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Feline Immunodeficiency Virus, a Model for Reverse Transcriptase-Targeted Chemotherapy for Acquired Immune Deficiency Syndrome

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Feline immunodeficiency virus (FIV), formerly called feline T-lymphotropic lentivirus, causes an immune deficiency in cats that is very similar to the acquired immune deficiency syndrome in humans (N. C. Pedersen, E. M. Ho, M. L. Brown, and J. K. Yamamoto, Science 235:790–793, 1987). We have examined the reverse transcriptase of this virus to determine whether it is similar enough to the reverse transcriptase of the human immunodeficiency virus type 1 (HIV-1) to enable its use as a model for chemotherapy for acquired immune deficiency syndrome. The FIV reverse transcriptase is similar to that of HIV-1 in sensitivity to the noncompetitive inhibitor phosphonoformate (K_i, 0.3 μM) and relative insensitivity to phosphonoacetate. This enzyme was also sensitive to two competitive inhibitors, the 5′-triphosphates of 2′, 3′-dideoxythymidine (K_i, 3.4 nM) and 3′-azido-3′-deoxythymidine (AZT; K_i, 6.2 nM). The ratios of K_i/K_m for these two competitive inhibitors are similar to the ratios calculated from previously reported data for the HIV-1 enzyme assayed under identical conditions. In contrast, the FIV enzyme is different from the reverse transcriptase of avian myeloblastosis virus in sensitivity to those inhibitors. The replication of FIV in Crandell feline kidney cells was inhibited by AZT; virus production was inhibited more than 95% by 1.0 μM AZT.

Identification of human immunodeficiency virus type 1 (HIV-1) as the causative agent in the acquired immune deficiency syndrome (AIDS) has stimulated the search for selective antiretroviral drugs and for retroviral components to utilize as chemotherapeutic targets (6, 21). Much of this work has focused on the retrovirus-encoded RNA-dependent DNA polymerase (reverse transcriptase [RT]). Among the most promising antiretroviral agents are several nucleoside analogs whose corresponding nucleotides are selectively targeted against RT. These include 3′-azido-3′-deoxythymidine (AZT) and 2′,3′-dideoxycytidine (ddCyd). AZT is metabolized by cellular enzymes to 3′-azido-3′-deoxythymidine-5′-triphosphate (N_3dTTP) which is the active form of the drug (4). This triphosphate is both a competitive inhibitor of the HIV-1 RT and a substrate which, upon incorporation into DNA, results in chain termination (16). Similarly, ddCyd is converted by cellular enzymes to the 5′-triphosphate (ddCTP) (15). The dideoxynucleoside 5′-triphosphates are well characterized as DNA chain terminators in bacterial systems (17, 18), and apparently the inhibition of HIV-1 replication by ddCTP is due to termination of DNA chains (10). Several other dideoxynucleosides have been shown to selectively block the replication of HIV-1 in cultured cells (1, 5, 7), and these are believed to work in a similar manner.

Most studies of the antiviral activities of these agents have been in cultured cells or with the HIV-1 RT. In vivo studies have been limited to trials in patients with AIDS. This has made it difficult to assess and compare potential anti-HIV drugs and chemotherapeutic strategies. An animal model that will enable both in vivo and in vitro studies is urgently needed.

Pedersen et al. (14) have recently described a feline lentivirus, feline immunodeficiency virus (FIV; formerly feline T-lymphotropic lentivirus) that might provide a useful model for studies of AIDS chemotherapy. FIV was isolated from cats with an immune deficiency syndrome that is similar to human AIDS in many respects (14, 20). Infection of pathogen-free kittens with FIV resulted in lymphadenopathy. FIV is morphologically similar to HIV-1, and its RT has a Mg^{2+} requirement that is similar to that of the HIV-1 RT. The work reported here was initiated to determine whether the FIV RT is similar enough to the HIV-1 RT in sensitivity to inhibitors to enable use of FIV as a model for studies of AIDS chemotherapy. In addition, the susceptibility of FIV to AZT has been examined.

MATERIALS AND METHODS

Chemicals. Phosphonoacetate (PAA) and phosphonoformate (PFA) were purchased from Sigma Chemical Co., St. Louis, Mo. AZT was a gift from Phillip A. Furman, Burroughs Wellcome Co., Research Triangle Park, N.C. The 5′-triphosphate of AZT (N_3dTTP) was kindly provided by Wayne Miller, also of the Burroughs Wellcome Co. Poly (rA)-oligo(dT)$_{10}$, 2′ 3′-dideoxythymidine 5′-triphosphate (dd TTP), and ddCTP were purchased from Pharmacia, Inc., Piscataway, N.J. The avian myeloblastosis virus (AMV) RT and nuclease-free bovine serum albumin were obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind. All other chemicals were reagent grade or better.

