Vaginal Microbicides: A Novel Approach to Preventing Sexual Transmission of HIV

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The AIDS epidemic continues its unrelentless expansion. According to the Joint United Nations Programme on HIV/AIDS, there are more than 40 million people living with HIV, and more than 15,000 new infections occur every day. One approach to curbing HIV is the development of topical microbicidal agents or microbicides. These are compounds designed to protect the body's mucosal surfaces from infection by sexually transmitted disease-causing pathogens, including HIV. Several candidates are in preclinical stages; however, only a handful have been tested in humans for safety, and even fewer are ready for clinical efficacy trials. In this update, we describe microbicide research and development, including preclinical screening algorithms, ideal properties, compounds in the pipeline, and future prospects. This review is based on a previous work, which has been updated to contain new information, especially regarding microbicide candidates in preclinical and clinical stages of development.

Introduction

Microbicides are compounds that, when applied topically, protect the body's mucosal surfaces from infection by sexually transmitted disease-causing pathogens. The main target of these agents has been HIV, the etiologic agent of AIDS. However, investigators are realizing that targeting other sexually transmitted pathogens (STPs), such as herpes viruses, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Treponema pallidum*, and even organisms causing bacterial vaginosis, may be easier and will ultimately result in a reduction of HIV transmission because the mucosal inflammation caused by these pathogens acts as a facilitating cofactor for HIV infection [1].

Why Are Microbicides Important?

More than 30 million adults and children worldwide have died from AIDS and 40 million are living with HIV. A new infection occurs somewhere in the world approximately every 6 seconds. More than 95% of new infections occur in developing countries where limited resources and cultural factors make containment of the epidemic difficult.

Most HIV infections are transmitted through heterosexual intercourse and, in many areas, women are disproportionately affected. Transmission of HIV from men to women is biologically more efficient compared to transmission from women to men. In many areas, women also lack the power to negotiate condom use.

The impact of the current level of HIV seroprevalence is enormous in terms of mortality, resource depletion, and human suffering. There is clearly an unmet need for treatment of individuals already infected. However, there is also a desperate need to prevent further infection.

Efforts to prevent HIV transmission have centered around three approaches: behavioral change (safer sex), the development of a vaccine, and development of a microbicide. Abstinence from intercourse is the surest way to prevent sexual transmission and the use of condoms and a careful choice of partners will reduce the possibility. However, for reasons of financial insecurity, fear of retaliation, or the desire for pregnancy, avoidance of sex and the use of condoms are not always feasible options. Progress in vaccine development has been disappointing .

The London School for Hygiene and Tropical Medicine estimates that if a microbicide 60% effective were used by 70% of women already being examined by the health services in developing countries, 2.5 million HIV infections could be avoided in 3 years. Work on microbicides has been ongoing since the 1980s, but it has recently picked up speed and increased funding because of the urgency of the HIV crisis and political pressure.

How Do Microbicides Work?

Targets for microbicidal intervention

Human immunodeficiency virus-1 infects the reproductive tract tissues mainly through transepithelial migration of infected Langerhans cells and seminal leukocytes or directly reaching dendritic cells, lymphocytes, and monocytes in the lamina propria or submucosa through epithelial disruption [2]. Genital epithelial cells may also play a role in HIV-1 sexual transmission by sequestering, protecting, and later transferring the virus to infectable immune cells [3].

All enveloped (Env) viruses encode Env proteins that choreograph the same general steps during entry into the host cell [4]. The virus attaches to the cell surface, allowing a second step involving Env-mediated engagement of a cellular receptor. A trigger event induces conformational changes in the Env proteins that lead to fusion between the viral and cellular membranes. As a consequence of fusion, the viral genome enters the cytoplasm and virus replication can proceed.

Soon after entry into permissive cells, reverse transcriptase acts to transcribe the viral RNA genome into a double-stranded DNA copy. This viral cDNA is the substrate for the virally encoded integrase, which catalyzes the covalent joining of the viral genome and the host cell chromosome.

Several compounds have been developed that target discrete steps of the virus entry process [4]. Microbicidal agents can inactivate cell-free or cell-associated HIV/STPs at the surface of the mucosa or in the lumen of the cavity covered by it, block HIV/STP cell entry, and inhibit HIV/STP replication and spreading.

Microbicidal inactivators

Exhibiting the first mechanism of action are surfactants, such as nonoxynol-9 (N-9), benzalkonium chloride, octoxynol-9, sodium dodecyl sulfate, and menfegol. These surfactants disrupt viral envelopes and cell membranes, displaying high antimicrobial activity in vitro. However, clinical trials of N-9–containing products have been disappointing [5–8]. The lack of proper contact between product and microbes, insufficient coverage of the cervicovaginal lining, and mucosal irritation/inflammation constitute possible reasons for their failure. An active immunoinflammatory reaction induced by N-9 at the cervicovaginal mucosa may have counterbalanced its potent virucidal effect by increasing the number of infectable target cells and disrupting the mucosal barrier.

Other membrane-active compounds are chlorhexidine, a widely-used antiseptic that binds to cell membranes through its biguanide group, and C31G, a broad-spectrum antimicrobial and spermicidal agent containing a equimolar mixture of two synthetic amphoteric compounds (an alkyl amine oxide and an alkyl betaine) [9,10]. C31G has been shown to have potent activity against Env viruses, a large number of gram-positive and gram-negative bacteria, and many fungi and yeasts, including *Candida* [11,12]. It is suspected that the mechanism of action involves binding to microbial surfaces through the polar head group of the amine oxide-betaine mixture and subsequent disruption of the microbial membrane by the alkyl portion of the molecule.

