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Purpose. The purpose of this study was to examine the effect of chronic AZT treatment on the *in vivo* tissue disposition of the phosphorylated AZT metabolites in healthy and retrovirus infected mice.

Methods. Female C57BL/6 mice at 12 weeks after inoculation with LP-BM5 murine leukemia virus as well as age-matched control animals were dosed subcutaneously with 25 mg/kg of AZT twice a day for 8 weeks. At the end of the 8-wk treatment (20 weeks post inoculation), each animal received a final AZT dosing solution containing [^{3}H]-AZT with a specific activity of 87 mCi/mmol. The levels of AZT and its phosphorylated metabolites were determined in tissues collected at different times after the last AZT administration using an analytical method coupling an ion-pair HPLC separation procedure with radioactivity detection following the separation.

Results. The tissue-to-plasma (T/P) AZT ratios in control mice could be ranked in the following order: kidneys > muscle ≅ spleen ≅ liver ≅ heart > lung ≅ thymus > lymph nodes >> brain. The distributions of AZT into lymph nodes, lung, and thymus tissues in infected mice increased significantly in comparison with those of control animals. Tissue AZT 5′-monophosphate (AZT-MP) profiles tended to parallel the AZT profiles in most tissues examined. Delays in the appearance of AZT 5′-diphosphate (AZT-DP) and AZT-TP were observed in all tissues tested. The conversion of AZT to AZT metabolites was found to be highest in the spleen and bone marrow samples from both control and infected animals. AZT-TP content was not detectable in any of the brain samples analyzed. The lymph nodes of the control animals showed poor ability to phosphorylate AZT to its active triphosphate moiety. This ability was significantly enhanced in infected animals.

Conclusions. Comparing these findings with those of our previous study performed following a single dose administration of AZT, the chronic AZT regimen had minimal effect on the *in vivo* tissue disposition of the phosphorylated AZT metabolites. The therapeutic implications of inadequate maintenance of the level of active AZT metabolite in the lymph nodes and the brain following chronic AZT treatment need to be further explored.

KEY WORDS: 3′-azido-3′-deoxythymidine; zidovudine; anabolism; *in vivo;* tissue disposition; mice; retrovirus infection.

INTRODUCTION

After having been in use for treating HIV infection for a decade, zidovudine (AZT) is still considered to be an essential component of antiretroviral regimens. Similar to other nucleoside based antiretrovirals and cancer chemotherapeutic agents, zidovudine needs to be converted intracellularly to phosphorylated metabolites by a series of cellular kinases in order to exert cytotoxic or antiviral activities. The cellular distribution and metabolism of AZT have been studied extensively in *in vitro* cell lines or in human peripheral blood mononuclear cells (PBMCs) (1–4). Unlike the endogenous nucleosides, AZT enters the cells primarily by passive diffusion. Once it is in the cells, AZT is first phosphorylated to AZT 5′-monophosphate (AZT-MP) by thymidine kinase. Thymidylate kinase is responsible for the conversion of AZT-MP to AZT 5′-diphosphate (AZT-DP). The enzyme that catalyzes the conversion of AZT-DP to AZT 5′-triphosphate (AZT-TP) is believed to be the nucleoside diphosphate kinase. These phosphorylated metabolites can be converted back to the parent drug. Because of their polarity, these metabolites are generally believed to be restricted to the inside of the cells and are not to be found in the extracellular fluid. Conventional pharmacokinetic studies determining the drug and metabolite levels in circulating plasma will not allow us to monitor and predict concentrations of the active phosphorylated metabolites inside target cells/tissues. This deficiency has resulted in lack of understanding of the *in vivo* disposition of the active phosphorylated AZT metabolites and has contributed in part to failure to optimize the therapeutic use of AZT and other nucleoside based therapeutic agents.

In our previous experiment (5), we found that the tissue disposition of AZT metabolites following a single dose administration of AZT was tissue-specific in mice and that experimentally induced chronic retrovirus infection resulted in changes in the distribution of AZT into the lymph nodes and in the disposition of the phosphorylated AZT metabolites in this important target tissue. Since antiretrovirals are generally taken chronically, in this research we aimed to examine the effect of chronic AZT treatment on the *in vivo* tissue disposition of the phosphorylated AZT metabolites in healthy and retrovirus infected mice. As in the previous experiment, this study was performed in female C57BL/6 mice as well as in the same strain of mice inoculated with LP-BM5 murine leukemia virus (MuLV).

