ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1994, p. 1573–1579
0066-4804/94/$04.00+0

Vol. 38, No. 7

Differential Antiviral Activities and Intracellular Metabolism of 3′-Azido-3′-Deoxythymidine and 2′,3′-Dideoxyinosine in Human Cells

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Received 6 December 1993/Returned for modification 21 March 1994/Accepted 10 May 1994

Dideoxynucleosides such as 3′-azido-3′-deoxythymidine (AZT) and 2′,3′-dideoxyinosine (ddI) can effectively inhibit the replication of human immunodeficiency virus (HIV) in T lymphoid cells. There is evidence that HIV can infect and replicate in other cells including monocytoid cells and macrophages. The present study compared the antiretroviral activities of ddI and AZT in three lineages of human cells, i.e., MOLT4 (T lymphocytoid, CD4$^+$), U937 (monocytoid, CD4$^+$), and HT1080 (fibroblastoid, CD4$^-$) cells. Feline leukemia virus, a retrovirus that causes immunodeficiency in cats, was used to infect the cells. The drug concentrations needed to reduce the viral p27 antigen titers in cell lysates by 50% (IC_{50}s) were determined. The data show that AZT and ddI inhibited viral replication in all three cell lines. The IC_{50}s of AZT were 0.02, 1.75, and 2.31 μM in MOLT4, HT1080, and U937 cells, respectively. For ddI, the IC_{50}s were 4.31, 9.52, and 43.5 μM, respectively. These data indicate differential antiviral activities of ddI and AZT in the different cells with the following rank order of drug sensitivity: MOLT4 > HT1080 > U937. A study of the intracellular metabolism of [^{3}H]AZT and [^{3}H]ddI shows that the antiretroviral activities of AZT and ddI in the three cell lines correlated with the levels of their intracellular triphosphate metabolites.

Infection of lymphoid cells by human immunodeficiency virus (HIV) results in immune suppression (32), and infection of monocyte cells and macrophages results in low and persistent viral production. Aberrant cytokine secretion by HIV-infected macrophages results in dementia (for a recent review, see reference 19). Infection of fibroblastoid cells is linked to the connective tissue disorders and/or weight loss and wasting observed in patients with AIDS (19, 23, 35, 37). Dideoxynucleoside analogs such as 3′-azido-3′-deoxythymidine (AZT) and 2′,3′-dideoxyinosine (ddI) inhibit the replication of HIV in human lymphoid cells (25) and suppress HIV replication in peripheral blood monocytoid cells and macrophages (5, 28–30). The antiviral activities of the dideoxynucleosides in target cells that are CD4$^-$, e.g., fibroblastoid cells, have not been established.

The present study compared the antiretroviral potencies of AZT and ddI in human lymphoid (MOLT4 [CD4$^+$]), monocytoid (U937 [CD4$^+$]), and fibroblastoid (HT1080 [CD4$^-$]) cell lines (33). The antiviral activity was measured by using a non-HIV retrovirus model, i.e., the feline leukemia virus (FeLV). FeLV is an attractive alternative to HIV, and its use avoids the biohazards associated with HIV. FeLV has been extensively studied because it is a common pathogen in cats and has been used as a retrovirus model for human immunosuppressive disorders (11–13, 31, 36, 38). A variety of human cell lines, including those used in the current investigation, are permissive to FeLV infection (35).

The replication of FeLV, as is the case for all retroviruses, requires reverse transcription by a virus-coded reverse transcriptase (RT) enzyme (11). Viral RT is inhibited by the 5′-triphosphate of dideoxynucleosides (21, 22, 31, 36, 38). AZT and ddI require intracellular metabolic activation to their active triphosphate analogs. The intracellular metabolism of AZT and ddI in MOLT4, U934, and HT1080 cells was investigated to elucidate the pharmacological basis of differential drug activity in these cells.

