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Anti-HIV Activity of Lecithinized Superoxide Dismutase

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Superoxide dismutase (SOD), is an enzyme used in the treatment of oxygen radical-related diseases. Lecithinization of SOD enhances its pharmacological activity. Lecithinized SOD (PC-SOD) inhibits human immunodeficiency virus type 1 and 2 in MT-4 cells. HIV-1 infected MT-4 cells were cultured for 5 days, in the presence of PC-SOD at various concentrations. MTT assay, Reverse transcriptase (RT) activity and p24 antigen production were measured. PC-SOD inhibited viral replication most effectively at 2500 U/ml, a concentration that did not affect cell viability, with EC50 value of 708 U/ml. PC-SOD treatment inhibited RT activity and p24 production in a dose dependent manner. Western blot analysis of the HIV-1-infected MT-4 cells treated with PC-SOD at 2500 U/ml did not detect any expression of viral proteins. Failure in inhibition of virus adsorption, proviral DNA and RNA synthesis, RT and proteinase enzyme activity suggests that the mechanism of action of PC-SOD is entirely different from the currently available anti-HIV drugs. To provide a rationale for combination therapy, we investigated the effect of PC-SOD with AZT or ddI or ddC or KNI-272 or dextran sulfate. PC-SOD shows synergistic interaction with all the drugs tested.

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Biologically Active Oligodeoxyribonucleotides--VI[1] : Structure-Activity Relationships of Anti-HIV-1 Hexadeoxyribonucleotides Bearing 3'- and 5'-End-Modifications

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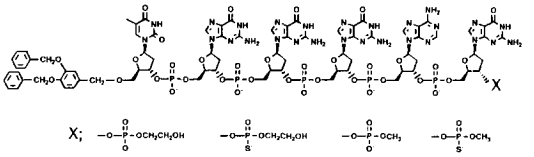
We have reported that the 5'-end-modified hexadeoxyribonucleotides (6mers), TGGGAG, have potent anti-HIV-1 activities *in vitro*. Among the 6mers bearing a variety of aralkyl groups at their 5'-ends, the 6mer bearing a 3,4-dibenzyloxybenzyl(DBB) group was most potent and furthermore showed less cytotoxicity.[1,2] 5'-End-modification entailed substitution of the 5'-oxygen atom by a sulphur atom or a nitrogen atom and the anti-HIV-1 activity of these 6mers was evaluated. In order to make these 6mers nuclease-resistant, the 3'-ends were modified. The 6mers bearing a DBB group at their 5'-ends and a variety of modified phosphate groups at their 3'-ends were tested for anti-HIV-1 activity. The presence of a hydroxyethylphosphate, hydroxyethylthiophosphate, methylphosphate or methylthiophosphate group at the 3'-end increased the anti-HIV-1 activity. The 6mer bearing a DBB group at the 5'-end and a hydroxyethylphosphate group at the 3'-end was found to be the preferred candidate in terms of anti-HIV-1 activity, synthesis and stability.

1) Part V: H. Hotoda et al. in preparation.
2) H. Hotoda et al. (1995) *Antisense Res. Dev.* **5**, 85.



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An Orally Bioavailable Sulfated Oligosaccharide Derivative That Inhibits Human Immunodeficiency Virus *In Vitro* and Provides Sustainned Drug Level in Mammals With Low-Anticoagulant Activity.

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This study provides an orally bioavailable oligosaccharide sulfate derivative (BSL-4) exhibiting anti-HIV activity *in vitro*. The compound is composed of penta-β(1-3)-glucoside with *n*-dodecyl aglycon and bulky lipophilic acyl groups introduced by esterification to hydroxyl groups of saccharide, and the remaining hydroxyl groups are sulfated. The EC50 value of BSL-4 against HIV-1IIIB in MT-4 cells was 0.8 μM, with no *in vitro* toxicity up to 147 μM. It inhibited the binding of 0.5β (anti-gp120 mAb) to MOLT-4/HIV-1IIIB cells by 95% at 30 μM, whereas it did not inhibit the binding of anti-Leu3a mAb to MOLT-4 cells. Surprisingly, BSL-4 was absorbed orally by mice and showed no prolongation of APTT (activated partial thromboplastin time) even at a dose of 60 mg/Kg. When BSL-4 (7.5 mg/Kg) was orally administered to mice, the concentration of the drug in sera at 8 h after treatment was 11 μM, as determined by anti-HIV activity. BSL-4 can also reduce the virus titer by two orders, and keep the same viability as the untreated control even after 35 days in culture of HIV-1IIIB infected MOLT-4 cells at 63 μM. By introducing the bulky lipophilic group to sulfated saccharide, we have synthesized a new orally bioavailable compound, whose appeal would seem to lie in the long lasting activity and non-anticoagulant property.

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A Novel Mutation (K70E) in HIV-1 Reverse Transcriptase Confers Resistance To PMEA *In Vitro*.

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9-(2-phosphonomethoxyethyl)adenine (PMEA), an acyclic nucleoside phosphonate analogue, is active against several retroviruses and herpesviruses and has shown anti-HIV activity in clinical trials. After serial passage of HIV-1 (IIIb) in MT2 cells in gradually increasing concentrations of PMEA, a virus with a >12-fold increase in IC_{50} value vs. PMEA as compared to IIIb was isolated. Sequence analyses of this PMEA-selected virus demonstrated the presence of a novel K70E substitution in reverse transcriptase. A recombinant virus carrying the K70E mutation was constructed and showed a 10-fold increase in IC_{50} value vs. PMEA as compared to IIIb. The K70E recombinant showed a 10-fold increase in IC_{50} value vs. 3TC as well, but showed wild-type susceptibility to AZT, ddC, ddI, d4T, foscarnet, and two additional phosphonates, PMPA and d4API. The K70E recombinant grew more slowly and to a lower maximum titer than the wild-type IIIb virus *in vitro*. Kinetic analyses of the K70E RT are underway. Selections for HIV resistance to PMPA and d4API are ongoing and results of those experiments will be presented.