MATERIALS AND METHODS

Chemicals and Reagents

AZT and AZT-TP were purchased from Moravek Biochemicals Inc. (Brea, CA) and were obtained from the AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases (Rockville, MD). AZT-MP was purchased from Moravek Biochemicals Inc. (Brea, CA). [methyl-^{3}H] AZT was obtained from the AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases (Rockville, MD). The radiolabelled AZT was purified with high performance liquid chromatography (HPLC) to a purity greater than 99%. All other reagents were of HPLC grade or of the highest grade commercially available.

Animals

Female C57BL/6 mice at 3–5 weeks of age (14–16 g) were obtained from Simosen Laboratory Inc. (Gilroy, CA). Animals were allowed to acclimate to a standard environment for two weeks before receiving any treatment. After this period,

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animals assigned to the virus infected group were injected intraperitoneally with 0.1 ml of LP-BM5 murine leukemia virus as described previously (6). Control groups consisted of uninfected age-matched animals.

Animal Experimentation

Chronic AZT treatment was initiated at twelve weeks after virus inoculation. Age-matched animals which did not receive virus inoculation were used in the study as controls. The chronic AZT regimens consisted of subcutaneous injection of 25 mg/kg dose of AZT twice a day, once between 8:00 and 10:00 am and again between 6:00 and 8:00 pm, for eight weeks. Following the eight-week AZT treatment, animals were injected with a final AZT morning dose (25 mg/kg) using dosing solution containing nonradioactive AZT and [^{3}H]-AZT with a specific activity of 87 mCi/mmol. At 30, 60 or 90 min after dosing, groups of four control mice were sacrificed and at 30 or 90 min after AZT administration, groups of four LP-BM5 MuLV inoculated mice were sacrificed via brief CO_2 exposure followed by cervical dislocation. Blood was collected from the inferior vena cava and plasma was separated. Liver, kidneys, spleen, heart, lung, thymus, major lymph nodes (axillary, superficial cervical, facial, inguinal, superior mesenteric, and iliac lymph nodes), brain and a piece of thigh muscle were collected and weighed in the order described. Saline flush of the cavities of femur and tibia was obtained for use as bone marrow samples and the cell numbers in these samples were counted with a haemocytometer. The tissues were processed immediately for the analysis of AZT and AZT metabolite levels according to the method described previously (5).

Data Analysis

A two-way ANOVA was used to compare the T/P ratios of AZT of different treatments (i.e., between study groups or among different sampling times after dosing). In this analysis, study group and sampling time were considered the main effects and the potential interaction between these effects was also examined. A p value < 0.05 for the main effects was considered to be statistically significant. However, when the interaction term was found to have a p value of less than 0.1, the following statistical tests were performed to examine differences between treatments. Differences associated with sampling times after dosing of control animals were determined with an one-way ANOVA followed by the Tukey's test. An unpaired two-sided t-test was used to compare data for infected animals collected at the two sampling times and for control and infected animals collected at the same sampling time.

The T/P ratios of AZT and AZT-MP determined at 90 min after dosing were compared using the ANOVA for repeated measurements. In this analysis, the study group was treated as the main effect and the chemical form (parent drug or metabolite) was considered the repeated measurements. The potential interaction between the study group and the chemical form was also examined. A p value < 0.05 for the main effect or for the repeated measurement was considered statistically significant. However, when the interaction term was found to have a p value less than 0.1, an unpaired two-sided t-test was used for comparisons between treatments (i.e., between different study groups or between AZT and AZT-MP).

Finally, an unpaired two-sided t-test was used to compare the ratios of tissue levels of AZT phosphates to AZT determined for control and infected animals at 90 min after dosing.

RESULTS

The time courses of the plasma levels of AZT and AZT metabolites are shown in Figure 1. The plasma AZT concentrations declined from about 36 μM at 30 min following the subcutaneous administration of the final AZT dose at the end of a 8-wk chronic AZT treatment to about 3 μM at 90 min after the final dose. Comparable plasma AZT levels were found in control and infected animals. The two hepatic metabolites, AMT and GAZT, and the monophosphorylated AZT were present in plasma samples at concentrations much lower than those of the parent drug. Minimal differences in AMT, GAZT or AZT-MP levels were observed between control and infected mice.