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MATERIALS AND METHODS

Chemicals. AZT (lot AJ-A1.2), AZT diphosphate (AZTDP) (lot 102-198-000), AZT triphosphate (AZTTP) (lot 102-191-000), and [*methyl*-^{3}H]AZT (specific activity, 14 Ci/mmol; lot 100-027-014) were gifts from the AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases (Rockville, Md.). ddI (lot 243-b-1) was a gift from the National Institutes of Health (Bethesda, Md.). ADP (lot 38F-7160), ATP (lot 116F-7080), hypoxanthine (lot 125F-0368), dAMP (lot 33H-7864), and aprotinin were purchased from Sigma Chemical Co. (St. Louis, Mo.), ddATP (lot 12H-0368) was from Pharmacia Biotech Inc. (Piscataway, N.J.), and [2′,3′-^{3}H]ddI (specific activity, 42 Ci/mmol; lot 111-156-042) was from Moravek Biochemicals Inc. (Brea, Calif.). The purities of the compounds were determined by high-pressure liquid chromatography (HPLC). AZT, AZTTP, ATP, hypoxanthine, dAMP, and ddI were >98% pure. AZTDP contained 5% AZT monophosphate (AZTMP) as an impurity, ADP contained 7% AMP, and ddATP contained 6% ddADP. The radiolabeled AZT and ddI were 99% pure. All other reagents were of HPLC grade and purchased from Fisher Scientific Co. (Cincinnati, Ohio). Cell culture supplies were purchased from Gibco Laboratories (Grand Island, N.Y.), fetal bovine serum was from HyClone Laboratories (Logan, Utah), Polybrene was from Aldrich Chemical Co. (Milwaukee, Wis.), and Nonidet P-40 was from Behring Diagnostics (La Jolla, Calif.).

Cell lines and virus. The human lymphoid MOLT4 cells and the human monocytoid U937 cells were obtained from Amer-

ican Type Culture Collection (Rockville, Md.). For these two cell lines, the tissue culture medium contained a 1:1 mixture of RPMI 1640 and Lebovitz-15 medium, 10% heat-inactivated fetal bovine serum, 2% of a stock solution of L-glutamine (29.2 mg/ml), and 1% of a stock solution of penicillin-streptomycin (5,000 U of penicillin and 5,000 μg of streptomycin per ml). The human fibroblastoid cells (HT1080) were a generous gift from Pradip Roy-Burman (University of Southern California). This cell line was maintained in α minimal essential medium containing 10% fetal bovine serum, 1% L-glutamine stock solution, and 1% penicillin-streptomycin stock solution. Cells were maintained at 37°C with 5% CO_2 and subcultured every 5 days. The doubling times were 51, 46, and 45 h for MOLT4, U937, and HT1080 cells, respectively.

The FeLV-C-Sarma strain of FeLV (gift from P. Roy-Burman) propagated on human HT1080 cells was used as a source of inoculum. To prepare viral stocks, the infected cells were cultured for 5 days after which the culture fluid was removed, aliquoted into 2-ml ampules, and stored at −70°C. The virus stock solution had an infection titer of 4.5×10^6 focus-forming units per ml, as determined on 81C cells (American Type Culture Collection) by the method of Fischinger et al. (8).

Viral infectivity study. Cells were plated in duplicate at concentrations of 10^5 cells per ml in 24-well culture dishes (Costar, Cambridge, Mass.) and infected with FeLV 18 to 20 h after plating. To facilitate attachment of the virus, the cells were pretreated with 4-μg/ml Polybrene solution. MOLT4 and U937 cells were grown in suspension, and HT1080 cells were grown as a monolayer. For MOLT4 and U937 cells, one-half of the cell suspension (500 μl) was removed and 500 μl of viral solution was added to each well. For HT1080 cells, one-half of the culture medium was removed and replaced with 500 μl of viral suspension. The cell culture medium was changed 4, 6, and 8 days after infection. On the 11th day following infection, the concentration of FeLV 27,000-Da core protein (p27) within the cells (in cell lysates) was determined. Cell pellets were lysed in 100 μl of an extraction buffer containing 0.05 M sodium chloride, 0.02 M Tris hydrochloride (pH 7.4), 10% aprotinin, 0.5% Nonidet P-40, and 0.5% sodium deoxycholate. Aprotinin, a protease inhibitor, was added to prevent degradation of the viral protein. The p27 concentration in 50-μl aliquots of cell lysates was determined with an enzyme-linked immunosorbent assay (ELISA) kit and measuring the color changes at 690 nm with an ELISA reader (Molecular Devices, Menlo Park, Calif.). Standard curves were constructed by using known dilutions of a stock solution of p27 at a concentration of 0.42 mg/ml (Pfizer Inc., Maywood, N.J.). Extracts from uninfected cells were used to correct for background.

A viral titration was performed to identify for subsequent drug concentration-effect studies the optimum viral dilution that would give satisfactory infection of the human cells and give viral antigen (p27) titer. MOLT4, HT1080, and U937 cells infected by the undiluted FeLV stock expressed intracellular p27 concentrations of 73, 56, and 57 ng/ml, respectively. The values dropped by about 50% with a 10-fold dilution of the stock virus for the HT1080 and U937 cells and with a 1,000-fold dilution for the MOLT4 cells. In order to maintain a constant virus challenge and to maintain a relatively high rate of infection, subsequent studies used a fivefold dilution of the virus stock (9×10^5 focus-forming units per ml).