Cells and virus. Uninfected and FIV-infected Crandell feline kidney (CrFK) cells were previously described (20). All cells were grown in L & M medium (composed of equal parts of Leibovitz L-15 and Dulbecco modified eagle medium, supplemented with 10% fetal bovine serum [heat inactivated for 1 h at 56°C], 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 2.0 mM L-glutamine). All cultures were maintained at 37°C with a humidified 5% CO_2 atmosphere.

FIV stocks were prepared from medium containing extracellular virus released from CrFK cells that had been infected with FIV for more than 21 days. Medium was removed from cultures that were near confluency in 175-cm^2 tissue culture flasks, and cells were removed by centrifugation at 500 × *g* for 5 min. Dimethyl sulfoxide was added to

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the supernatant to a final concentration of 10%, and aliquots were stored frozen at −80°C. For experiments, these stocks were thawed, diluted fivefold into fresh medium, and used for infection of CrFK cells as described below.

RT. The FIV RT used in these studies was obtained from detergent-treated virions. To prepare RT, the medium containing extracellular virus was removed from FIV-infected CrFK cells grown for 24 to 48 h to near confluency in 75-cm^2 tissue culture flasks (1×10^7 to 2×10^7 cells per flask). This medium was centrifuged at $500 \times g$ for 5 min to remove any intact cells, and the supernatant was then centrifuged at $35,000 \times g$ for 60 min to pellet virions. The supernatant was discarded, and the viral pellet was suspended in 50 mM Tris hydrochloride, pH 8.5–20 mM dithiothreitol–160 mM KCl–0.33% Triton X-100 (disruption buffer). The yield was typically 1 to 2 U per 75-cm^2 flask (1 U is the amount of enzyme required to incorporate 1 nmol of dTMP per min into poly (rA)-oligo$(dT)_{10}$ at 37°C).

RT assay. RT was assayed as described by Cheng et al. (3), with poly(rA)-oligo$(dT)_{10}$ as the template-primer. Reactions were carried out in a volume of 50 or 100 μl and contained 50 mM Tris hydrochloride, pH 8.5, 10 mM dithiothreitol, 0.05% Triton X-100, 250 μg of bovine serum albumin (nuclease free) per ml, 6 mM $MgCl_2$, 80 mM KCl, 20 μM [*methyl*-^{3}H]dTTP, 0.5 A_{260} U of poly(rA)-oligo$(dT)_{10}$ per ml, and enzyme. Samples (35 or 40 μl) were taken at the indicated times and spotted onto filters (no. 3, 2.3-cm diameter; Whatman, Inc., Clifton, N.J.) that had been presoaked with 5% trichloroacetic acid–1% sodium pyrophosphate. Filters were processed as previously described (13). They were dried, washed four times (at least 1 h each) with 5% trichloroacetic acid–1% sodium pyrophosphate and twice with 95% ethanol, dried, and then counted in Liquifluor (Dupont, NEN Research Products, Boston, Mass.). In all experiments, 6 mM $MgCl_2$ was used to enable direct comparison to results from other studies of the HIV-1 RT (3, 19).

Drug sensitivity of FIV replication. The ability of FIV to replicate in the presence or absence of AZT was monitored by quantitation of virion-associated RT with an approach similar to that used by Mitsuya et al. (11). Cultures of CrFK cells (1×10^6 to 2×10^6 cells per 25-cm^2 flask) were infected with 2 ml of freshly thawed and diluted FIV stock (described above); virus was allowed to adsorb for 30 min, and then an additional 2 ml of medium was added containing AZT to give the indicated final concentration. Medium was replaced every 2 to 3 days with fresh medium containing the indicated concentration of AZT. When cultures reached confluency, cells were removed by trypsinization, diluted 50-fold, and reseeded into new flasks. Samples were taken for assay of virion-associated RT at the indicated times. For these determinations, cells were always at or near confluency and had been growing for 48 h since the latest change of culture medium. Medium was removed from cultures and centrifuged to remove cells and debris, and then virus was pelleted as described above. Pellets were suspended in disruption buffer and frozen at −80°C. When these samples were taken, cells were also trypsinized and aliquots were taken to determine cell number by counting in a hemacytometer. All data reported here are determinations from triplicate cultures for each condition.