Table 1 summarizes the T/P ratios of AZT for control and infected animals at different times after the final AZT dose at the end of a 8-wk chronic AZT treatment. The T/P AZT ratios (g tissue/ml plasma) in control mice could be ranked in the following order: kidneys >> muscle ≅ spleen ≅ liver ≅ heart > lung ≅ thymus > lymph nodes >> brain. The extent of AZT distribution into bone marrow was difficult to compare with that into other tissues because we were uncertain how many bone marrow cells would be equivalent to 1 g of tissue weight. The distribution of AZT into lymph nodes, thymus, and lung tissues in infected mice increased significantly in comparison with that of control animals.

Table 2 summarizes the T/P ratios of AZT-MP for control and infected animals at 90 min after the final AZT dose at the end of a 8-wk chronic AZT treatment period. The most

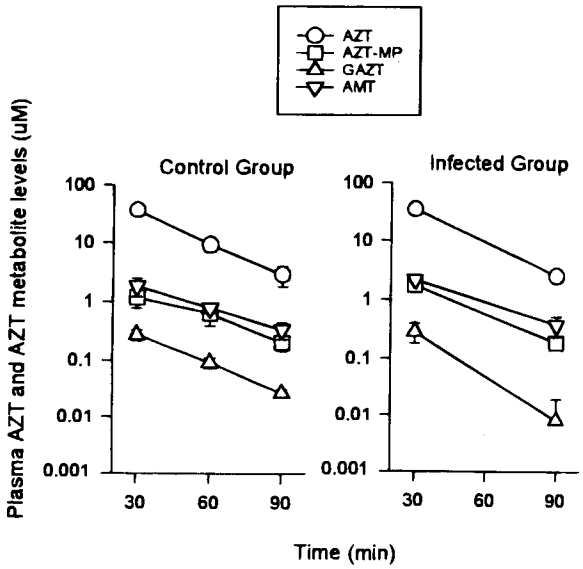

Fig. 1. *Plasma concentration-time profiles of AZT and its metabolites.* Semi-logarithmic plots of plasma levels of AZT and AZT metabolites of control and LP-BM5 MuLV inoculated mice as a function of time following the subcutaneous administration of the final AZT dose at the end of a 8-wk chronic AZT treatment. Each point represents the average of 4 mice and the cross-vertical bars represent one standard deviation of the mean. Key: AZT, 3′-azido-3′-deoxythymidine; AZT-MP, AZT 5′-monophosphate; AMT, 3′-amino-3′-deoxythymidine; GAZT, AZT 5′-glucuronide.

Table 1. T/P Ratios of AZT at Different Times After Dosing

	Bone marrow	Lymph nodes	Spleen	Thymus	Brain	Liver	Kidneys	Lung	Heart	Muscle
Control										
30 min	0.838	0.439	0.742	0.391	0.078	0.605	2.47	0.306	0.701	0.550
	±0.042	±0.093	±0.035	±0.031	±0.004	±0.028	±0.29	±0.234	±0.109	±0.091
60 min	0.958	0.334[b]	0.592[b]	0.477	0.101	0.663	2.74	0.487	0.619	0.671
	±0.302	±0.071	±0.102	±0.089	±0.029	±0.080	±0.39	±0.113	±0.123	±0.126
90 min	0.926	0.257[b]	0.554[b]	0.352	0.112[b]	0.569	1.97	0.371	0.595	0.646
	±0.167	±0.149	±0.063	±0.108	±0.049	±0.133	±0.28	±0.279	±0.036	±0.245
Infected										
30 min	1.64	0.702[a]	0.763	0.520[a]	0.078	0.673	2.02	0.643[a]	0.639	0.557
	±0.54	±0.521	±0.033	±0.094	±0.006	±0.110	±0.74	±0.012	±0.052	±0.114
90 min	0.722[b]	0.697[a]	0.695	0.461[a]	0.164[b]	0.642	2.46	0.506[a]	0.623	0.762
	±0.280	±0.126	±0.099	±0.120	±0.055	±0.193	±0.35	±0.145	±0.023	±0.289

