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Reverse Transcriptases from Human Immunodeficiency Virus Type 1 (HIV-1), HIV-2, and Simian Immunodeficiency Virus (SIV_{MAC}) Are Susceptible to Inhibition by Foscarnet and 3′-Azido-3′-Deoxythymidine Triphosphate

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Reverse transcriptases from human immunodeficiency virus type 1 (HIV-1), HIV-2, and simian immunodeficiency virus (SIV) were investigated with respect to susceptibilities to the reverse transcriptase inhibitors foscarnet and 3′-azido-3′-deoxythymidine triphosphate (AZTTP). The different reverse transcriptases had the same sensitivity to foscarnet (50% inhibition at 0.10 to 0.16 μM). The K_i values for AZTTP were 0.01 to 0.02 μM for HIV-1 reverse transcriptase and 0.02 to 0.03 μM for HIV-2 and SIV_{MAC} reverse transcriptases.

Several different strains of human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS), have been isolated, and genomic analysis demonstrates a range of variation between 2 and 25% (7). Another human immunodeficiency virus (HIV-2), isolated from West African AIDS patients, is more closely related to simian immunodeficiency virus (SIV; formerly called STLV-III) than to HIV-1 (2, 3).

Since reverse transcriptase (RT) inhibitors have potential in antiviral therapy, it is of great interest to compare RTs from different HIV strains in terms of their susceptibility to these inhibitors. Assays of RT susceptibility to different inhibitors may also be a more exact method of determining differences in sensitivity than the use of inhibition of virus replication in cell culture, since host cell types and culture conditions directly influence the rate of virus replication. We have investigated the respective RT susceptibilities of HIV-1, HIV-2, and SIV_{MAC} (either in disrupted virions or as the purified enzyme) to foscarnet and 3′-azido-3′-deoxythymidine triphosphate (AZTTP).

RT inhibition assays were performed as described earlier (10). A 50-μl reaction mixture contained 100 mM Tris hydrochloride (pH 7.6), 100 mM KCl, 4 mM $MgCl_2$, 4 mM dithiothreitol, 5 μg of bovine serum albumin fraction V, 0.5 μg of $(rA)_n \cdot (dT)_{12-18}$, and 7.2 μM [^{3}H]dTTP (specific activity, 1,535 cpm/pmol) in determinations of inhibition by foscarnet, and [^{3}H]dTTP concentrations varied between 2 and 8 μM in kinetic studies (specific activity, 1,382 to 5,527 cpm/pmol), in which K_i values for AZTTP were determined. A 10-μl enzyme preparation, adjusted to contain 0.005 U of RT (1 U is the amount of enzyme that catalyzes the incorporation of 1 nmol of dTMP in 10 min at 37°C), was added, and the reaction mixture was incubated for 45 min at 37°C. We confirmed all data by performing two separate experiments.

Inhibition by foscarnet (which is a noncompetitive inhibitor [11]) and AZTTP (which is a competitive inhibitor with respect to dTTP [6, 9, 10]) of several HIV-1 isolates, HIV-2, and SIV was determined; the results are presented in Table 1. K_i values were determined from double-reciprocal plots of velocity versus substrate concentration at different amounts of inhibitor. The inhibitory activity of AZTTP was monitored over time, and the rate was linear up to 45 min (data not shown). The 50% inhibitory concentrations of foscarnet varied between 0.10 and 0.16 μM, and the K_i values for AZTTP were 0.01 to 0.04 μM for HIV-1 and 0.02 to 0.03 μM for HIV-2 and SIV_{MAC}. The results for AZTTP inhibition of HIV-2 RT do not explain the cell culture data reported by Richman (8), who found that inhibition of HIV-2 replication in MT-2 cells required a 300-fold higher concentration of 3′-azido-3′-deoxythymidine (AZT) than inhibition of HIV-1 replication did. These results have not been confirmed, but two recent reports, one presenting similar data for AZT inhibition of SIV and HIV-2 in helper T cells (5) and the other presenting similar inhibition constants for AZTTP on

TABLE 1. Inhibition of HIV-1, HIV-2, and SIV_{MAC} RTs by foscarnet and AZTTP

Isolate	K_m (μM dTTP)	Foscarnet concn (μM) giving 50% inhibition	AZTTP K_i (μM)
HIV-1 (*E. coli*)[a]	3.0	0.20	0.01
HIV-1 (HTLV-IIIB)[b]	6.0	0.11	0.02
HIV-1 (I 32)[c]	2.4	0.11	0.01
HIV-2 (LAV-II)[d]	10.4	0.14	0.03
HIV-2 (SBL 6669)[c]	6.0	0.16	0.02
SIV_{MAC}[d]	4.6	0.10	0.02
HIV-1[e]			
Median	5.1	0.22	0.02
Range	0.6–7.1	0.14–0.35	0.01–0.03
HIV-1[f]	4.9		0.013
HIV-1[g]	1.2		0.002
HIV-1[h]	2.8		0.04

[a] HIV-1 RT expressed in *E. coli* was a kind gift from K. Moelling.
[b] RT from HIV-1 (HTLV-IIIB) was purified as described earlier (12).
[c] HIV-1 (I 32) and HIV-2 (SBL 6669) were isolated by E.-M. Feny and B. Åsjö and tested as disrupted virions.
[d] $LAV\text{-}II_{ROD}$ and $SIV_{MAC\ 251}$ were grown in HUT-78 cells and tested as disrupted virions. $SIV_{MAC\ 251}$ was originally designated HTLV-4 (4).
[e] Eleven HIV-1 isolates from patients with AIDS or AIDS-related complex were tested as disrupted virions.
[f] Data from reference 1.
[g] Data from reference 10.
[h] Data from reference 9.

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RT from HIV-1 and SIV (B. F. H. Eriksson, R. F. Schinazi, and C. K. Chu, Abstr. Antiviral Res. 9:84, 1988), are both in agreement with our own results (Table 1).

The K_i values found for HIV-2 and SIV_{MAC} correspond to earlier reports for HIV-1 (1, 9, 10). Published K_i values for AZTTP have varied between 0.002 and 0.04 μM. The high concentration of AZT needed for inhibition of HIV-2 replication in cell culture could be explained by the small amount of phosphorylated AZT within the cell or the different rate of replication of HIV-1 and HIV-2 in infected cells. The present results indicate that RTs from HIV-1, HIV-2, and SIV_{MAC} are similar in their sensitivity to inhibition by foscarnet and AZTTP.

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