Dideoxynucleosides Are Less Inhibitory In Vitro against Human Immunodeficiency Virus Type 2 (HIV-2) than against HIV-1

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The antiviral activities of various dideoxynucleosides against LAV strains of human immunodeficiency viruses type 1 (HIV-1) and type 2 (HIV-2) were evaluated. Significantly more 3'-azido-3'-deoxythymidine was required to inhibit the replication of HIV-2 than HIV-1 in three human cell lines. HIV-2 also appeared more resistant than HIV-1 to other dideoxynucleosides. These results suggest that dideoxynucleosides may be less effective in vivo for HIV-2 infection and that a broader range of clinical isolates of human retroviruses should be examined for drug susceptibility in vitro.

Human immunodeficiency virus (HIV) is the etiologic agent of the acquired immunodeficiency syndrome (AIDS) (1, 9, 12). The first serotype of HIV, designated HIV-1, is responsible for the current pandemic of AIDS.

The most potent inhibitors of replication of HIV-1 yet identified are dideoxynucleosides, which include 3'-azido-3'-deoxythymidine (AZT), azidothymidine, zidovudine, 2',3'-dideoxycytidine (ddC), and 2',3'-dideoxyadenosine (ddA) (13, 14). To become active, these drugs must be phosphorylated by host cells, and as 5'-triphosphates they inhibit the viral reverse transcriptase and may terminate viral DNA chain elongation (4, 8). AZT reduces morbidity and delays mortality in patients with AIDS and the AIDS-related complex (6, 15, 17). The drug also increases CD4⁺ cell counts, improves cutaneous delayed hypersensitivity, reduces the frequency of serious opportunistic infections, and reduces levels of viral p24 antigenemia; however, it does not significantly reduce the rate of recovery of virus from peripheral blood samples from treated patients (6, 15, 17). ddC is under phase 1 investigation in similar patient populations.

More recently, a second human lentivirus, HIV-2, was isolated from patients with AIDS and the AIDS-related complex (2, 3). The circulation of HIV-2 is now focused in West Africa. The genome of HIV-2 has been cloned and sequenced (10). HIV-2 is serologically distinct from HIV-1, and its genome hybridizes poorly with DNA probes prepared from HIV-1 (2, 3, 10). Nevertheless, the two viruses can produce similar disease, resemble each other morphologically, share CD4⁺ cell lymphotrophism and cytopathogenicity, and possess homologous structural and replicative proteins (10) including reverse transcriptase, the target of inhibition by dideoxynucleosides. In this report, these compounds, especially AZT, are shown to be less effective antiviral agents in vitro against HIV-2 than against HIV-1.

MATERIALS AND METHODS

Cells. The human T-lymphoblastoid cell line CCRF-CEM (7), the human monoblastoid cell line U937 (16), and the human T-cell leukemia virus type 1-transformed human T-lymphoblastoid cell line MT-2 (11) were obtained and propagated in RPMI 1640 medium containing 100 U of penicillin G per ml, 100 µg of streptomycin per ml, 2 mM glutamine, and 10% fetal bovine serum (Hyclone Laboratories, Inc., Logan, Utah).

Viruses. The LAV-1 strain of HIV-1 and the LAV-2 (I-502) strain of HIV-2 were generously provided by F. Barre-Sinoussi, J.-C. Chermann, and L. Montagnier, Institut Pasteur, Paris, France. The viruses have been passaged in CEM cells. Cell-free virus pools with a titer of $10^{8.0}$ 50% tissue culture infective doses per ml for HIV-1 and $10^{7.0}$ 50% tissue culture infective doses per ml for HIV-2 were prepared in CEM cells and titrated in MT-2 cells by terminal dilution in 96-well microtiter plates (T. Haertle, C. J. Carrera, J. S. McDougal, L. C. Sowers, D. D. Richman, and D. A. Carson, *J. Biol. Chem.*, in press).

Drugs. AZT was provided by S. Lehrman, Burroughs-Wellcome Co., Research Triangle Park, N.C. ddC was provided by I. Sim, Hoffmann-La Roche, Inc., Nutley, N.J. ddA and 3'-deoxythymidine (dT) (Sigma Chemical Co., St. Louis, Mo.), 2',3'-dideoxyguanosine (ddG) (Pharmacia Fine Chemicals, Piscataway, N.J.), and 2',3'-dideoxyinosine (ddI) (Calbiochem-Behring, La Jolla, Calif.) were purchased. These compounds were diluted in culture medium.

