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Kinetic Analysis of the Inhibition of Human Immunodeficiency Virus Type 1 (HIV-1) Reverse Transcriptase by R82150, a TIBO analog. E.V. Connell, K.B. Frank and I.S. Sim. Roche Research Center, Nutley, NJ, USA.

The reverse transcriptase (RT) of HIV-1 is an attractive target for antiviral chemotherapy and several inhibitors of this replicase have been shown to be effective antiviral agents. We have used recombinant DNA-derived RT to determine the inhibition kinetics of a recently described member of the TIBO (tetrahydro-imidazo [4,5,1-jk] [1,4]-benzodiazepin-2 (1H)-thione) family of compounds, R82150, a potent inhibitor of HIV-1 replication *in vitro*. Employing a variety of homopolymer template-primers we were able to demonstrate noncompetitive inhibition of dNTP utilization by R82150. K_I values of 100, 300, 570 and 890nM were determined for dATP, dGTP, TTP and dCTP, respectively, in the presence of saturating concentrations of the appropriate template-primer. Noncompetitive inhibition of poly(rC)·oligo(dG) utilization by R82150 was also observed in the presence of saturating levels of dGTP (K_I=280nM). In contrast, R82150 at concentrations as high as 100μM did not inhibit utilization of dATP by human DNA polymerase α, β or γ in studies using activated DNA as template. This compound, unlike the dideoxynucleosides which are currently being evaluated in the clinic for the treatment of HIV infection, does not appear to act as a substrate analog, but may interact allosterically with a heretofore unrecognized site of HIV-1 RT.

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Mechanism of the Inhibitory Effect of 4'-Azido-thymidine (ADRT) on the Replication of Human Immunodeficiency Virus In Vitro. M.S. Chen, R. Suttmann, C. Bach, J.C. Wu, E.J. Prisbe, M.J. McRoberts, and D. Crawford-Ruth. Institute of Cancer and Developmental Biology, Syntex Research, Palo Alto, CA 94303.

The thymidine analog 4'-azidothymidine (ADRT) is a potent and selective inhibitor of the replication of human immunodeficiency virus 1 (HIV-1) in vitro. The nature of this selectivity was investigated by studying the metabolic pathways of ADRT. ADRT triphosphate was a potent inhibitor against HIV-1 reverse transcriptase with a Ki value of 8.5 nM versus the Km value of 2 µM for TTP. ADRT triphosphate was a poor inhibitor against DNA polymerases α and β; Ki values were 62.5 µM and 150 µM, respectively, versus Km values of 2.8 µM and 2.7 µM for TTP. Competitive inhibition patterns were found in all enzymatic analyses. ADRT was phosphorylated efficiently to ADRT triphosphate in human peripheral blood lymphocytes. The maximum concentrations and half-lives of ADRT triphosphate and AZT triphosphate were similar in both A3.01 cells (Alex cells) and H9 cells. In U937 cells the maximum concentration of ADRT triphosphate was 500% higher than AZT triphosphate. The half-life of ADRT triphosphate was 6 hr, while that of AZT triphosphate was 3 hr in U937 cells. ADRT triphosphate was the major phosphorylated metabolite of ADRT in ADRT-labelled cells, where as AZT monophosphate was the major phosphorylated metabolite in those labelled with AZT. ADRT was found to be internally incorporated into host DNA, in contrast to AZT which is a DNA chain teminator. The extent of ADRT monophosphate substitution for TMP in DNA was 1 in 6979 for A3.01 cells after incubation with 2.9 µM ADRT for 24 hr. DNA elongation stops when two ADRT molecules are incorporated adjacent to one another. This DNA chain termination required HIV reverse transcriptase and is due to the high affinity of ADRT triphosphate toward the HIV reverse transcriptase.