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Evaluation of the Anti-HIV Activity of Natalizumab—An Antibody Against Integrin alpha4

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Integrins have been involved in the mechanism of viral entry and infection. Recently, alpha4beta7 integrin has been shown to serve as a coreceptor for HIV-1, as signalling mediated by gp120-alpha4beta7 interaction led to LFA-1 activation, a process associated to increased virus production and virus transfer (Arthos, Nat. Immunology 2008). Thus, targeting alpha4beta7 integrin could provide a putative treatment for HIV-1 infection.

Antagonism of alpha4 integrin has been validated as a therapeutic approach for the treatment of inflammatory diseases, with one agent, natalizumab (Tysabri®), approved for the treatment of multiple sclerosis and Crohn's disease. We evaluated the effect of natalizumab in HIV-1 infection in cell culture, using a standard drug-screening assay. Anti-HIV activity in MT-4 cells and peripheral blood mononuclear cells (PBMC) was determined after acute infection with HIV-1.

Functionality of natalizumab in cell culture was evaluated in monocytic U937 cells by adhesion assays. Attachment of U937 cells to VCAM-1 coated wells (endothelial ligand for alpha4beta1 and alpha4beta7) was blocked by natalizumab in a dose dependent manner (IC_{50} of 0.1 mg/ml) suggesting that alpha4 integrins could be effectively blocked by natalizumab. Conversely, natalizumab did not affect the replication of HIV-1 NL4-3 or BaL strains in MT-4 cells expressing CXCR4 or CCR5.

To model alpha4beta7 activation, PBMC from healthy donors were cultured in the presence of retinoic acid prior to HIV infection. However, natalizumab did not affect HIV replication in PBMCs irrespective of retinoic acid preincubation. Interaction of gp120 with alpha4beta7 was shown to be mediated by a tripeptide (LDV) in the V2 loop of gp120, a peptide motif that mimics the structure presented by the natural ligands of alpha4beta7. This peptide motif is found in two out of the four HIV-1 strains tested.

From our results, we concluded that natalizumab did not have anti-HIV activity in cell culture. Thus, alpha4 containing integrins appear not to be essential cofactors for HIV replication.

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Antiviral Activity of Unithiolum Against the Human Immunodeficiency Virus and Herpes Simplex Virus

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HIV destroys the immune system of human organism and opens the gate to the opportunistic infections. AIDS-associated herpes simplex viruses (HSV) cause widely distributed and clinically severe diseases. The important problem is the search of effective compounds against to HIV and HSV-1. The drug preparation 1,2-dithiol-3-propylsulfonate sodium – unithiolum (UT) is usually used in a medical practice as an antidote in case of poisoning by thiols poisons (Hydrargyrum, Arsenic et al.). UT could destroy disulfide bridges –SH-group. Our previous investigations have shown that UT has demonstrated the antiviral activity towards human and avian influenza viruses. The scope of the present work was to study antiviral efficacy of UT towards HIV and HSV-1 types. The antiviral activity of UT against the HSV-1 was studied on primarily trypsinized culture of chick embryos cells (CEC) in vitro. Anti-HIV activity of UT against the HIV-1 (strain III B) was studied on cells C8166 in vitro. A model MT-4 cells has been used to evaluate the anti-viral activity against HIV-1 (strain MT-4B/III). Cell cultures were preheated by the UT, incubated for 30–60 min at 37 °C and infected by HIV-1. An inhibitory effect has been determined at the 5th day of infected cultures growth according to immunoenzyme quantification of HIV-specific antigen and infectious HIV titers. The UT in dose 115 mg/ml was inhibited by 50% and in dose 145 mg/ml was inhibited on 80% the reproduction of the virus HIV-1 (strain III B). Selection index of UT was equal to 17 in this cells model of C8166. UT taken in dose 100 mg/ml inhibited the reproduction of the HIV-1 (strain MT-4B/III) by 2.5 lg ID_{50} in cells MT-4. The UT taken in dose 2.5 mg/ml has inhibited HSV-1 reproduction in primarily tripsinized culture of CEC by 43%. The preparation taken in dose 5 mg/ml has inhibited HSV-1 reproduction in this virus to 60%. The results of the present study allow us to state that UT demonstrates antiviral activity both against the HIV-1 and the HSV-1 in vitro.

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A Novel Small-molecule CCR5 Agonist, ESN-196, with Potent R5 HIV-1 Activity

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CCR5 antagonists such as maraviroc (Selzentry™) represent a new class of recently approved HIV entry inhibitors. However, viral resistance has emerged as mutations in gp120 permits HIV entry via antagonist-bound receptor. An alternate strategy impervious to such resistance is to down-regulate CCR5 from the cell surface. Here, we demonstrate that a novel small-molecule CCR5 agonist effectively inhibits HIV-1 infection in PBMCs by selective internalization of the CCR5 receptor.

The compound affinity for CCR5 was measured using both [^{125}I]MIP-1alpha and [^{125}I]RANTES radioligand binding assays. Agonist-induced receptor internalization was visualized using YFP-tagged CCR5 receptor whereas agonist-induced receptor desensitization was measured using the cAMP-HTRF assay. The efficacy of ESN-196 to inhibit infection of recombinant HIV-1 was measured in a luciferase reporter assay in MAGI-CCR5 cells and finally evaluated in PHA-stimulated PBMCs and freshly isolated monocytes. IC_{50} values for the inhibition of viral replication were calculated from p24 Ag production. Maraviroc was always included as reference compound.

