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Cooperativity of a HIV-1 Protease Inhibitor (SKF 108922) with AZT in Acute and Chronic HIV-1 Infections in vitro. D. M. Lambert, H. Bartus, G. B. Dreyer, T. D. Meek, B. W. Metcalf and S. R. Petteway, Jr., Depts. of Antiinfectives, and Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 USA.

Synthetic peptide analog inhibitors of HIV-1 protease inhibit the spread of infectious virus in acutely infected T-cells and production of infectious virions in chronically infected cells. These inhibitors work predominantly late in the infectious cycle while AZT works early. To investigate whether they exhibited additive or synergistic drug interaction effects, SKF 108922, an HIV-1 protease inhibitor, was compared with AZT. Antiviral activity of these compounds was evaluated in three separate in vitro assays. (1) In acute infection of Molt-4 cells with HIV-1 strain IIIB, co-treatment with these compounds demonstrated additivity affording a 2-3-fold enhancement of activity of both compounds. (2) In co-cultivation experiments with Molt-4 cells and chronically infected H9 cells, SKF 108922 demonstrated potent synergy with AZT. Whereas, with a chronically infected CEM cell line, the interaction was additive. (3) AZT treatment of the H9/IIIB chronic cell line alone demonstrated no inhibitory effect, but in comparison SKF 108922 was potently inhibitory. Co-treatment of H9/IIIB chronically infected cultures with both SKF 108922 and AZT showed activity similar to SKF 108922 alone. These data suggest that the antiviral effect of AZT is primarily manifest on acute infection. In contrast, HIV-1 protease inhibitors exert a potent antiviral effect on both acute and chronic infections. Since AZT and SKF 108922 have different viral targets and inhibit different stages of infection, it is likely that co-treatment with AZT and a protease inhibitor could have an additive or synergistic effect in vivo. The in vitro data to be presented support this concept.

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Biochemical Characterization of HIV-1 Reverse Transcriptases with Mutations Associated with DDI Resistance and Collateral AZT Sensitivity.

Furman, PA[1], JL Martin[1], BA Larder[2], and JE Wilson[1]; [1]Division of Virology, Burroughs Wellcome Co., Research Triangle Park, NC, USA, [2]Division of Molecular Sciences, Wellcome Research Laboratories, Beckenham, Kent, England

Genomic analysis of isolates obtained from AIDS patients treated with ddI following long-term AZT treatment revealed a mutation at position 74 associated with decreased sensitivity to ddI. Molecular clones were constructed by site-directed mutagenesis and subcloned from the M13 vector into an over-expression vector, pKK233, for maximum expression of protein. The protein was purified by means of immunoaffinity chromatography and biochemical and kinetic characterizations were done. Reverse transcriptase L74V catalyzed substrate dNTP's as efficiently as wild type with no change in K_m. Inhibitor analysis revealed that there is four- to five- fold increase in K_i for ddNTP's . The mutation at position 74 is also known to supress the effect of the AZT resistance mutation at position 215. Molecular clones were constructed by site-directed mutagenesis to contain both of these mutations. Kinetic analysis with substrates and inhibitors for this mutant will be discussed.