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SHORT COMMUNICATIONS

Inhibition of HIV reverse transcriptase by 2′,3′-dideoxynucleoside triphosphates

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Human immunodeficiency virus [HIV, human T-lymphotropic virus type III (HTLV-III)/lymphadenopathy-associated virus (LAV)] is the leading etiologic candidate for Acquired Immune Deficiency Syndrome (AIDS) [1]. Among the agents that have been shown to be active against the replication of HIV in cell culture are 3′-azidothymidine [2], 2′,3′-dideoxynucleosides [3], Suramin [4], ammonium-21-tungsto-9-antimonate [5], and phosphonoformate [6]. HIV reverse transcriptase is the target for inhibition by these compounds [4–6] or their triphosphate metabolites [2, 3]. Some of these compounds have been under clinical trials for testing in AIDS patients, but no conclusive results are available.

The importance of finding agents that could inhibit the replication of HIV prompted us to study the effects of several potential inhibitors against HIV reverse transcriptase.

Materials and methods

Radioactive deoxynucleoside triphosphates (dNTPs) were purchased from New England Nuclear, Inc., while template-primers, ddTTP, ddCTP, ddATP and ddGTP were obtained from P.L. Biochemicals, Inc. Bovine serum albumin, dithiothreitol (DTT), KCl, dTTP, dATP, dCTP, dGTP, $MgCl_2$, glycerol and Tris–HCl were from the Sigma Chemical Co. DE-52 cellulose was obtained from Whatman, Inc.

Purification of HIV reverse transcriptase. Reverse transcriptase was purified from the lysate of HIV by using the procedure of Chandra *et al.* [7] with the following modifications: (1) The lysate was dialyzed against 10 mM Tris–HCl, pH 8.3, 1 mM DTT, and 10% glycerol, and applied to a DE-52 column that had been equilibrated with the same buffer. The column was eluted with a gradient of 10 mM Tris–HCl, pH 8.3, to 50 mM Tris–HCl, pH 7.9, 0.3 M KCl in 1 mM DTT and 10% glycerol. (2) The buffer used for dialysis prior to P-11 column chromatography was 10 mM Tris–HCl, pH 7.5, 1 mM DTT and 10% glycerol. The enzyme solution was applied to a phosphocellulose column (P-11) equilibrated with the same buffer. The column was eluted with a gradient of 10 mM Tris–HCl, pH 7.5, to 50 mM Tris–HCl, pH 7.9, 0.7 M KCl in 1 mM DTT and 10% glycerol. The purified enzyme was dialyzed against 20 mM Tris–HCl, pH 7.5, 10% glycerol and 1 mM DTT followed by concentration in an Amicon ultrafiltration cell. The purified enzyme was stored at −70° until use.

Enzyme assay. The assay for HIV reverse transcriptase measured the incorporation of tritiated dNTP into the acid-precipitable fraction. The assay mixture contained 42 mM Tris–HCl, pH 7.5, 83 mM KCl, 4.2 mM $MgCl_2$, 4.2 mM DTT, 0.125 O.D. units of template, 0.025 O.D. units of primer, and a preparation of enzyme in a total volume of 60 μl. After incubation at 37° for various periods of time, 10 μl was removed and processed as reported previously [8].

Results and discussion

Figure 1 shows the inhibition pattern of ddATP against dATP with poly(dU)-oligo$(dA)_{12\text{-}18}$ as a template-primer. The K_m for dATP was 3.9 μM, whereas the K_i for ddATP against dATP was 13 μM with competitive kinetics.

Table 1 shows the inhibition constants of the compounds against HIV reverse transcriptase. The kinetic constants

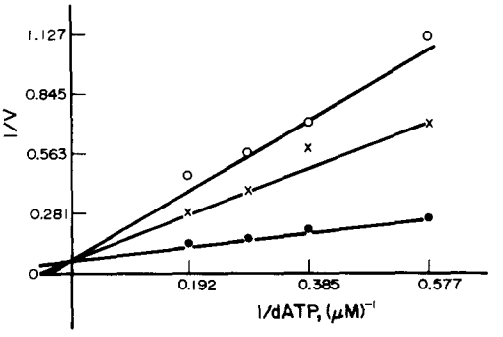

Fig. 1. Inhibition patterns of ddATP against dATP for the HIV reverse transcriptase. Enzyme assay is described in Materials and Methods. Poly(dU)-oligo$(dA)_{12\text{-}18}$ was used as template-primer. V (initial rate) is expressed as cpm/min of incubation. Key: (●) velocity in the absence of ddATP, and (×) and (○) ddATP at 31.03 and 62.06 μM respectively.

