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Photoaffinity labeling of HIV reverse transcriptase: Synthesis and utilization of 2',3'-dideoxy uridylate analogs bearing aryl(trifluoromethyl)diazirine moiety

T. Yamaguchi and M. Saneyoshi

Department of Biological Sciences, Teikyo University of Science, Uenohara, Yamanashi 409-01, Japan

In order to investigate the recognition of substrates with HIV reverse transcriptase and other DNA polymerase molecules, synthesis and characterization of a photoaffinity labeling reagent for these enzymes, 2',3'-dideoxy-*E*-5-[4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]styryl]UTP (**TDSddUTP**), was undertaken.

In the presence of 0.025 mM Mg^{2+} ion, photoaffinity labeling experiment showed that **TDSddUTP** could preferentially bind to the dTTP binding site in 66-kDa subunit of the p66/p51 heterodimeric enzyme under irradiation by near-ultraviolet light (365 nm). On the other hand, in the presence of 4 mM Mg^{2+} or 0.05 mM Mn^{2+}, **TDSddUTP** was incorporated into the 3'-end of the primer strand by reverse transcriptase, and then the generated photoreactive primer was able to bind to the 66-kDa subunit of HIV-1 reverse transcriptase by photo-irradiation. Thus, it was demonstrated that **TDSddUTP** should be an useful tool for studying of the substrates binding site of HIV-1 reverse transcriptase.

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Potent Inhibition of HIV-1 Replication *In Vivo* by a Novel Inhibitor of HIV-1 Fusion. MICHAEL A. USSERY, OWEN L. WOOD, DENNIS D. BROUD, MATTHEW A. BACHO, STEVEN C. KUNDER, SUSAN F. PAPERMASTER, DENNIS M. LAMBERT[1], SHAWN BARNEY[1], and PAUL L. BLACK. U.S.F.D.A., Rockville, MD, and [1]Trimeris, Inc., Research Triangle Park, NC, USA.

T-20 (pentafuside, DP-178), a 36-mer synthetic peptide derived from the HIV-1 gp41 transmembrane protein, is a selective and potent inhibitor of HIV-1 fusion (IC_{50}=1 ng/ml) and infection *in vitro*. Pharmacokinetic studies in rodents revealed a surprisingly long half-life in circulation ($t_{1/2}$=2.4 hr), with sustained levels above the IC_{50} for at least 6 hr. Therefore, we tested the ability of T-20 to inhibit HIV-1 replication *in vivo* in the HuPBMC-SCID mouse model of HIV-1 infection. Female SCID mice (5-7 wk old) received $5X10^7$ adult human PBMC I.P. Two weeks after reconstitution, mice were infected I.P. on day 0 with 10^3 $TCID_{50}$ HIV-1 9320 (passed in PBMC, AZT-sensitive isolate A018, D. Richman). Treatment with T-20 was I.P., bid, for total daily doses of 2, 20, or 200 mg/kg, beginning on day -1. The extent of infection in blood cells, splenocytes, lymph nodes (LN), and peritoneal cells (PC) was assayed by quantitative coculture with human PBMC blasts 1 wk later. Infectious HIV-1 was recovered from **none** of the tissues from the animals treated with the 200 mg/kg/day dose. Furthermore, the two lower doses of T-20 reduced the recovery of HIV-1 from cells in PC, LN, and blood, but not from spleen. Additionally, viral load was quantitated by the NASBA assay, and the results were generally consistent with those of cocultures. However, low levels of HIV-1 RNA were detected in the plasma and cells of some, but not all, animals treated with the high dose of T-20. Antiviral effects also correlated with the protection of human CD4 lymphocytes (assayed by flow cytometry) from the cytotoxic effects of HIV infection, with a dose-dependent restoration of the CD4/CD8 ratio by T-20. These results support the potential therapeutic efficacy of T-20 and this novel class of antiviral agents.

