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Broad spectrum antiretroviral activity of 2′,3′-dideoxynucleosides

(lentiviruses/Kirsten murine sarcoma virus/antiviral agents)

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ABSTRACT Certain dideoxynucleosides have been shown to markedly inhibit the infectivity of human T-lymphotropic virus type III/lymphadenopathy-associated virus, the causative agent of acquired immunodeficiency syndrome (AIDS). Our present studies demonstrate that these drugs are broad spectrum antiretroviral agents capable of inhibiting the infectivity of evolutionarily divergent mammalian type C and animal lentiviruses. Under some conditions, virus infectivity could be inhibited by more than six orders of magnitude. However, the potency of these agents was shown to be greatly influenced by cell-specified determinants. Drug exposure during the initial 24 hr was almost as effective as prolonged treatment on the inhibition of a single cycle of virus infection and expression. Moreover, virus infection was shown directly to be inhibited at the level of proviral DNA synthesis. Thus the time period during which reverse transcription and provirus integration occur is the critical period required for drug action. Our findings have implications concerning strategies to be considered in attempts to utilize 2′,3′-dideoxynucleosides in control and treatment of retrovirus-induced diseases of animals and humans.

In the past few years, efforts aimed at prevention and control of retroviral-induced diseases have gained increasing importance with accumulating evidence that such viruses are responsible for human as well as animal diseases. A potentially useful strategy has arisen from the application of a group of nucleoside analogues first synthesized over 20 years ago (1–5). These dideoxynucleosides were shown to inhibit the retroviral reverse transcriptase (6–8) as well as cellular DNA polymerases β and γ, while polymerase α was relatively resistant (8–12). Initial studies aimed at demonstrating selective inhibition of viral replication by these drugs showed relatively modest effects (7, 8). Recently, however, 3′-azido-3′-deoxythymidine (N_3dThd; sometimes referred to as AZT) (13) and virtually all of the 2′,3′-dideoxynucleosides (14) analyzed were found to powerfully and selectively inhibit replication of human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV),[§] the retrovirus causative of acquired immunodeficiency syndrome (AIDS).

The AIDS virus has been shown to be closely related to animal lentiviruses (15–17), whereas early investigations of these antiviral drugs focused upon distantly related type C viruses. Thus, it is not known whether the reported differences in their antiviral effects relate to host cell or viral specificities. Recently Mitsuya and his colleagues have shown that these nucleoside analogues act as chain terminators of the reverse transcription products of the incoming HTLV-III/LAV (18), although this need not be the only mechanism for antiretroviral activity. Our present studies demonstrate a broad spectrum of antiretroviral activity by these agents, but show considerable cell variation in the potency of different agents. Under optimal conditions, the selective inhibition of even a single cycle of retroviral infection can be so profound as to suggest that these agents may have broad application in the control of diseases induced by retroviruses of animals and humans (19–26).

MATERIALS AND METHODS

Cells and Viruses. Animal lentiviruses, caprine arthritis encephalitis virus (CAEV) and equine infectious anemia virus (EIAV), were grown in Himalayan tahr (goat) ovary and equine dermis cells, respectively. The murine C type transforming retrovirus, Kirsten murine sarcoma virus (Ki-MuSV), with its associated amphotropic helper virus, amphotropic murine leukemia virus (amp-MuLV), was grown in NIH 3T3 cells. Other cells utilized include rat (NRK Cl13) and human (501T) fibroblast lines.

Nucleosides. The compounds 2′,3′-dideoxyadenosine, 2′,3′-dideoxyinosine, 2′,3′-dideoxycytidine, and 2′-deoxycytidine were purchased from Calbiochem-Behring, whereas 2′,3′-dideoxyguanosine and 2′,3′-dideoxythymidine were purchased from Pharmacia. S. N. Lehrmann and D. Barry (Wellcome Research Laboratories, Research Triangle Park, NC) kindly provided N_3dThd (BWA509U). Fig. 1 illustrates the structures of the dideoxynucleosides, including N_3dThd (AZT), as well as normally utilized 2′-deoxyadenosine.

Infectivity Assays. For measurement of CAEV and EIAV infectivity, cells were grown in 24-well microtiter trays and pretreated for 4–5 hr with the indicated drug. Serial 10-fold dilutions of virus were added to wells (4 wells per dilution), the trays were incubated at 37°C for 1 hr, and drug-containing medium was added to each well. At 2 weeks cultures were observed for cytopathic effect, and/or the culture fluids were tested for viral antigen by RIA. Drug effectiveness was determined by comparing the virus titer on drug-treated as opposed to untreated cells. Virus titer was defined as the reciprocal of the endpoint dilution at which ≈50% of the wells were virus positive.