Data are mean ± one standard deviation of four mice. T/P ratios are presented as nmol/10^{10} cells over nmol/ml plasma for bone marrow samples and as nmol/g tissue over nmol/ml plasma for the rest of tissues.
[a] Significantly different from that determined at the respective times after dosing of the control animals, $p < 0.05$.
[b] Significantly different from the corresponding values in the same study group at 30 min after dosing, $p < 0.05$.

substantial changes in the T/P ratios after the addition of a phosphate group were observed in the spleen and bone marrow samples (from 0.926 ± 0.167 for AZT to 4.42 ± 0.797 for AZT-MP in bone marrow and from 0.554 ± 0.063 for AZT to 2.60 ± 1.38 for AZT-MP in the spleen). The most notable changes in infected animals also were observed in the spleen samples (from 0.695 ± 0.099 for AZT to 2.94 ± 0.167 for AZT-MP). None of the tissues examined showed statistically significant differences in T/P AZT-MP ratios between control and infected animals.

The tissue concentration-time profiles of AZT and AZT metabolites in spleen, lymph node, brain, and bone marrow samples of control animals following the subcutaneous administration of the final AZT dose at the end of eight weeks of chronic AZT treatment are shown in Figure 2. Tissue concentration-time profiles of AZT-MP tended to parallel the AZT profiles in these tissues and in other tissues examined. Declines in AZT-DP and AZT-TP levels as a function of time seemed to occur at a slower rate in most tissues than did declines in AZT and AZT-MP levels. AZT-TP content was not quantifiable in any of the brain samples analyzed.

The tissue concentration-time profiles of AZT and AZT metabolites in spleen, lymph node, brain, and bone marrow samples of retrovirus inoculated animals are shown in Figure 3. For the limited time points collected, the overall profiles of AZT and AZT metabolites were comparable to those observed in the control animals. The phosphorylated AZT metabolite levels in the pooled lymph nodes of the infected animals were significantly higher than in the pooled lymph nodes of the control animals (1.97 ± 0.415 vs. 0.607 ± 0.065 nmol/g tissue and 0.234 ± 0.065 vs. 0.056 ± 0.050 nmol/g tissue for AZT-MP at 30 and 90 min time points, respectively; 0.209 ± 0.051 vs. 0.010 ± 0.017 nmol/g tissue and 0.101 ± 0.007 nmol/g tissue vs. non-quantifiable levels for AZT-TP at 30 and 90 min time points, respectively). No significant differences between AZT metabolite levels in control and infected animals were found in most other tissues examined.

The ratios of tissue levels of AZT-MP to AZT and of AZT-TP to AZT at 90 min after the final AZT dose at the end of a 8-wk chronic AZT treatment are summarized in Table 3. The highest ratios of both AZT-MP to AZT and AZT-TP to AZT were observed in the spleen and bone marrow samples. The AZT-TP to AZT ratios in an important lymphoid tissue, the thymus, were higher than those in other non-lymphoid tissues examined. In control animals, AZT-TP levels were not quantifiable in the lymph nodes collected at 90 min after dosing. Very low AZT-TP/AZT ratios were observed in the lymph nodes of control animals at 30 and 60 min after dosing (0.001 ± 0.001 and 0.0005 ± 0.001 at 30 and 60 min after dosing, respectively). These ratios increased significantly in the infected animals (0.005 ± 0.003 and 0.057 ± 0.022 at 30 and 90 min after dosing, respectively).

Table 2. T/P Ratios of AZT-MP at 90 min After Dosing

	Bone marrow	Lymph nodes	Spleen	Thymus	Brain	Liver	Kidneys	Lung	Heart	Muscle
Control	4.42[a]	0.250	2.60[a]	0.505[a]	0.117	1.32[a]	2.58[a]	0.355	0.727	0.374
	±0.80	±0.161	±1.38	±0.120	±0.024	±0.06	±0.44	±0.252	±0.034	±0.162
Infected	2.14	0.947	2.95[a]	0.920[a]	0.178	1.26[a]	3.29[a]	0.611	0.582	0.438
	±1.45	±0.407	±0.17	±0.304	±0.034	±0.03	±0.53	±0.207	±0.188	±0.166

Data are mean ± one standard deviation of four mice. T/P ratios are presented as nmol/10^{10} cells over nmol/ml plasma for bone marrow samples and as nmol/g tissue over nmol/ml plasma for the rest of tissues.
[a] Significantly different from the respective T/P ratios of AZT at 90 min after dosing (AZT data are shown in Table 1), $p < 0.05$.

