

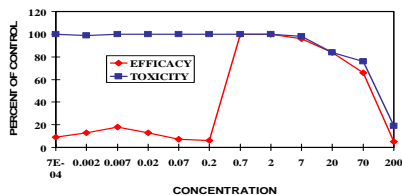
In Vitro Strategies for the Inhibition of Sexually Transmitted Diseases: Preclinical Development of Topical Mono- and Combination Microbicide Therapies

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ABSTRACT

One of the current challenges in anti-infective drug discovery is the inhibition of the sexual transmission of infectious organisms between sexual partners. We have developed a series of microtiter-based, high-throughput assays to evaluate the ability of anti-infective compounds to be used as topical microbicides alone or in combination with agents active against the same or different infectious organisms. These assays include cell based and biochemical assays that quantify the inhibition of replication of the infectious organism. These assays have served to define a variety of classes of active inhibitors, including polyanionic molecules, surfactants, natural products, peptides, proteins, heterocycles, virucidal agents and other anti-infective agents. Efficacy and toxicity (especially against common flora of the vagina, such as *Lactobacillus*) of candidate compounds is determined in conditions that mimic the type of environment in which the compounds will be required to work, including the effects of pH and mucopolysaccharides, as well as other conditions involving time of infection, treatment schedule and multiplicity of infection. An integral part of the assessment of any topical microbicide candidate is demonstration of appropriate range and mechanism of action compatible with a topical microbicide. Range of action assays evaluate the ability of candidate compounds to act against a variety of wild-type, drug-resistant, laboratory-derived and clinical strains of virus. Mechanism of action assays, encompassing both biochemical/enzymatic and cell-based assays, are employed to further define the activity of the compound in intact cells. Assays are performed to determine the relative ease of selecting for drug resistant virus strains in culture and to define the interactions of the compounds when used in combination with other active agents. Finally, candidates can be assessed in animal models for *in vivo* efficacy. Representative results obtained with a variety of compounds will be presented.

IN VITRO ANTIVIRAL ACTIVITY OF ISIS 5320



Classes of Potential Topical Microbicides

- | | | |
|-------------------------------|---|---|
| Polyanionic molecules | Peptides | Heterocycles |
| Naphthalene sulfonates | natural and synthetic surface active peptides | polyamines (bicyclams) |
| sulfated polysaccharides | CPFs | monensins |
| polycarboxylates | gp41 peptides (T20, T21) | porphyrins |
| polyoxymetalates | lexitropsin and related analogs | diaminoacidones |
| Surfactants | Proteins | Reverse Transcriptase Inhibitors |
| neutral and charged compounds | negatively charged albumins | dideoxynucleotides |
| Natural Products | cyanovirin-N | acyclic phosphonates (PMEA, PMPA) |
| tannins | | Virucidal Agents |
| plant lectins | | Zinc Finger Inhibitors |
| betulinic acid derivatives | | |
| macrolides | | |

Primary Screening Assays

CD4-dependent ME180 Assay
CD4-dependent Ghost X4R5 Assay
Standard XTT-based antiviral Assays
Assays with and without mucin
CONRAD Assays (VBA, CAIA)
Virucidal Assays
Toxicity to *Lactobacillus*

Range of Action Assays

Activity against clinical strains of HIV in fresh human peripheral blood cell cultures
Activity against diverse SI and NSI strains
Activity against virus subtypes
Activity against strains with different tropism
Activity against other STD Agents
Activity against H2O₂-secreting *Lactobacillus* sp.

Mechanism of Action Assays

Attachment Inhibition
Fusion Inhibition
Ghost X4 Assay
Ghost R5 Assay
Attachment Fusion Complex Assay
Ligand binding and displacement assays
Virucidal Assays

Combination Therapy

MacSynergy II-based evaluation of the combination of two and three compounds to define their antiviral and toxicity interactions.

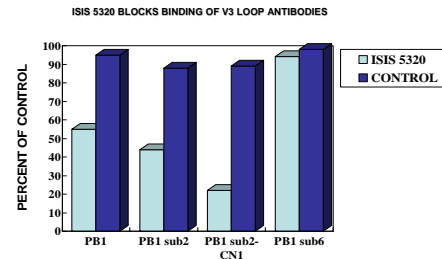
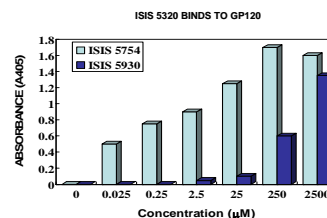
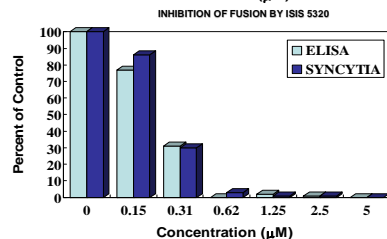
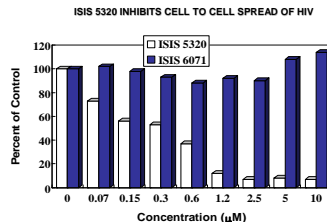
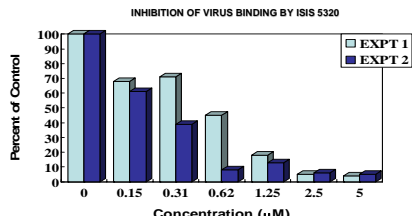
- Synergy
- Additivity
- Antagonism
- Synergistic toxicity

Resistance

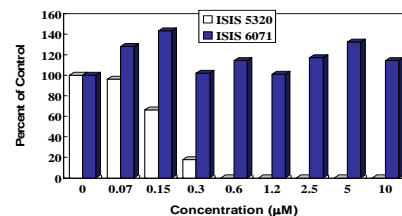
Inhibition of drug resistant strains in CD4-dependent and CD4-independent topical microbicide screening assays. Selection of drug resistant strains in target cells by sequential virus passage in the presence of topical microbicide.

Animal Models

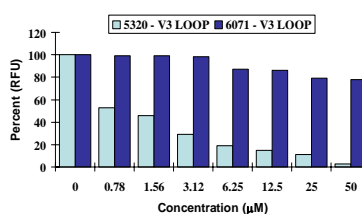
Non-human primate models of infection using SIV or SHIV as challenge virus with rectal or vaginal route of infection



ISIS 5320 Inhibits Syncytium Formation in CEM-SS Cocultivation Assay



ISIS 5320 Binds to the V3 Loop of GP120



ANTIBODY

Sexually Transmitted Disease Efficacy Evaluations

Viruses: HIV-1, HIV-2, HSV-1, HSV-2, HCMV, HBV, HCV

Bacteria/Fungi: *Gardnerella vaginalis*, *Mycoplasma hominis*, *Mobiluncus curtisi*, *Prevotella corporis*, *Haemophilus ducreyi*, *Candida albicans*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*