91

REPLICATION AND INFECTIVITY OF HIV IN CHRONICALLY-INFECTED MACROPHAGES AND LYMPHOCYTES IS SELECTIVELY BLOCKED BY PROTEASE INHIBITORS

CF. Perno*, A. Bergamini+, CD. Pesce*, M. Capozzi+, S. Thaisrivongs, G. Tarpley**, G. Zon++, G. Rocchi+, E. Garaci*, R. Calio'*.** * Dept.Exp. Medicine, and +Dept. Public Health, Univ. Rome Tor Vergata, Italy; **Upjohn Co., Kalamazoo, MI, USA; ++Linx Therapeutics, Foster City, CA, USA

Cells of monocyte/macrophage lineage (M/M) infected by HIV represent a crucial reservoir of the virus within the body. Since inhibition of HIV replication in such cells is quite important in the therapeutic approach to the HIV infection, we assessed the activity of U-75875 protease inhibitor and of phosphorothioate antisense in this cellular model and in chronically-infected T-lymphocytes. Inhibition of HIV replication can be achieved by both U-75875 and antisense in *de novo*-infected M/M and lymphocytes at concentrations at least 1,000 fold lower than those toxic. U-75875, but not antisense, was also able to inhibit HIV production and infectivity in chronically-infected M/M and lymphocytes, with an ED_{50} of 3uM and 1uM respectively. Such effect of U-75875 is maintained overtime for at least 21 days of culture, and is characterized by a >100,000 fold drop of virus titer. Furthermore, either macrophage- or granulocyte-macrophage colony stimulating factor (cytokines that enhance HIV replication in M/M) do not affect the activity of protease inhibitor in chronically-infected M/M. Interestingly, quantitative PCR analysis demonstrates that HIV-DNA is not affected by U-75875 either if the drug is added before virus challenge (*de-novo* infection) or 9-10 days after (chronical infection), while AZT is clearly active if given before (but not after) virus challenge. Finally, virus antigen expression on the surface of chronically-infected M/M is also not affected by the treatment with U-75875. Taken all together, these data strongly support the efficacy of protease inhibitors in chronically-infected cells, and suggest their utility in patients infected by HIV.

92

IN VITRO ISOLATION OF BICYCLAM-RESISTANT VARIANTS OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

M. Baba,[1] N. Yamamoto,[2] R. Pauwels,[2] Z. Debyser,[2] S. Shigeta,[1] E. De Clercq,[2] G. Bridger,[3] G. Henson,[3] and M. Abrams[3]
[1]*Department of Microbiology, Fukushima Medical College, Fukushima 960-12, Japan,* [2]*Rega Institute, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium, and* [3]*Johnson Matthey Pharmaceutical Research, Pennsylvania 19380, U.S.A.*

We have recently described bicyclams as a novel series of human immunodeficiency virus type 1 (HIV-1) inhibitors that appear to be targeted at a viral uncoating event (De Clercq *et al., Proc. Natl. Acad. Sci. USA* 89: 5286-5290, 1992). JM2763, a representative derivative of the bicyclams, inhibits HIV-1 replication in MT-4 cells at a 50% effective concentration of 0.1-1 µg/ml. HIV-1 variants resistant to the bicyclams have been isolated from a clinical HIV-1 isolate (A018A) following several passages of the virus in cell culture in the presence of the drug. These variants were more than 100-fold less sensitive to JM2763 as compared to the reference strain ($HTLV\text{-}III_B$). However, no cross-resistance was observed to 3'-azido-3'-deoxythymidine (AZT), 1-ethoxymethyl-5-ethyl-6-(phenylthio)-uracil (E-EPU) or dextran sulfate. An uncoating assay, based on the sensitivity of [5-^{3}H]uridine-labeled virus particles to ribonuclease A treatment, revealed that JM2763 inhibited the uncoating of $HTLV\text{-}III_B$ but not of the drug-resistant HIV-1 variants.