ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1996, p. 1270–1273 Vol. 40, No. 5
0066-4804/96/$04.00+0

Comparative Kinetic Analyses of Interaction of Inhibitors with Rauscher Murine Leukemia Virus and Human Immunodeficiency Virus Reverse Transcriptases

JULIE M. CHERRINGTON,[1] MICHAEL D. FULLER,[1] ANDREW S. MULATO,[1] SIMON J. W. ALLEN,[1] STEVEN C. KUNDER,[2] MICHAEL A. USSERY,[2] ZHIGNEW LESNIKOWSKI,[3,4] RAYMOND F. SCHINAZI,[3,4] JEAN-PIERRE SOMMADOSSI,[5] AND MING S. CHEN[1]*

Gilead Sciences, Foster City, California 94404[1]; Food and Drug Administration, Rockville, Maryland 20857[2]; Georgia Research Center for AIDS and HIV Infections, Veterans Affairs Medical Center (Atlanta), Decatur, Georgia 30033[3]; Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30322[4]; and Division of Clinical Pharmacology, Department of Pharmacology, Center for AIDS Research, University of Alabama at Birmingham, Birmingham, Alabama 35294[5]

Received 20 November 1995/Returned for modification 29 December 1995/Accepted 26 February 1996

The inhibitory effects of several nucleoside triphosphate analogs on Rauscher murine leukemia virus (RMuLV) and human immunodeficiency virus (HIV) type 1 reverse transcriptases (RTs) were studied. With RNA as the template, the apparent K_m and apparent K_i values of HIV RT toward its substrates and inhibitors are 12 to 500 times lower than the corresponding values for RMuLV RT. However, the K_i/K_m ratios (inhibition efficiencies) for HIV and RMuLV RTs are similar for AZTTP (zidovudine triphosphate), d4TTP [3′-deoxythymidine-2′-ene-(3′-deoxy-2′,3′-didehydrothymidine) triphosphate], PMEADP [9-(2-phosphonylmethoxyethyl)adenine diphosphate], FIAUTP [1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-iodouracil triphosphate], and HPMPCDP [(*S*)-1-(3-hydroxy-2-phosphylmethoxypropyl)cytosine diphosphate]. With DNA as the template, the K_m values are similar for HIV and RMuLV RTs. However, the K_i/K_m values of HIV and RMuLV RTs are significantly different for ddCTP, ddATP, and 3TCTP (2′,3′-dideoxy-3′-thiacytidine). The RTs of RMuLV and HIV are sufficiently different from one another that the kinetic inhibition constants for a particular antiviral compounds should be determined to indicate whether anti-RMuLV activity is likely to be predictive for the anti-HIV activity of the compound. This information, in conjunction with species-specific drug metabolism differences and tissue culture antiviral activity, is important in determining the suitability of a particular animal model.

Rauscher murine leukemia virus (RMuLV) (15) has been pursued as a murine antiretroviral drug screening model for human immunodeficiency virus (HIV) infection in humans. Several features of RMuLV make it an attractive model: RMuLV is infectious and pathogenic in adult animals, it has a short incubation period, and RMuLV splenomegaly is proportional to the virus titer (5). Furthermore, AZT (zidovudine) and PMEA [9-(2-phosphonylmethoxyethyl)adenine] have been shown to provide antiviral protection in mice infected with RMuLV (10a, 16). Reverse transcriptase (RT) is the target of antiviral nucleoside and nucleoside phosphonate analogs after their phosphorylation to deoxynucleoside triphosphate (dNTP) equivalents. Therefore, a kinetic analysis of interactions between these two RTs and various inhibitors was conducted.

The triphosphates of d4T [3′-deoxythymidine-2′-ene-(3′-deoxy-2′,3′-didehydrothymidine)] and 3TC (2′,3′-dideoxy-3′ thiacytidine) were synthesized by the method of Ludwig and Eckstein (12). The triphosphates of FIAU [1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-iodouracil] and the analogous diphosphates of PMEA and HPMPC [(*S*)-1-(3-hydroxy-2-phosphylmethoxypropyl)cytosine] were synthesized as described previously (3, 4). All other dNTPs were obtained commercially.