Novel acylcarnitine analogues have proved to be potent inactivators of HIV and sperm, without showing significant vaginal irritation potential. These compounds are also effective against *C. trachomatis*, *N. gonorrhoeae*, *Haemophilus ducreyi*, and *Candida albicans* [13].

Based on the reported susceptibility of HIV, other STPs, and spermatozoa to low pH, investigators have designed acid-buffering gels that maintain a low vaginal pH, even after ejaculation [14,15]. BufferGel (ReProtect, Inc., Baltimore, MD) and AcidForm (TOPCAD, Chicago, IL) are undergoing clinical testing.

Another innovative product that uses the vagina's natural defenses is a lactobacilli-containing suppository called Lactin V (Osel, Inc., Santa Clara, CA) [16]. Under normal circumstances, H_2O_2-producing lactobacilli, in conjunction with other natural defenses, are thought to keep vaginal growth of pathogenic bacteria under control. Furthermore, HIV has also been shown to be susceptible to H_2O_2 attack [17].

Antimicrobial peptides are another example of natural substances with good potential to be developed as vaginal microbicides. Gramicidins and magainins represent two of the most studied antimicrobial peptides, whereas defensins, retrocyclins, bactenecin, and protegrins are just emerging [18–23].

Cell-entry inhibitors

There are similarities in the way some viruses and bacteria interact with their host/target cells. These common molecular pathways may also be important in sperm-egg interaction. These commonalities have led investigators to search for compounds that could inhibit multiple pathogens and, in some cases, human sperm at the cell-entry level.

Sulfated compounds, in particular polymers, were the first reported to inhibit HIV and other STPs. Dextran sulfate, pentosan sulfate, heparin, and fucoidan have long been recognized as active anti-HIV agents [24•]. Carrageenan, a naphthalene sulfonic acid derivative, dextrin-2-sulfate, cellulose sulfate (CS), and polystyrene sulfonate (PSS) are in clinical trials [25–28]. All of these sulfate compounds inhibit HIV and herpes in vitro and most inhibit chlamydial and gonococcal growth. PRO2000 (Indevus Pharmaceuticals, Lexington, MA), CS, and PSS have been shown to possess contraceptive properties. CS and PSS also display antiviral activity against papillomaviruses.

Other polyanions have been considered as potential microbicides [29]. These polyanions block CD4/glycoprotein (gp)-120 interaction, impeding the first step in virus-cell recognition. Some of these polyanions may also induce dead-end gp-41 six-helix bundle formation [30]. In the case of herpes viruses, they compete with cellular glycosaminoglycans, similar to heparan sulfate, for viral attachment proteins [31].

Polyanionic dendrimers are highly branched macromolecules that can carry a high density of peripheral functional (*eg*, anionic) groups, which determine the properties of the molecule. Polyanionic dendrimers can inhibit the replication of HIV by interfering with virus absorption and later steps [32]. They have also been described to be active against herpes viruses [33]. SAMMA, another anionic polymer, derived from the condensation of mandelic acid, is also a very potent inhibitor of herpes viruses and HIV and has activity against *C. trachomatis*, *N. gonorrhoeae*, and sperm [34,35]. Cellulose acetate phthalate is a well-characterized compound

displaying antimicrobial activity against an array of STPs, including HIV, which appears to be inhibited through virucidal and receptor-blocking mechanisms [36,37].

Cyanovirin-N, an 11-kDa protein isolated from cyanobacteria, inhibits HIV infection in vitro with high potency. Although not completely elucidated, its mechanism of action appears to reside in its ability to block high-mannose oligosaccharides [38]. Similar to cyanovirin-N, actinohivin, a protein isolated from actinomycetes, also displays potent anti-HIV activity against T-cell and macrophage-tropic isolates [39]. Another antiviral protein with a unique mechanism of action, involving inactivation of ribosomes by removal of a single adenosine from ribosomal RNA, is pokeweed antiviral protein [40].

Beta cyclodextrins are soluble heptasaccharides that attack lipophilic molecules, such as cholesterol, to their hydrophobic core. In contact with cells and Env viruses, especially HIV, they extract cholesterol from their membranes altering their architecture and function [41]. Lipid rafts are particularly affected. Several studies have demonstrated that HIV-1 uses lipid rafts to enter, assemble, and bud out of cells and demonstrated that β cyclodextrins, given enough time and concentration, can interfere with these processes blocking HIV infection [42,43].

CD4, a cell-surface receptor predominantly expressed by T lymphocytes, is one receptor HIV uses to get into the host cells; however, leukocytic chemokine receptors CCR5 and CXCR4 are equally important. Galactosylceramide, heparan-sulfate proteoglycan, and cell-adhesion molecules may also act as coreceptors and, in the case of CD-negative cells, are perhaps the main receptors for HIV entry. After the attachment phase takes place, a fusion event mediated by the viral protein gp-41 ensues. Attachment coreceptors and fusion peptides are the target of intense research for the development of new therapeutics [44••]. Compounds targeting gp-120, galactosylceramide, CCR5, CXCR4, and gp-41 have been studied in clinical trials [44••].

