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Effect of Specific Amino Acid Changes on the Anti-HIV Activity of NNRTIs: Confirmation of NNRTI-Resistant Virus Subgrouping and Selection of Compounds for Combination Therapy. R.W. Buckheit, Jr., V. Fliakas-Boltz and L.A. Pallansch. Southern Research Institute-Frederick Research Center, Frederick, MD USA.

The nonnucleoside RT inhibitors (NNRTIs) include the most potent and selective agents targeted against HIV-1. We have recently described the existence of discrete subgroups of NNRTI-resistant virus isolates. These subgroups are defined by the specific amino acid change in the RT and the cross-resistance patterns exhibited by the virus isolates to other members of the pharmacologic class of NNRTIs. The subgrouping patterns have been used to determine if a rational combination of NNRTIs might be selected for therapeutic use which would require the presence of incompatible mutations in the RT in order for resistance to both anti-HIV agents to develop. In order to further examine and expand the subgroups of NNRTI-resistant viruses, we evaluated a wide variety of NNRTIs against both reverse transcriptase and virus isolates with defined single amino acid changes in the RT. The data accumulated provide support for the existence of at least four distinct subgroups of NNRTI-resistant virus isolates. The first subgroup includes viruses with the mutations L100I, K101E and K103N and viruses with these mutations were cross-resistant to each of the 12 NNRTIs evaluated. Subgroup II is composed of virus isolates resistant to calanolide A and its analogs. These virus isolates have mutations V90I, T139I and P225S and are not cross-resistant to any of the remaining NNRTIs. Subgroup III virus isolates have the Y181C mutation and are cross-resistant to each of the NNRTIs, with the exception of calanolide A. Calanolide A exhibits a ten-fold enhanced activity against virus isolates with the Y181C mutation and remains sensitive to viruses with the mutations Y181C and K103N. The antiviral activity of calanolide A is further enhanced when the Y181C mutation is present with mutations conferring AZT resistance. Subgroup IV consists of virus isolates with the mutation P236L. These virus isolates remain sensitive to all of the NNRTIs and exhibit enhanced sensitivity to the NNRTI compounds thiazolobenzimidazole and oxathiin carboxanilide. Evaluation of the agents against RT with defined mutations provide further support for the cross-resistance patterns described above. Cross-resistance data suggest the therapeutic potential of calanolide A in combination with either HEPT or a diarylsulfone. Whereas most combinations of NNRTIs have resulted in the rapid selection of drug-resistant isolates, the combination of calanolide A and diphenylsulfone may be effective at inhibiting the replication of HIV-1 without rapid selection of resistant virus isolates.

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Unique Purine Crossover Activation Pathway for the Potent Anti-HIV Agent 1592U89.
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1592U89 ((1S,4R)-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol), is a potent and selective inhibitor of HIV replication *in vitro*. The ultimate active species generated from 1592U89 has been found to be the 5'-triphosphate of its guanine analog (-)-carbovir (CBV), a potent inhibitor of HIV reverse transcriptase, and not the triphosphate of 1592U89 itself. Distinctly different anabolic pathways for 1592U89 and CBV were demonstrated. CBV-TP is formed from 1592U89 by two unusual enzymatic steps. Human adenosine phosphotransferase, which prefers AMP as phosphate donor, phosphorylates 1592U89 (K_m = 3.2 mM, V_{max} 55% that of adenosine) but not CBV. The resultant 1592U89-MP is then converted to CBV-MP and on to CBV-TP. In contrast, CBV is a good substrate for 5'-nucleotidase (phosphate donor IMP) whereas 1592U89 is not. Mycophenolic acid increased IMP (100-fold) and CBV-TP formation from CBV (75-fold) but did not enhance 1592U89 anabolism to CBV-TP in $CD4^+$ CEM cells. 1592U89 is a poor substrate for adenosine deaminase (0.00003% of adenosine rate). In 1592U89-treated CEM cells, intracellular CBV levels are <2% those of 1592U89. This amount of CBV is insufficient to generate the CBV-TP levels observed after 1592U89 treatment. Thus, 1592U89 is not a prodrug of CBV. The adenosine deaminase inhibitor EHNA did not inhibit CBV-TP formation from CBV or 1592U89. However, the adenosine/adenylate deaminase inhibitor 2'-deoxycoformycin selectively inhibited 1592U89 anabolism to CBV-TP and reversed the antiviral activity of 1592U89. 1592U89 was also anabolized to CBV-TP in normal human peripheral blood lymphocytes. The anti-HIV activity of 1592U89 is mediated through the dGTP analog CBV-TP *via* an intracellular purine crossover pathway that occurs at the monophosphate level and not at the nucleoside level. Intrinsic to this pathway is the N^6-cyclopropylamino modification of the 1592U89 purine ring. The existence of this unique activation pathway enables 1592U89 to overcome the deficiencies of CBV (which include low oral bioavailability and minimal brain penetration) while maintaining potent and selective anti-HIV activity.