Drug concentration-effect relationship. Cells (5×10^4) were treated with AZT or ddI beginning 24 h before viral infection. The drug concentration ranged from 0.001 to 200 μg/ml. Drug-free medium was used in the controls. On 4, 6, and 8 days after viral infection, one-half of the culture medium was replaced by medium containing fresh drug. Cells and culture fluids were harvested 11 days after infection and analyzed for p27 content.

The effects of AZT and ddI on cell growth were also investigated. Cell counts were performed with a hemocytometer, and cell viability was determined on the basis of trypan blue exclusion.

Intracellular metabolism. AZT is sequentially phosphorylated to the monophosphate (AZTMP), diphosphate (AZTDP), and triphosphate (AZTTP) by cellular enzymes thymidine kinase, thymidylate kinase, and deoxynucleoside diphosphate kinase, respectively (9). ddI is anabolized to its monophosphate (ddIMP), followed by amination to form ddAMP, which is subsequently converted to the diphosphate (ddADP) and the triphosphate (ddATP) (1, 16, 17). The 5′-triphosphates of AZT and ddI inhibit the RT of HIV by chain termination and by acting as a competitive inhibitor of the endogenous deoxynucleoside 5′-triphosphates for the RT of HIV (6, 9, 26). In humans, degradation of AZT occurs primarily by extracellular glucuronidation in the liver (39). ddI is degraded in cells by the purine nucleoside phosphorylase to hypoxanthine, which is subsequently converted to IMP, AMP, ADP, and ATP via the established purine nucleotide pathways (12).

The intracellular metabolism of AZT and ddI was studied by using cells in exponential growth phase, i.e., 1 day after subculture. A 1-ml aliquot of cell suspension (10^6 cells) was centrifuged, and the cell pellet was resuspended in 1 ml of medium containing 5 μCi of [^{3}H]AZT (equivalent to 0.37 μM) or 15 μCi of [^{3}H]ddI (equivalent to 0.38 μM). After 6 h of incubation, the cells were centrifuged at $300 \times g$ at 4°C for 5 min, and the harvested cell pellet was washed three times with 10 ml of ice-cold phosphate-buffered saline to remove the residual drug. Extraction of intracellular unchanged drug and metabolites was done with acetonitrile as described by Au et al. (2). Ice-cold acetonitrile (500 μl) was added to the cell pellets to precipitate the macromolecules. This step was immediately followed by the addition of 500 μl of distilled water to extract the cellular contents. The suspension was centrifuged, and the supernatant was collected, evaporated to dryness, and reconstituted with 200 μl of distilled water.

HPLC analysis. The concentration of the metabolites was quantitated by HPLC fractionation and liquid scintillation. AZT, ddI, and their metabolites were separated by gradient elution, using a Partisil-10 SAX column (Whatman Inc., Clifton, N.J.). The HPLC instrument consisted of an automated gradient controller (model 680; Waters, Milford, Mass.), a solvent delivery pump (Waters 501), and an HP 3394A integrator (Hewlett-Packard, Palo Alto, Calif.). The separation of AZT and metabolites was similar to that of Perno et al. (30) with modifications in the elution program. The eluting buffers were 0.01 M ammonium phosphate (pH 3.6) (buffer A) and 0.6 M ammonium phosphate (pH 3.8) (buffer B). The analytes were eluted with buffer A for 15 min, elution by a convex gradient to buffer B for 10 min, followed by isocratic elution by buffer B for 30 min. The flow rate was 2.0 ml/min. The ddI analysis was similar to that of Hartman et al. (12) with modifications in the buffer constituents. Buffer A was 0.03 M ammonium phosphate (pH 4.8), and buffer B was 0.7 M ammonium phosphate (pH 4.6) plus 10% acetonitrile. The elution program was 5 min of buffer A followed by a 10-min convex gradient to 75% buffer A and 25% buffer B, 15-min convex gradient to 100% buffer B, and 15-min isocratic elution with 100% buffer B. The flow rate was 1.7 ml/min.

The retention times of the labeled compounds were calibrated by using nonlabeled drug and metabolites, i.e., AZT, AZTDP, AZTTP, ddI, ADP, ATP, and hypoxanthine. The

retention time of AZTMP was determined from the elution profile of AZTDP which contained AZTMP as an impurity. Similarly, the retention times of AMP and ddADP were obtained from the elution profiles of ADP and ddATP. Because of the unavailability of ddAMP, the monophosphate was obtained by the degradation of ddATP during storage as a saline solution at room temperature for 96 h. The retention times of AZT, AZTMP, AZTDP, and AZTTP were 6.5, 15, 22.5, and 35 min, respectively. The retention times of hypoxanthine, AMP, ddI, ddAMP, ADP, ddADP, ATP, and ddATP were 4.7, 6.5, 9.0, 11, 19, 23.5, 27, and 33 min, respectively.

