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Phosphatidylazidothymidine and Phosphatidyl-ddC: Assessment of Uptake in Mouse Lymphoid Tissues and Antiviral Activities in Human Immunodeficiency Virus-Infected Cells and in Rauscher Leukemia Virus-Infected Mice

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During the early stages of human immunodeficiency virus (HIV) infection, although symptoms are absent and viral replication in peripheral blood mononuclear cells is low, substantial levels of HIV replication can be documented in lymphoid tissue [G. Pantaleo, C. Graziosi, J. F. Demarest, L. Butini, M. Montroni, C. H. Fox, J. M. Orenstein, D. P. Kotler, and A. S. Fauci, Nature (London) 362:355–358, 1993, and J. Embretsen, M. Zupancic, J. L. Ribas, A. Burke, P. Racz, K. Tenner-Tacz, and A. T. Haase, Nature (London) 362:359–362, 1993]. This observation suggests that earlier treatment of HIV infection may be indicated and that strategies for enhancing drug targeting to the lymphoid tissue reservoirs of HIV infection may be beneficial. To address this issue, we synthesized dioleoylphosphatidyl-ddC (DOP-ddC) and dipalmitoylphosphatidyl-3′-azido-3′-deoxythymidine (DPP-AZT), phospholipid prodrugs which form lipid bilayers and which are readily incorporated into liposomes. The anti-HIV activity of DOP-ddC was similar to that of ddC in HIV type 1-infected HT4-6C cells, but DPP-AZT was considerably less active than AZT in HT4-6C cells. Liposomes containing DOP-[^{3}H]ddC or DPP-[^{3}H]AZT administered intraperitoneally to mice produced greater levels of total radioactivity over time in plasma, spleen, and lymphoid tissue relative to the results with [^{3}H]ddC and [^{3}H]AZT, respectively. DPP-AZT administered intraperitoneally in liposomes as a single daily dose to mice infected with Rauscher leukemia virus prevented increased spleen weight and reverse transcriptase levels in serum with a dose-response roughly comparable to that of AZT given continuously in the drinking water. DOP-ddC, DPP-AZT, and lipid conjugates of other antiretroviral nucleosides may provide higher levels of drug over time in plasma and in lymph nodes and spleen, important reservoirs of HIV infection, and may represent an interesting alternative approach to antiviral nucleoside treatment of AIDS.

In early human immunodeficiency virus (HIV) infection, patients exhibit an initial period of viremia and then a prolonged period of clinical latency during which viremia is relatively low and the number of infected cells in the blood is minimal (3, 17). Recently, lymphoid tissue from patients with early or intermediate HIV infection, as evidenced by CD4 cell counts of either >500 or 200 to 400/mm^3, was found to be heavily infected, demonstrating that HIV replicates preferentially in lymphoid tissue early in the course of disease (3, 17).

Dideoxynucleosides inhibit the replication of HIV and other retroviruses (2, 5, 6, 16, 21–23). Zidovudine (3′-azido-3′-deoxythymidine [AZT]) prolongs survival in patients with advanced AIDS and decreases the frequency and severity of opportunistic infections (5), but it is associated with anemia and neutropenia (6, 18, 22, 23). Zalcitabine (ddC [2′,3′-dideoxycytidine]) is also a potent inhibitor of HIV replication; it decreases serum p24 levels and improves CD4 cell counts in vivo (13–15, 24). However, ddC therapy is associated with a dose-related sensory peripheral neuropathy which limits its utility as a single agent (14, 24).

To enhance the uptake and retention of AZT and ddC in lymph nodes, spleen, and other HIV target tissues, we synthesized phospholipid prodrugs of these compounds for in vitro antiviral evaluation in HIV-infected cells since liposome-associated materials are readily taken up by tissues with elements of the reticuloendothelial system. Liposomal dispersions of tritium-labeled dipalmitoylphosphatidyl-AZT (DPP-AZT) and dioleoylphosphatidyl-ddC (DOP-ddC) were prepared, and pharmacokinetic and tissue distribution studies were carried out in mice. Finally, liposomal DPP-AZT was administered once daily to mice infected with Rauscher leukemia virus (RLV), and its dose-dependent effects on disease parameters were determined and compared with those of AZT given continuously in the drinking water.