Concentrated 1,000× HIV type 1 (HIV-1) virions were purchased from Advanced Biotechnologies (Columbia, Md.). These virions were disrupted with 0.2% Triton X-100 and used as the source of HIV RT. RMuLV virions were harvested from RMuLV-infected SC-1 cell culture supernatant. The supernatant (250 ml) was clarified at 400 × *g* for 10 min and then filtered through a 0.45-μm-pore-size filter. The virions were collected by centrifugation at 100,000 × *g* for 90 min. The RMuLV pellet was resuspended in 2 ml of 20 mM Tris-HCl (pH 7.5), 20% glycerol, 1 mg of bovine serum albumin per ml, 5 mM β-mercaptoethanol, and 1% Nonidet P-40 and used as the source of RMuLV RT. The activities of HIV RT and RMuLV RT remained linear for at least 1 h during the assay.

Enzyme assays for HIV RT were carried out as described by Cherrington et al. (3) using either activated calf thymus DNA, poly(rA) · oligo(dT)$_{12–18}$ or a defined DNA-RNA primer template. One unit of RT is designated as the amount of enzyme activity that incorporates 1 nmol of [^{3}H]dNTP into acid-insoluble form in 60 min at 37°C. The defined DNA-RNA primer template used for the RT assays was derived as follows. The oligonucleotides 5′GATCCTCTCTTCTCTCTGTCAAATCC CACGTTACATGGTCAG3′ and 5′TCGACTGACCATGTA ACGTGGGATTTGACAGAGAGAAGAGAG3′ were annealed and ligated into pGEM3Z that had been digested with *Bam*HI and *Sal*I. The resultant recombinant plasmid was linearized with *Hin*dIII and then transcribed with T7 RNA polymerase, generating an RNA molecule of 86 nucleotides. This RNA template was annealed to the DNA primer 5′TGACC ATGTAACGTG3′ and used as the primer template for RNA-directed DNA synthesis by RT. This specifically designed

* Corresponding author. Phone: (415) 572-6560. Fax: (415) 573-4890.

TABLE 1. Kinetic constants against HIV RT and RMuLV RT with activated calf thymus DNA as a template

RT	K_m (μM)[a]			K_i (μM)[b]							
	dTTP	dCTP	dATP	AZTTP	d4TTP	ddCTP	3TCTP	ddATP	PMEADP	FIAUTP	HPMPCDP
HIV	2.1	4.6	4.6	0.51 (0.24)	0.19 (0.091)	0.53 (0.12)	2.3 (0.50)	0.87 (0.19)	0.98 (0.21)	3.6 (1.7)	21 (4.6)
RMuLV	3.2	2.5	3.5	0.38 (0.12)	0.99 (0.31)	3.0 (1.2)	19 (7.6)	8.6 (2.5)	0.81 (0.23)	1.4 (0.44)	14 (5.6)

[a] Values are averages from at least three experiments. The average standard error for K_m values is 15%.
[b] The average standard error for K_i values is 14.6%. Numbers in parentheses are K_i/K_m ratios.

primer template allowed the incorporation of 13 molecules of dATP, 7 molecules of dCTP, and 8 molecules of dTTP during reverse transcription. The K_m of this primer template was determined to be 136 nM (dNTP at 1 μM for HIV RT); reactions carried out to determine apparent K_i values of various inhibitors contained three times (408 nM) the K_m concentration of this primer template. RMuLV RT was found to have a similar K_m toward this DNA-RNA primer template (dNTP at 60 μM). RMuLV RT assays were conducted as described for HIV RT except that $MnCl_2$ was substituted for $MgCl_2$ to a final concentration of 5 mM, 0.0005 U of RMuLV RT was used per 60 μl of reaction mixture with DNA as the primer template, 0.0007 U of enzyme was used in reaction mixtures with poly(rA) · oligo$(dT)_{12–18}$, and 0.00004 U of enzyme was used in reaction mixtures with the defined-sequence DNA-RNA primer template (3). All of the K_i, K_m, apparent K_i, and apparent K_m determinations were performed as previously described (3, 4), and kinetic constants were determined by fitting the initial rate data to the KinetAsyst program (6).