The compound ESN-196 demonstrates potent and selective affinity for the CCR5 receptor (Ki, 0.8 nM). This compound potently internalized YFP-tagged CCR5 and, correspondingly, desensitized CCR5 signalling. Furthermore, ESN-196 demonstrated a high potency of inhibition in viral entry in MAGI-CCR5 cells (IC_{50}: 90 nM). The compound was active in PBMCs and monocytes cultures (IC_{50} between 20 and 200 nM) against various R5 strains and clinical R5 isolates, comparable to the activity range of maraviroc. ESN-196 also remained active against an in vitro generated maraviroc-resistant R5 virus. No cytotoxicity up to 100 μM was observed.

Thus, ESN-196 is a unique CCR5 agonist compound with potent activity in inhibiting R5 HIV-1 replication in PBMCs and monocytes. Small-molecule CCR5 agonists offers significant advantages over the known peptide agonists where clinical utility is limited, in part, due to pharmacokinetic liabilities. Moreover, the concept of agonist-induced CCR5 receptor internalization may offer potential advantages over the receptor-blockade (i.e. antagonist) approach.

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Human Immunodeficiency Virus Type 1 Does Not Escape From Novel Single-Stranded DNAzyme Expression-Mediated Inhibition

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Recently, several groups reported that the antiviral activity of shRNA targeting the HIV-1 gene is abolished due to the emergence of viral quasispecies harboring a point mutation in the shRNA target region. This finding is particularly relevant for viruses that exhibit significant genetic variation due to error-prone replication machinery, and the risk might be more severe for RNA viruses and retroviruses than for DNA viruses. On the other hand, ribozyme technologies are also major tools for inactivating genes in gene therapy. One model, termed deoxyribozyme (Dz), is especially useful because it can bind and cleave any single-stranded RNA at purine/pyrimidine junctions.

In the present paper, we describe the inhibition of HIV-1 replication by a lentiviral vector-transduced DNAzyme. Human immunodeficiency virus type 1 (HIV-1) reverse transcription was used to construct a DNAzyme expression vector against the HIV-1 env V3 loop. As initiation of HIV-1 reverse transcription requires the formation of a complex containing viral RNA, tRNALys-3, and reverse transcriptase, we included a HIV-1 primer binding site and tRNALys-3 at the 3′-end of its RNA transcript in the expression vector, thus enabling the synthesis of single-stranded DNA. We demonstrated that lentiviral vector-mediated DNAzyme expression suppressed HIV-1 replication in SupT1 cells. Furthermore, HIV-1 arrests escape from novel single-stranded DNAzyme expression-mediated inhibition in long-term-assay. Such lentiviral vector-mediated DNAzyme anti-genes are promising tools for HIV-1 gene therapy in the treatment of HIV/AIDS.

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In Silico Screening for Anti-HIV-1 Compounds Targeting to Human Cyclin T1

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Considering the drug resistance and side effects of long-term HAART, it is still mandatory to develop anti-HIV-1 drugs with novel mechanisms of action. At present, entry inhibitors, reverse transcriptase inhibitors, an integrase inhibitor, and protease inhibitors are available for the treatment of HIV-1 infection. However, the drugs targeting the transcription from HIV-1 proviral DNA to mRNA have not been developed yet. The HIV-1 transcription process is essential for virus replication and is regulated by a complex composed of p-TEFb (cyclin T1/CDK9), Tat, and TAR RNA. Thus, the formation of this complex could be a potential target for the inhibition of HIV-1 replication. The crystal structure of cyclin T1 (protein data bank ID:2pk2) has been reported. Furthermore, the crystal structure of a complex composed of cyclin T1, Tat, and TAR RNA (protein data bank ID:2w2h) has recently been elucidated. These structures provide essential templates for the design of potential inhibitors. We examined *in silico* screening of compound libraries to identify potential inhibitors of cyclin T1 using the molecular docking simulation software MOE. The molecular surface of cyclin T1 was analyzed and searched for potential sites to which compounds could bind. Then, the docking simulation of cyclin T1 with the library compounds was performed. Approximately 200,000 compounds were screened, and 124 compounds having the optimum docking scores were selected. Consequently, most compounds were assumed to interact with the amino acid residues composed of the Tat/TAR RNA binding surface. We are currently evaluating the anti-HIV-1 activity of the selected compounds in vitro and further screening small-molecule compounds that bind to the Tat/TAR RNA binding surface of cyclin T1.

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Predicted Models of Resistance and Hypersensitivity Conferred by Natural Polymorphisms of HIV-1 Integrase

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Viral resistance remains a significant factor in the treatment of HIV patients and the development of anti-HIV drugs. In addition to resistant viral mutants that arise from drug-induced pressure, natural polymorphisms have been reported in isolated viral strains and suggested to influence susceptibility to drugs. Although less common than in protease and reverse transcriptase, inter- and intra-subtype diversity is evident in integrase from drug-naïve viral strains. In this study, the effect of natural polymorphisms in altering the binding affinity of inhibitors to integrase was evaluated via in silico molecular modelling. In total, the binding of 10 integrase inhibitors described in recent literature including Raltegravir and Elvitegravir to subtype B and subtype C integrase models was scored before and after the insertion of single or multiple point mutations. Findings from this study detail the inhibitors that are more susceptible to natural polymorphisms than others; demonstrate that both single and multiple point mutations can confer hypersensitivity to certain inhibitors; and identify several differences between the docking of compounds to subtype B and C mutants. These findings could be of relevance in the design of future inhibitors and to personalised antiviral treatment.

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Synergistic Inhibition of Bovine Leukemia Virus Replication In Vitro by Ribavirin and alpha-Interferon

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Bovine leukemia virus (BLV) is an oncogenic retrovirus that infects cattle. It is classified in the human T-cell leukemia virus (HTLV) group, although BLV mainly infects B cells rather than T