MATERIALS AND METHODS

Synthesis of DOP-ddC and DPP-AZT. DOP-ddC and DPP-AZT were synthesized by coupling dioleoyl- or dipalmitoylphosphatidic acid to ddC or AZT, respectively, by using triisopropylbenzenesulfonyl chloride in dry pyridine as described previously (8, 10). The respective compounds were purified on a column of silica gel (Kieselgel 60; E. Merck, Darmstadt, Germany), eluted with 8 to 20% methanol in

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chloroform as described previously, and lyophilized from cyclohexane, and purity was estimated to be >97% by high-pressure liquid chromatography, thin-layer chromatography, and nuclear magnetic resonance imaging (8, 10).

Cells and HIVs. HeLa cells transformed with the CD4 gene (HT4-6C cells) were obtained from Bruce Chesebro, Hamilton, Mont. The effects of the antiviral compounds on HIV replication were measured by a plaque reduction assay (11). Briefly, monolayers of HT4-6C cells were infected with 100 to 300 PFU of virus per well in 24-well microdilution plates. DOP-ddC or DPP-AZT was incorporated into sonicated vesicles as described previously (9, 10). The vesicles consisted of dioleoylphosphatidylcholine (DOPC), dioleoylphosphatidylglycerol (DOPG), and cholesterol (Chol) at a molar ratio of DOPC/DOPG/Chol/drug of 50/10/30/10. Liposome preparations were stored at 4°C after preparation and were used within 10 days, although the preparations are stable at 4°C for in excess of 6 months (4). Various concentrations of ddC, DOP-ddC, AZT, and DPP-AZT were added as indicated to Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Lipid controls matched for total lipid content (DOPC/DOPG/Chol; 60/10/30 mol%) were incubated similarly. After 3 days at 37°C the monolayers were fixed with 10% formaldehyde solution in phosphate-buffered saline and stained with 0.25% crystal violet, and plaques were quantified.

The human lymphoblastoid cell line CEM-CCRF was obtained from the American Type Culture Collection (Rockville, Md.). The cells were infected with HIV-1_{LAV} (L. Montagnier, Paris, France) as noted previously (9). The respective drugs were added at the noted concentrations, and after 3 days of incubation the effects of the drugs were assessed by measurement of p24 antigen in the cell-free supernatant by an enzyme-linked immunosorbent assay (Abbott, North Chicago, Ill.) as described previously (9). The effects of the drugs on viable cell numbers were determined after 3 days by fluorescence-activated cell sorting of cells stained with propidium iodide as reported previously (9).

Distribution of tritium-labeled antiviral nucleosides in mouse tissue. DOP-[5,6-^{3}H]ddC and DPP-[5′-^{3}H]AZT were synthesized as described previously (7), and liposomes consisting of DOPC/DOPG/Chol/DOP/ddC[5,6^{3}H] (59/10/30/1 mol%) were prepared by four cycles of extrusion through stacked polycarbonate filters having a pore diameter of 200 nm (12). In another case, liposomes consisting of hydrogenated egg phosphatidylcholine (EPC-1; Asahi Chemical Ind. Ltd., Tokyo, Japan) and DPP-[^{3}H]AZT (9/1; molar ratio) were prepared by sonication at 65°C as described previously (9, 10). [5,6-^{3}H]ddC or [5′-^{3}H]AZT and liposomes containing DOP-[^{3}H]ddC or DPP-[^{3}H]AZT were injected intraperitoneally into mice at a dose of 3 mg of ddC (or its molar equivalent as DOP-ddC) per kg of body weight or 10 mg of AZT (or its molar equivalent of DPP-AZT) per kg of body weight. The mice were sacrificed at the indicated times, plasma and tissues were removed and homogenized in saline, and aliquots were processed for liquid scintillation counting. Tissues included spleen, lymph node (mesenteric), liver, lung, small intestine, and sciatic nerve. The results are expressed as the mean ± standard deviation nanomoles of (n = 3) [^{3}H]ddC or [^{3}H]AZT metabolites per gram of tissue.