Quantitation of the nonlabeled drug and metabolites was done by monitoring A_{254} (Waters 441 absorbance detector). For the quantitation of the radiolabeled drug metabolites, fractions of HPLC eluents were collected every minute with a fraction collector (Frac 100; Pharmacia Fine Chemicals, Piscataway, N.J.). Radioactivities in fractions corresponding to the retention times of drugs and metabolites were determined by scintillation counting with an LSC Tricarb 1600 TR liquid scintillation analyzer (Packard Instrument Co., Meriden, Conn.). The counting efficiency of tritium was 67%.

Data analysis. The drug concentration-effect relationship was analyzed by computer fitting the following equation of the fractional E_{max} model (15) to the experimental data:

$$E = E_0\left(1 - \frac{C^n}{K^n + C^n}\right) \quad (1)$$

where E is the effect of AZT or ddI, defined as the ratio of the p27 concentration in the drug-treated infected cells to that in the untreated infected cells, E_0 is the baseline effect in the absence of the drug, C is the concentration of the drug, K is the drug concentration for 50% inhibition of viral replication (IC_{50}), and n is the curve shape parameter.

Analysis of variance, Mann-Whitney nonparametric tests and repeated t tests were used to identify the statistical significance of the differences between the means (Minitab Statistical Software, State College, Pa.). The correlation between parameters was evaluated by linear regression, and the significance of the correlation was evaluated by comparing the value of the correlation coefficient to zero. A P value less than or equal to 10% was considered significant, since no α value had been set a priori and because of the small sample size (three cell lines) for the experiments (4).

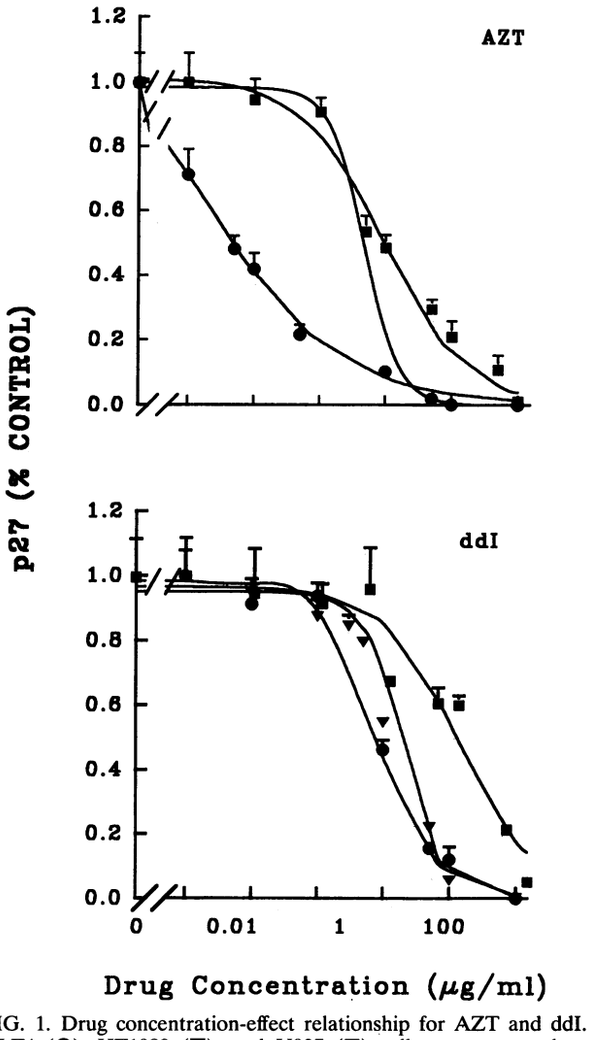

FIG. 1. Drug concentration-effect relationship for AZT and ddI. MOLT4 (●), HT1080 (▼), and U937 (■) cells were exposed to various AZT or ddI concentrations beginning 24 h before infection with FeLV. The concentrations of p27 in the cell lysates on the 11th day after viral infection were determined by ELISA and expressed as percent control. Each point is the mean + 1 standard deviation of three determinations. The curves are the computer-fitted relationship according to equation 1.

RESULTS

Drug concentration-effect relationship. AZT and ddI inhibited viral replication in all three cell lines. As shown in Fig. 1, the extent of inhibition was dependent on the drug concentration and was well described by a sigmoidal relationship, with a maximal effect approaching 100%. A comparison of the drug concentration-effect curves of AZT and ddI in the three cell lines and their IC_{50}s (summarized in Tables 1 and 2) indicates that the MOLT4 lymphoid cells were the most sensitive and the U937 monocytoid cells were the least sensitive, whereas the HT1080 fibroblastoid cells showed an intermediate sensitivity to these drugs. Within individual cell lines, AZT was more potent than ddI on a molar basis. On the basis of their IC_{50}s, AZT was 5-, 16-, and 200-fold more effective than ddI in HT1080, U937, and MOLT4 cells, respectively.