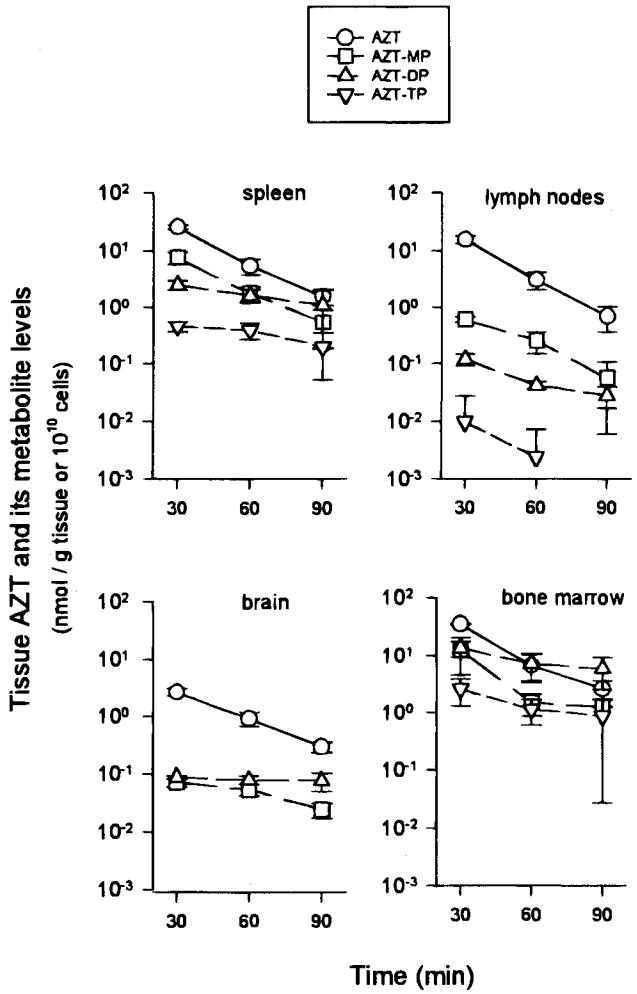

Fig. 2. *Tissue concentration-time profiles of AZT and AZT metabolites of control animals.* Semi-logarithmic plots of tissue levels of AZT and AZT metabolites of C57BL/6 mice as a function of time following the subcutaneous administration of the final AZT dose at the end of a 8-wk chronic AZT treatment. Tissue levels of AZT and AZT metabolites are presented in nmol/10^{10} cells for bone marrow samples and in nmol/g tissue for the other tissues. Each point represents the average of 4 mice and the cross-vertical bars represent one standard deviation of the mean. Key: AZT, 3′-azido-3′-deoxythymidine; AZT-MP, AZT 5′-monophosphate; AZT-DP, AZT 5′-diphosphate; AZT-TP, AZT 5′-triphosphate.