RESULTS

Sensitivity of FIV RT to inhibitors. The data in Table 1 show the activities of several inhibitors of the FIV RT. These inhibitors were also evaluated with the AMV RT, and results were compared with previously reported data for the HIV-1 RT. The two pyrophosphate analogs, PFA and PAA, were chosen for these comparisons because PFA is a potent and selective inhibitor of the HIV-1 RT, whereas PAA is relatively inactive (19). Results in Table 1 show that the FIV RT was also sensitive to PFA, with a concentration inhibiting RT activity by 50% (IC_{50}) nearly identical to the value for the HIV-1 RT determined by Vrang and Öberg (19). Also like HIV-1 RT, FIV RT was relatively insensitive to PAA. The two nucleotides chosen for these comparisons were ddTTP and N_3dTTP (15). The choice of ddTTP rather than ddCTP was owing to the use of poly(rA)-oligo$(dT)_{10}$ as template-primer. As expected, ddCTP did not inhibit the FIV RT in this reaction (Table 1). The IC_{50} for inhibition of the FIV RT by ddTTP was slightly lower than the value reported by Cheng et al. (3) for the HIV-1 RT. With N_3dTTP, the IC_{50} for the FIV RT was somewhat higher than the value reported for the HIV-1 RT. With all these inhibitors, the IC_{50}s obtained for the FIV RT were more similar to the published values for the HIV-1 RT than to the values we obtained for the AMV RT (Table 1).

TABLE 1. Inhibition of RTs by pyrophosphate analogs and nucleotide analogs

Addition	IC_{50} (nM) for RT[a]:		
	FIV	HIV-1	AMV
PFA	450 ± 70	500[b]	>5,000
PAA	~500,000	>500,000[b]	>10^6
ddTTP	25 ± 5	<50[c]	100 ± 20
N_3dTTP	150 ± 20	40[c]	>400
ddCTP	>1,000		ND[d]

[a] The IC_{50}s were obtained from dose-response curves of RT activity versus log concentration. Data are from at least two separate experiments for each inhibitor. Within each experiment, determinations were made in duplicate following 60-min incubations. The data were obtained from reactions containing 0.05 to 0.20 U of FIV RT or 0.10 U of AMV RT per 100 μl. Values for the HIV-1 RT are from previously reported data and are included for comparison.
[b] From Vrang and Öberg (19).
[c] From Cheng et al. (3).
[d] ND, Not determined.

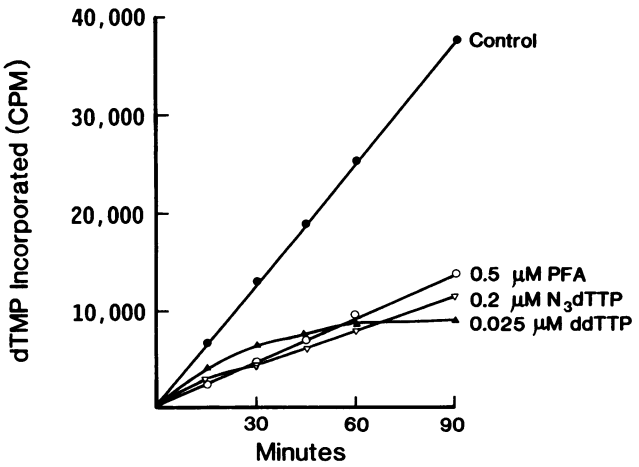

FIG. 1. Time course of FIV RT activity in the presence or absence of inhibitors. To reaction mixtures (250 μl) containing equal amounts of FIV RT were added the final concentrations indicated. At indicated times, 35-μl samples were withdrawn and spotted onto filters, and radioactivity was quantitated. The specific activity of [^{3}H]dTTP was 314 cpm/pmol.