The K_m values for the natural substrates and the K_i values of the inhibitors for HIV RT and RMuLV RT with activated calf thymus DNA as the template are shown in Table 1. The K_m values for dATP, dTTP, and dCTP are very similar between the two enzymes. The K_i/K_m ratios (inhibition efficiencies) for the eight inhibitors against the two RTs fall into two general classes. The K_i/K_m values for PMEADP, AZTTP, FIAUTP, HPMPCDP, and d4TTP are similar (within 4-fold) for the two enzymes, while the K_i/K_m values for ddCTP, ddATP, and 3TCTP are significantly different (10- to 15-fold) between the two enzymes.

The apparent K_m values for the natural substrates and the apparent K_i values of the inhibitors for HIV RT and RMuLV RT obtained with the defined sequence DNA-RNA primer template are shown in Table 2. The apparent K_m values determined for dNTP substrates are very different between HIV RT and RMuLV RT. The apparent K_m values of RMuLV RT for dATP, TTP, and dCTP are 14 to 43 times higher than the apparent K_m values of HIV RT for these dNTPs. In contrast to the results with the DNA template, the apparent K_i values obtained with the RNA template for all eight inhibitors are very different between the two enzymes, being 10 to 500 times higher for RMuLV RT than for HIV RT. The largest difference in apparent K_i values for the two enzymes was measured for 3TCTP. Schinazi et al. (19) have shown that RT from another murine retrovirus, Moloney murine leukemia virus, is naturally "resistant" to 3TC because it carries a valine in place of methionine at the position in its RT corresponding to 3TC-"sensitive" amino acid 184 in HIV RT. While DNA sequence information concerning this region of RMuLV RT is not available, the extremely high apparent K_i value of RMuLV RT for 3TCTP might suggest that this position is also a valine, isoleucine, or other amino acid rather than methionine in RMuLV RT.

Interestingly, when the RNA template is used, the apparent K_i/apparent K_m ratios for the eight inhibitors against the two RTs fall into the two general classes defined by the DNA template results. The apparent K_i/apparent K_m values for PMEADP, AZTTP, FIAUTP, HPMPCDP, and d4TTP are similar (within 3-fold) for the two enzymes, while the ratios for ddCTP, ddATP, and 3TCTP are significantly different (4.5 to 32-fold) between the two enzymes. Although dideoxycytidine (ddC) and dideoxyinosine (ddI) are strong inhibitors of HIV replication in cell culture, higher concentrations are required to inhibit RMuLV in tissue culture or in vivo (2). While intracellular metabolism differences between murine and human cells account for much of this difference, the data presented here might suggest that this is in part due to the fact that ddCTP and ddATP (the active metabolite of ddI) are poorer inhibitors of RMuLV RT than of HIV RT.

Since there was such a large difference in apparent K_i and apparent K_m values between HIV and RMuLV RTs when the defined-sequence DNA-RNA primer template was used, a second RNA template was used for the experiment. Poly(rA) · oligo$(dT)_{12–18}$ was used as the template primer to determine the K_m value of TTP and the K_i values of AZTTP, FIAUTP, and d4TTP. Results of these experiments are presented in

TABLE 2. Kinetic inhibition constants against RMuLV RT and HIV RT with RNA as a template[a]

Template and RT	K_m (μM)[b]			K_i (μM)[c]							
	dTTP	dCTP	dATP	AZTTP	d4TTP	ddCTP	3TCTP	ddATP	PMEADP	FIAUTP	HPMPCDP
RNA											
HIV	0.087	0.14	0.051	0.0081 (0.093)	0.042 (0.48)	0.054 (0.39)	0.10 (0.71)	0.031 (0.61)	0.012 (0.24)	0.078 (0.89)	0.83 (5.9)
RMuLV	3.7	2.1	2.2	0.89 (0.24)	3.0 (0.81)	8.6 (4.1)	50 (24)	5.5 (2.5)	0.59 (0.27)	0.98 (0.27)	11 (5.2)
Poly(rA) · oligo$(dT)_{12–18}$											
HIV	2.6			0.012 (0.0046)	0.023 (0.0089)					0.066 (0.025)	
RMuLV	38			0.18 (0.0047)	0.47 (0.012)					0.91 (0.024)	

[a] K_m and K_i values obtained with the heteropolymeric RNA-DNA template primer are apparent K_m and K_i, respectively. The relationships of K_i, K_m, apparent K_i, and apparent K_m are as follows: apparent $K_i = K_i[(K_a/A) + 1]$, and apparent $K_b = K_b[(K_b/A) + 1]$, where K_a is the limiting Michaelis constant for substrate A, A is the concentration of substrate A, and K_b is the limiting Michaelis constant for substrate B.
[b] Values are averages from at least three experiments. The average standard error for K_m values is 18%.
[c] The average standard error for K_i values is 19%. Numbers in parentheses are K_i/K_m ratios.