Antiviral nucleoside treatment of RLV-infected mice. (i) DPP-AZT liposome preparation. For in vivo studies in RLV-infected mice, the liposome formulation consisted of 30 mol% DPP-AZT and 70 mol% EPC-1. The aqueous vehicle for the liposomes was 20 mM sodium phosphate (pH 7.4) and 250 mM sorbitol. A total of 382.8 mg of DPP-AZT and 747 mg of EPC-1 were dissolved in approximately 50 ml of chloroform in a round-bottom flask. The chloroform was removed by rotary evaporation in vacuo, and the flask was placed under high vacuum overnight. The dry lipid film was suspended with 60 ml of 20 mM sodium phosphate (pH 7.4)–250 mM sorbitol by vortexing. The suspension was microfluidized in an M-110S microfluidizer (Microfluidics, Newton, Mass.) at an inlet pressure of 80 lb/in^2 for five passes at 50 to 60°C. After the last pass, the liposome formulation was filtered through a sterile 0.2-μm-pore-size filter. The final formulation contained 6.38 mg of DPP-AZT per ml and 12.45 mg of EPC-1 per ml. This formulation was used directly for the 10-mg/kg dose group and was diluted with aqueous vehicle for the lower-dose groups.

(ii) Mice. Female BALB/c mice were obtained from Charles River Laboratories, housed at five per cage in microisolator cages, and supplied with sterile water and food pellets ad libitum. At 5 weeks of age, the mice were inoculated intraperitonealy with 0.1 ml of spleen homogenate from RLV-infected mice. Mice were monitored for health, food, and water daily.

(iii) RLV. The RLV isolate used was originally obtained from J. T. Rankins of the U.S. Army Medical Research Institute for Infectious Diseases. Subsequent passages were produced by making 10% homogenates from the spleens of mice that were inoculated intraperitoneally (i.p.) with RLV 21 days earlier. Titers were determined by the XC plaque assay (20). Nine groups of 10 mice each were inoculated i.p. with 100 μl (1.2×10^6 PFU/ml) of RLV stock.

(iv) Cells. The SC-1 and XC cells used in the XC plaque assay were obtained from the American Type Culture Collection and were grown in minimal essential medium (Gibco) supplemented with 10% fetal bovine serum and antibiotics.

(v) Experimental groups. Two days after the mice were inoculated, treatment began and was continued for 21 days. DPP-AZT was given i.p. once daily, and AZT was given in the drinking water. Treatment groups are indicated. Bottles of AZT solution were changed every other day, and the volumes consumed were recorded for dose calculations. The body weights of the mice were recorded at the conclusion of the experiment to calculate the average daily doses.

(vi) Sample collection. The disease parameters, measured 23 days after inoculation, were spleen weight and reverse transcriptase (RT) activity in serum. After the mice were anesthetized i.p. with ketamine (100 mg/kg) and xylazine (10 mg/kg), blood was collected for assay of RT activity. After cervical dislocation, body and spleen weights were recorded.

(vii) RT assay. Mouse sera were assayed for RT activity to evaluate the effect of treatment and to correlate the results with spleen weight data. A 10-μl serum sample was mixed with 140 μl of RT assay cocktail in 96-well plates. The RT assay cocktail contained the ingredients at the indicated following final concentrations: 50 mM Tris-HCl (pH 8.3), 20 mM dithiothreitol, 0.6 mM $MnCl_2$, 60 mM NaCl, 0.05% Nonidet P-40, 20 μg of poly(rA:dT) per ml, and 30 μM [^{3}H]dTTP (specific activity, 63 Ci/mmol). The reaction mixture was incubated for 2 h at 37°C, and then 50 μl of the reaction mixtures were spotted onto DEAE paper divided into 1-in (2.54-cm) squares. After the paper was dried, it was washed twice in 5% phosphate dibasic buffer, once in distilled water, and twice in 95% ethanol. After the paper squares were thoroughly dried they were analyzed with a scintillation counter.