The proliferation of the MOLT4 and HT1080 cells was not affected by the highest concentration of AZT or ddI (100 μg/ml), and the cell viability remained >90%. In comparison, the two drugs were toxic to the U937 cells, with a 99 and 22% reduction in cell count at a 100-μg/ml concentration of AZT and ddI, respectively.

Intracellular metabolism of AZT and ddI. A preliminary study to determine the relationship between metabolite formation and drug incubation time was conducted in MOLT4 cells. Increasing the incubation time from 6 to 24 h did not change the concentration of AZT and AZTTP or the ratio of AZTTP to AZT (Table 3). Subsequent studies were conducted using the 6-h incubation time.

Figure 2 shows the HPLC-radioactivity profiles of AZT and metabolites in the three cell lines and Table 1 summarizes the results. The intracellular level of AZT was significantly higher in the MOLT4 cells than in the U937 cells, but the differences between HT1080 and either MOLT4 or U937 cells were not significant. The major intracellular AZT metabolite was AZTMP, which accounted for 93, 89, and 96% of the total nucleotide pool in the MOLT4, HT1080, and U937 cells,

TABLE 1. Comparison of anti-FeLV activity of AZT with its intracellular metabolism[a]

Cell line	IC_{50} (μM)	AZTTP concn (pmol/10^6 cells)	AZT concn (pmol/10^6 cells)	AZTTP/AZT ratio	AZTMP/AZTTP ratio
MOLT4	0.02 ± 0.006^1	0.159 ± 0.041^1	0.069 ± 0.019^1	2.52 ± 1.10^1	27.3 ± 7.43^1
HT1080	1.75 ± 0.86^2	0.054 ± 0.022^2	$0.032 \pm 0.011^{1,2}$	1.88 ± 0.91^2	$19.4 \pm 5.82^{1,2}$
U937	2.31 ± 1.3^3	0.020 ± 0.007^3	0.022 ± 0.02^2	1.40 ± 0.81^3	55.9 ± 24.7^2

[a] MOLT4, HT1080, and U937 cells (10^6 cells per ml) were incubated with 5 μCi of [^{3}H]AZT (0.57 μM) for 6 h. Afterwards, AZT and metabolites were extracted from the cells and analyzed by HPLC and liquid scintillation. The IC_{50}s represent the concentrations of AZT required to produce a 50% reduction of FeLV p27 antigen titer in cell lysates. Data represent means ± standard deviations of three separate experiments. Intracellular concentrations were standardized to the starting cell numbers. Means with different numbered superscripts (1, 2, or 3) are significantly different ($P \leq 0.1$) on the basis of repeated t tests and Mann-Whitney nonparametric test. Means with the same numbered superscript are not statistically different. The P values obtained by analysis of variance for the different values are as follows: 0.05 for IC_{50}s, 0.002 for AZTTP concentrations, 0.08 for AZT concentrations, and 0.01 for AZTMP/AZTTP ratios; the AZTTP/AZT ratios were not significantly different by analysis of variance.

respectively. The AZTMP levels were between 32 to 62 times that of AZT. Degradation of dideoxynucleoside monophosphate to dideoxynucleoside during processing has been shown previously (14). Degradation of AZTMP might have contributed to the high AZT levels. The ratios of AZTDP to AZTTP were similar for the three cell lines (1.01 in MOLT4, 1.17 in HT1080, and 0.98 in U937). However, the actual levels of AZTDP and AZTTP in the MOLT4 cells were significantly higher than that in the U937 cells (Fig. 1).

Figure 2 shows the HPLC-radioactivity profiles of ddI and metabolites in the three cell lines. There was inadequate resolution between ddI and ddAMP and between hypoxanthine and AMP. In lymphoid cells, ddAMP is not a major metabolite and is formed at a similar rate as those of ddADP and ddATP (1). The ddATP level was about 3% of the ddI level, suggesting an insignificant contribution of the ddAMP peak to the ddI concentrations. Likewise, in human lymphoid cells treated with dideoxyadenosine, which is converted to ddI and its subsequent metabolites, the concentration of AMP produced was about twofold that of ADP (16). On the basis of this known relationship and the ADP level detected in this study, the contribution of AMP to the hypoxanthine plus AMP peak was calculated to be insignificant (3 to 4%). Table 2 summarizes the levels of intracellular ddI and metabolites. The ddATP concentrations in all three cell lines were two- to threefold higher than the ddADP concentration. The MOLT4 cells had a significantly higher ddATP level than that of the U937 cells. The levels of hypoxanthine plus AMP contributed to about 34% of the total intracellular metabolite concentration, whereas the ddATP levels were 2% of the total metabolite pool, indicating a higher intracellular degradation rate of ddI than its phosphorylation rate.