Focus formation by Ki-MuSV was assayed as previously described (27). Replication of type C helper virus was analyzed by testing tissue culture fluids for the mouse leukemia virus structural protein, p30, by competition RIA (28).

RIAs. The replication of CAEV, EIAV, and type C helper virus was monitored by determining the levels of purified *gag*

Abbreviations: AIDS, acquired immunodeficiency syndrome; HTLV-III, human T-lymphotropic virus III; LAV, lymphadenopathy-associated virus; N_3dThd, 3′-azido-3′-deoxythymidine; CAEV, caprine arthritis encephalitis virus; EIAV, equine infectious anemia virus; Ki-MuSV, Kirsten murine sarcoma virus; amp-MuLV, amphotropic murine leukemia virus.

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§Recently, a subcommittee of the International Committee on the Taxonomy of Viruses has proposed that the causative retrovirus of AIDS be designated as human immunodeficiency virus, HIV [(1986) *Science* **232,** 697, and *Nature (London)* **321,** 10].

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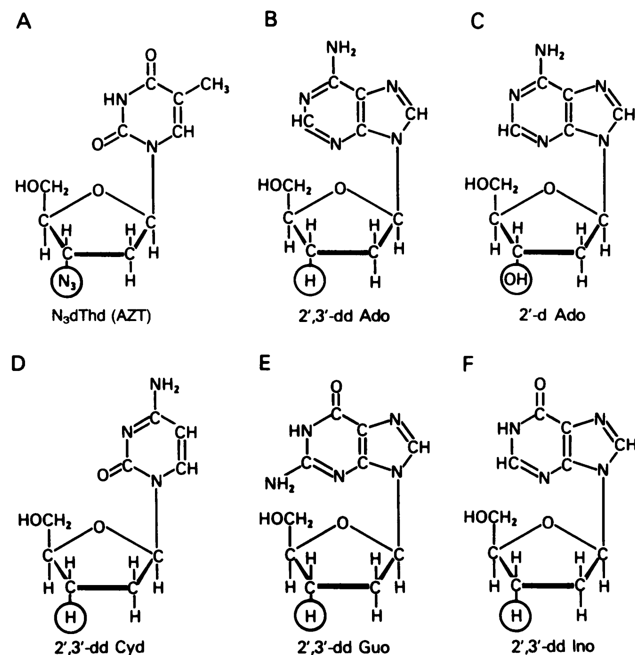

FIG. 1. Structure of dideoxynucleosides. Abbreviations: N_3dThd (AZT), 3'-azido-3'-deoxythymidine; 2',3'-ddAdo, 2',3'-dideoxyadenosine; 2'-dAdo, 2'-deoxyadenosine; 2',3'-ddCyd, 2',3'-dideoxycytidine; 2',3'-ddGuo, 2',3'-dideoxyguanosine; 2',3'-ddIno, 2',3'-dideoxyinosine. The key residues at the 3'-position on the deoxyribose moiety are circled. 2',3'-Dideoxythymidine (2',3'-ddThy) is identical to AZT except that a -H is substituted for the $-N_3$ at the 3'-position of deoxyribose.

protein (p28, p24, and p30, respectively) present in clarified culture supernatants obtained from drug-treated and control cultures. Limiting antibody was incubated with serial dilutions of competing antigen followed by the addition of 10,000 cpm of purified iodinated viral protein (29). The immune complexes were precipitated by the addition of excess antispecies immunoglobulin, collected by centrifugation, and the radioactivity of the pellets was determined as previously described (30).

Southern Blot Analysis of the Effect of N_3dThd on Proviral DNA Synthesis. Unintegrated Ki-MuSV DNA and amp-MuLV DNA was obtained by Hirt extraction (31) of rat NRK C113 cells 24 and 72 hr after infection at a multiplicity of approximately 1 focus forming unit per cell. Half of the cultures were exposed to 100 μM N_3dThd throughout the experiment. After electrophoresis, the DNA was transferred to nitrocellulose and hybridized (at 42°C for 16 hr) with a murine leukemia virus long terminal repeat (LTR) probe (32) in 50% formamide/0.02% (wt/vol) of bovine serum albumin, Ficoll, and polyvinyl pyrrolidone/100 μg of sonicated salmon sperm DNA per ml/10% dextran sulfate/2.5× SSC (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7).

