

### Evaluation of the Anti-HIV Activity of Natalizumab—An Antibody Against Integrin $\alpha 4$

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Integrins have been involved in the mechanism of viral entry and infection. Recently,  $\alpha 4\beta 7$  integrin has been shown to serve as a coreceptor for HIV-1, as signalling mediated by gp120- $\alpha 4\beta 7$  interaction led to LFA-1 activation, a process associated to increased virus production and virus transfer (Arthos, Nat. Immunology 2008). Thus, targeting  $\alpha 4\beta 7$  integrin could provide a putative treatment for HIV-1 infection.

Antagonism of  $\alpha 4$  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  $\alpha 4\beta 1$  and  $\alpha 4\beta 7$ ) was blocked by natalizumab in a dose dependent manner ( $IC_{50}$  of 0.1 mg/ml) suggesting that  $\alpha 4$  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  $\alpha 4\beta 7$  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  $\alpha 4\beta 7$  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  $\alpha 4\beta 7$ . 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,  $\alpha 4$  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 trypsinized 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 [<sup>125</sup>I]MIP-1 $\alpha$  and [<sup>125</sup>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 ( $K_i$ , 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  $\mu$ M was observed.