Correlation of anti-FeLV activity with intracellular phosphorylation. Tables 1 and 2 compare the IC_{50}s of AZT and ddI in the three cell lines with the intracellular concentrations of AZTTP and ddATP. The rank orders of the triphosphate levels of the two drugs in the three cell lines were identical to the rank order of their antiviral activities, i.e., MOLT4 > HT1080 > U937. The IC_{50}s of AZT and ddI were inversely correlated with their respective triphosphate levels in these cells (Fig. 3).

The catabolic rates of ddI calculated as the ratio of hypoxanthine plus AMP to ddI were similar in the three cell lines. There was no correlation between the catabolic rates with the ddATP concentration ($r = 0.5$, $P > 0.1$; data not shown).

DISCUSSION

Numerous studies providing comparative data on the antiviral activities of various dideoxynucleosides were done with different lymphoid cell lines, assay systems, multiplicities of infection, and endpoints (for a review, see reference 13). Comparison of data between studies requires caution. The present investigation compared the antiviral activities of AZT and ddI simultaneously in three different human cell types, i.e., lymphoid cells, monocytoid cells, and fibroblastoid cells, using a feline retrovirus model. The evaluations were conducted concurrently and under the same experimental conditions. We used cells of different lineages that are potential targets of HIV infection. These cells were infected with FeLV at a constant infectious dose. FeLV has the advantage of being nonbiohazardous and highly infective to each of these cell lines and therefore permitted side-by-side comparison of antiviral activity based on almost equal virus expression.

AZT and ddI inhibited replication of FeLV in all three cell lines. The IC_{50}s for viral inhibition were at least 10- to 20-fold lower than the drug concentrations needed to cause cytotoxicity. The anti-FeLV effect was therefore not a secondary effect of the drug-induced cytotoxicity. The effective AZT and ddI concentrations to prevent FeLV infection were different for

TABLE 2. Comparison of anti-FeLV activity of ddI with its intracellular metabolism[a]

Cell line	IC_{50} (μM)	ddATP concn (pmol/10^6 cells)	ddI+ddAMP concn (pmol/10^6 cells)	ddATP/(ddI + ddAMP) ratio	(Hyp[b] + AMP)/(ddI + ddAMP) ratio
MOLT4	4.31 ± 1.29^1	0.0061 ± 0.001^1	0.196 ± 0.087	0.033 ± 0.008	0.627 ± 0.201
HT1080	$9.52 \pm 3.93^{1,2}$	$0.0051 \pm 0.001^{1,2}$	0.192 ± 0.006	0.0263 ± 0.004	0.609 ± 0.089
U937	43.5 ± 40.9^2	0.0042 ± 0.001^2	0.156 ± 0.021	0.0236 ± 0.004	0.673 ± 0.085

[a] MOLT4, HT1080, and U937 cells (10^6 cells per ml) were incubated with 15 μCi of [^{3}H]ddI (0.36 μM) for 6 h. Afterwards, ddI and metabolites were extracted and analyzed by HPLC and liquid scintillation. The IC_{50}s represent the concentrations of ddI required to produce a 50% reduction in the FeLV p27 antigen titer in cell lysates. Data represent means ± standard deviations of three separate experiments. Intracellular concentrations were standardized to the starting cell numbers. Means with different numbered superscripts (1 or 2) are significantly different ($P \leq 0.1$) on the basis of repeated t tests and Mann-Whitney nonparametric test. Means with the same numbered superscript are not statistically different. Note that ddI plus ddAMP and hypoxanthine (Hx) plus AMP are represented as a summation because of the inadequate resolution of the peaks. The P values obtained by analysis of variance for the different values are as follows: 0.02 for IC_{50}s and 0.1 for ddATP concentrations; the other values are not significantly different by analysis of variance.

[b] Hyp, hypoxanthine.