RESULTS

Spectrum of Inhibitory Activity of Dideoxynucleosides Against Animal Lentiviruses. HTLV-III/LAV replication has been shown to be potently inhibited by dideoxynucleosides such as 2',3'-dideoxycytidine and N_3dThd, whereas a related analogue, 2',3'-dideoxythymidine, was markedly less effective (13, 14). We sought to determine whether lentiviruses, to which the AIDS virus is related (15–17), were inhibited by these agents. CAEV, like HTLV-III/LAV, is cytopathic in permissive cells, such as Himalayan tahr ovary. In contrast, EIAV induces no detectable cytopathic effect in equine dermis cells (33).

When CAEV in tissue culture fluids was assayed at 2 weeks following infection in the presence or absence of drug, we observed an excellent correlation between CAEV detectable by RIA and cultures showing cytopathic effect. As shown in Fig. 2, several of the 2',3'-dideoxynucleosides were very effective in inhibiting CAEV replication. In some cases, virus titers were decreased by more than five orders of magnitude. The minimum concentrations required in our assay system to achieve this degree of inhibition were 30 μM for 2',3'-dideoxyguanosine, 1.5 μM for 2',3'-dideoxycytidine, and 50 μM for 2',3'-dideoxyadenosine and 2',3'-dideoxyinosine. In the same assay, 2',3'-dideoxythymidine and N_3dThd were almost completely ineffective, as were the normal nucleosides, thymidine and 2'-deoxycytidine. At the drug concentrations tested, there was no obvious inhibition of cell growth by any of the compounds analyzed (Fig. 1).

We observed striking differences in the effectiveness of several of these compounds when tested against EIAV titered in equine dermis cells. Fig. 3 compares the effect of different concentrations of 2',3'-dideoxyadenosine, 2',3'-dideoxycytidine, and N_3dThd on the replication of CAEV in tahr cells and EIAV in equine cells. It is apparent that while CAEV was completely unaffected by up to 30 μM N_3dThd, EIAV was inhibited by nearly five orders of magnitude by this concentration of the same drug. Thus, EIAV titered in equine dermis cells, as previously shown for HTLV-III/LAV in human lymphocytes (13), appeared to be very sensitive to inhibition by N_3dThd. Less marked differences in drug potency were observed with the other two drugs (Fig. 3, *A* and *B*). 2',3'-Dideoxythymidine, as shown with HTLV-III/LAV (14) and CAEV (Fig. 2) was also ineffective in inhibiting the infectivity of EIAV (data not shown).

Antiretroviral Effectiveness of Dideoxynucleosides Is Cell Specified. The results presented above suggested either that distinct retroviruses exhibited very different sensitivities to specific nucleoside analogues or that the variations in sensitivity observed might result from differences in cellular properties. To investigate these possibilities, we compared the ability of different dideoxynucleosides to inhibit the

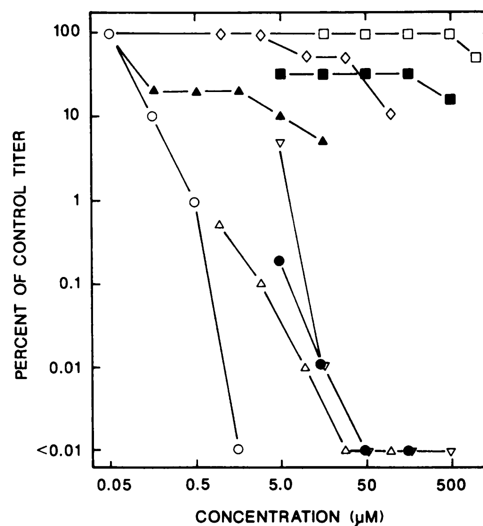

FIG. 2. Inhibition of CAEV replication by dideoxy- and normal nucleosides. Himalayan tahr ovary cells were grown in cluster 24 trays, treated for 4 hr with the indicated concentrations of nucleoside, and infected with serial dilutions of virus. Infected wells were refed with medium containing nucleoside and the presence of virus was determined by RIA 2 weeks later. Effectiveness of drug treatment was defined by the decrease in the viral titer compared with the control viral titer. -△-, 2',3'-ddGuo; -○-, 2',3'-ddCyd; -▽-, 2',3'-ddAdo; -□-, 2',3'-ddThy; -●-, 2',3'-ddIno; -◇-, N_3dThd; -■-, 2'-dAdo; -▲-, 2-deoxycytidine. See legend to Fig. 1 for abbreviations.