RESULTS

The effects of ddC and DOP-ddC were examined in HIV-infected HT4-6C cells. ddC and DOP-ddC reduced HIV

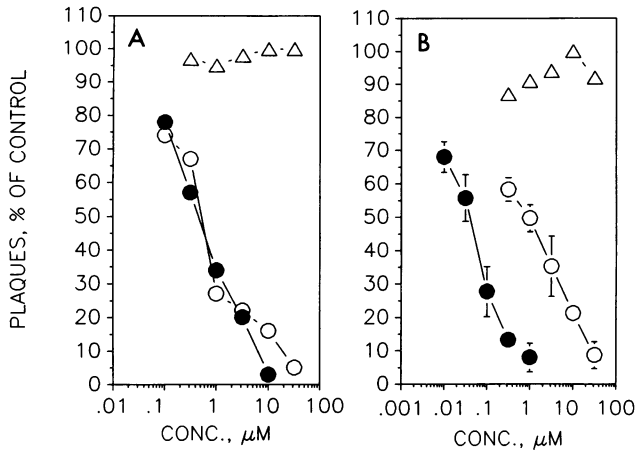

FIG. 1. Inhibition of HIV type 1 replication in HT4-6C cells. (A) Closed circles, ddC; open circles, DOP-ddC; open triangle, lipid control (n = 2). (B) Closed circles, AZT; open circles, DPP-AZT; open triangles, lipid control. Values are means ± standard deviations (n = 3).

plaque formation by 50% (50% inhibitory concentration [IC_{50}]) at 0.6 and 0.4 μM, respectively (Fig. 1). A lipid control without the liponucleotide and matched for total lipid content had no effect on plaque formation. With AZT and DPP-AZT, IC_{50}s of 0.03 and 1.0 μM, respectively, were observed (Fig. 1).

In CEM-CCRF cells infected with HIV-1 the IC_{50} of ddC was 0.05 μM, whereas it was 0.52 μM with DOP-ddC; AZT reduced p24 by 50% at 0.11 μM, while DPP-AZT was less effective, with an IC_{50} of 4.0 μM (Table 1). The toxicities of the antiretroviral nucleosides and their corresponding lipid prodrugs were determined in rapidly dividing CEM-CCRF cells stained with propidium iodide and were analyzed by fluorescence-activated cell sorting. The amount of drug required to reduce the viable cell number by 50% (TC_{50}) was determined. In all cases, the percentage of cells which were viable remained greater than 90% to 3,160 μM drug concentrations, and the observed reduction in viable cell number was due solely to the reduced cell number. ddC was more toxic than DOP-ddC, with TC_{50}s of 2.6 and 31.6 μM, respectively. The in vitro selectivity indices for ddC and DOP-ddC were comparable at 52 and 61, respectively (Table 1). DPP-AZT toxicity was difficult to assess because the vehicle was toxic at doses greater than the dose equivalent to a DPP-AZT dose of 316 μM. At the highest dose, 3,160 μM, equivalent to 31.6 mM total lipid, the vehicle reduced the viable cell numbers by 64%; the presence of 3,160 μM DPP-AZT in the vehicle at this concentration further reduced viable cell numbers by 30%.

To assess retention of the drugs in plasma and tissues, 3 mg of [^{3}H]ddC per kg and the molar equivalent of DOP-[^{3}H]ddC were administered to mice by i.p. injection, and the levels of [^{3}H]ddC and its metabolites were determined by liquid scintillation counting at the indicated times in plasma, spleen, and lymph node (Fig. 2) and in other tissues. Parallel studies were done with [^{3}H]AZT (10 mg/kg) and DPP-[^{3}H]AZT in two different liposome formulations. Free ddC levels were highest in plasma at 15 min (15 μM), and declined rapidly to 0.34 and 0.10 μM at 4 and 6 h, respectively. In contrast, the peak level of DOP-ddC in plasma was more than three times higher (49 μM) at 1 h. DOP-ddC declined more gradually than ddC to 7.9 and 6.0 μM at 4 and 6 h, respectively. In lymph node, peak drug levels were 11.1 nmol/g for ddC versus 4.3 nmol/g for DOP-ddC. However, DOP-ddC levels in lymph node declined much more slowly, to 2.9 and 2.4 nmol/g at 4 and 6 h, respectively, versus 0.18 and 0.12 nmol/g, respectively, for ddC. At 24 h, DOP-ddC levels were 0.21 nmol/g. The levels of ddC and DOP-ddC in sciatic nerve were low and not statistically different at the times studied. For example, at 6 h, sciatic nerve ddC levels were 0.12 ± 0.13 (n = 3) versus 0.04 ± 0.04 (n = 3) for DOP-ddC ($P > 0.05$). With AZT and DPP-AZT in two different liposomal formulations, the profiles of the concentrations in plasma and tissues were generally similar to those observed with ddC and DOP-ddC. AZT and DPP-AZT levels in sciatic nerve were not assessed because peripheral neuropathy is not a major side effect of AZT treatment in humans.