A few monoclonal antibodies against HIV Env proteins have broad potent neutralizing activity. b12 inhibits most HIV-1 strains tested in vitro [45]. The engineered CD4-immunoglobulin G2 molecule (PRO-542) is also broadly reactive [46]. Vaginal infection studies in rhesus macaques suggest that 1000- to 1-million–fold higher concentrations of entry inhibitors are needed to block vaginal transmission than are effective against the same virus in vitro [47,48]. Reasons for this difference and perhaps some of the failures seen in animal studies are the viral inoculum size, the need to cover the entire mucosal surface, the possible existence of multiple mechanisms of infection, and the clearance of the inhibitor from the vaginal vault by leakage and degradation [49].

Replication and cell-processing inhibitors

Once STPs enter their host cells, they multiply and grow using cellular resources and molecules. These intracellular steps represent another target for antimicrobial intervention. Nucleoside and non-nucleoside reverse transcriptase inhibitors are being used in combination with protease inhibitors for anti-HIV multiple drug therapy (*ie*, highly active antiretroviral therapy).

Bromo-methoxy derivatives of zidovudine, classic nucleoside reverse transcriptase inhibitors, have proved to be good candidates for vaginal microbicides, displaying anti-HIV, spermicidal, and nonirritating properties [50]. Stampidine, a novel aryl phosphate derivative of stavudine and another nucleoside reverse transcriptase inhibitor, is also being developed as a microbicide [51].

The acyclic nucleoside phosphonate derivative tenofovir is a highly selective anti-HIV compound that has been shown to be effective in preventing simian immunodeficiency virus transmission in a vaginal infection animal model [52].

The compound thiocarboxanilide (*ie*, UC-781) is a potent and selective anti-HIV non-nucleoside reverse transcriptase inhibitor that may display virucidal activity, inactivating the virus at contact [53]. Because it has proved to be safe after vaginal applications, does not require intracellular activation, and shows poor bioavailability (*ie*, low systemic absorption), UC-781 represents a good candidate for a vaginal microbicide [54].

Two other tight-binding non-nucleoside HIV-1 reverse transcriptase inhibitors are TMC-120 and the oxopyrimidine derivatives (*ie*, DABOs) [55,56]. Similarly to UC-781, these two agents display virus-inactivating properties and are capable of protecting cells from HIV infection many days after being removed from the culture (memory effect).

The two zinc-fingers of the HIV p7 nucleocapsid protein are essential for virus replication, thus compounds that interact with these motifs have antiviral properties. Because of the possible interference with multiple steps of the HIV life cycle, zinc-finger inhibitors are worth considering as topical microbicidal candidates [57].

Attributes of an Ideal Product

Some attributes of an ideal product are fairly clear-cut: it must be safe and effective. However, even these characteristics vary by the intended population of users. What is safe and effective in a population of women having intercourse using a microbicide once or twice a week may be very different from what is safe and effective in a population of sex workers having sex using a microbicide several times a day, and this may be different from the safety of a product in rectal use.

Other attributes, by their nature, are culture sensitive. For example, studies have shown differing preferences for certain formulations (*eg*, films and gels) over others, depending on the area studied [58]. The less detectable a product, the better. However, the issue of discreet use is a difficult one. In some areas a woman who is discovered to be using this type of product is at risk for repercussions much more severe compared to those repercussions resulting from an attempt to negotiate microbicide (or condom) use before the sex act.

