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Vaginal microbicides and the prevention of HIV transmission

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Abstract

Worldwide, nearly half of all individuals living with HIV are now women, who acquire the virus largely by heterosexual exposure. With an HIV vaccine likely to be years away, topical microbicide formulations applied vaginally or rectally are being investigated as another strategy for HIV prevention. A review of preclinical and clinical research on the development of microbicides formulated to prevent vaginal HIV transmission yielded 118 studies: 73 preclinical and 45 clinical. Preclinical research included in-vitro assays and cervical explant models, as well as animal models. Clinical research included phase I and II/IIb safety studies, and phase III efficacy studies. Whereas most phase I and phase II clinical trials have found microbicide compounds to be safe and well tolerated, phase III trials completed to date have not demonstrated efficacy in preventing HIV transmission. Topical microbicides are grouped into five classes of agents, based on where they disrupt the pathway of sexual transmission of HIV. These classes include surfactants/membrane disruptors, vaginal milieu protectors, viral entry inhibitors, reverse transcriptase inhibitors, and a fifth group whose mechanism is unknown. The trajectory of microbicide development has been toward agents that block more specific virus—host cell interactions. Microbicide clinical trials face scientifically and ethically complex issues, such as the choice of placebo gel, the potential for viral resistance, and the inclusion of HIV-infected participants. Assessment of combination agents will most likely advance this field of research.

Introduction

According to recent UNAIDS estimates, in 2007 more than 33 million people were living with HIV and approximately 2·5 million people were newly infected.[1] Worldwide, nearly half of all individuals living with HIV are now women, who acquire the virus largely by heterosexual exposure.[1–3] Many women, because of limited economic options and gender inequality, cannot reliably negotiate sexual encounters, leaving them vulnerable to unwanted pregnancy and sexually transmitted infections (STIs), including HIV. With clinical deployment of a safe and effective HIV vaccine still likely to be years away, topical microbicide formulations that are applied vaginally or rectally are receiving increasing attention as another strategy for HIV prevention.

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Conflicts of interest JJ has received funding to conduct the following National Institutes of Health-sponsored microbicide clinical trials: HPTN 049 (phase I safety and acceptability study of the vaginal microbicide 6% cellulose sulfate gel among HIV-infected women), HPTN 050 (phase I safety and acceptability study of the vaginal microbicide agent PMPA gel), HPTN/MTN 059 (phase II expanded safety and acceptability study of the vaginal microbicide 1% tenofovir gel) and will soon begin MTN 001 (phase II adherence and pharmacokinetics study of oral and vaginal preparations of tenofovir). She has served as Investigator of Record for the Bronx-Lebanon Hospital Center Clinical Research Site in New York City for all of these trials and was protocol co-chair for HPTN 059. BC assisted with HPTN 059 as a clinical fellow. Gilead was a co-sponsor of HPTN 050 and HPTN/MTN059, and CONRAD a co-sponsor of HPTN 049. Both Gilead and CONRAD are co-sponsors of MTN 001. CONRAD is a non-profit reproductive health organisation and currently holds the Investigational New Drug (IND) for tenofovir gel. Neither JJ or BC have any financial relationships with any companies involved in HIV products, including Gilead.

Until recently, an incomplete understanding of key steps in the sexual transmission of HIV hindered the science of microbicides. Most of the agents that were first developed, including surfactants and acidifying agents, act non-specifically, either by disrupting viral and cellular membranes, or creating a more hostile environment in the genital tract for viral transmission. Progress in understanding how HIV gains entry into the host and establishes lasting infection has permitted the development of compounds that target specific viral—host cell interactions and has allowed for a more tailored approach to microbicide development. At least ten reverse transcriptase inhibitors and 16 entry inhibitor agents have been investigated or are currently being investigated in preclinical or clinical microbicide trials.[4] The development of these agents will be reviewed here, with a brief overview of current research delineating the sexual transmission of HIV.

