initial exposure. Multiple daily exposures also increased the exposure duration that was required to elicit levels of epithelial damage similar to those seen after the initial exposure. However, *in vitro* cytotoxicity experiments, which were conducted to explore potential parallels between *in vitro* and *in vivo* assays of microbicide safety, conversely demonstrated that HeLa cells became increasingly sensitive to the presence of N-9 after multiple exposures. These multiple exposure studies are now being expanded to include more acute exposure frequencies and assessments of immune cell recruitment as a measure of local inflammation subsequent to repeated exposures to topical agents.

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Resistance Developed Against Alamethicin an Antimicrobial Peptide in *Enterococcus faecalis* is Directly Proportional to its Concentration

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Enterococcus faecalis has become one of the most notable nosocomial pathogens in the last decade. *E. faecalis* a food borne pathogen or called as an opportunistic pathogen in hospitals is becoming resistant to most of the antibiotics used in daily use to preserve food or to combat diseases. So resistance is a major issue, we are trying to tackle resistance issue using computer aided design (CAD) approach. For computer aided designing, Structure–Activity Relationship (SAR) should be understood. So to find SAR between peptide and phospholipid bilayer of target, a variety of resistant mutants were developed. To develop resistant mutants of *E. faecalis*, sensitive strain of *E. faecalis* was grown in nutrient broth (pH 7.0) with increasing concentration of Alamethicin corresponding to 4,6 8 and 10 times the IC_{50} of sensitive strain. The control was set up without peptide in culture broth. The cells grown in different concentration of peptide were plated and single colonies were picked. The cultures were subcultured ten times to confirm that the resistance developed was stable. The inhibitory concentration of Alamethicin against both sensitive strain and resistant mutants was calculated using broth dilution assay. All sensitive strain and resistant mutants were gram stained. The inhibitory concentration for sensitive strain and resistant mutants was found to be 5.0 μg/μl, 10.6 μg/μl, 15.2 μg/μl, 20.1 μg/μl, and 25.8 μg/μl, respectively. A linear relationship was seen in IC_{50} of all resistant mutants with increasing concentration of Alamethicin. One other important observation come to seen was that the solvent used for Alamethicin solution, was found to have the inhibitory activity against *E. faecalis* resistant mutants with maximally inhibiting the highly resistant mutant (Ten time resistant mutant) where as no significant inhibition in case of sensitive strain. After gram staining of both sensitive strain and resistant mutants significant difference was found in morphological features. The sensitive strains were found in short straight chain but the resistant mutants show aggregation or clumps formation. The aggregation in resistant mutants increased with increasing concentration of Alamethicin. Further studies are going on.

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Using the Conrad Testing Algorithm to Evaluate the Cytotoxicity and Anti-HIV-1 Activity of Candidate Microbicide Compounds

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Microbicide testing conducted for the CONRAD Program at the Drexel University College of Medicine identifies compounds that may be used to reduce or eliminate the risk of human immunodeficiency virus type 1 (HIV-1) sexual transmission. Ideal compounds would have little or no *in vitro* cytotoxicity and fast-acting activity against multiple strains and subtypes of cell-free and cell-associated HIV-1. This *in vitro* testing algorithm includes assays designed to screen approximately 15 compounds per month. Testing begins with a cytotoxicity screen (CTS) to assess the impact of each compound on cell viability and to guide the selection of concentrations to be used in antiviral testing. Activity against infectious HIV-1 is measured using viral infection inhibition (VII) assays, in which each compound is evaluated for the ability to inhibit target cell infection by HIV-1 strains IIIB (X4 phenotype) or BaL (R5 phenotype). Finally, compounds are assessed for their ability to interfere with cell-to-cell (CTC) HIV-1 transmission. Additional assays can be used to evaluate combinations of two agents for additive or synergistic activity against HIV-1. The goal of this work is to identify compounds that have *in vitro* characteristics indicative of their potential as anti-HIV-1 microbicide agents. The CONRAD testing algorithm was used to evaluate over 825 compounds between May 2001 and November 2009. A number of agents were shown to have high selectivity indices (little or no cytotoxicity and consistently high activity in all three viral assays). These efforts will greatly facilitate the discovery of new compounds that can be used globally as topical microbicides.

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Clinical Failures of Select Polyanionic Microbicide Candidates may be Predicted by In Vitro Enhancement of HIV-1 Infection

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Increasing efforts are being directed toward the development of topical vaginal products, called microbicides, which will be used to reduce or eliminate the risk of human immunodeficiency virus type 1 (HIV-1) sexual transmission. Polyanionic compounds, which interact non-specifically with HIV-1 gp120 to block infection, were among the first agents evaluated clinically for their potential as microbicide agents. Unfortunately, Phase III clinical trials involving polyanion-containing formulations (Carraguard and Ushercell) demonstrated that these products were ineffective and may have, in some instances, increased the risk of HIV-1 infection. These findings precipitated reassessments of the *in vitro* activities of these agents to determine if variables that can affect agent safety and efficacy had been overlooked during pre-clinical testing. One such variable is product retention and loss following topical application in the female reproductive tract. By mimicking product loss *in vitro*, we showed that several polyanionic compounds, including those involved in clinical trial failures, caused enhancement of HIV-1 infection following compound removal, despite their potent antiviral activity when introduced simultaneously with the viral challenge. The presence and magnitude of this effect was

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.

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

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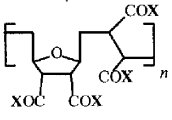
The synthetic polycarboxylic compounds, imitating the principle of furan-derived and negative charged structures alternating in the polymeric backbone of nucleic acids, early explored as interferon inducing agonists of viral genome and stimulators of antiviral immunity in vivo, have been modified by 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

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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.

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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-