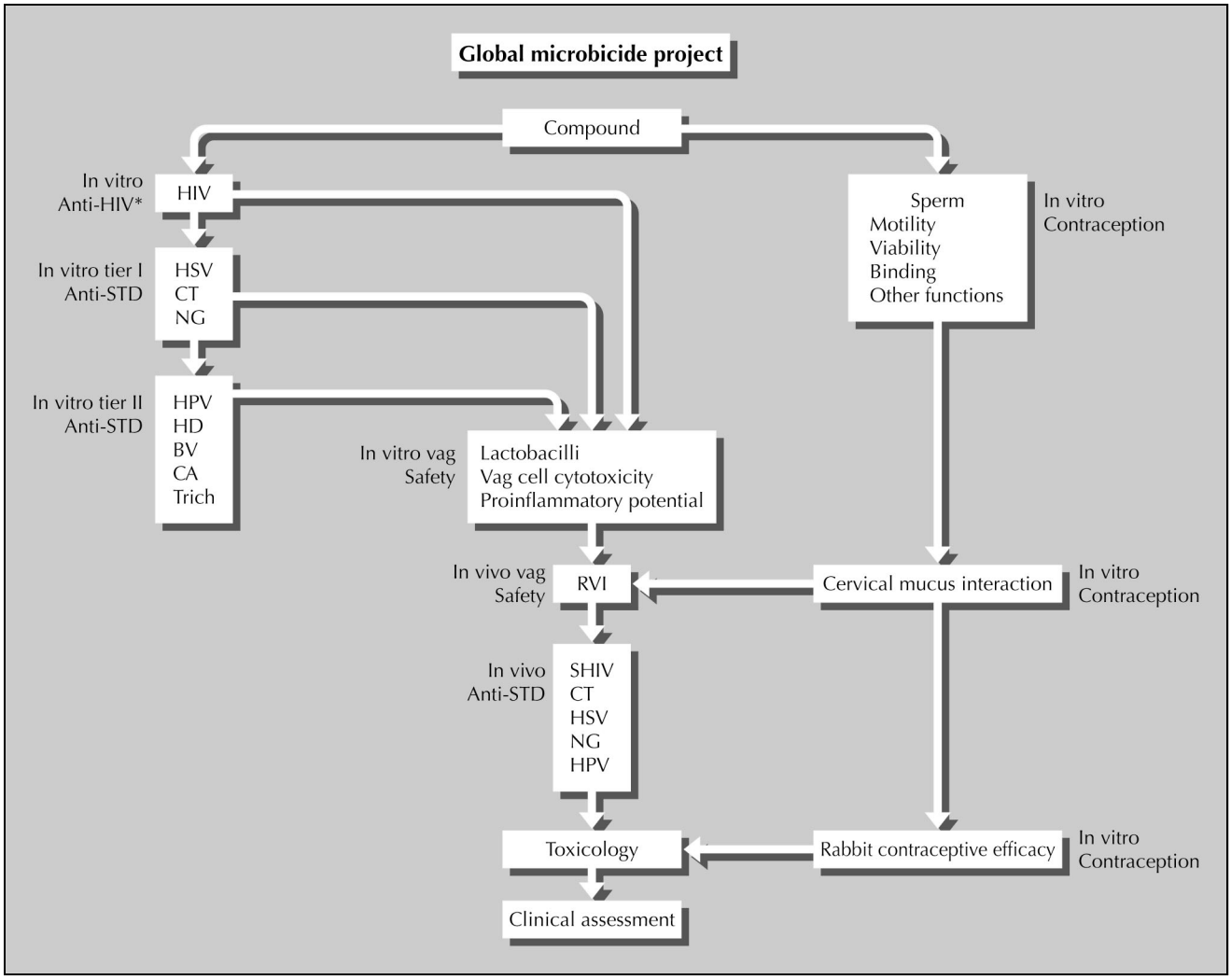

Figure 1. Screening algorithm for microbicides with or without contraceptive activity. *—see additional algorithm (Fig. 2); BV—bacterial vaginosis pathogens; CA—*Candida albicans*; CT—*Chlamydia trachomatis*; HD—*Haemophilus ducreyi*; HPV—human papilloma virus; HSV—herpes simplex virus; NG—*Neisseria gonorrhoeae*; RVI—rabbit vaginal irritation; SHIV—simian immunodeficiency virus/HIV chimeric virus; STD—sexually transmitted disease; Trich—*Trichomonas vaginalis*; vag—vaginal.

There has been debate regarding whether microbicides should be contraceptives. There is a market for both types of products. Women spend most of their reproductive lives trying to avoid pregnancy and, in high-risk situations, all of their reproductive lives trying to avoid infections. Therefore, a product with both capabilities is desirable. However, there is also a need for a product that is microbicidal alone, to allow pregnancy in women desirous of it. Both of these types of products are in development.

What Is Needed to Get a Microbicide on the Market?

Preclinical evaluation

The preclinical evaluation of a microbicide is based on the demonstration of its anti-STP, antisperm, and mucosal safety properties [59]. Detailed recommendations for the conduct of preclinical studies have been written by the International Working Group on Microbicides and will be published in 2004.

Figure 1 shows the screening algorithm used by the Global Microbicide Project (GMP) of CONRAD [60]. Briefly, a microbicidal agent needs to demonstrate potent activity against HIV and, preferably, other STPs, such as herpes virus and *Chlamydia* and *Neisseria* organisms, and prove that it is safe for topical use, displaying no mucosal irritation or inflammatory properties. These features are evaluated first in vitro and then in animal models. Because vaginal microbicides will be applied intravaginally and will come in contact with sperm, antisperm contraceptive properties need to be assessed in vitro and in animal models. Vaginal absorption and systemic acute toxicology should be determined before clinical trials can be started.

Because of the importance of AIDS among sexually transmitted diseases and the focus of microbicide development on HIV-transmission prevention, we have developed