Antiviral drug assays. Inhibition of syncytium formation was assayed in 96-well microtiter plates using 6×10^4 MT-2 cells per well infected with 0.1 50% tissue culture infectious doses of virus per cell in medium containing serial 1/2-log₁₀ dilutions of drug (Haertle et al., in press). Inhibition of the yield of virus infectivity in CEM cells and U937 cells was determined by incubating cells in serial 1/2-log₁₀ dilutions of drug for 24 h in the wells of a 96-well microtiter plate with 6×10^4 cells per well and then adding a cell-free virus suspension to yield a final multiplicity of infection of 1 50% tissue culture infective dose per cell for 60 min at 37°C. The cells were then washed three times by centrifugation and suspended in medium containing the drug. After incubation at 37°C for 5 days, cell-free medium was assayed for infectivity by terminal dilution in MT2 cells.

RESULTS AND DISCUSSION

The inhibition by AZT of the production of cytopathology in MT-2 cells required more than 300-fold more drug for HIV-2 than HIV-1 (Table 1). ddC was threefold less effective against HIV-2 than against HIV-1, but it still showed efficacy at less than 0.1 µM. The other dideoxynucleosides were 10-fold less effective against HIV-2 than against HIV-1.

The inhibition of yield of infectious virus by these drugs in two different CD4⁺ human cell lines was then examined (Fig. 1). In both lines, AZT was significantly less effective

FIG. 1. Inhibition of yield of infectious HIV-1 (●) and HIV-2 (○). Similar results were obtained in duplicate experiments. Uninfected cell viability as measured by cell numbers and trypan blue vital staining was not significantly affected by the drugs at the concentrations used.

against HIV-2 than HIV-1, and ddC and ddA were equivalent or somewhat less effective against HIV-2 in these two cell lines.

Animal retroviruses have been shown to be more sensitive (murine) or less sensitive (caprine) than HIV-1 to AZT. Such differences have been attributed to different levels of host cell thymidine kinase activity which is necessary to generate the active AZT triphosphate (5, 8). Replication of HIV-1 has been shown not to be susceptible to dideoxynucleosides in primary human macrophages, because these cells are deficient in deoxynucleoside kinase activities (15a). The differences observed between these two viruses in this study, however, occurred when compared in the same cells when three different cell lines were used. Previous reports of HIV-1 susceptibility to dideoxynucleosides have indicated very similar levels of susceptibility to those obtained in this study; however, only a narrow range of HIV-1 strains has been evaluated (8, 13, 14). More variation in susceptibility among wild-type HIV-1 strains may be documented in the future.

The increased resistance of HIV-2 to dideoxynucleosides, especially AZT, which inhibited the virus poorly below 10 μ M, is of great concern. This virus causes life-threatening

disease (2, 3) and only 1 to 2 μ M AZT is readily attainable in the plasma (17). ddC remains potent against both viruses; however, this drug has only just entered clinical evaluation. The results reported in this study suggest that the reverse transcriptase of HIV-2 may have different substrate specificities from those of the same enzyme of HIV-1, that AZT may be ineffective therapy for HIV-2 infection, and that the relative susceptibility of multiple additional isolates of HIV-1 and HIV-2 to dideoxynucleosides should be documented. Each of these possibilities merits further investigation.

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LITERATURE CITED

1. Barre-Sinoussi, F., J. C. Chermann, J. Rey, M. T. Nugebe, S. Chamaret, J. Gruet, C. Dauge, C. Axler-Blin, F. Vezinek-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier. 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome. *Science* **220**:868-871.
2. Brun-Vezinet, F., C. Katlama, D. Roulot, L. Lenoble, M. Alizon, J. J. Madjar, M. A. Rey, P. M. Girard, P. Yeni, F. Clavel, S. Gadelles, and M. Harzic. 1987. Lymphadenopathy-associated virus type 2 in AIDS and AIDS-related complex. *Lancet* **i**:128-132.
3. Clavel, F., D. Guetard, F. Brun-Vezinet, S. Chamaret, M. A. Rey, M. O. Santos-Ferriera, A. G. Laurent, C. Dauge, C. Katlama, C. Rouzioux, D. Klatzmann, J. L. Champalimaud, and L. Montagnier. 1986. Isolation of a new human retrovirus from West African patients with AIDS. *Science* **233**:343-346.
4. Cooney, D. A., M. Dalal, H. Mitsuya, J. B. McMahon, M. Nadkarni, J. Balzani, S. Broder, and D. G. Johns. 1986. Initial studies on the cellular pharmacology of 2',3'-dideoxycytidine, an inhibitor of HTLV-III infectivity. *Biochem. Pharmacol.*

TABLE 1. MIC inhibiting virus-induced syncytium formation in MT-2 cells

Drug	MIC ^a (μ M) for:	
	HIV-1	HIV-2
AZT	0.3	>100
ddC	0.01	0.03
ddA	3	30
ddI	1	10
ddG	10	100
ddT	>100	>100

^a The lowest concentration of drug to inhibit syncytium formation 3 days after infection is indicated. Similar results were obtained in four separate experiments, each involving the use of duplicate wells for each drug dilution.

