tivates HIV by binding to glycan residues on gp120. However, the prohibitive cost of mass producing and isolating this protein negate its deployment in the developing world. By exploiting the multivalency affect we have developed a synthetic lectin capable of binding to gp120. We present surface Plasmon resonance binding of small molecules to recombinant gp120 HIV BaL as a function of pH. Based on affinity and ease of synthesis, one lead compound was chosen. Synthesis of a monomer and incorporation at different feed ratios yielded polymers at 25, 50 and 75 mol%. Antiviral activity was tested in two R5-tropic, well-characterized, reference strains of HIV-1 isolated from acute, sexually transmitted infections and grown in peripheral blood mononuclear cells: HIV-1 DU156 (Clade C) and HIV-1 TRO (Clade B) as well as a pseudotyped CXCR-4 topic HIV-1 WEAU (Clade B) produced in 293T/17 cells. The EC_{50} for the 75 and 50 mol% were in the 1 to 5 μg/mL range (∼10 nM). The 75 mol% exhibited slightly higher activity against DU156, while the activity for the 50 mol% was similar across all three strains. Cytotoxicity against the vaginal cell line, Vk2/E6E7 vaginal cell lines after 24 h suggests these compounds may be safe and warrant further investigation as a vaginal lumen active entry inhibitor.

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Induction of HIV-1 Gene Expression in Human Monocytic Cells by the Failed Microbicide Carrageenan Occurs Through Activation of Toll-like Receptor 4 (TLR4)

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Microbicides are intended for use by women in order to reduce or eliminate the risk of human immunodeficiency virus type 1 (HIV-1) sexual transmission. Carrageenan is a polyanionic compound that was among the first agents to undergo clinical evaluations as a microbicide. Unfortunately, despite the potent *in vitro* antiviral activity of carrageenan and the documented *in vivo* safety of Carraguard (the topical vaginal formulation containing the active ingredient carrageenan), Phase III clinical trials of Carraguard concluded that it was ineffective in preventing HIV-1 transmission. These results have prompted investigations into the mechanisms that underlie the failure of carrageenan. Previous studies of carrageenan as a food additive demonstrated its ability to act as an agonist for toll-like receptor 4 (TLR4), a pattern recognition receptor involved in initiating innate immune responses during pathogen invasion. Based on these observations, we hypothesized that the anti-HIV-1 activity of carrageenan may be offset by its capacity for increasing HIV-1 gene expression through stimulation of TLR4. In experiments in which human monocytic U-937 cells were transiently transfected with an HIV-1 long terminal repeat (LTR) reporter vector, carrageenan induced LTR activity to a level similar to that of LPS, which is a natural ligand of TLR4. These results suggest that the presence of carrageenan may facilitate HIV-1 infection of uninfected cells or increase the magnitude of viral replication in cells already infected with HIV-1. Experiments are now underway to fully characterize carrageenan as a TLR4 agonist in the context of HIV-1 infection and the clinical failure of Carraguard.

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SEVI and Semen Impair the Anti-HIV-1 Activity of Drugs and Microbicides

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Currently, an enormous effort is made to develop vaginal microbicides to prevent sexual transmission of HIV. Thus far, however,microbicides have generally failed to reduce the rate of HIV-1 transmission. We have recently shown that human semen potently enhances HIV-1 infection and demonstrated that fragments of the prostatic acid phosphatase form amyloid like aggregates (termed "SEVI") that contribute to this effect (Muench et al., 2007).

Since HIV-1 containing semen is the major vehicle for sexual HIV-1 transmission worldwide it is highly relevant whether it affects the efficacy of preventive agents. To test this we examined the effect of semen and SEVI on the antiviral potency of a large number of antiretrovirals and first generation microbicides with various modes of action. We found that SEVI and semen substantially reduced the susceptibility of HIV-1 to these agents. For example, concentrations of polyanion based microbicides usually sufficient to block untreated HIV-1 infection had little if any inhibitory effect on virus that was briefly exposed to semen or SEVI.

Our data demonstrate that many microbicides and antiretrovirals are ineffective in inhibiting semen treated HIV-1infection. Thus, to develop potent preventive drugs or microbicides it will be critical to identify those that are effective against the virus in the presence of semen—the major vector of HIV-1 transmission.

Reference

Muench, et al., 2007. Cell 131.

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Nonoxynol-9 (N-9), After Repeated Applications, Does not Result in Cumulative Damage to the Murine Cervicovaginal Epithelium

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The disappointing clinical failures of four topical vaginal microbicides have provided new insights into factors that impact microbicide safety and efficacy. Specifically, the greater risk for HIV-1 acquisition in association with multiple uses of an N-9-containing product has highlighted the importance of application frequency as a variable during pre-clinical microbicide development, particularly in animal model studies. To evaluate an association between application frequency and N-9 safety, a mouse model of cervicovaginal microbicide safety was used. This model system, which is used to assess changes in epithelial integrity and immune cell recruitment following exposure to microbicidal agents, was shown to be predictive of clinical toxicity following a single N-9 exposure and concentration-dependent toxicity of the microbicide Savvy (C31G), which was also removed from clinical trials. In multiple exposure experiments using this model, the initial application of N-9 (aqueous, 1%) caused considerable damage to the cervical epithelium (as previously published). Subsequent daily exposures were characterized by diminished cervical toxicity relative to the

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