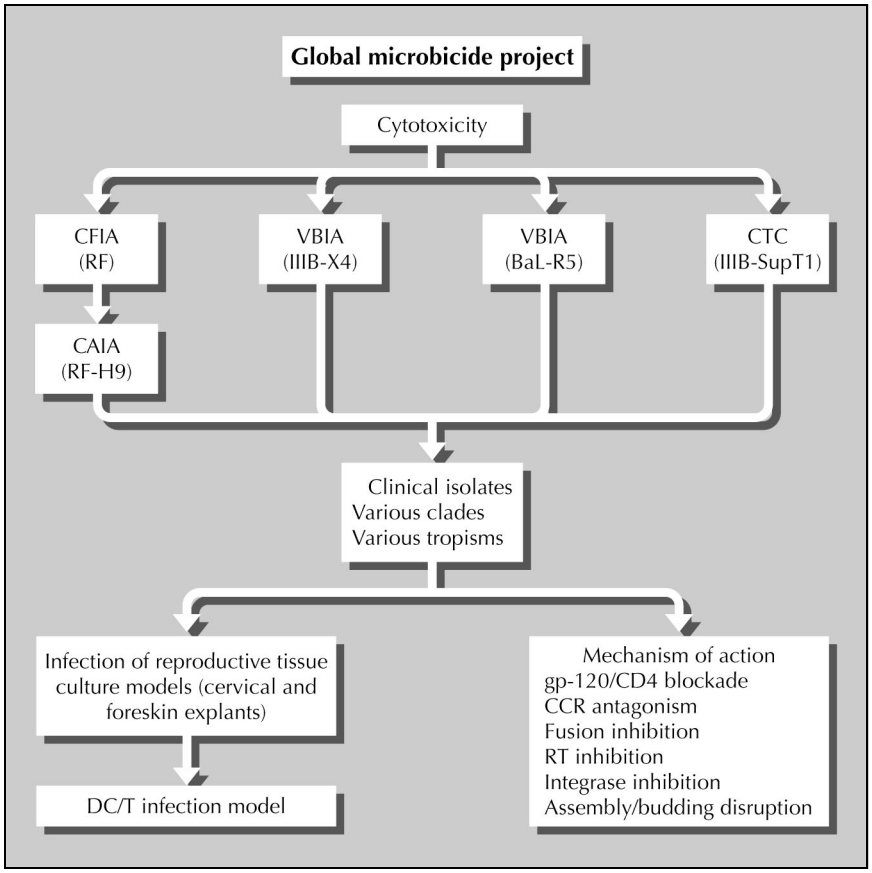

Figure 2. Anti-HIV screening algorithm for microbicide candidates. CAIA—cell-associated inactivation assay; CCR—chemokine receptor; CFIA—cell-free inactivation assay; CTC—cell-to-cell transmission assay; DC/T—dendritic cell/T lymphocyte; RT—reverse transcriptase; VBIA—viral-binding inhibition assay. (HIV-1 strains are indicated in parenthesis.)

an additional algorithm to evaluate the candidate's anti-HIV properties in detail. The compounds are tested against cell-free and cell-associated virus, lympho- and monocytotropic strains, and lab-adapted and clinical isolates from different clades. In-vitro models for prevention of infection in reproductive tissues and determination of the mechanism of action of the compound are the last steps of our screening algorithm (Fig. 2).

Clinical trials

Once initial preclinical testing has shown promise, trials in humans or clinical trials may begin. Permission for these trials must be granted by the relevant regulatory body (*eg*, through an Investigational New Drug application from the US Food and Drug Administration) and by an ethical review panel. In vitro and in vivo testing continues during the conduct of clinical trials.

Clinical testing is traditionally divided into three phases. Phase I includes the initial use of the product in small numbers of humans and focuses on evaluations of the safety of the product. Phase I studies involve assessing patient symptoms, findings on naked-eye examination of the genitalia, and examination of the genitalia under magnification (colposcopy). Studies generally progress from once-daily application by sexually abstinent women and men for 7 to 14 days to twice-daily application by sexually active couples. Participants initially are HIV-uninfected, but eventually participants include HIV-infected women and men. Spreading studies may be carried out. Rectal safety studies may also be conducted

Phase II studies are larger and provide additional safety information and initial information regarding effectiveness. Phase III studies are large trials involving thousands of participants conducted to demonstrate the ability of the product to prevent disease. Detailed recommendations for the conduct of microbicide trials have been published by the International Working Group on Microbicides [61••].

Research on microbicides entails consideration of a number of issues of less concern in studies of other types of products. One consideration is simply the extremely urgent need for a product. Because of this urgent need, it is possible that phase II studies will be proposed as a run-in to phase III, rather than as a separate phase. Another consideration is the choice of comparator. Typically, a new drug is compared to a placebo, if no other active drug exists, or to an active drug if one is marketed. There are no marketed microbicides, but the use of a placebo group is complicated by the fact that any vaginally administered product may affect HIV transmission, despite containing no ingredients with specific activity against the virus. For example, this type of product may reduce HIV transmission by reducing the mechanical trauma and epithelial disruption associated with intercourse or increase HIV transmission by virtue of irritation caused by the inactive ingredients of the placebo. The use of a group receiving no treatment, meaning a group in which participants are not administered a study product but are

counseled, similar to all of the participants, to use condoms with each act of intercourse, has been suggested. However, participants in this type of group may alter their risk-taking behavior (*eg*, by reducing the frequency of intercourse or the number of partners or by increasing condom use) in response to their awareness that they are not using a product. A reduction in HIV incidence resulting from these factors in this group would make it more difficult to show that the microbicide used by participants in the other groups was able to reduce HIV.

Estimated Cost and Sources of Funding and Market Size

It has been estimated that the world market for microbicides may be approximately $1.8 billion. The Rockefeller Foundation has estimated that bringing one or more microbicides to market by 2010 will require roughly $775 million. Funds are being provided by private sources, such as the Bill and Melinda Gates Foundation, government sources, and small biotechnology firms. However, worldwide funding for microbicide research at the end of 2002 totaled $343 million, leaving a gap in excess of $400 million.

What Is Happening at the Moment and Who Is Doing It?

As of February 2004, there were 17 products in clinical trials. Thirteen of these products were in phase I trials, eight were in phase I/II, II, or II/III trials, and three products were in phase III trials (carrageenan, C31G, and cellulose sulfate; Alliance for Microbicide Development web site).

Most of the research is being done by the public sector and small companies. In 2000, the Consortium for Industrial Collaboration in Contraceptive Research of CONRAD Program established the GMP (www.conrad.org). The GMP works in for-profit and not-for-profit partnerships to screen lead compounds in animal studies and the lab, in addition to clinical testing. As of February 2004, GMP was supporting, fully or in part, seven clinical studies on three products, including two HIV-prevention studies involving cellulose sulfate. GMP also supports the development of research centers in developing countries.

The HIV Prevention Trials Network (www.hptn.org), a program of the National Institute of Allergy and Infectious Disease of the National Institutes of Health, is a collaborative clinical trials network established to evaluate the safety and efficacy of nonvaccine prevention interventions. The HIV Prevention Trials Network has sponsored four phase I studies on four different products and is preparing a phase II/IIb HIV prevention study of PRO2000 and BufferGel. The Population Council (New York, NY; www.populationcouncil.org) has carried out multiple safety studies on carrageenan and will begin a phase III HIV-prevention study in South Africa in 2004. Biosyn, Inc. (Huntingdon Valley, PA; www.biosyninc.com) is a specialty pharmaceutical company focused on the development of novel drugs in the areas of infectious disease and reproductive health. The company has carried out five trials involving C31G and plans to begin an HIV-prevention trial in 2004. UC-781, the company's other microbicide, is entering a phase I clinical trial.