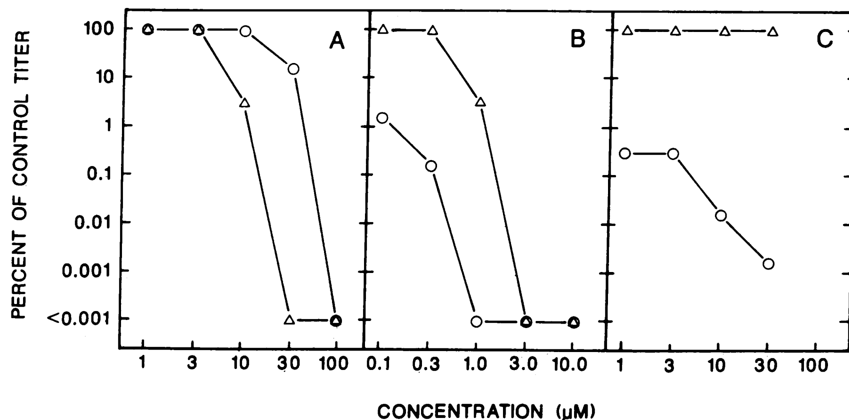

FIG. 3. Comparative inhibition of caprine and equine lentiviruses by dideoxynucleosides. EIAV was measured in equine dermis cells and CAEV was measured in Himalayan tahr ovary cells, as described for Fig. 2. *A*, *B*, and *C* illustrate the effect of different concentrations of 2′,3′-dideoxyadenosine, 2′,3′-dideoxycytidine, and N_3dThd, respectively. ○, Titers of EIAV; △, Titers of CAEV.

infectivity of evolutionarily divergent retroviruses (15) in the same assay cells. As summarized in Table 1, drug effectiveness correlated well with the assay cell utilized and was completely independent of the virus tested. In other studies, we tested the ability of N_3dThd, 2′,3′-dideoxycytidine and 2′,3′-dideoxyadenosine to inhibit the replication of amp-MuLV in cells of human, mouse, and rat origin. The degree of inhibition observed varied by up to five orders of magnitude among the three cell lines analyzed. For example, 2′,3′-dideoxycytidine effectively inhibited amp-MuLV replication in human cells but had no effect on either NIH 3T3 or NRK C113 cells, while N_3dThd was very potent in inhibiting amp-MuLV replication in the mouse and rat cells, and was moderately effective in the human fibroblast cells (data not shown). Thus, we conclude that cellular determinants predominate in determining dideoxynucleoside potency.

In earlier studies, it was shown that 2′,3′-dideoxythymidine must be phosphorylated in order to inhibit *in vitro* activity of the reverse transcriptase of retroviruses (7, 9). Thus, the differences in potency might reflect differences in the levels of the necessary kinase(s) in cells of different tissues or species. Other cellular properties, such as nucleoside pool size and rates of transport into and out of the cell could also be important, because they would be expected to influence the concentration of the drug available to the kinase(s).

Dideoxynucleosides Inhibit Retrovirus Infection at an Early Step. In the above studies, retrovirus infectivity was quantitated under conditions that allowed multiple cycles of replication and virus spread. To more precisely measure the capacity of these agents to inhibit a single cycle of retrovirus integration and expression, we investigated their action on focus formation by Ki-MuSV. It is well established that only a single cycle of virus integration is required for a cell infected by this replication-defective transforming retrovirus to grow as a focus of transformed cells (27).

Following treatment with either 2′,3′-dideoxythymidine or N_3dThd, mouse NIH 3T3 and rat NRK C113 cells were exposed to Ki-MuSV. As shown in Fig. 4, N_3dThd profoundly inhibited a single cycle of Ki-MuSV infection, reducing focus formation by $>10^6$-fold on NIH 3T3 cells and by as much as 10^4-fold in NRK C113 cells. Even at the highest N_3dThd concentration, there was no obvious impairment of cell proliferation. Under identical conditions, 2′,3′-dideoxythymidine did not cause inhibition of Ki-MuSV focus formation in either cell type (Fig. 4). When we compared the effects of the same drugs on amp-MuLV replication by measuring virus present in tissue culture supernatants by RIA at 2 weeks following infection, there was a corresponding and even more striking inhibition (data not shown).

In order to define the time period critical for drug treatment, NRK C113 cells were exposed to 30 μM N_3dThd for different times before and/or after Ki-MuSV infection. As shown in Fig. 5, maximum inhibition was observed when the drug was present throughout the time of the experiment (treatment 5). However, very marked inhibition was seen

Table 1. Dideoxynucleoside inhibition of retrovirus replication is cell-specified

		% of control virus titers		
Assay cell	Virus	30 μM N_3dThd*	5 μM ddCyd	30 μM ddAdo
Tahr ovary	amp-MuLV	100	<0.005	<0.001
Tahr ovary	CAEV	100	<0.005	<0.005
Equine dermis	amp-MuLV	0.2	0.003	2.0
Equine dermis	EIAV	0.03	<0.001	0.3

*The indicated drug concentration was added 4 hr before infection with either amp-MuLV, CAEV, or EIAV. Control virus titers were determined with untreated cultures. The drug was kept in the culture medium for 2 weeks at which time the culture supernatants were tested for the presence of viral antigen by RIA.