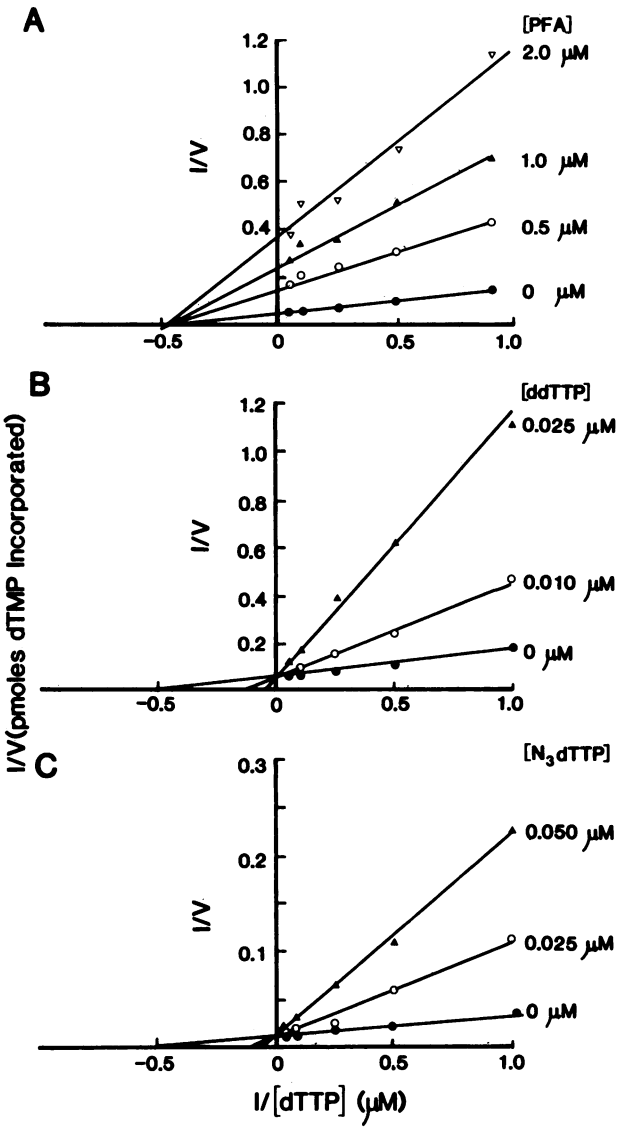

FIG. 2. Determination of K_i values for inhibition of FIV RT. RT activity was quantitated from reactions incubated for 30 min at 37°C with [dTTP] varied from 1.0 to 20 μM. Values reported are averages from duplicate determinations.

The activities of PFA, ddTTP, and N_3dTTP against the FIV RT were studied in more detail. The time course of the reaction in the presence of these inhibitors is shown in Fig. 1. In the presence of ddTTP or N_3dTTP, the reaction deviated from linearity by 15 to 30 min. This is similar to results with the HIV-1 RT (3) and is consistent with the known abilities of these nucleotides to work as chain terminators. Inhibition of RT by PFA did not cause deviation from linearity in the time course.

Kinetic analyses of the reactions with each of these three inhibitors were also performed (Fig. 2). The K_m for dTTP was determined to be 2.2 μM for the FIV RT. This is slightly lower than the previously reported values of 5 μM (3), 3.6 μM (2), and 2.8 μM (16) for the HIV-1 RT. The inhibition of FIV RT by PFA was noncompetitive, and the K_i was determined to be 0.3 μM. This is very close to the K_i of 0.4 μM for inhibition of HIV-1 RT by PFA as reported by Vrang and Öberg (19). Although the reactions deviated from linearity in the presence of ddTTP or N_3dTTP, K_i values were estimated using 30-min reactions to enable direct comparison to the data of Cheng et al. (3). Both of these nucleotides are competitive inhibitors of the FIV RT, as they are for the HIV-1 RT. The K_i values of 3.4 nM for ddTTP and 6.2 nM for N_3dTTP are lower than the values of Cheng et al.; however, the ratios of K_i/K_m for these two competitive inhibitors are nearly identical to those calculated from the data of Cheng et al. (3). These values are somewhat different from those of St. Clair et al. (16) and of Chen and Oshana (2), although the reaction conditions used in those studies are different from those used here. These determinations and comparisons are summarized in Table 2.

TABLE 2. Comparison of inhibition constants determined for FIV and HIV-1 RTs[a]

Inhibitor	K_i (nM) for RT:		K_i/K_m for RT[b]:	
	FIV	HIV-1	FIV	HIV-1
PFA	300 ± 40	400[c]	0.14	0.08[c]
ddTTP	3.4 ± 1.2	7 ± 4[d]	0.0015	0.0017[d]
		30[e]		0.0083[e]
N_3dTTP	6.2 ± 1.8	13 ± 5[d]	0.0028	0.0032[d]
		40 ± 2[f]		0.014[f]

[a] Values for the FIV RT were calculated from the data on Fig. 2 with a non-competitive inhibition equation for PFA and a competitive inhibition equation for ddTTP and N_3dTTP. Data are from three determinations each for PFA and ddTTP and five determinations for N_3dTTP. Previously reported values for the HIV-1 RT are presented for comparison.