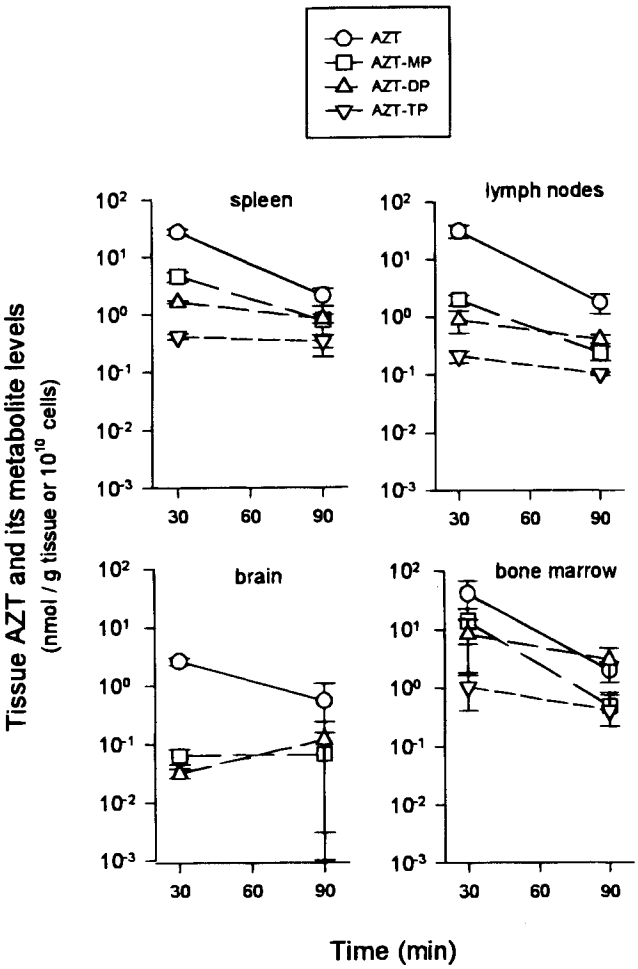

Fig. 3. *Tissue concentration-time profiles of AZT and AZT metabolites of LP-BM5 MuLV inoculated animals.* Semi-logarithmic plots of tissue levels of AZT and AZT metabolites of LP-BM5 MuLV inoculated mice as a function of time following the subcutaneous administration of the final AZT dose at the end of a 8-wk chronic AZT treatment. Tissue levels of AZT and AZT metabolites are presented in nmol/10^{10} cells for bone marrow samples and in nmol/g tissue for the other tissues. Each point represents the average of 4 mice and the cross-vertical bars represent one standard deviation of the mean. Key: AZT 3′-azido-3′-deoxythymidine; AZT-MP, AZT 5′-monophosphate; AZT-DP, AZT 5′-diphosphate; AZT-TP, AZT 5′-triphosphate.

DISCUSSION

In this study, we demonstrated that the distribution of AZT and the disposition of phosphorylated AZT metabolites in mice following chronic AZT treatment were tissue-specific and that chronic retrovirus infection resulted in minimal changes in either the tissue distribution of AZT or the tissue disposition of the phosphorylated AZT metabolites. These results provide further understanding of the *in vivo* tissue disposition of AZT and AZT metabolites and the effects thereon of chronic retrovirus infection, furnishing perspective on the possible contribution of tissue-specific formation and elimination of AZT metabolites to therapeutic problems associated with the use of AZT.

In this study as well as in previous investigation after a single dose AZT administration (5), the T/P ratios of AZT-MP in bone marrow and spleen were much higher than the corresponding ratios of AZT. Consistently, the ratios of AZT-MP/AZT in these two tissues were found to be higher than those in other tissues. This suggests that AZT is more efficiently converted to AZT-MP in the spleen and bone marrow, although preferential accumulation of AZT-MP in the spleen and bone marrow could also be possible. Literature information on thymidine kinase, the catalyst responsible for the conversion of AZT to AZT-MP, tends to support our hypothesis that the formation of AZT-MP is tissue specific. In eucaryotic cells, two different thymidine kinases have been found, thymidine kinase 1 and thymidine kinase 2 (7). High levels of thymidine kinase 1 activity have been found in proliferating and malignant cells and a considerably lower level of thymidine kinase activity observed in resting cells has been attributed to the presence of

Table 3. Ratios of Tissue Levels of AZT phosphates to AZT at 90 min After Dosing

	Bone marrow	Lymph nodes	Spleen	Thymus	Brain	Liver	Kidneys	Lung	Heart	Muscle
Control										
AZT-MP/AZT	0.344	0.076	0.333	0.111	0.083	0.171	0.094	0.071	0.088	0.042
	±0.040	±0.037	±0.167	±0.041	±0.028	±0.005	±0.0085	±0.0074	±0.008	±0.008
AZT-TP/AZT	0.296	0.0005[a]	0.166	0.075	N.Q.[b]	0.0145	0.0023	0.0024	0.0019	0.0067
	±0.216	±0.0011	±0.002	±0.055		±0.0050	±0.0028	±0.0002	±0.0008	±0.0043
Infected										
AZT-MP/AZT	0.229[c]	0.116	0.359	0.131	0.077	0.157	0.095	0.0096[c]	0.070	0.046
	±0.046	±0.030	±0.137	±0.063	±0.047	±0.013	±0.023	±0.011	±0.016	±0.007
AZT-TP/AZT	0.338	0.057[c]	0.167	0.046	N.Q.[b]	0.0094	0.0007	0.0043	0.0022	0.0040
	±0.048	0.022	±0.016	±0.029		±0.0088	±0.0007	±0.0026	±0.0055	±0.0031

