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Combinations of AZT and Analogs of TIBO Act Synergistically to Inhibit HIV-1 Infection In Vitro. R.W. Buckheit, Jr., J.Germany-Decker, E.L. White, L. Ross, L. B. Allen and W.M. Shannon. Southern Research Institute, Birmingham, AL, USA

The toxicity of 3'-azido-3'-deoxythymidine (AZT) and the appearance of drug resistant mutants emphasizes the importance of the development of alternative strategies for the therapy of AIDS patients. Combination antiviral chemotherapy provides an attractive therapeutic strategy since the dose of the individual agents may be lowered to reduce toxicity and limit the development of drug-resistant mutants. A second pharmacological class of reverse transcriptase inhibitors, derivatives of tetrahydro- imidazo[4,5,1-jk]-[1,4]-benzodiazepin-2(1H)-thione (TIBO), potently and selectively inhibit the replication of HIV-1 in cell culture. In combination with AZT, the two TIBO derivatives, R82913 and R86183 exhibit a high degree of synergy in cell culture based antiviral assays. Our findings suggest that the TIBO compounds act at a site on the HIV-1 RT molecule distinct from the site recognized by AZT and that the combination of RT inhibitors are more potent when administered together. The significant activity exhibited by both AZT and the TIBO analogs suggests that combination chemotherapy with these agents would be beneficial to AIDS patients and would allow lower nontoxic antiviral concentrations of AZT to be employed. The results of classical biochemical analysis of these combinations at the level of the proposed antiviral target, reverse transcriptase, will also be presented. R82913 and R86183 were a gift from Janssen Research Foundation (Beerse, Belgium).

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AZT is Not Synergistic with Either ddI, ddC, or Carbovir at Their Proposed Site of Action, the Reverse Transcriptase. E. L. White, L. J. Ross, W. B. Parker, and W. M. Shannon. Southern Research Institute, Birmingham, AL USA.

The nucleoside analogues AZT, ddC, and ddI are clinically useful in the treatment of individuals infected with human immunodeficiency virus type 1 (HIV-1). A fourth nucleoside analogue, carbovir, has been shown to be capable of protecting cells in culture from the cytopathic effects of HIV-1. Substantial evidence has been published showing that these four compounds act, after conversion to their 5'-triphosphate, by inhibiting the viral reverse transcriptase (E.C.2.7.7.49). (In the case of ddI, the active metabolite is presumed to be the 5'-triphosphate of ddA.) When tested in combination, AZT acts synergisticaly with any of the three other nucleoside analogues in protecting cells infected with HIV-1. Since it has been generally assumed that the retroviral reverse transcriptase is similar to other DNA and RNA polymerases in having only one deoxynucleotide binding site, the observation of synergy for these analogues is surprising. With the standard assay for the RNA-directed DNA polymerase activity of reverse transcriptase which uses a synthetic homopolymer and a single deoxynucleotide substrate, it has been impossible to test for synergy at the enzyme level for these combinations. We have used an assay containing ribosomal RNA to test these combinations directly at the enzyme level. In addition, we have assessed the effect of these combinations on the other polymerase activity of reverse transcriptase, DNA-directed DNA synthesis. The combinations were evaluated by classical kinetic methods described by Irwin H. Segel in **Enzyme Kinetics** (1975, John Wiley & Sons, Inc., NY). Combinations of AZTTP with ddATP, AZTP with ddCTP, and AZTTP with carbovirTP were not synergistic on either the RNA or DNA template. These results indicate that the synergy seen with combinations of anti-HIV nucleoside analogs is not due to the synergistic inhibition of either polymerase activity of HIV-1 reverse transcriptase. **Partially supported by NIH grant RO1 AI29157 and UO1-AI26054.**