TABLE 1. Effects of ddC, AZT, DOP-ddC, and DPP-AZT on HIV replication in vitro[a]

Drug	IC_{50} (μM) for HT4-6C cells	CEM-CCRF cells		
		IC_{50} (μM)	TC_{50} (μM)	Selectivity index
ddC	0.6	0.05	2.6	52
DOP-ddC	0.4	0.52	31.6	61
AZT	0.03	0.11	700	6,360
DPP-AZT	1.0	4.0	NM	

[a] Antiviral and toxicity assays were carried out as described in Materials and Methods. TC_{50}, 50% toxic concentration, μM; NM, not meaningful. Selectivity index represents toxicity divided by efficacy (TC_{50}/ED_{50}). Determination of DPP-AZT toxicity was not meaningful because the liposome vehicle itself inhibits cell division by 64% at a 31.6 mM total lipid concentration (equivalent to 3.16 mM DPP-AZT). DPP-AZT at 3.16 mM reduced the viable cell numbers by an additional 30%. Thus, the TC_{50} of DPP-AZT is probably >3,160 μM, but the results are difficult to interpret because of vehicle toxicity.

The areas under curve (AUC) from time zero to infinity of liposomal formulations of DOP-[^{3}H]ddC and DPP-[^{3}H]AZT and the corresponding free nucleosides in plasma and various tissues were calculated (Table 2). The AUC of DOP-ddC in spleen and lymph node were 107 and 3.8 times greater, respectively, than those observed with ddC; the AUC in plasma was 14 times greater with DOP-ddC. In sciatic nerve the AUC of DOP-ddC was higher than the AUC observed with free [^{3}H]ddC, but the differences were not statistically significant. DOP-ddC AUC values were also increased relative to those for ddC in the liver, lung, and small intestine (50-, 4.3-, and 5.9-fold greater than those for ddC, respectively). DPP-[^{3}H]AZT administered in sonicated unilamellar liposomes consisting of DOPC/DOPG/Chol/DPP-AZT (50/10/30/10 molar ratio) gave similar results. The AUC of DPP-[^{3}H]AZT in plasma was increased 34-fold relative to that of AZT, and the AUCs of DPP-AZT in the spleen and lymph node were 65- and 5.2-fold higher than those of AZT, respectively. AUCs of DPP-AZT in liver, lung, and small intestine were 33-, 3.4-, and 7.3-fold greater than those of AZT, respectively (Table 2). Generally similar results were obtained when the liposomal formulation consisted of EPC-1/DPP-AZT (9/1 molar ratio), except that the AUC in liver was lower than that observed with DOPC/DOPG/Chol/DPP-AZT liposomes (460 versus 1,351 nmol · h/g). The increased AUC values in tissues appear to be a result of the increased AUC values of the respective lipid prodrug nucleosides in plasma.

To compare the in vivo activity of liposomal DPP-AZT with that of free AZT, we treated mice infected with RLV starting 2 days after infection with either a single i.p. daily dose of DPP-AZT or AZT given continuously in the drinking water. The average spleen weight for the group of untreated mice on day 23 was 1,482 ± 412 mg, whereas it was 93 ± 60 mg for