Large pharmaceutical companies have been reluctant to enter the field because of what is perceived to be an expensive development process and a small market. If proof of concept can be shown and with appropriate incentives, large pharmaceutical companies may eventually elect to take an active role in microbicide development.

Of critical importance to the field of microbicide research are the contributions made by the Alliance for Microbicide Development (www.microbicide.org), a nonprofit coalition formed in 1998 to offer leadership and coordination to the microbicide field; the International Working Group on Microbicides, an informal group of approximately 20 experts from governmental and nongovernmental organizations formed in 1994 to help coordinate microbicide research; and the International Partnership for Microbicides (www.ipmmicrobicides.org), which includes in its objectives increasing the efficiency of microbicide development by increasing funding, identifying gaps in research, leveraging partnerships between public and private sector parties, and increasing microbicide awareness.

How Does the Future Look?

The first phase III trial of a microbicide is likely to begin in 2004 and be completed perhaps in 2007. If a second efficacy trial is required for approval, the availability of a microbicide will be delayed further. It has been said that, with regard to microbicide development, "delay is measured in deaths."

The main challenges to microbicide development are inadequate understanding of HIV transmission, uncertainty regarding which host cells to target, complexities of the genital and rectal environments, lack of adequate animal models, and inadequate funding. In addition to developing the product, planning should begin to ensure product introduction and availability.

Conclusions

A microbicide suitable for vaginal and rectal use to prevent infection by HIV and other STPs is urgently needed. Even a partially effective microbicide could have an important effect in reducing the rate of HIV infection. Microbicides could work by inactivating STPs, blocking their entry into cells, or inhibiting their replication and spreading. An ideal microbicide should be highly active against HIV and other STPs, fast and long acting, nonirritating to the epithelial lining, nontoxic for the vaginal microflora, and not absorbed. The ideal microbicide may or may not display contraceptive properties. Although single compounds would be preferable from a toxicologic and regulatory

point of view, combination products may be the only way to attain all these properties. We have developed a screening algorithm to evaluate the antimicrobial, antisperm, and mucosal-safety properties of microbicidal candidates (Fig. 1 and 2). Vaginal absorption and systemic toxicity should also be determined.

Clinical assessment of microbicides entails consideration of many specific issues. All of the study participants must be counseled to use condoms. Several candidates that work by different mechanisms are in the pipeline and clinical effectiveness trials will begin in 2004. It has been estimated that bringing one or more microbicides to the market by 2010 will require more than $775 million and a concerted effort between the private and public sectors.

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References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Moodley P, Connolly C, Sturm AW: **Interrelationships among human immunodeficiency virus type 1 infection, bacterial vaginosis, trichomoniasis, and the presence of yeasts.** *J Infect Dis* 2002, **185:**69–73.
2. Miller CJ, Shattock RJ: **Target cells in vaginal HIV transmission.** *Microbes Infect* 2003, **5:**59–67.
3. Wu Z, Chen Z, Phillips DM: **Human genital epithelial cells capture cell-free human immunodeficiency virus type 1 and transmit the virus to CD4+ cells: implications for mechanisms of sexual transmission.** *J Infect Dis* 2003, **188:**1473–1482.
4. Pierson TC, Doms RW: **HIV-1 entry inhibitors: new targets, novel therapies.** *Immunol Lett* 2003, **85:**113–118.
5. Kreiss J, Ngugi E, Holmes K, *et al.*: **Efficacy of nonoxynol-9 contraceptive sponge use in preventing heterosexual acquisition of HIV in Nairobi prostitutes.** *JAMA* 1992, **268:**477–482.
6. Roddy RE, Zekeng L, Ryan KA, *et al.*: **A controlled clinical trial of nonoxynol-9 film to reduce male-to-female transmission of sexually transmitted diseases.** *N Engl J Med* 1998, **339:**504–510.
7. Stephenson J: **Widely used spermicide may increase, not decrease risk of HIV transmission.** *JAMA* 2000, **284:**949.
8. Van Damme L, Ramjee G, Alary M, *et al.*: **Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomised controlled trial.** *Lancet* 2002, **360:**971–977. *[Published erratum appears in Lancet* 2002, **360:**1892.]
9. Chantler E: **New and existing spermicides with virucidal properties.** In *Heterosexual Transmission of AIDS.* Edited by Alexander NJ, Gabelnick HL, Spieler JM. New York: Wiley-Liss; 1990:303.
10. Corner AM, Dolan MM, Yankell SL, *et al.*: **C31G, a new agent for oral use with potent antimicrobial and antiadherence properties.** *Antimicrob Agents Chemother* 1998, **32:**350–353.
11. Calis S, Yulug N, Sumnu M, *et al.*: **A non-antibiotic antimicrobial mixture (C31G): evaluation of the antimicrobial efficiency of C31G on vaginal cultures.** *Boll Chim Farm* 1992, **131:**335–338.
12. Corner AM, Dolan MM, Yankell SL, Malamud D: **C31G, a new agent for oral use with potent antimicrobial and antiadherence properties.** *Antimicrob Agents Chemother* 1988, **32:**350–353.
13. Savle PS, Gandour RD, Doncel GF: **Development of novel, carnitine analogues as topical microbicidal spermicides.** *US Pat* 2003, **6:**656.
14. Olmsted SS, Dubin NH, Cone RA, *et al.*: **The rate at which human sperm are immobilized and killed by acidity.** *Fertil Steril* 2000, **73:**687–693.
15. Garg S, Anderson RA, Chany CJ 2nd, *et al.*: **Properties of a new acid-buffering bioadhesive vaginal formulation (ACIDFORM).** *Contraception* 2001, **64:**67–75.
16. Antonio MA, HillierSL: **DNA fingerprinting of lactobaccilus crispatus strain CTV-05 by repetitive element sequence-based PCR analysis in a pilot study of vaginal colonization.** *J Clin Microbiol* 2003, **41:**1881–1887.
17. Klebanoff SJ, Kazazi F: **Inactivation of human immunodeficiency virus type 1 by the amine oxidase-peroxidase system.** *J Clin Microbiol* 1995, **33:**2054–2057.
18. Bourinbaiar AS, Lee-Huang S: **Comparative in vitro study of contraceptive agents with anti-HIV activity: gramicidin, nonoxynol-9, and gossypol.** *Contraception* 1994, **49:**131–137.
19. Reddy KV, Shahani SK, Meherji PK: **Spermicidal activity of Magainins: in vitro and in vivo studies.** *Contraception* 1996, **53:**205–210.
20. Cole AM, Lehrer RI: **Minidefensins: antimicrobial peptides with activity against HIV-1.** *Curr Pharm Des* 2003, **9:**1463–1473.
21. Risso A: **Leukocyte antimicrobial peptides: multifunctional effector molecules of innate immunity.** *J Leukoc Biol* 2000, **68:**785–792.
22. Tokunaga Y, Niidome T, Hatakeyama T, *et al.*: **Antibacterial activity of bactenecin 5 fragments and their interactions with phospholipid membranes.** *J Pept Sci* 2001, **7:**297–304.
23. Bellm L, Lehrer RI, Ganz T: **Protegrins: new antibiotics of mammalian origin.** *Expert Opin Investig Drugs* 2000, **9:**1731–1742.
24. • DeClerq E: **Current lead natural products for the chemotherapy of human immunodeficiency virus (HIV) infection.** *Med Res Rev* 2000, **5:**323–349.

