compound-specific, dependent on the interval between compound removal and virus challenge, and dependent on HIV-1 co-receptor usage. Compounds that enhanced HIV-1 infection in this assay increased levels of HIV-1 infection up to 10-fold. More detailed studies are now underway to determine the mechanism responsible for this enhancement effect, and to determine the contributions of this effect to the clinical failures of these agents.

doi:10.1016/j.antiviral.2009.02.159

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A Macromolecular Basis for Microbicides Dual Protecting Against HIV and Cytomegalovirus Infection

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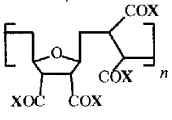
The synthetic polycarboxylic compounds, imitating the principle of furan-derived and negative charged structures alternating in the polymeric backbone of nucleic acids, early explored as interferon inducing agonists of viral genome and stimulators of antiviral immunity in vivo, have been modified by side-groups to amplify the direct antiviral potency in vitro, particularly against human immunodeficiency (HIV) and cytomegalo (CMV) viruses. This modulation was targeted to membrane locus to block earliest steps of viral entry. We developed combinations of structure-specific lipophil- and electrostatic-activating strategies using for the modifications both cage-hydrocarbon (rimantadine/camphor-like) vectors and sulfate anionic species, related by negative charge to the HIV used extracellular sites of CCR5/CXCR4 or to CMV-sensitive heparansulfate receptors of cells. The new generations of antiviral substances (AVS) has been designed, synthesized, and evaluated on HIV-1 and CMV experimental models in vitro (examples on fig/tab). The both factors of the structure-functional modulation (lipotropic and anionic) were found are effective tools for an amplification of the microbicidal activity against HIV and CMV (dominantly depended on electric charge modulation). In view the fact, that CMV is one of most danger opportunistic co-factor of HIV/AIDS pathogenesis, the obtained data can become a platform for further advance in new generation microbicides, promising for a combined prevention of the sexual transmitted infections. And the multipoint-active macromolecular basis is most preferable for virus drug resistance prevention.

Acknowledgement: to the Projects ISTC#3272; RFBR06-04-89402/NWO#047.017.026.



Where the side-groups are **-X =**
-OH/-O⁻ Na⁺, carboxylic acid (**CA**), slight anionic, in part negative charged
-NH-Spacer$_1$-Adamantane (**Ad**), cage-tricyclic mebranotropic
-NH-Spacer$_2$-Norbornene (**Nb**), exo-, cage-bicyclic, mebranotropic
-O-Spacer$_3$-SO$_3^-$ Na+, sulfoacid (**SA**), strong anionic, full negative charged

AVS code	Various kind side groups (X), mol. ratio, CA : Ad : Nb : SA	Cytotoxicity, CC_{50}, μg/ml		Selectivity Index $SI = CC_{50}/EC_{50}$	
		MT-4[a]	HFC[b]	HIV[c]	CMV[d]
ÀS. 470	1.00 : 0.00 : 0.00 : 0.00	> 1000	3500	> 37	350
ÀS. 473	0.94 : 0.06 : 0.00 : 0.00	950	2500	---	25
ÀS. 632	0.93 : 0.07 : 0.00 : 0.00	1000	2400	**730**	240
ÀS. 504	0.92 : 0.00 : 0.08 : 0.00	≥ 1000	1700	**≥ 1250**	17
ÀS. 677	0.86 : 0.00 : 0.08 : 0.06	> 1000	1440	> 139	**1400**
ÀS. 678	0.79 : 0.00 : 0.08 : 0.13	> 800	1420	> 242	**1400**
ÀS. 679	0.67 : 0.00 : 0.08 : 0.25	> 800	500	> 258	500
ÀS. 688	0.60 : 0.00 : 0.00 : 0.40	> 2000	3000	**> 680**	**5500**

[a] in human lymphoblastoid MT-4 cells culture, trypan blue test;
[b] in human embryo lung diploid fibroblast cells primary culture, trypan blue test after 3 days;
[c] **HIV-1**, EVK strain, in MT-4 cells culture, simultaneously with treatment, p24 immunoblot test after 24 h.
[d] **CMV**, AD-169 strain, 1h post treatment by AS in HFC, plaque formation test after 5 days;
Anti-CMV viricidal, preventive and therapeutic schemes data are represented in M. Pavlova et al. report

doi:10.1016/j.antiviral.2009.02.160

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CADA, a Potential Anti-HIV Microbicide that Specifically Targets the Cellular CD4 Receptor