TABLE 3. Effect of exposure time on the intracellular levels of AZT metabolites[a]

Exposure time (h)	Concn (pmol/10^6 cells)[b,c]				AZTTP/AZT ratio[b]
	AZT	AZTMP	AZTDP	AZTTP	
6	0.036 (0.034–0.039)	3.024 (2.561–3.532)	0.143 (0.104–0.181)	0.164 (0.134–0.193)	4.456 (3.929–4.983)
24	0.034 (0.034–0.035)	4.616 (4.365–4.867)	0.147 (0.146–0.148)	0.157 (0.151–0.163)	4.865 (3.973–4.865)

[a] MOLT4 cells were incubated with 5 μCi of [^{3}H]AZT (0.37 μM) for 6 or 24 h. Afterwards, AZT and metabolites were extracted from the cells and quantitated by HPLC and liquid scintillation.
[b] Data represent the averages of two determinations in a single experiment, with the range indicated in parentheses.
[c] Intracellular concentrations were corrected for the increase in cell number over 6 and 24 h.

the three cell lines; the two drugs were most effective in inhibiting FeLV replication in the lymphoid cells and least effective in the monocytoid cells, with an intermediate effect in the fibroblastoid cells. Our finding that AZT was superior to ddI in all three cell lines on an equimolar basis is consistent with the previous finding that pyrimidine 2′,3′-dideoxynucleosides are more active antiviral agents in vitro than their purine analogs (25). In humans, active doses of AZT and ddI are similar.

AZT and ddI at concentrations up to 0.4 mM were not toxic

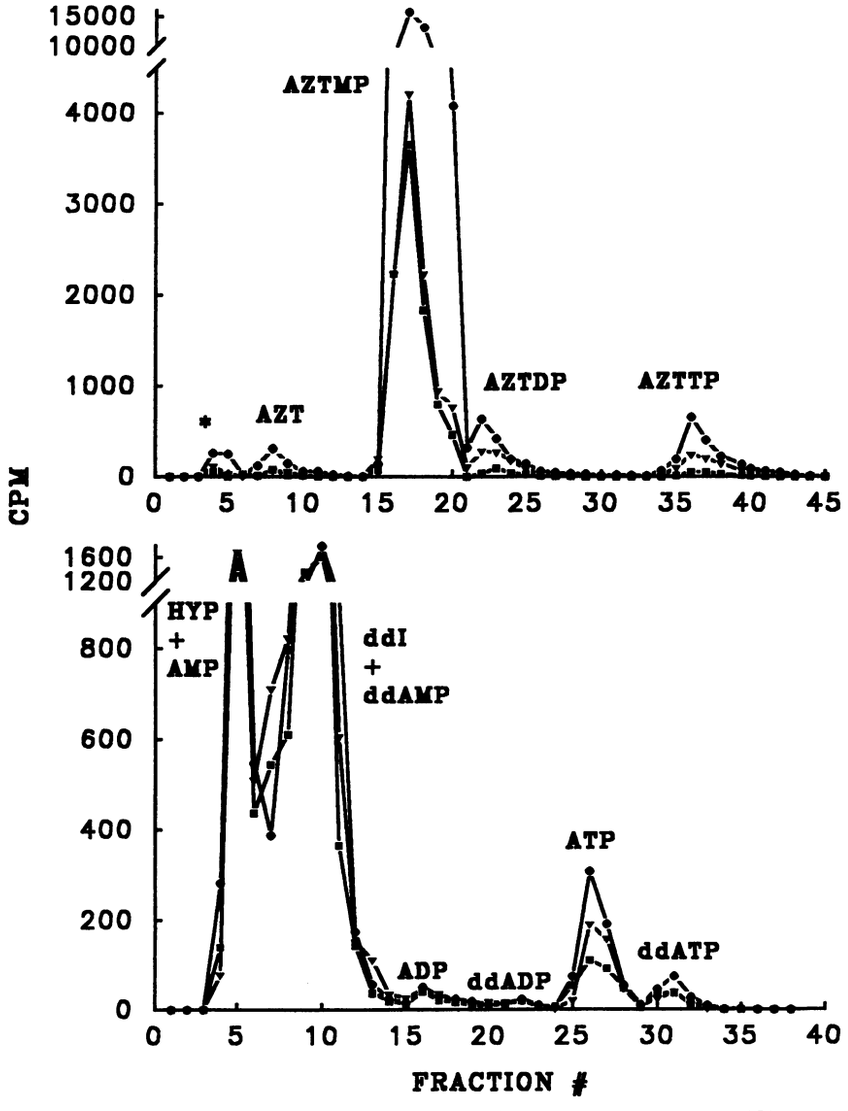

FIG. 2. HPLC-radioactivity profile of intracellular AZT (top) and ddI (bottom) and metabolites. MOLT4 (●), HT1080 (▼) and U937 (■) cells (10^6 cells per ml) were incubated with [^{3}H]AZT or [^{3}H]ddI for 6 h. Extraction and HPLC analyses were done as described in Materials and Methods. *, Unknown. HYP, hypoxanthine.

