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Capture of HIV-1 gp120 and virions by lectin-immobilized polystyrene nanospheres: Potential approach toward prevention of HIV-1 infection

T. Hayakawa*, T. Nitkawa*, A. Kishida*, M. Akashi*, and M. Baba*
*Division of Human Retroviruses, Center for Chronic Viral Diseases, Faculty of Medicine, Kagoshima University, Kagoshima 890, Japan, and *Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering, Kagoshima University, Kagoshima 890, Japan.

It has been shown that HIV-1 gp120 strongly interacts with some lectins. In particular, concanavalin A (con A) has a high affinity for gp120. Thus, gp120 and HIV-1 virions are expected to be effectively captured by lectins if they are immobilized to certain materials. Polymeric particles are often used as a material for immobilization of biomolecules such as antibodies and enzymes. Among them, polystyrene particles are quite useful, since monodispersed particles can be prepared easily. However, immobilization of biomolecule to polystyrene particles is difficult without nonspecific adsorption of other biomolecules. To overcome this problem, we have recently developed a technology on hydrophilic polymer chain-coated polystyrene nanospheres (NSs). Based on the technology, we have immobilized con A on the surface of polystyrene NSs and examined for their capturing activity for gp120 and virions. Polystyrene NSs covered with poly(methacrylic acid) were prepared by copolymerization of styrene with poly(*tert*-butylmethacrylayte) macromonomer and acid hydrolysis. Resulting NSs (mean diameter: 400 nm) were dispersed well in water. The immobilization of con A was performed by using water soluble carbodiimide. The surface of the obtained NSs (con A-NSs) were found to be fully covered with con A (0.3 $\mu g/cm^2$). The interaction of con A-NSs with HIV-1 was determined by the reduction of gp120 level and viral infectivity of HIV-1 suspensions after 60-min incubation at room temperature. The con A-NSs were also observed electronmicroscopically. Con A-NSs achieved 95 and 77% reduction of gp120 level and viral infectivity at a concentration of 0.5 mg/ml, respectively, indicating the effective capture of gp120 and virions.

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Topical and Combination Use of the Anti-HIV Phosphorothioate Oligonucleotide ISIS 5320 Halliday, S.M., Russell, J.D., Wyatt, J.R., Ecker, D.J. and Buckheit, R.W., Jr. Southern Research Institute, Frederick, MD., ISIS Pharmaceuticals, Carlsbad, CA.

The phosphorothioate oligonucleotide ISIS 5320 is a potent inhibitor of HIV infection *in vitro*. The oligonucleotide forms a parallel-stranded, tetrameric guanosine quartet (G-quartet) structure that specifically binds to the HIV envelope glycoprotein (gp120) and inhibits both cell-to-cell and virus-to-cell infection at submicromolar concentrations. Cell-based mechanism of action studies demonstrate that the compound inhibits the binding of infectious virus and virus-infected cells to both CD4-expressing and non-CD4 expressing target cells by binding to the cationic V3 loop of the envelope glycoprotein. The tetramer inhibits the infection of fresh human peripheral blood cells by biologically diverse clinical HIV-1 isolates, including strains representative of the various clades found worldwide. ISIS 5320 exhibits additive activity in combination with protease inhibitors, ddC, and α-APA; additive to slightly synergistic activity with AZT, ddI, and TIBO; and highly synergistic activity with the neutralizing antibody 2F5. ISIS 5320 prevents infection of the cervical epithelial cell line ME180, suggesting that the compound may effectively act as a topical microbicide to prevent the sexual transmission of HIV.

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Subcutaneous efficacy testing of two antisense oligonucleotides, HYB0184 and HYB0286, in SCID-hu-PBL Reconstituted HIV-Infected SCID mice

D.C. Quenelle[1], L.B. Allen[1], L. Pallansch [2], K. Keith [1], S. Li [1], K. Wright[1], G. Triggs [1], R. Harris [1], A. Brazier [1] and S. Agrawal [3], [1]Southern Research Institute, Birmingham, AL and [2]Frederick, MD USA and [3]Hybridon, Inc., Worcester, MA USA

Two oligonucleotides, HYB0184 and HYB0286, were evaluated in the SCID-hu-PBL HIV model. SCID mice were injected with 1 x 10^8 human peripheral blood leukocytes intraperitoneally on Day -7. Each animal was anesthetized and injected on Day 0 with 10,000 $TCID_{50}$ of HIV, strain III_B via intraperitoneal catheter. Drug therapy was initiated 4 hours postvirus inoculation by the subcutaneous route and was administered every 24 hours from Day 0 through Day 7. On Day 8, each animal was anesthetized for collection of blood, spleen and peritoneal wash samples for HIV analysis. Samples were analyzed for CPE following co-cultivation of sample dilutions with MT-2 cells and also by PCR analysis of plasma samples. The peritoneal wash samples had a mean log titer of 2.50 (n=8) for diluent treated infected mice. The HYB0184 (n=8) and HYB0286 (n=8) treated mice had peritoneal wash mean log titers of 1.50 and 1.00, respectively, which was statistically significant for HYB0286 with a probability = 0.04 by the Mann-Whitney **U** test. The spleen samples had a mean virus titer of 1.38 for the diluent injected mice while the HYB0184 and HYB0286 treated mice had mean log titers of 1.00 and 0.57, respectively. PCR analysis of plasma samples did not reveal statistically significant differences between groups of samples.

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Efficacy, Pharmacokinetics and *In Vivo* Anti-HIV Activity of UC781 Buckheit, R.W., Jr., Hollingshead, M., Stinson, S. and Bader, J.P. Southern Research Institute, Frederick, MD, National Cancer Institute, Bethesda, MD, USA

Structure-activity relationships of a series of compounds related to the nonnucleoside reverse transcriptase inhibitor oxathiin carboxanilide have been described. Three new analogs (UC040, UC82, and UC781) inhibited laboratory and clinical isolates of HIV-1 in both established and fresh human cells. Virus isolates with the amino acid changes L100I, K103N, V106I and Y181C in the reverse transcriptase were partially resistant to these compounds. However, UC781 inhibited these virus isolates at low nontoxic concentrations, presenting a broad *in vitro* therapeutic index. As with other NNRTIs, each of the compounds synergistically interacted with AZT to inhibit HIV-1 replication. UC781 possesses a favorable pharmacokinetic profile in mice with a high level of oral bioavailability. Plasma concentrations reached maximum levels within 2 to 4 hours of oral administration and remained in excess of those required for *in vitro* anti-HIV activity for at least twenty-four hours after a single oral dose. When evaluated in a murine hollow fiber implant model of HIV infection, UC781 dosed orally or parenterally was able to completely suppress HIV replication in this model system, providing evidence of the *in vivo* efficacy of the compound.