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The cyclotriazadisulfonamide (CADA) compounds are a new class of specific CD4-targeted HIV entry inhibitors. The anti-HIV activity of CADA correlated with its ability to specifically down-modulate the cell surface expression of the CD4 receptor in human cells. Here, we evaluated its potential as an anti-HIV microbicide. T-cell lines, and human and macaques PBMCs were treated with CADA and infected with HIV-1, HIV-2, and SIV strains and isolates, and the EC_{50} was calculated from the p24 or p27 viral antigen content in the supernatant. For the measurement of surface CD4 expression, cells were incubated with CADA, stained with anti-CD4 mAbs and analysed by flow cytometry. CADA down-regulated the CD4 expression in immature monocyte-derived dendritic cells (MO-DC) and exerted a clear anti-HIV-1 activity in MO-DC/T cell co-cultures. It showed consistent antiviral activity against viruses of HIV-1 group M (A, B, C, D, A/E, F, G) and group O, and also against various HIV-2 strains. In addition, CADA potently inhibited $SIVmac_{251}$ infection of PBMCs isolated from macaques. Comparable results were obtained in human cells. Flow cytometric analysis demonstrated a significant and dose-dependent down-regulation of CD4 expression at the cell surface of simian PBMCs after treatment with CADA. CADA showed synergistic activity when evaluated in combination with various other anti-HIV drugs, and with the candidate microbicide cellulose acetate 1,2-benzenedicarboxylate (CAP), an enteric coating polymer for capsules and tablets. Finally, a gel formulation of CADA in hydroxyethyl cellulose (HEC 1.5%) was developed and tested against several isolates, showing a preservation of the antiviral potency of CADA. In summary, our data indicate that CADA may qualify as a potential anti-HIV microbicide drug candidate for the prevention of the sexual transmission of HIV.

doi:10.1016/j.antiviral.2009.02.161

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The Development of HIV-1 NCP7 Inhibitors as Components in Combination Topical Microbicides

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The HIV-1 nucleocapsid protein (NCp7) has been identified as a potential antiviral target based on its broad range of function in virus replication. The highly conserved NCp7 protein of HIV contains two copies of the zinc finger motif Cys(X)2Cys(X)4His(X)4Cys(CCHC). NCp7 plays a pivotal role during both the early and late phases of HIV-1 replication, being required for the function of the reverse transcriptase, integrase and protease as well as the packaging of the RNA genome into maturing virions. Mutations in the Zn-chelating and/or non-chelating residues have been shown to result in loss of NCp7-mediated functions, rendering the virus noninfectious. Thus, the central role of the NCp7 protein makes it an attractive target for not only therapeutic drug development but also in the development of preventatives to inhibit the sexual transmission of HIV since effective NCp7-targeted com-

pounds would directly inactivate both cell-free and cell-associated virions. Based on data obtained on a series of small molecule thioester inhibitors of NCp7, three lead compounds (designated 19, 89 and 247) were chosen for further elucidation of their microbicide potential. We have utilized standard in vitro assays for the development of vaginal microbicides as a means to define the most potent lead thioester microbicide candidate to be used in combination with other topical microbicides in preclinical and clinical development. These data indicate that the thioesters result in inactivation of all clinical strains of virus tested in fresh human PBMCs and monocyte-macrophages, including subtype C and E strains which predominate in sub-Saharan Africa and South East Asia. These data would indicate that the biological activity of the NCp7 inhibitors was not dramatically affected by the presence of semen or vaginal fluids. Additionally, the NCp7's appear to have a memory effect that reduces virus production substantially for 21 days after initial exposure and resistant virus is unable to be selected for due to the barrier in infectivity only after 4 or 5 passages of selection. These characteristics make the NCp7 inhibitors attractive candidates as part of a combination microbicide product with other molecules that possess a different mechanism of action.

doi:10.1016/j.antiviral.2009.02.162

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Development of a Long Lasting Combination Microbicide Product Consisting of Highly Potent Compounds Exhibiting Multiple Mechanisms of Action