- 35:2065-2068.
5. Dahlberg, J. E., H. Mitsuya, S. B. Blam, S. Broder, and S. A. Aaronson. 1987. Broad spectrum antiretroviral activity of 2',3'-dideoxynucleosides. *Proc. Natl. Acad. Sci. USA* **84**:2469-2473.
6. Fischl, M. A., D. D. Richman, M. H. Grieco, M. S. Gottlieb, P. A. Volberding, O. L. Laskin, J. M. Leedom, J. E. Groopman, D. Mildvan, R. T. Schooley, G. G. Jackson, D. T. Durack, D. King, and the AZT Collaborative Working Group. 1987. The efficacy of 3'-azido-3'-deoxythymidine (azidothymidine) in the treatment of patients with AIDS and AIDS-related complex: a double-blind placebo-controlled trial. *N. Engl. J. Med.* **317**: 185-191.
7. Foley, E. G., H. Lasarus, S. Farber, B. G. Uzman, B. A. Boone, and R. E. McCarthy. 1965. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. *Cancer* **18**:522-529.
8. Furman, P. A., J. A. Fyfe, M. H. St. Clair, K. Weinhold, J. L. Rideout, G. A. Freeman, S. Nusinoff-Lehrman, D. P. Bolognesi, S. Broder, H. Mitsuya, and D. W. Barry. 1986. Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. *Proc. Natl. Acad. Sci. USA* **83**:8333-8337.
9. Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Hanes, T. J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, P. Foster, and P. D. Markham. 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* **224**:500-503.
10. Guyader, M., M. Emerman, P. Sonigo, F. Clavel, L. Montagnier, and M. Alizon. 1987. Genome organization and transactivation of the human immunodeficiency virus type 2. *Nature (London)* **326**:662-669.
11. Harada, S., Y. Koyanagi, and N. Yamamoto. 1985. Infection of HTLV-III/LAV in HTLV-1 carrying cells MT-2 and MT-4 and application in a plaque assay. *Science* **229**:563-566.
12. Levy, J. A., A. D. Hoffman, S. M. Kramer, J. A. Landis, J. M. Shimabukuro, and L. S. Oshiro. 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. *Science* **225**:840-842.
13. Mitsuya, H., and S. Broder. 1986. Inhibition of the *in vitro* infectivity and cytopathic effect of human T-lymphotrophic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by 2',3'-dideoxynucleosides. *Proc. Natl. Acad. Sci. USA* **83**:1911-1915.
14. Mitsuya, H., K. J. Weinhold, P. A. Furman, M. H. St. Clair, S. Nusinoff-Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, and S. Broder. 1985. 3'-Azido-3'-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus *in vitro*. *Proc. Natl. Acad. Sci. USA* **82**:7096-7100.
15. Richman, D. D., M. A. Fischl, M. H. Grieco, M. S. Gottlieb, P. A. Volberding, O. S. Laskin, J. M. Leedom, J. E. Groopman, D. Mildvan, M. S. Hirsch, G. G. Jackson, D. T. Durack, S. Nusinoff-Lehrman, and the AZT Collaborative Working Group. 1987. The toxicity of 3'-azido-3'-deoxythymidine (azidothymidine) in the treatment of patients with AIDS and AIDS-related complex: a double-blind, placebo-controlled trial. *N. Engl. J. Med.* **317**:192-197.
- 15a. Richman, D. D., R. S. Kornbluth, and D. A. Carson. 1987. Failure of dideoxynucleosides to inhibit HIV replication in cultured human macrophages. *J. Exp. Med.* **166**:1144-1149.
16. Sundstrom, C., and K. Nilsson. 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U937). *Int. J. Cancer* **17**:565-577.
17. Yarchoan, R., R. W. Klecker, K. J. Weinhold, P. D. Markham, H. K. Lyerly, D. T. Durack, E. Gelmann, S. Nusinoff-Lehrman, R. M. Blum, D. W. Barry, G. M. Shearer, M. A. Fischl, H. Mitsuya, R. C. Gallo, J. M. Collins, D. P. Bolognesi, C. E. Myers, and S. Broder. 1986. Administration of 3'-azido-3'-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS-related complex. *Lancet* **i**:575-580.