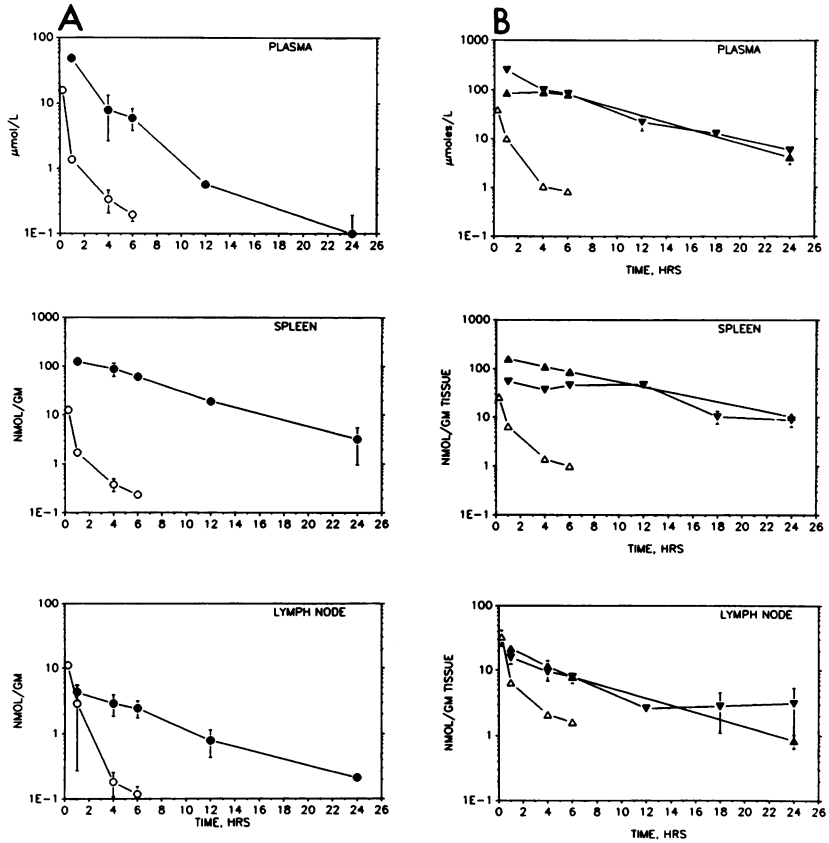

FIG. 2. Levels of [^{3}H]ddC and DOP-[^{3}H]ddC, [^{3}H]AZT, and DPP-[^{3}H]AZT in the plasma, spleen, and lymph nodes of mice after i.p. administration. (A) open circles, [^{3}H]ddC; closed circles, DOP-[^{3}H]ddC. (B) open triangles, [^{3}H]AZT; closed inverted triangles, DPP-[^{3}H]AZT in liposomes consisting of EPC-1/DPP-AZT (9/1 molar ratio); closed triangles, DPP-[^{3}H]AZT in liposomes consisting of DOPC/DOPG/Chol/DPP-AZT (50/10/30/10 molar ratio).

uninfected normal mice (Table 3). DPP-AZT given at 10 mg/kg/day inhibited the increase in spleen weight, with a group average weight of 411 ± 290 mg ($P < 0.01$ versus lipid vehicle), while AZT given continuously via the drinking water at 8.8 mg/kg/day resulted in an average spleen weight of 479 ± 328 mg ($P < 0.001$ versus untreated mice). The doses which inhibited the increase in spleen weight by 50% were 5.7 and 7.3 mg/kg/day for AZT and DPP-AZT, respectively. The difference was not statistically significant. Serum RT levels were also lower with AZT or DPP-AZT treatment; 50% lower serum RT activity versus that in the appropriate control was observed at a dosage of 4 mg/kg/day for both treatments (data not shown).

TABLE 2. AUC from time zero to infinity of free antiviral nucleosides and liposomal liponucleotides in plasma and tissues after i.p. administration to mice[a]

Tissue	AUC (nmol · h/g of tissue)				
	ddC	DOP-ddC	AZT	DPP-AZT/ EPC-1	DPP-AZT/ DOPC
Plasma	10.98	156	34.1	1,284	1,168
Lymph node	9.71	36.8	27.1	129	141
Spleen	9.16	983	22.4	959	1,475
Liver	10.72	537	41.4	460	1,351
Lung	9.12	39.5	52.9	170	179
Small intestine	12.94	76.9	24.4	218	178
Sciatic nerve	2.60	6.07	ND	ND	ND

[a] $AUC_{0-\infty}$ values were determined by using RSTRIP II software. Liposomal preparations of DOP-[^{3}H]ddC and DPP-[^{3}H]AZT were prepared as described in Materials and Methods. ddC was used at a dose of 3 mg/kg, and AZT was used at a dose of 10 mg/kg. The corresponding lipid prodrugs were administered at the respective molar equivalent doses. ND, not determined.