Data are mean ± one standard deviation of four mice.
[a] AZT-TP levels were not quantifiable in the samples collected at 90 min after dosing; data collected at 60 min after dosing are listed in the table for comparison.
[b] AZT-TP levels were not quantifiable.
[c] Significantly different from the respective values of the control animals, $p < 0.05$.

thymidine kinase 2 (7). Tissue-specific thymidine kinase activity attributed to differences in the tissue distribution of these two isozymes has been previously reported. In research to determine thymidine kinase activity in several rat tissues, including liver, kidney, brain, spleen, and bone marrow, it was found that thymidine kinase activity in the spleen and bone marrow was significantly higher than that in other tissues (8,9). In enzyme kinetic studies conducted in our laboratory (unpublished data), a substantially higher relative catalytic efficiency (Vmax/km) of the conversion of AZT to AZT-MP was observed in mouse spleen cytosolic preparations than in mouse liver and brain preparations. These limited thymidine kinase activity studies show results consistent with our hypothesis that AZT could be more efficiently converted to the monophosphorylated metabolite in tissues with high thymidine kinase activity, such as the spleen and bone marrow.

As was observed following single-dose administration of AZT, the disposition of the active triphosphate moiety also was tissue specific following chronic AZT treatment. The ratios of tissue levels of AZT-TP to AZT at 90 min after the final AZT administration are presented in Table 3. The data are presented in ratios to correct for differences in AZT distribution into different tissues and time-dependent changes in tissue levels of the precursor. Presenting the data in ratios also reduces the inter-animal variability resulting from differences in tissue distribution of AZT among animals. In addition, this presentation format allows the comparison of AZT metabolite data collected from bone marrow (absolute values were expressed in nmol/10^{10} cells whereas ratios were dimensionless values) with those in other tissues (absolute values were expressed in nmol/g tissue whereas ratios were dimensionless values). Because of delays in the distribution of AZT into a number of tissues and in the appearance of AZT-TP in most tissues, only ratios at 90 min are presented and used as an approximate measurement for comparison of the extent of conversion of AZT to its metabolites. Differences in tissue disposition of AZT and AZT metabolites also became apparent when these ratios were compared among tissues. The highest ratios of conversion to the active triphosphate moiety were also found in the spleen and bone marrow samples after eight weeks of AZT treatment. This outcome could have resulted from more efficient conversion of AZT to the active or cytotoxic metabolites or from preferential accumulation of these metabolites in these tissues. In combination with a cell- or tissue-specific defense mechanism, the observed tissue-specific disposition of the cytotoxic AZT metabolites could contribute to the development of bone marrow toxicity following chronic AZT treatment.

The cells in the lymph nodes, one of the most important target cells/tissues in retrovirus infection, did not efficiently convert AZT to its active moiety in control animals. AZT-TP/AZT ratios were lower than those of most tissues examined. However, these ratios increased significantly in the diseased animals. These changes are likely to be attributable to changes in the activity of the enzymes responsible for the addition of the third phosphate group because when the ratios of AZT metabolites to their respective immediate precursors were estimated, only the ratios of AZT-TP/AZT-DP exhibited the same magnitude of change (data not shown).

Despite an observed increase in the brain AZT and AZT-MP concentrations in infected animals receiving the chronic AZT regimen used in this study over those observed after single dose administration (5), AZT-TP content was still not quantifiable in any of the brain samples analyzed. Inability to detect the presence of AZT-TP in brain tissue could have resulted from an inherent deficiency in the brain's ability to phosphorylate AZT-DP to form AZT-TP since the absolute AZT-DP levels detected in the brain were higher than or comparable to those in a number of other tissues where quantifiable levels of AZT-TP could be found. Inadequate maintenance of AZT-TP levels in the brain could therefore be responsible for failure to control neurological disorders associated with HIV infection following AZT treatment.