TABLE 3. In vitro activity against HIV and RMuLV

Compound[a]	IC_{50} (μM)[b]	
	HIV[c]	RMuLV[d]
AZT	0.2	0.0019
PMEA	10	0.30
3TC	1.2	120
ddC	1.4	3.7
ddI	6.5	14
d4T	8.0	0.077

[a] HPMPC and FIAU were used only to probe the active sites of HIV RT and RMuLV RT, and they were not active against HIV at 100 μM. HPMPC and FIAU have not been tested against RMuLV.
[b] IC_{50}, 50% inhibitory concentration.
[c] MT2 cells infected with HIV IIIb.
[d] Syncytial inhibition by drug in murine SC-1 cells infected with RMuLV (1).

Table 2. The K_m and K_i values for TTP and AZTTP, respectively, are in agreement with literature values for HIV RT obtained with poly(rA) · oligo$(dT)_{12–18}$ (15). As predicted from the kinetic constants determined by using the defined sequence DNA-RNA primer template, the K_m value for TTP was 14-fold higher for RMuLV RT than for HIV RT and the K_i values against the three inhibitors exhibited a similar 13- to 23-fold differential as well. Therefore, the K_i and K_m results obtained with two different DNA-RNA primer templates were consistent.

AZT (14), PMEA (7, 8), 3TC (18), ddC (13), ddI (13), and d4T (11) show selective anti-HIV activity in cell culture. All of these compounds are able to serve as alternate substrates for the natural dNTP by HIV RT (17). These compounds are incorporated into the viral DNA and subsequently result in termination of viral DNA synthesis. AZT and PMEA (10a, 14) also have been shown to exert an antiviral effect in mice infected with RMuLV. The data presented here show that both of these inhibitors are active against HIV and RMuLV RTs when DNA or RNA is used as the template.

Included in this study were two additional antiviral compounds, HPMPC, a compound exhibiting antiviral activity against a broad range of DNA viruses (8), and FIAU, which has demonstrated anti-hepatitis B virus activity (10). These were included to probe the active sites of these two RTs and to determine whether weak inhibitors of HIV RT would also be weak inhibitors of RMuLV RT. Indeed, this was the case, as shown in Tables 1 and 2.

The kinetic inhibition data generated from these eight inhibitors and their interactions with HIV RT and RMuLV RT support the rationale for the activity of AZT, PMEA, and d4T against HIV and RMuLV replication. Certainly, this has been demonstrated for AZT and PMEA not only in cell culture but also in vivo. In contrast, HPMPCDP and FIAUTP are relatively weak inhibitors of HIV RT, and because the free nucleosides are poor inhibitors of HIV replication in cell culture (Table 3) and weak inhibitors of RMuLV RT, they might not be expected to provide antiviral protection for RMuLV-infected mice. ddCTP, ddATP, and 3TCTP inhibit HIV RT with good potency, and ddC (13), ddI (13), and 3TC (18) also demonstrate anti-HIV activity in cell culture. However, ddCTP, ddATP, and 3TCTP are weak inhibitors of RMuLV RT, and, not unexpectedly, ddC and ddI have correspondingly weaker anti-RMuLV activity in cell culture (2). On the basis of the RT experiments described here, one might predict that d4T would exert a better antiviral effect against RMuLV than 3TC does. It is important to keep in mind that inhibition of the target enzyme, RT, addresses only part of a molecule's ability to function as an antiviral agent in tissue culture or in vivo. For example, the metabolism of AZT, d4T, ddI, and ddC was shown (9, 21) to be different in activated and in resting peripheral blood mononuclear cells. The extent of metabolism of nucleoside drugs to the triphosphate form has also been shown to be different between murine and human cells. Therefore, clearly the efficiency of the metabolism of the parent molecule to its active moiety in specific cell types is critical as well. In our laboratory, endogenous nucleotide pools did not differ markedly between murine SC-1 cells (TTP, 51.5 pmol/10^6 cells; dCTP, = 26.5 pmol/10^6 cells; dATP, 56.2 pmol/10^6 cells; and dGTP, 13.4 pmol/10^6 cells) and the human T-cell line A3.01 (TTP, 49.6 pmol/10^6 cells; dCTP, 28.8 pmol/10^6 cells; dATP, 15.3 pmol/10^6 cells; and dGTP, 5.52 pmol/10^6 cells [20a]). Our recent data also show that 3TC is a poor inhibitor of RMuLV replication in cell culture (50% inhibitory concentration = 120 μM), while d4T is a potent inhibitor of RMuLV replication (50% inhibitory concentration = 0.077 μM) (Table 3).