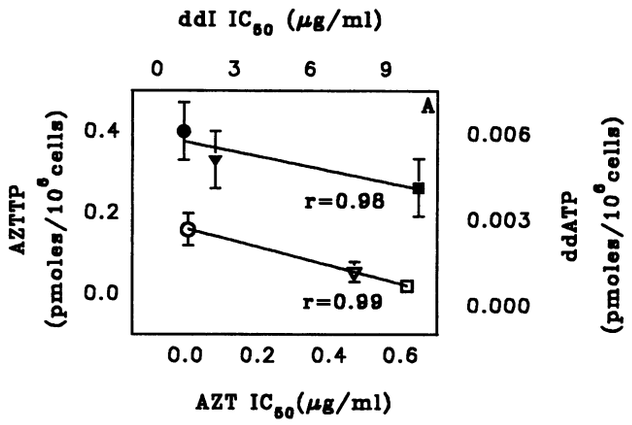

FIG. 3. Relationship of intracellular AZT and ddI metabolism and drug activity. The IC_{50}s of AZT (open symbols) and ddI (solid symbols) in MOLT4 (circle), HT1080 (triangle), and U937 (square) cells were plotted against the intracellular AZTTP and ddATP levels. *r* is correlation coefficient obtained by linear regression. The correlations for AZT ($P \leq 0.05$) and ddI ($P \leq 0.1$) were significant. Each point is the mean ± standard deviation of three determinations.

to the lymphoid and fibroblastoid cells but were toxic to the monocytoid cells. The selective toxicity of AZT in the monocytoid cells may be related to the significantly higher accumulation of AZTMP in the monocytoid cells than in the lymphoid and fibroblastoid cells (Table 1). Accumulation of AZTMP causes feedback inhibition of thymidylate kinase and has been associated with the cytotoxicity of AZT (9). The reason for the ddI cytotoxicity in the monocytoid cells is unclear.

Hao et al. (10) have shown that the differences in the abilities of the dideoxynucleosides to generate their corresponding 5′-triphosphates correlated strongly with their antiviral activities in lymphoid cells. Balzarini et al. (3) showed that phosphorylation of the dideoxynucleosides was highly species dependent, with a substantially higher phosphorylation and antiviral activity in murine lymphoid cells than in human lymphoid cells. Results of the present study show that the antiretroviral activities of AZT and ddI in human lymphoid, monocytoid, and fibroblastoid cells correlated with the levels of their active triphosphate metabolites.

We observed differences between the intracellular and extracellular AZT and ddI concentrations. AZT enters cells mainly by passive diffusion (40). Domin et al. (7) have shown that ddI permeates human erythrocytes partially by a nucleobase carrier and partially by passive diffusion. Assuming a mean cellular volume of 1 pl (11), the intracellular concentrations of AZT were calculated to be 0.064, 0.03, and 0.022 μM and the concentrations of ddI were 0.20, 0.19, and 0.19 μM, in the MOLT4, HT1080, and U937 cells, respectively. The corresponding extracellular concentrations were 0.37 μM for AZT and 0.36 μM for ddI. The low binding of the two drugs to protein in plasma (27, 34) rules out protein binding in the extracellular fluid as a cause of the large differences between the intracellular and extracellular AZT and ddI concentrations. The lower intracellular drug concentrations suggest that AZT and ddI were not transported against a concentration gradient.

In summary, our data show that AZT and ddI protected $CD4^+$ lymphoid and monocytoid cells as well as $CD4^-$ fibroblastoid cells from FeLV infection. The drug activity was variable and dependent on the cell lineage and correlated qualitatively with the triphosphate levels in these cells. The three cell types, i.e., lymphoid, monocytoid, and fibroblastoid, showed differential drug uptake and metabolic activation. Our data further suggest that optimal antiviral concentrations of AZT and ddI may not be achieved at all potential sites of HIV replication. This conclusion is consistent with the observation that AZT fails to completely suppress the HIV during long-term therapy or effectively inhibit viral infection during prophylactic treatment (18, 20, 24). Evaluation of anti-HIV drug efficacy needs to include all cell types and not be limited to lymphoid cells. Further studies to define the kinetics of drug uptake and metabolism to the active triphosphate analogs in the target cells are warranted.

ACKNOWLEDGMENTS

This work was supported in part by research grants 1R01 AI28757 and 1R01 AI29133 from the National Institute of Allergy and Infectious Diseases, NIH, DHHS, research grant 1R01 CA56295, and a Research Career Development Award (K04 CA01497) to J.L.-S. Au from the National Cancer Institute, NIH, DHHS, E. Mukherji was supported in part by a fellowship from the Berlex Corporation.

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