An updated review of natural products displaying anti-HIV properties, some of which are being developed and tested as microbicides.

25. Bourne N, Bernstein DI, Ireland J, *et al.*: **The topical microbicide PRO 2000 protects against genital herpes infection in a mouse model.** *J Infect Dis* 1999, **180:**203–205.
26. Anderson RA, Feathergill K, Diao X, *et al.*: **Evaluation of poly (styrene-4-sulfonate) as a preventive agent for conception and sexually transmitted diseases.** *J Androl* 2000, **21:**862–875.
27. Anderson RA, Feathergill KA, Diao XH, *et al.*: **Preclinical evaluation of sodium cellulose sulfate (Ushercell) as a contraceptive antimicrobial agent.** *J Androl* 2002, **23:**426–438.
28. Stafford MK, Cain D, Rosenstein I, *et al.*: **A placebo-controlled, double-blind prospective study in healthy female volunteers of dextrin sulfate gel.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1997, **14:**213–218.
29. Schols D, Pauwels R, Desmyter J, De Clercq E: **Dextran sulfate and other polyanionic anti-HIV compounds specifically interact with the viral gp120 glycoprotein expressed by T cells persistently infected with HIV-1.** *Virology* 1990, **175:**556–561.
30. Neurath AR, Strick N, Jiang S, *et al.*: **Anti-HIV-1 activity of cellulose acetate phthalate: synergy with soluble CD4 and induction of "dead-end" gp41 six-helix bundles.** *BMC Infect Dis* 2002, **2:**6.
31. Herold BC, Bourne NG, Marcellino D, *et al.*: **Poly(sodium 4-styrene sulfonate): an effective candidate topical antimicrobial for the prevention of sexually transmitted diseases.** *J Infect Dis* 2000, **181:**770–773.
32. Witvrouw M, Fikkert V, Pluymers W, *et al.*: **Polyanionic (ie, polysulfonate) dendrimers can inhibit the replication of human immunodeficiency virus by interfering with both virus adsorption and later steps (reverse transcriptase/integrase) in the virus replicative cycle.** *Mol Pharmacol* 2000, **58:**1100–1108.