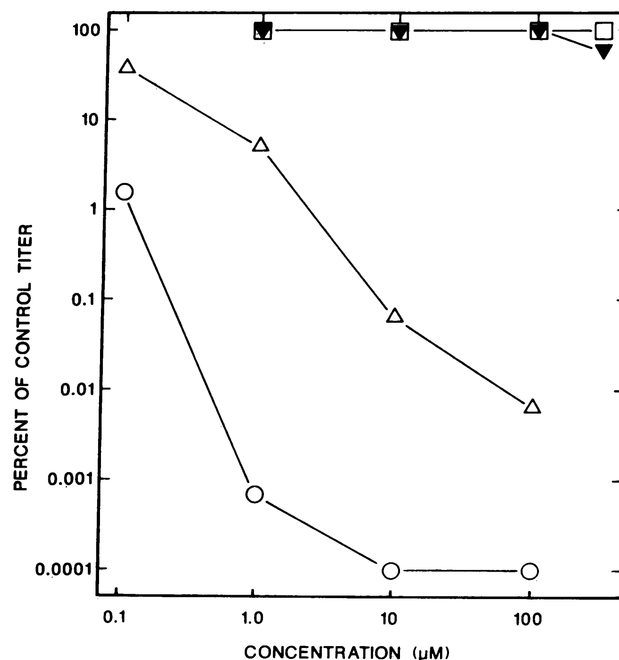

FIG. 4. Comparative inhibition of Ki-MuSV focus formation in both mouse and rat cells by N_3dThd and 2′,3′-dideoxythymidine. NRK C113 and NIH 3T3 cells that had been treated with the indicated concentrations of drug for 4 hr were infected with serial dilutions of virus. Following infection, the cultures were refed with medium containing drug, and foci were counted at 2 weeks. -□-, 2′,3′-dideoxythymidine on NRK C113 cells; -▼-, 2′,3′-dideoxythymidine on NIH 3T3 cells; -△-, N_3dThd on NRK C113 cells; and -○-, N_3dThd on NIH 3T3 cells.

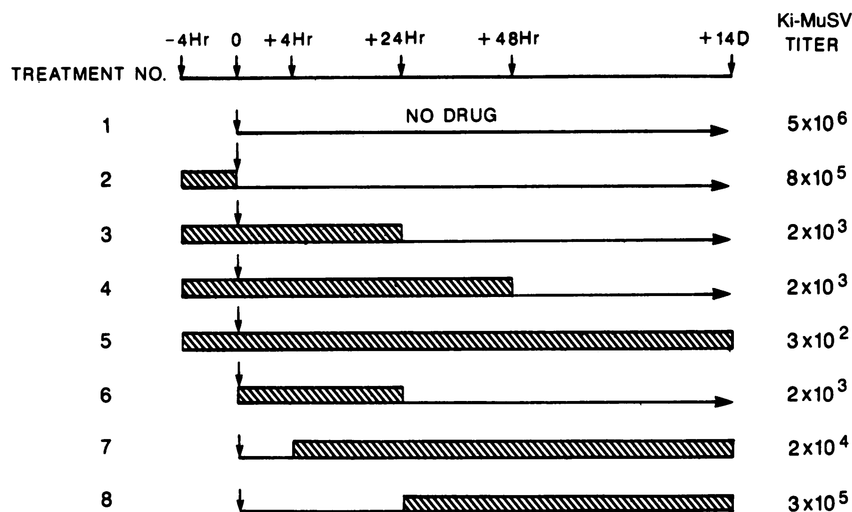

FIG. 5. Effect of varying time of exposure to N_3dThd on Ki-MuSV titer in NRK C113 cells. A series of identical titrations was done, as described for Fig. 4, where all of the plates of a particular titration were exposed to 30 μM N_3dThd for the indicated time. For each treatment regimen (left) the exposure time is indicated by the hatched bar, and the resulting titer is shown at the right.

when the drug exposure was limited to the first 24 hr (treatment 6). Pretreatment for 4 hr alone (treatment 2) led to only a modest effect, as did delay for 24 hr prior to drug addition (treatment 8). All of these findings established that 2',3'-dideoxynucleosides can effectively block the establishment of persistent retrovirus infection by cell exposure during the critical period during which reverse transcription and provirus integration would normally be expected.