[b] The K_m for dTTP was 2.2 μM for the FIV RT; for calculations with the HIV-1 RT, the K_m determined in the cited report was used.

[c] From Vrang and Öberg (19).

[d] From Cheng et al. (3).

[e] From Chen and Oshana (2).

[f] From St. Clair et al. (16).

Inhibition of FIV replication by AZT. The effect of AZT upon replication of FIV in CrFK cells is shown in Fig. 3. When infections were carried out in the presence of 10 μM AZT, there was no detectable virus production. Virus production was also undetectable at 50 μM AZT (data not shown). At 1.0 μM AZT, the replication of FIV was inhibited by 99% on day 21 postinfection and by 95% on day 28.

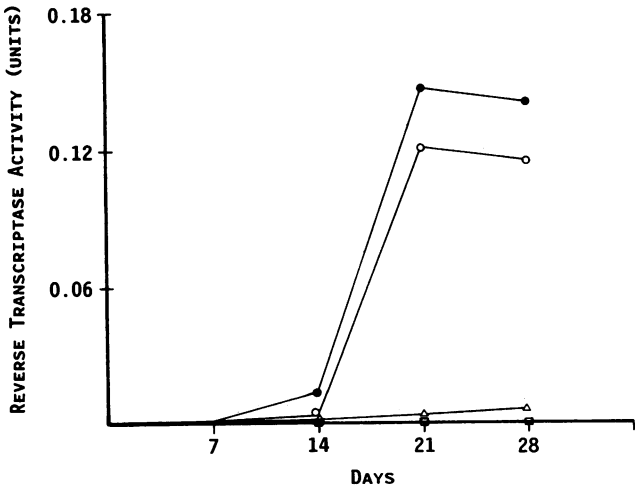

FIG. 3. Inhibition of FIV replication by AZT. Cultures were treated throughout infection with 10 (□), 1.0 (△), or 0.1 (○) μM AZT or with no AZT (●). Virus replication was measured from production of virion-associated RT.

In the presence of 0.1 μM AZT, replication was inhibited by 15 to 20%. This susceptibility of FIV to AZT is similar to that of HIV-1, which is completely inhibited by 1 to 3 μM AZT (11).

DISCUSSION

FIV has now met several important criteria necessary for its use as a model for studies of AIDS chemotherapy. It is a lentivirus and is morphologically similar to HIV-1, it was isolated from cats with an immune deficiency similar to AIDS in humans, and it causes a lymphadenopathy in infections of pathogen-free kittens (14, 20). It was previously shown that the RT of FIV has a requirement for Mg^{2+} that is similar to that of HIV-1 but distinguishable from the RT of feline leukemia virus or Maedi/visna virus (14, 20). The results we report here show that the FIV and HIV-1 RTs are nearly identical in sensitivity to the active forms of several antiviral agents, including two (AZT and PFA) that selectively inhibit replication of HIV-1. The FIV RT is different from the AMV RT in sensitivity to these inhibitors. In addition, the concentration of AZT required to inhibit replication of FIV is similar to that required to inhibit replication of HIV-1. With these similarities to HIV-1, FIV should be useful for in vitro studies of RT-targeted antiviral agents. Its suitability as a model for in vivo studies will depend on whether the pharmacological properties of these compounds in cats and their metabolism in feline cells and tissues are similar to the events in humans. For drugs targeted to sites other than RT, the suitability of FIV as a model will depend on the similarities between HIV-1 and FIV in other appropriate properties.

There is no animal model that fulfills the needs for study of potential drugs and strategies to use in the treatment or management of AIDS. Studies with HIV-1 are limited to in vitro studies and to patients with AIDS. Although the simian immunodeficiency virus (8) is similar to HIV-1 and a suitable model for many studies of AIDS, the limitation in number of animals and high cost preclude large-scale investigation and comparison of different drugs and different approaches. Similar situations limit the use of HIV-1 infections of chimpanzees or of the recently described mice that have a transplanted human immune system (9, 12). There are several lentiviruses of large animals (caprine arthritis encephalitis virus, equine infectious anemia virus, Maedi/visna virus) that might be useful for some studies but are not suitable models for chemotherapy of AIDS. This feline model should be better suited for such studies. It appears that suitable numbers of naturally infected animals are available, and pathogen-free kittens can be infected with FIV. It is hoped that the availability of this model will facilitate evaluation of inhibitors of virus replication for treatment or management of AIDS in humans and FIV in cats.

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