33. Bourne N, Stanberry LR, Kern ER, *et al.*: **Dendrimers, a new class of candidate topical microbicides with activity against herpes simplex virus infection.** *Antimicrob Agents Chemother* 2000, **44:**2471–2474.
34. Zaneveld LJ, Anderson RA, Diao XH, *et al.*: **Use of mandelic acid condensation polymer (SAMMA), a new antimicrobial contraceptive agent, for vaginal prophylaxis.** *Fertil Steril* 2002, **78:**1107–1115.
35. Herold BC, Scordi-Bello I, Cheshenko N, *et al.*: **Mandelic acid condensation polymer: novel candidate microbicide for prevention of human immunodeficiency virus and herpes simplex virus entry.** *J Virol* 2002, **76:**11236–11244.
36. Neurath AR: **Microbicide for prevention of sexually transmitted diseases using a pharmaceutical excipient.** *AIDS Patient Care STDS* 2000, **14:**215–219.
37. Neurath AR, Strick N, Li YY: **Anti-HIV-1 activity of anionic polymers: a comparative study of candidate microbicides.** *BMC Infect Dis* 2002, **2:**27.
38. Shenoy SR, O'Keefe BR, Bolmstedt AJ, *et al.*: **Selective interactions of human immunodeficiency virus-inactivating protein cyanovirin-N with high-mannose oligosaccharides on gp120 and other glycoproteins.** *J Pharmacol Exp Ther* 2001, **2:**704–710.
39. Chiba H, Inokoshi J, Okamoto M, *et al.*: **Actinohivin, a novel anti-HIV protein from an actinomycete that inhibits syncytium formation: isolation, characterization, and biological activities.** *Biochem Biophys Res Commun* 2001, **282:**595–601.
40. Irvin JD, Uckun FM: **Pokeweed antiviral protein: ribosome inactivation and therapeutic applications.** *Pharmacol Ther* 1992, **55:**279–302.
41. Brown D: **Structure and function of membrane rafts.** *Int J Med Microbiol* 2002, **291:**433–437.
42. Liao Z, Cimakasky LM, Hampton R, *et al.*: **Lipid rafts and HIV pathogenesis: host membrane cholesterol is required for infection by HIV type 1.** *AIDS Res Hum Retroviruses* 2001, **17:**1009–1019.
43. Nguyen DH, Hildreth JE: **Evidence for budding of human immunodeficiency virus type 1 selectively from glycolipid-enriched membrane lipid rafts.** *J Virol* 2000, **74:**3264–3272.
44.•• Blair WS, Lin PF, Meanwell NA, *et al.*: **HIV-1 entry: an expanding portal for drug discovery.** *Drug Discov Today* 2000, **5:**183–194.
In this article, the mechanisms of HIV entry and its sequentially distinct steps (attachment, receptor/coreceptor interaction, and fusion) are reviewed in the context of representative antiviral agents that interfere with key steps in this process.
45. Burton DR, Pyati J, Koduri R, *et al.*: **Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody.** *Science* 1994, **266:**1024–1027.
46. Allaway GP, Davis-Bruno KL, Beaudry GA, *et al.*: **Expression and characterization of CD4-IgG2, a novel heterotetramer that neutralizes primary HIV type 1 isolates.** *AIDS Res Hum Retroviruses* 1995, **11:**533–539.
47. Veazey RS, Shattock RJ, Pope M, *et al.*: **Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120.** *Nat Med* 2003, **9:**343–346.
48. Veazey RS, Klasse PJ, Ketas TJ, *et al.*: **Use of a small molecule CCR5 inhibitor in macaques to treat simian immunodeficiency virus infection or prevent simian-human immunodeficiency virus infection.** *J Exp Med* 2003, **198:**1551–1562.
49. Moore JP, Shattock RJ: **Preventing HIV-1 sexual transmission: not sexy enough science or no benefit to the bottom line?** *J Antimicrob Chemother* 2003, **52:**890–892.
50. Jan ST, Shih MJ, Venkatachalam TK, *et al.*: **Synthesis of dual function (5R, 6R)-and (5S,6S)-5 bromo-6-methoxyl-5, 6-dihdro-AZT-5'-(para-bromophenyl methoxyalaninyl phosphate) as novel spermicidal and anti-HIV agents.** *Antivir Chem Chemother* 1999, **10:**39–46.
51. D'Cruz OJ, Samuel P, Waurzyniak B, Uckun FM: **Development and evaluation of a thermoreversible ovule formulation of stampidine, a novel nonspermicidal broad-spectrum anti-human immunodeficiency virus microbicide.** *Biol Reprod* 2003, **69:**1843–1851.
52. Pauwels R, De Clercq E: **Development of vaginal microbicides for the prevention of heterosexual transmission of HIV.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1996, **11:**211–221.
53. Balzarini J, Naesens L, Verbeken E, *et al.*: **Preclinical studies on thiocarboxanilide UC-781 as a virucidal agent.** *AIDS* 1998, **12:**1129–1138.
54. Borkow G, Salomon H, Wainberg MA, Parniak MA: **Attenuated infectivity of HIV type 1 from epithelial cells pretreated with a tight-binding non-nucleoside reverse transcriptase inhibitor.** *AIDS Res Hum Retroviruses* 2002, **18:**711–714.
55. Di Fabio S, Van Roey J, Giannini G, *et al.*: **Inhibition of vaginal transmission of HIV-1 in hu-SCID mice by the non-nucleoside reverse transcriptase inhibitor TMC120 in a gel formulation.** *AIDS* 2003, **17:**1597–1604.
56. Pani A, Musiu C, Loi AG, *et al.*: **DABOs as candidates to prevent mucosal HIV transmission.** *Antivir Chem Chemother* 2001, **12(Suppl 1):**51–59.
57. Turpin JA: **Considerations and development of topical microbicides to inhibit the sexual transmission of HIV.** *Expert Opin Investig Drugs* 2002, **11:**1077–1097.
58. Coggins C, Elias CJ, Atisook R, *et al.*: **Women's preferences regarding the formulation of over-the-counter vaginal spermicides.** *AIDS* 1998, **12:**1389–1391.
59. Doncel GF: **Chemical vaginal contraceptives: preclinical evaluation.** In *Barrier Contraceptives, Current Status and Future Prospects.* Edited by Mauck CK, Cordero M, Gabelnick HL, et al. New York: Wiley-Liss; 1994:147–162.
60. Mauck C, Doncel G: **An update on vaginal microbicides.** *Curr Infect Dis Rep* 2001, **3:**561–568.
61.•• Mauck C, Rosenberg Z, Van Damme L: **Recommendations for the clinical development of topical microbicides: an update.** *AIDS* 2001, **15:**857–868.
This review compiles the recommendations of a group of experts in regard to how to design and implement microbicide clinical trials.