The known ability of the phosphorylated 2',3'-dideoxynucleosides to cause DNA chain termination *in vitro* led us to directly investigate the effect of the drugs on proviral DNA synthesis *in vivo*. We analyzed the effect of N_3dThd on synthesis of unintegrated proviral Ki-MuSV and amp-MuLV DNAs in acutely infected NRK cells. As shown in Fig. 6, readily detectable amounts of unintegrated linear forms of both viral DNAs were observed at both 24 and 72 hr postinfection in the absence of drug. The relative increase in unintegrated Ki-MuLV DNA at 72 hr probably reflects its ability to infect

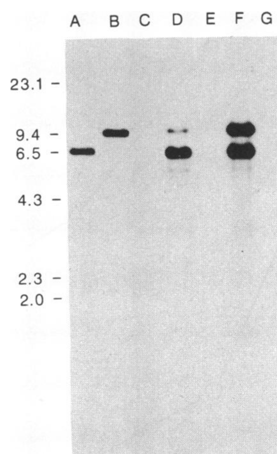

FIG. 6. N_3dThd inhibits synthesis of Ki-MuSV and amp-MuLV proviral DNA. NRK C113 cells were infected with Ki-MuSV and an amphotropic helper virus at a multiplicity of about 1 focus forming unit per cell. Half of the cultures were maintained in 100 μM N_3dThd and at 1 and 3 days postinfection Hirt-extracted DNA was isolated, electrophoresed, blotted onto nitrocellulose, and hybridized with a nick-translated probe derived from the long terminal repeat (LTR) of MuLV (34). The lanes were loaded as follows: A, 700 pg of cloned Ki-MuSV; B, 700 pg of cloned MuLV; lanes C and E, Hirt-extracted DNA from N_3dThd-treated cells infected for 1 and 3 days, respectively; lanes D and F, Hirt-extracted DNA from untreated cells infected for 1 and 3 days, respectively; lane G, Hirt-extracted DNA from an uninfected NRK cell culture.

other cells and become amplified relative to the replication-defective Ki-MuSV genome. Additional DNA species showing faster mobilities were consistent with the expected forms of the supercoiled Ki-MuSV and amp-MuLV DNA (Fig. 6, lanes D and F). In striking contrast, N_3dThd-treated cultures showed no detectable unintegrated proviral DNA at either time point following virus infection. This reflects at least a 300-fold reduction in the synthesis of full-length genomic viral DNA under conditions of drug treatment. Thus, our findings directly establish that 2',3'-dideoxynucleosides effectively block retroviral infection at the level of proviral DNA synthesis.

DISCUSSION

2',3'-Dideoxynucleosides were first synthesized over 20 years ago (1–4). Some of these agents were shown to be effective *in vitro* inhibitors of *Escherichia coli* (35) and mammalian DNA polymerases (9–12), as well as reverse transcriptases (6–8). Because of the modest effects seen with 2',3'-dideoxythymidine in inhibiting Rous sarcoma virus in chicken cells (6) and MuSV and MuLV in rodent cells (8), efforts to further utilize these agents were not actively pursued. Recently, other 2',3'-dideoxynucleosides, but not 2',3'-dideoxythymidine, have been shown to be potent inhibitors of HTLV-III/LAV infectivity in tissue culture (13, 14). Our present studies demonstrate that 2',3'-dideoxynucleosides are broad spectrum antiretroviral agents. They were able to reduce infectivity of animal lentiviruses and evolutionarily divergent mammalian type C retroviruses by several orders of magnitude under optimal conditions with minimal impairment in cellular growth properties.

We did observe striking differences in drug potency among different cell lines tested. Although the triphosphate derivatives of the 2',3'-dideoxynucleosides are known to be their active forms in *in vitro* reactions, triphosphates are not taken up by cells and, hence, the nonphosphorylated analogues have been utilized in these cell culture studies. Thus, variation in effectiveness of the 2',3'-dideoxynucleosides from cell line to cell line may reflect variations in endogenous levels of the relevant kinases and the corresponding phosphorylases. The active phosphorylated analogues are thought to act as chain terminators once they are incorporated into the nascent chain during DNA synthesis (36–38). Indeed, Mitsuya *et al.* (18) have shown that phosphorylated dideoxynucleotides terminate DNA chains synthesized *de novo* by HTLV-III/LAV reverse transcriptase. There is some evidence that the level of a specific kinase is correlated with the effectiveness of a particular dideoxynucleoside. The conversion of 2',3'-dideoxynucleoside to phosphorylated derivatives is slow both in *E. coli* (36) and in most mammalian cells (12).