TABLE 3. Effect of treatment with AZT or DPP-AZT on spleen weight in RLV-infected BALB/c mice[a]

Treatment group (dosage [mg/kg/day])	Spleen wt (mg)
Healthy mice	93 ± 60[b]
DPP-AZT (10)	411 ± 290[c]
DPP-AZT (3)	1,068 ± 504
DPP-AZT (1)	1,324 ± 532
DPP-AZT (0.3)	1,374 ± 341
Liposomal vehicle	1,228 ± 336
AZT (8.8)	479 ± 328[b]
AZT (2.7)	1,106 ± 635
AZT (1)	1,211 ± 590
No treatment	1,482 ± 412

[a] Liposomal DPP-AZT and liposome vehicle were administered by i.p. injection once daily; AZT was given continuously in the drinking water. DPP-AZT doses are expressed as the molar equivalent of AZT. Data are means ± standard deviations ($n = 10$) for every group except healthy mice ($n = 3$). Statistical comparisons were made by the Student-Newman-Keuls multiple comparison test (Instat 2, V2.04; GraphPad Software, San Diego, Calif.).

[b] $P < 0.001$ versus no treatment.

[c] $P < 0.01$ versus liposomal vehicle treatment.

RLV studies were not done with ddC and DOP-ddC because murine cells do not phosphorylate ddC readily (1); therefore, the mouse has been regarded as a poor model for use in the evaluation of the antiviral activity of ddC (19).

DISCUSSION

DOP-ddC and DPP-AZT were synthesized chemically by conjugating phosphatidic acid to the 5′-hydroxyl of ddC and were shown to be active in HIV-infected cells in vitro. DOP-ddC activity was equal to that of ddC in HIV-infected HT4-6C cells (IC_{50}, 0.4 to 0.6 μM), while DPP-AZT was considerably less active than AZT. In CEM-CCRF cells, both lipid prodrugs were substantially less active than the corresponding nucleosides. The reasons for the marked difference in the antiviral activities of AZT and ddC in HT4-6C cells versus those in CEM-CCRF cells are most likely due to differences in uptake, intracellular compartmentalization, and metabolism to the triphosphate forms of the nucleosides in the two cell types. Both liponucleotides were slightly more active in HT4-6C cells than in CEM cells. Detailed studies of comparative cell metabolism would be required to evaluate these differences.

DOP-ddC appears to be less toxic than ddC in vitro. The toxicity of DPP-AZT was technically difficult to determine because the lipid vehicle inhibits cell division at lipid levels above 3.16 mM (316 μM DPP-AZT). DPP-AZT itself appears to be relatively nontoxic in vitro since 3.16 mM liponucleotide reduced viable cell number only by an additional 30%. If one is to use lipid conjugates of antiviral nucleosides for targeting key tissues in patients with AIDS, it is important to find lipid groups which provide optimal antiviral activity and cell toxicity profiles.

DOP-ddC and DPP-AZT form lipid bilayers and can be readily incorporated into liposomes. We found that i.p. administration of liposomal DOP-[^{3}H]ddC to mice results in a 107-fold increased AUC of tritium relative to that of [^{3}H]ddC in the spleen and a 3.8-fold increase in the AUC in the lymph node without a statistically significant increase in the level of ddC in the target (sciatic nerve). Generally similar pharmacokinetic results were obtained with DPP-[^{3}H]AZT in two different liposomal formulations. The increased AUC values of DOP-ddC and DPP-AZT in most tissues appeared to be driven in part by the increased AUC in plasma. The AUC values in the liver and spleen exceeded the AUC values in plasma, and the apparent increase in tissue penetration was probably due to liposome uptake by Kupffer cells and spleen macrophages.

DPP-AZT administered in liposomes once a day to mice infected with RLV provided antiviral effects on spleen weight and serum RT levels equivalent to those of AZT administered continuously in the drinking water. This indicates that the increased levels of DPP-AZT noted in tissue can result in the intracellular formation of biologically active AZT, presumably AZT-triphosphate. We have shown previously that phosphatidyl-[^{3}H]AZT is converted to AZT-triphosphate in CEM cells in vitro (7).

We hypothesize that phospholipid prodrugs of antiviral nucleosides may be useful in improving the efficacies and reducing the toxicities of the antiviral agents used in treating HIV infection by providing increased amounts of drug in the spleen and lymph nodes, important sites of local HIV replication during the latent period of AIDS (3, 17). Further evaluations are needed to define the optimal prodrug structure, formulation, and means of administration before further studies in animals are undertaken.

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