The data presented here show that the RTs of RMuLV and HIV are distinct from one another and may interact similarly or differently with a particular antiviral compound. Our data underscore the need to compare antiviral susceptibilities of target enzymes between HIV and a proposed model virus in order to correctly interpret in vitro and/or in vivo animal model results. This information as well as intracellular nucleotide metabolism differences between animal model species and humans can be used to predict a model's utility for evaluating a particular drug. The development and evaluation of animal models for antiretroviral treatment requires several stages to provide useful information on a practical basis. As an initial screen, RMuLV provides retroviral targets shared with HIV, e.g., RT and protease. We examined RT inhibitors in the Rauscher model for its validation; indeed, the validity of the model is dependent upon demonstrating that antiviral drugs act the same way against the same targets in the model and in HIV infections in vivo. A small-animal model such as RMuLV also permits examination of the pharmacokinetics of the drug, as well as possible toxicity, and the effects upon an immune system, together with antiviral efficacy. We select successful candidate drugs from the Rauscher model for further evaluation in the human peripheral blood mononuclear cell-severe combined immunodeficiency (HuPBMC-SCID) model. In the HuPBMC-SCID model, the in vivo activity of the agent against HIV may be examined. For comparison, the Rauscher model allows large experimental groups, providing enhanced statistical significance in a model system which does not involve a human pathogen requiring extensive safety precautions, as does HIV. The Rauscher model is far less labor-intensive as well as much less expensive. These criteria establish these two models in a two-tier hierarchy in our laboratory. Validation of a small-animal model for in vivo testing of compounds exhibiting in vitro anti-HIV activity would be very useful for the timely development of novel potent therapeutics.

This work was supported in part by an appointment (S.C.K.) to the Postgraduate Research Program at the Center for Drug Evaluation and Research administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration.

We gratefully acknowledge Paul L. Black for helpful discussions and Bradford A. Hall and Susan Papermaster for their excellent technical assistance.

REFERENCES

1. **Black, P. L., M. B. Downs, M. G. Lewis, M. A. Ussery, G. B. Dreyer, S. R. Petteway, Jr., and D. M. Lambert.** 1993. Antiretroviral activities of protease inhibitors against murine leukemia virus and simian immunodeficiency virus in tissue culture. Antimicrob. Agents Chemother. **37:**71–77.

2. **Black, P. L., M. A. Ussery, J. T. Rankin, Jr., and M. A. Chirigos.** 1990. Therapeutic activity of reverse transcriptase inhibitors in murine retrovirus models. Ann. N.Y. Acad. Sci. **616:**486–489.
3. **Cherrington, J. M., S. J. W. Allen, N. Bischofberger, and M. S. Chen.** 1995. Kinetic interaction of the diphosphates of 9-(2-phosphonylmethoxyethyl)adenine and other anti-HIV active purine congeners with HIV reverse transcriptase and human DNA polymerases α, β, and γ. Antiviral Chem. Chemother. **6:**217–221.
4. **Cherrington, J. M., S. J. W. Allen, B. H. McKee, and M. S. Chen.** 1994. Kinetic analysis of the interaction between the diphosphate of (S)-1-(3-hydroxy-2-phosphylmethoxypropyl)cytosine, ddCTP, AZTTP, and FIAUTP with human DNA polymerases β, and γ. Biochem. Pharmacol. **48:**1986–1988.
5. **Chirigos, M. A.** 1964. Studies with the murine Rauscher leukemogenic virus. III. An in vitro assay for antiviral agents. Cancer Res. **24:**1035–1041.
6. **Cleland, W. W.** 1979. Statistical analysis of enzyme kinetic data. Methods Enzymol. **63:**6002–6010.
7. **DeClercq, E., A. Holy, I. Rosenberg, T. Sakuma, J. Balzarini, and P. C. Maudal.** 1986. A novel selective broad spectrum anti-DNA virus agent. Nature (London) **323:**464–467.
8. **DeClercq, E., T. Sakuma, R. Pauwels, J. Balzarini, I. Rosenberg, and A. Holy.** 1987. Antiviral activity of phosphonylmethoxyalkyl derivatives of purines and pyrimidines. Antiviral Res. **8:**261–272.
9. **Gao, W.-Y., T. Shirasaka, D. G. Johns, S. Broder, and H. Mitsuya.** 1993. Differential phosphorylation of azidothymidine, dideoxycytidine, and dideoxyinosine in resting and activated peripheral blood mononuclear cells. J. Clin. Invest. **91:**2326–2333.
10. **Korba, B. E., and J. L. Gerin.** 1992. Use of a standardized cell culture assay to assess activities of nucleoside analogs against hepatitis B virus replication. Antiviral Res. **19:**55–70.