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In the absence of an effective HIV vaccine, topical microbicides represent an important strategy for preventing the sexual transmission of HIV, the predominant mode of HIV transmission worldwide. Women now account for 46% of all adults living with HIV worldwide. The dynamics of the epidemic demand the development of safe, effective and acceptable female-controlled chemical and physical barrier methods, including topical microbicides, to reduce HIV transmission. An approved vaginal microbicide does not yet exist despite extensive development efforts. Thus far, three microbicide candidates have failed in human clinical trials, raising the hurdle for other microbicides in development. Although the microbicide products in clinical trials are tested as single agents, current thinking suggests that a combination product will be the required ideal microbicide. Our laboratories have been actively pursuing the development of combination microbicides that include different classes of molecules targeting multiple steps in the HIV replication cycle. Our strategy focuses on the development of a long lasting microbicide which prevents HIV infection and replication at multiple steps through the development of a combination product which will be formulated and delivered in an optimal fashion to place the right drug(s) at the right concentration at the right place at the right time. Agents under development include the pyrimidinediones (inhibition of both virus entry and reverse transcription), the phosphorothioate oligonucleotide ISIS 5320 (inhibition of virus attachment and fusion via binding to the V3 loop of gp120), and the thioester NCp7 zinc finger inhibitors (direct inactivation of cell-free and cell-associated HIV through removal of the coordinated zinc in NCp7). We have evaluated the in vitro activity of combinations of these agents in a variety of microbicide specific virus transmission assays in order to define and prioritize appropriate combination therapy strategies. Evaluations include the ability of the combination products to inhibit virus replication in PBMCs, activity in a microbicidal transmission sterilization assay, and other virus entry inhibition assays.

doi:10.1016/j.antiviral.2009.02.163

Oral Session 4: Herpesviruses and Poxviruses

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Inhibition of Herpesvirus Replication With 5-Iodo-4′-Thio-2′-Deoxyuridine

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A series of 4′-thionucleosides was synthesized and their antiviral activity was evaluated against orthopoxviruses and herpesviruses. We have reported previously that one analog, 5-iodo-4′-thio-2′-deoxyuridine (4′-thioIDU), exhibited good antiviral activity both in vitro and in vivo against two orthopoxviruses. This compound also has good activity against many of the herpesviruses. It inhibited the replication of herpes simplex virus type 1 and type 2 (HSV-1, HSV-2), and varicella-zoster virus with EC_{50} values of 0.4 μM, 0.5 μM, and 2 μM, respectively. It also inhibited the replication of human cytomegalovirus (HCMV) with an EC_{50} of 5.9 μM, but did not selectively inhibit Epstein-Barr virus, either variant of human herpes virus-6, or human herpesvirus-8. While some acyclovir-resistant strains of HSV-1, and -2 were comparatively resistant to 4′-thioIDU, it retained some activity against these strains (4–12 μM). Some ganciclovir resistant strains of HCMV also exhibited reduced susceptibility to the compound, and appeared to be related to the specific mutations in the DNA polymerase since it was fully active in an HCMV strain that lacked UL97 kinase activity. The activity of this molecule was also evaluated in mice infected intranasally with the MS strain of HSV-2. Twice daily oral administration of 4′-thioIDU at 5 mg/kg, 10 mg/kg or 30 mg/kg was initiated 24 h, 48 h, or 72 h after infection. Although there was no decrease in final mortality rates, the mean day of death was increased significantly ($P < 0.05$) in all animals receiving 4′-thioIDU even when therapy was delayed 72 h post infection. The highest dose of the compound was the most effective and increased the mean day of death irrespective of treatment delay ($P < 0.001$). Studies presented here suggest that 4′-thioIDU is a good inhibitor of some herpesviruses as well as orthopoxviruses and warrants further study as a therapy for these infections.

Acknowledgements: Supported by contract NO1-AI-30049 and grant 1-U54-AI-057157 from the NIAID, NIH.

doi:10.1016/j.antiviral.2009.02.164

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Selection and Characterization of (S)-1-[3-Hydroxy-2-(Phosphonomethoxypropyl)-2,6-Diaminopurine [HPMPDAP] Resistant Camelpox Viruses

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The acyclic nucleoside phosphonate (ANP) family of drugs shows promise as therapeutics for treating poxvirus infections by interfer-