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Anti-HIV-1 Activity of a Novel Nonnucleoside Reverse Transcriptase Inhibitor of HIV-1 (SJ3366 a pyrimidinedione analog) Used in Combination With Other Inhibitors of HIV-1 Replication

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Monotherapy has had limited success in the treatment of human immunodeficiency virus (HIV) infection. The rapid replication rate and subsequent viral diversity of HIV leads to the emergence of drug-resistant viral strains which is linked to failure of single-agent therapy. Potent and rationally designed chemotherapy combining protease inhibitors and nucleoside analogs can dramatically slow the progression of HIV infection in patients. In the present study SJ3366 a nonnucleoside reverse transcriptase inhibitor which inhibits HIV-1 replication at concentrations below 1 nM and exhibits a therapeutic index of greater than 4,000,000, was evaluated in combination with a variety of other mechanistically diverse inhibitors of HIV replication. Such *in vitro* studies provide insights into whether certain drug combinations yield synergistic antiviral activity or, more importantly, antagonistic antiviral activity or synergistic cytotoxicity. SJ3366 exhibited synergistic antiviral interactions with the nucleoside reverse transcriptase inhibitor ddI, and in triple combination with the nucleoside reverse transcriptase inhibitor AZT and nonnucleoside reverse transcriptase inhibitor nevirapine. All other tested compounds yielded additive interactions. Studies are underway to determine the effect of the combinations on viruses resistant to SJ3366. No evidence of either combination toxicity or antagonistic antiviral activity was detected with any of the tested compounds. These findings suggest promising new therapies for the treatment of HIV infection.

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Phosphorylation of anti-HIV nucleotides AZT, d4T and ddNTP by Nucleoside Diphosphate Kinase

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Nucleoside Diphosphate Kinase catalyzes the addition of the third phosphate to cellular nucleotides according the reaction: $N_1TP + N_2DP \Longleftrightarrow N_1DP + N_2TP$ with usually ATP as N_1TP. This reaction constitutes the last step in the intracellular activation of antiviral nucleosides analogs. We address the question of the reactivity of NDP kinase for nucleotides analogs used in anti HIV-therapies. The reaction involves a phospho-enzyme intermediate (E~P). We use the protein fluorescence to follow the kinetic of phosphotransfer from NTP to E and from E~P to NDP. We show that AZT-diphosphate and ddTDP are poor substrates for NDP kinases with a loss in catalytic efficiency in the 10^4 to 10^5 range. Presteady state experiments demonstrate that the rate of phosphorylation of the enzyme by antiviral ddNTP is dramatically decreased. D4T (2'3'dideoxy, 2'3'didehydro thymidine, stavudine) is a better substrate than AZT for NDP kinase with a catalytic efficiency, compared to AZT, increased by a factor of 10 to 50. The X-ray structure of the cocrystal of a NDP kinase variant with d4T-triphosphate gives a rational for its high affinity. The metabolic activation pathways for D4T and AZT will be discussed.

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Anti-HIV-1 Activity of PMEA, PMPA (9-(2-phosphonylmethoxypropyl)adenine) and AZT Against HIV-1 Subtypes A, B, C, D, E, F, G and O

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The global spread of HIV-1 subtypes other than subtype B in developing and industrialized countries highlights the need to assess the activity of new and upcoming anti-HIV agents against non-subtype B viruses. Moreover, the genetic variability of the LTR and *pol* genes of HIV-1 subtypes point to possible differences in replicative properties and susceptibility to antiretroviral treatments between subtypes. We assessed the susceptibility of subtypes A, B, C, D, E, F, G, and O to PMEA, PMPA and AZT in peripheral blood mononuclear cells (PBMC). At least three different isolates for each subtype were studied. For most isolates the treatment histories of the patients were unknown, but considering their country of origin and year of collection (1992-1993) it may be assumed that these isolates originate from untreated patients. The activity of the antiretroviral compounds against the different subtypes was determined by viral p24 antigen measurement and reverse transcriptase enzyme (RT) activity. The results show that all non-B subtype isolates of HIV-1 were similar in their drug susceptibility to PMEA, PMPA and AZT as compared to subtype B isolates. This study suggests that PMEA and PMPA should retain their activity *in vivo* against HIV-1 strains other than subtype B.

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Antiviral Amino Acid Carbamate Derivatives of AZT

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A series of tryptophan and phenylalanine carbamates of AZT was prepared and evaluated for anti-HIV-1 activity in PBMCs. These compounds showed antiviral (HIV-1) activity in the low μM range and were not toxic at concentrations >100 μM. The carbamates compounds did not exhibit inhibitory activity toward HIV-1 reverse transcriptase or integrase. The stereochemical requirement for the amino acid side chain does not appear to be specific, since the L-tryptophan derivatives were found to be more potent than the D-tryptophan derivatives, while the D-phenylalanine derivatives were found to be more potent than the L-phenylalanine derivatives. Neither the methyl ester nor methyl amide carbamates were converted by cell lysates to detectable amounts of AZT. However, unlike the methyl amides, the methyl esters were rapidly hydrolyzed presumably by carboxyesterase to the corresponding free acids. Although the antiviral activity of this series is in the low μM range, their inability to either inhibit HIV-1 reverse transcriptase or integrase or serve as AZT prodrugs suggests that they may act through a mechanism not previously utilized by nucleoside based antivirals.