The enzyme that catalyzes the conversion of AZT-DP to AZT-TP, nucleoside diphosphate kinase, has been shown to have at least two isoforms present in a number of animal species, including mice (10), rats (11), and humans (10). Of the two isoforms (α and β) isolated from the rat, the α-isoform, the major form detected in most rat tissues, varied in levels from one tissue to another whereas the β-isoform was detectable mainly in the brain and testes (12). Differences in the substrate

specificity of these two isoforms were observed in the same study. Therefore, the tissue specific disposition of AZT-TP observed in our studies is likely to be attributable to variations in the tissue distribution of different isoforms of this cellular kinase. Because the conversion of the diphosphate derivatives to the triphosphate moiety of most nucleoside analogues is mediated by the same enzyme, tissue specific disposition of the active triphosphate moiety of other nucleoside-based therapeutic agents is also plausible.

In this investigation, the clinical manifestations observed in animals inoculated with LP-BM5 MuLV were similar to those described previously (13). In infected animals, spleens frequently weighed 0.5 g or more and were filled with nodular masses of lymphoid cells. In addition, reductions in the number of white blood cells, erythrocytes, and platelets in the blood were observed in the inoculated animals (data not shown). The AZT regimen employed in this study did not result in apparent improvement in the clinical manifestations associated with MAIDS. Inability to observe clinical improvement following the chronic AZT treatment is possibly attributable to the significant delay in the initiation of AZT treatment following virus inoculation (14,15). Minor changes in the disposition of AZT metabolites and AZT were observed in a number of tissues in the LP-BM5 MuLV infected animals following chronic AZT treatment. The most noteworthy change was observed in the lymph nodes. Significant increases in the distribution of AZT into the lymph nodes (an increase of about two-fold) and in the conversion of AZT to the triphosphate form of AZT in this important target tissue (a more than 5-fold increase in AZT-TP/AZT ratios) were observed in the retrovirus inoculated animals.

It needs to be emphasized that the tissue concentrations of AZT and the phosphorylated AZT metabolites reported in this study as well as in most tissue disposition studies represent the apparent tissue drug concentrations attributed to the presence of drugs in the tissue and in residual tissue blood. "Blood-free" tissue drug concentrations are difficult to obtain experimentally, but could be approximated with information on blood drug concentrations and volume fraction of blood in a tissue (16). Corrected "blood-free" tissue drug concentrations were not reported in our study because plasma rather than blood drug concentrations were determined. Estimation of "blood-free" tissue drug concentrations was made by assuming that the plasma-to-blood concentration ratio is one. It was found that the measured tissue AZT concentrations tended to overrepresent the "blood-free" tissue drug concentrations and the measure AZT metabolite concentrations underrepresented the "blood-free" tissue metabolite concentrations. The most significant deviations between the average and "blood-free" tissue drug were found in tissues having the highest blood content (i.e, lung and liver). Our conclusions were minimally affected by these deviations.

In summary, compared with our previous study employing a single-dose administration of AZT, the chronic AZT regimen used in this study (25 mg/kg twice a day for eight weeks) did not result in significant changes in the tissue disposition of the phosphorylated AZT metabolites. Inadequate maintenance of active AZT metabolite levels in the lymph nodes and the brain and high levels of the active AZT metabolite in bone marrow samples were observed in this study. Moreover, advanced-stage retrovirus infection in LP-BM5 MuLV infected animals appeared to have minimal effects on the tissue distribution of AZT and tissue disposition of AZT metabolites in most of the tissues examined but to have resulted in significant changes in the AZT and AZT metabolite levels in the lymph nodes. Extrapolation to humans of results obtained from control and retrovirus inoculated mice following the regimen employed in this study are complicated by factors such as species differences in the activities of cellular kinases, time of the initiation of AZT administration or concomitant use of other antiretrovirals in HIV-infected individuals, and different cellular responses to the mouse and human retroviruses. The therapeutic implications of inadequate maintenance of the active AZT metabolite in the lymph nodes and the brain following chronic AZT treatment need to be further explored.

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