10a. **Kunder, S. C., et al.** Unpublished data.

11. **Lin, T. S., R. F. Schinazi, and W. H. Prusoff.** 1987. Potent and selective in vitro activity of 3′-deoxythymidine-2′-ene-(3′-deoxy-2′,3′-didehydrothymidine) against human immunodeficiency virus. Biochem. Pharmacol. **36:**2713–2718.
12. **Ludwig, J., and F. Eckstein.** 1989. Rapid and efficient synthesis of nucleoside 5′-O-(1-thiotriphosphates), 5′-triphosphates and 2′,3′-cyclophosphorothioates using 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one. J. Org. Chem. **54:** 631–635.
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. N. 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. **Rauscher, F. L.** 1962. A virus induced disease of mice characterized by erythrocytopoiesis and lymphoid leukemia. J. Natl. Cancer Inst. **29:**515–543.
16. **Ruprecht, R. M., L. G. O'Brien, L. D. Rossoni, and S. Nusinoff-Lehrman.** 1986. Suppression of mouse viraemia and retroviral disease by 3′-azido-3′-deoxythymidine. Nature (London) **323:**467–469.
17. **Schinazi, R. F.** 1993. Competitive inhibitors of human immunodeficiency virus reverse transcriptase, p. 151–180. *In* P. S. Anderson, G. L. Kenyon, and G. R. Marshall (ed.), Perspectives in drug development and design, vol. 1. ESCOM, Leiden, The Netherlands.
18. **Schinazi, R. F., C. K. Chu, A. Peck, A. McMillan, R. Mathis, D. Cannon, L.-S. Jeong, J. W. Beach, W.-B. Choi, S. Yeola, and D. C. Liotta.** 1992. Activities of the four optical isomers of 2′,3′-dideoxy-3′ thiacytidine (BCH-189) against human immunodeficiency virus type 1 in human lymphocytes. Antimicrob. Agents Chemother. **34:**1061–1064.
19. **Schinazi, R. F., R. M. Lloyd, Jr., M.-H. Nguyen, D. L. Cannon, A. McMillan, N. Ilksoy, C. C. Chu, D. C. Liotta, H. Z. Bazmi, and J. W. Mellors.** 1993. Characterization of human immunodeficiency viruses resistant to oxathiolane-cytosine nucleosides. Antimicrob. Agents Chemother. **37:**875–881.
20. **St. Clair, M. H., C. A. Richards, T. Spector, K. J. Weinhold, W. H. Miller, A. J. Langolis, and P. A. Furman.** 1987. 3′-Azido-3′-deoxythymidine triphosphate as an inhibitor and substrate of purified human immunodeficiency reverse transcriptase. Antimicrob. Agents Chemother. **31:**1972–1977.

20a. **Zhang, H., and M. Ussery.** Unpublished results.

21. **Zhu, Z., H.-T. Ho, M. J. M. Hitchcock, and J.-P. Sommadossi.** 1990. Cellular pharmacology of 2′,3′-didehydro-2′,3′-dideoxythymidine (D4T) in human peripheral blood mononuclear cells. Biochem. Pharmacol. **39:**R15–R19.