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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152

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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153

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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154

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