Variations in the levels of deoxycytidine kinase were reported to vary over a several 100-fold range in a series of human, mouse, and caprine cells (39). This level is particularly low in NIH 3T3 cells where we determined that 2′,3′-dideoxycytidine was ineffective at inhibiting retrovirus infectivity.

N_3dThd was also a potent inhibitor of retrovirus infectivity in many of the cell lines tested. Interestingly, its spectrum of activity was different from that of most of the 2′,3′-dideoxynucleosides. For example, it was particularly effective in rodent cell lines, NIH 3T3 and NRK, where the 2′,3′-dideoxynucleosides were almost without effect. In Himalayan tahr ovary cells, on the other hand, N_3dThd lacked activity, and the 2′,3′-dideoxynucleosides, except for 2′,3′-dideoxythymidine, were highly potent. It is not fully understood whether N_3dThd uses the same enzymes as the corresponding 2′,3′-dideoxynucleosides to become phosphorylated, although Furman *et al.* (40) have shown that triphosphorylated N_3dThd acts as a chain terminator. Other variables that might influence cell responsiveness to different nucleoside analogues could include nucleotide pool size as well as the rate of uptake of a particular compound.

We established that the first 24 hr of infection was the time period of drug exposure critical for its effectiveness. This corresponds to the time during which reverse transcription and proviral integration would be expected, consistent with the postulated action of these agents on the reverse transcription process. In a direct test of this, we showed that N_3dThd-treatment of susceptible cells resulted in the profound inhibition of the synthesis of unintegrated proviral DNA (Fig. 6). No inhibition of virus expression was observed if drug exposure was delayed 24 hr, implying little or no ability of these agents to inhibit expression of the integrated provirus. The magnitude of inhibition of a single cycle of virus infection was shown to be up to greater than six orders of magnitude, a profound effect considering the lack of detectable inhibition of cell proliferation under the same conditions.

The efficacy of these agents in cell culture is consistent with their potential applicability in the treatment or control of a variety of retroviral-induced diseases of animals and humans. However, our findings of major differences in susceptibility to these drugs among cell lines indicate a need for a systematic analysis of the response to dideoxynucleosides at the tissue and species level. This type of approach may reveal a combination of these agents that would most likely be effective in inhibiting retrovirus infection and spread. The tremendous potency of these agents in inhibiting a single round of virus infectivity further argues that a likely *in vivo* application would be in short term prophylaxis under conditions of high risk of retrovirus transmission, or in diseases in which target cell regeneration is possible and prevention of viral spread to uninfected target cells could still confer a benefit to the host.