Table 1. Kinetic constants for HIV reverse transcriptase

Template-Primer	Substrate	K_m (μM)	Inhibitor	K_i (μM)
Poly(A)-Oligo$(dT)_{10}$	[^{3}H]dTTP	3.6	ddTTP	0.03
			IdUTP	1.8
Poly(dA)-Oligo$(dT)_{12\text{-}18}$	[^{3}H]dTTP	7.2	ddTTP	0.09
			dUTP	2.5
Poly(U)-Oligo$(dA)_{12\text{-}18}$	[^{3}H]dATP	2.3	ddATP	5.75
Poly(dU)-Oligo$(dA)_{12\text{-}18}$	[^{3}H]dATP	3.9	ddATP	13
Poly(rC)-Oligo$(dG)_{12\text{-}18}$	[^{3}H]dGTP	1.5	ddGTP	0.03
Poly(dC)-Oligo$(dG)_{12\text{-}18}$	[^{3}H]dGTP	2.3	ddGTP	0.07
Poly(dG)-Oligo$(dC)_{12}$	[^{3}H]dCTP	9.3	ddCTP	0.9

(both K_m and K_i values) increased only slightly when the templates used were changed from RNA to DNA, indicating that the inhibition of both steps of reverse transcriptase activity by the triphosphates of 2′,3′-dideoxynucleosides was equally effective. This has great significance in terms of inhibition of HIV replication.

Mitsuya and Broder [3] ranked the inhibitory activities of 2′,3′-dideoxynucleosides against the cytopathic effect of HIV as 2′,3′-dideoxycytidine > 2′,3′-dideoxyadenosine > 2′,3′-dideoxyguanosine > 2′,3′-dideoxythymidine. However, as shown in Table 1, the order of inhibitory activities against HIV reverse transcriptase was ddTTP ≃ ddGTP > ddCTP > ddATP. This lack of correlation between anti-HIV activities of the 2′,3′-dideoxynucleosides and the ability of their triphosphate derivatives to inhibit HIV reverse transcriptase may result from several factors:

(1) The efficiency of the phosphorylation of 2′,3′-dideoxynucleosides to their triphosphate derivatives and the affinities of the triphosphate derivatives to HIV reverse transcriptase may both vary independently. Two compounds, 2′,3′-dideoxycytidine and 3′-azidothymidine, have been shown to be phosphorylated to their triphosphate derivatives in HIV infected and mock infected cell cultures [9–11]. Also the artificial templates/primers used in these assays may not truly reflect the affinities of these triphosphates to HIV reverse transcriptase inside the infected cells.
(2) Metabolic inactivation by deamination (e.g. 2′,3′-dideoxycytidine and its monophosphate derivative are potential substrates for enzymic deamination, 2′,3′-dideoxyadenosine was deaminated by adenosine deaminase at 26% of the rate of deamination of adenosine) and phosphorolysis of the nucleosides could be different.
(3) In addition to their triphosphate derivatives, unknown metabolites of 2′,3′-dideoxynucleosides may also contribute to their anti-HIV activities. One of the anabolites of 2′,3′-dideoxycytidine was reported to be 2′,3′-dideoxycytidine-choline [9, 10].

In summary, the triphosphates of 2′,3′-dideoxythymidine and 2′,3′-dideoxyguanosine were the most potent inhibitors of RNA to DNA synthesis with the other compounds in the following order: ddTTP ≃ ddGTP > IdUTP > ddATP. All the inhibitors showed a slightly lower degree of activity against DNA to DNA synthesis catalyzed by HIV reverse transcriptase.

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Effect of daunorubicin on dihydropyridine-sensitive binding sites in cardiac sarcolemma

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Calcium ion movement through voltage-activated channels in the cardiac cell membrane in response to voltage changes is largely responsible for control of the contractile mechanisms in the heart [1]. Any agent that alters the movement of calcium across the cardiac cell membrane can be expected to influence cardiac contractile force and rate. Agents that are positive inotropes and chronotropes and increase the movement of calcium through calcium channels in the cell membrane include the beta-receptor stimulating catecholamines, cyclic AMP, and histamine [1]. The anthracycline antitumor agents, e.g. doxorubicin or daunorubicin, have been shown to inhibit many aspects of cardiac function. They noncompetitively block the positive inotropic or chronotropic action of isoproterenol and histamine [2, 3]. Cardiac sarcolemmal membrane systems are affected adversely by anthracyclines. For example, doxorubicin decreases the activity of adenyl cyclase [4] and calmodulin [5] *in vitro*. Attempts to reverse the acute cardiotoxicity of carminomycin with isoproterenol, digoxin or calcium infusion in the isolated rat heart showed that it can be performed best by an increase in the extracellular calcium ion concentration [6]. In a recent report, Villani *et al.* [7] showed that doxorubicin can acutely inhibit the increase in contractile force produced by increasing con-