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Antiviral activity of a sulfated polysaccharide extracted from the marine *Pseudomonas* and marine plant *Dinoflagellata* against human immunodeficiency viruses and other enveloped viruses

K. Hashimoto,[1] E. Kodama,[1] S. Mori,[1] J. Watanabe,[1] M. Baba,[2] K. Okutani,[3] M. Matsuda,[3] and S. Shigeta.[1]

[1]Department of Microbiology, Fukushima Medical College, Fukushima 960-12, [2]Faculty of Medicine, Kagoshima University, Kagoshima 890, and [3]Faculty of Agriculture, Kagawa University, Kagawa 761-07, Japan

A natural sulfated mucopolysaccharide (OKU40) extracted from a marine plant *Dinoflagellata* and an artificial sulfated polysaccharide (OKU41) prepared from a marine *Pseudomonas* displayed antiviral activities against several enveloped viruses. OKU40 and OKU41 were found to be homogenous in electrophoresis and had a molecular weight of 8.0×10^6 and 5.0×10^5, respectively. The sulfation rate of OKU40 and OKU41 was 8.9% and 5.4%, respectively. Each OKU40 and OKU41 inhibited the cytopathic effect of HIV-1, HIV-2 and zidovudine-resistant HIV-1 in MT-4 cells at similar concentrations to those of dextran sulfate (MW: 5000) (IC_{50}: 0.86-1.95 μg/ml), whereas these compounds did not affect the growth and viability of mock-infected MT-4 cells at concentrations up to 500 μg/ml. These compounds proved inhibitory not only to HIV-1 and HIV-2 but also to other enveloped viruses, i.e. HSV-1, FluV -A and -B, RSV, and MSLV. OKU40 and OKU41 suppressed syncytium formation induced by cocultivation of MOLT-4/III_B and MOLT-4 cells at concentrations higher than 20 μg/ml. Although OKU41 inhibited the binding of HIV-1 to the host cells and the binding of anti-gp120 monoclonal antibody to HIV-1 gp120, OKU40 did not inhibit these bindings, suggesting that the mechanism of anti-HIV activity of OKU40 and OKU41 may be primarily due to the inhibition of virus-cell fusion and viral adsorption to the host cells, respectively. Furthermore, these compounds did not inhibit to the blood coagulation process at a concentration that was significantly inhibitory to HIV replication.

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Directed Generation and Characterization of Clinically Important, Resistance Associated Mutants of HIV-1 Protease

Th. Klimkait, C. Sonderegger, F. Stauffer, B. Poncioni, J. Mestan
Pharmaceutical Research, Ciba-Geigy, Ltd., Basle, Switzerland

The unique aspartic protease of HIV-1, responsible for particle maturation and infectivity belongs currently to the most appealing targets for antiviral therapy against HIV. The enzyme of a well characterized proviral clone, pNL4-3, was adapted to the general HIV-1 sequence consensus. Based on this 'wild type' we generated a PCR- and M13-based cloning cassette for the introduction of complex point mutations into the protease gene. The changes resemble those patterns, reported to be critically involved in the emergence of viral resistance against clinically relevant protease inhibitors. A comparison of the most frequent variants reported by Roche, Merck, Vertex and with CGP 61755 revealed a dramatic impact of certain mutations on the speed of replication, on viral protein processing, and on particle infectivity. Evaluation of he last parameter is far from trivial, since a decrease of virion-associated RT activity and of infectivity complicates the accurate quantification of viral gene activity and particle release; therefore complementing, cell based methodologies were employed. A persistent clinical benefit despite the emergence of 'protease resistant' variants has been recently observed *in vivo*. By the use of complex but defined point mutants of a known, infectious HIV-1 clone may we hope to be able to correlate certain *in vitro* characteristics of the mutated protease with the clinical activity profile of specific protease inhibitors. Moreover, molecularly defined mutants are currently being used to find and characterize viral resistance classes and, as a consequence, to identify therapeutically beneficial combinations of HIV protease inhibitors.