1. Robins, M. J. & Robins, R. K. (1964) *J. Am. Chem. Soc.* **86,** 3585–3586.
2. Horwitz, J. P., Chua, J. & Noel, M. (1964) *J. Org. Chem.* **29,** 2076–2078.
3. Horwitz, J. P., Chua, J., Noel, M. & Donatti, J. T. (1967) *J. Org. Chem.* **32,** 817–818.
4. Doering, A. M., Jansen, M. & Cohen, S. S. (1966) *J. Bacteriol.* **92,** 569–574.
5. Lin, T.-S. & Prusoff, W. H. (1978) *J. Med. Chem.* **21,** 109–112.
6. Smoler, D., Molineux, I. & Baltimore, D. (1971) *J. Biol. Chem.* **246,** 7697–7700.
7. Faras, A. J., Taylor, J. M., Levinson, W. E., Goodman, H. M. & Bishop, J. M. (1973) *J. Mol. Biol.* **79,** 163–183.
8. Furmanski, P., Bourguignon, G. J., Bolles, C. S., Corombos, J. D. & Das, M. R. (1980) *Cancer Lett.* **8,** 307–315.
9. Wagar, M. A., Evans, M. J. & Huberman, J. A. (1978) *Nucleic Acids Res.* **5,** 1933–1946.
10. Ono, K., Ogasawara, M. & Matsukage, A. (1979) *Biochem. Biophys. Res. Commun.* **88,** 1255–1262.
11. Krokan, H., Schaffer, P. & DePamphilis, M. L. (1979) *Biochemistry* **18,** 4431–4443.
12. Wagar, M., Evans, M. J., Manly, K. F., Hughs, R. G. & Huberman, J. A. (1984) *J. Cell. Physiol.* **121,** 402–408.
13. Mitsuya, H., Weinhold, K. J., Furman, P. A., St. Clair, M. H., Lehrman, S. N., Gallo, R. C., Bolognesi, D., Barry, D. W. & Broder, S. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7096–7100.
14. Mitsuya, H. & Broder, S. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1911–1915.
15. Chiu, I.-M., Yaniv, A., Dahlberg, J. E., Gazit, A., Skuntz, S. F., Tronick, S. R. & Aaronson, S. A. (1985) *Nature (London)* **317,** 366–368.
16. Gonda, M. A., Wong-Staal, F., Gallo, R. C., Clements, J. F., Narayan, O. & Gilden, R. V. (1985) *Science* **227,** 173–177.
17. Sonigo, P., Alizon, M., Staskus, K., Klatzmann, D., Cole, S., Danos, O., Retzel, E., Tiollais, P., Haase, A. & Wain-Hobson, S. (1985) *Cell* **42,** 369–382.
18. Mitsuya, H., Jarrett, R. F., Matsukura, M., Di Marzo Veronese, F., DeVico, A., Sarngadharan, M. G., Johns, D. G., Reitz, M. S. & Broder, S. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2033–2037.
19. Poiesz, B. J., Ruscetti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. & Gallo, R. C. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 7415–7419.
20. Barré-Sinoussi, F., Chermann, J. C., Rey, F., Nugeybe, M. T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinek-Brun, F., Rouzioux, C., Rozenbaum, W. & Montagnier, L. (1983) *Science* **220,** 868–870.
21. Gallo, R. C., Salahuddin, S. Z., Popovic, M., Shearer, G. M., Kaplan, M., Haynes, B. F., Palker, T. J., Redfield, R., Oleske, J., Safai, B., White, G., Foster, P. & Markham, P. D. (1984) *Science* **224,** 500–502.
22. Daniel, M. D., King, N. W., Letvin, N. L., Hunt, R. D., Sehgal, P. K. & Desvosiers, R. C. (1984) *Science* **223,** 602–605.
23. Kanki, P. J., McLane, M. F., King, N. W., Letvin, N. L., Hunt, R. D., Sehgal, P., Daniel, M. D., Desvosiers, R. C. & Essex, M. (1985) *Science* **228,** 1199–1201.
24. Daniel, M. D., Letvin, N. L., King, N. W., Kannagi, M., Sehgal, P. K., Hunt, R. D., Kanki, P. J., Essex, M. & Desvosiers, R. C. (1985) *Science* **228,** 1201–1204.
25. Kanki, P. J., Barin, F., M'Boup, S., Allan, J. S., Romet-Lemonne, J. L., Marlink, R., McLane, M. F., Lee, T.-H., Arbeille, B., Denis, F. & Essex, M. (1986) *Science* **232,** 238–243.
26. Gessain, A., Vernant, J. C., Maurs, L., Barin, F., Gout, O. & de The, G. (1985) *Lancet* **ii,** 407–410.
27. Aaronson, S. A. & Rowe, W. P. (1970) *Virology* **42,** 9–19.
28. Hino, S., Stephenson, J. R. & Aaronson, S. A. (1976) *J. Virol.* **18,** 933–941.
29. Greenwood, F. C., Hunter, W. M. & Glover, J. S. (1963) *Biochem. J.* **89,** 114–123.
30. Dahlberg, J. E., Tronick, S. T. & Aaronson, S. A. (1980) *J. Virol.* **33,** 552–530.
31. Hirt, B. (1967) *J. Mol. Biol.* **26,** 365–369.
32. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
33. Malmquist, W. A., Barnett, D. & Becvar, C. S. (1973) *Arch. Virol.* **42,** 361–370.
34. Lowy, D. R., Rands, E., Chattopadhyay, S. K., Garon, C. F. & Hager, G. L. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 614–618.
35. Toji, L. & Cohen, S. S. (1970) *J. Bacteriol.* **103,** 323–328.
36. Atkinson, M. R., Deutscher, M. P., Kornberg, A., Russell, A. F. & Moffett, J. G. (1969) *Biochemistry* **8,** 4897–4904.
37. Toji, L. & Cohen, S. S. (1969) *Proc. Natl. Acad. Sci. USA* **63,** 871–877.
38. Byars, N. & Kidson, C. (1975) *Biochemistry* **14,** 3159–3164.
39. Cooney, D. A., Dalal, M., Mitsuya, H., McMahon, J. B., Nadkarni, M., Balzarini, J., Broder, S. & Johns, D. G. (1986) *Biochem. Pharmacol.* **35,** 2065–2068.
40. Furman, P. A., Fyfe, J. A., St. Clair, M. H., Weinhold, K., Rideout, J. L., Freeman, G. A., Lehrman, S. N., Bolognesi, D. P., Broder, S., Mitsuya, M. & Barry, D. W. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8333–8337.