The sexual transmission of HIV and microbicide strategies

The sexual transmission of HIV is not uniformly efficient. The type of sexual activity and the phase of disease affect the risk of transmission. Initial estimates of transmission rates per coital act have ranged from 0·0003 to 0·008,[5–9] with insertive vaginal intercourse associated with lower estimates and receptive anal intercourse associated with estimates as high as 0·01 or 1%.[10] The role of anal intercourse in heterosexual transmission is less well described and the frequency might be greater than previously thought.[11,12]

Recent investigation has also shown that the rate of sexual transmission depends on cofactors such as circumcision status, genital ulcer disease, and phase of disease.[13] High serum HIV-1 concentrations during the acute infection period increases the probability of male-to-female heterosexual transmission by up to eight to tenfold.[14] A study of Ugandan serodiscordant couples found the rate of HIV-1 sexual transmission per coital act within 2·5 months after seroconversion of the index partner to be 0·0082, or almost 1%.[15] Although these per-act estimates for HIV-1 transmission risk are not particularly high, the cumulative risk of sexual activity over an extended period of time—with prolonged viral shedding, frequent sexual contact, inflammation or ulcerative lesions of the genital tract, or having sex during a particularly high-risk period, such as acute infection—makes the sexual transmission of HIV-1 increasingly efficient.

Male circumcision status also affects the efficiency of transmission. Three recent randomised clinical trials in Africa have shown that circumcision decreases the risk of female-to-male HIV transmission by 50–76%.[16–18] Uncircumcised men might acquire HIV at higher rates than circumcised men because of the presence of key target cells in human foreskin: macrophages expressing CD4 receptors and dendritic cells expressing dendritic-cell C-specific intercellular adhesion molecular-3-grabbing non-integrin (DC-SIGN), a mediator of HIV entry into CD4 cells.[16,19]

By contrast with human foreskin, the intact vaginal epithelium and endocervix each present a different challenge to the entry pathway of the HIV virion. Although vaginal epithelial cells have limited permeability to particles greater than 30 nm (HIV virion is 80–100 nm),[20] HIV seems to enter the superficial layers of the squamous epithelium by diffusing across a concentration gradient,[21] and sequesters itself on the surface of epithelial cells until it can infect other cell types, particularly CD4+ helper cells and Langerhans cells, both of which are found in mucosal epithelium.[22,23] One recent study using an ex-vivo human organ culture system found that HIV simultaneously enters both intraepithelial CD4+ T cells and Langerhans cells within 2–3 h of challenge and survives within Langerhans cells for 3 days.[24] Thus, finding microbicide agents that can disrupt the virus envelope before the initial attachment of the virus to epithelial target cells has been one of the earliest strategies for microbicide development.

The acidity of the vaginal canal is protective against a variety of bacteria and viruses, including *Chlamydia trachomatis*, *Haemophilus ducreyi*, and herpes simplex virus (HSV) type 2.[25–27] The presence of normal commensal vaginal flora, particularly lactobacilli, and an acidic vaginal pH has been correlated with a decrease in HIV proliferation[28] as well as a decrease in HIV acquisition.[29–31] The development of compounds that protect the acidic vaginal milieu, either by buffering the neutralising effect of semen or maintaining sufficient lactobacilli production in the vaginal canal has been a second strategy in microbicide development.

The subepithelial layer of the genital mucosa is a very favourable environment for HIV replication. Dendritic cells, macrophages, and T cells all densely populate the subepithelial stromal tissues of the male and female genital tract and the rectum. Each of these cell types expresses CD4, CCR5, and in lesser quantities, CXCR4 receptors, making them all vulnerable to HIV-1 binding and entry. Recent research has shown that CCR5-tropic HIV is sexually transmitted more frequently than CXCR4-tropic virus.[32–34] Epithelial disruption caused by, for example, genital ulcerative disease such as HSV,[35] dry sex,[36] or trauma[37,38] therefore increases susceptibility to HIV-1.[39] The formulation of microbicide candidates as a protective gel is common to the development of many agents and has the theoretical benefit of minimising mucosal breaks. Additionally, finding specific agents that block viral binding, entry, or viral replication have been other strategies.

The single layer of columnar epithelium lining the endocervix is vulnerable to disruption,[40] and the cervical transformation zone between the squamous and columnar cells contains many HIV target cells near the surface.[41] The intact endocervix has the capacity to block infection of cell-associated and cell-free HIV and resists internalisation of viral particles, most likely because of a physical barrier created by cervical mucus. Additionally, antiviral proteins contained in the cervical mucus, such as secretory leucocyte protease inhibitor, and high levels of natural ligands to CXCR4 and CCR5 might block HIV-1 binding to local CD4+ cells.[42,43] Mimicking or augmenting these natural ligands is a fourth modality under investigation.

Microbicide development strategies have also had to account for the ways in which differences in the vaginal and rectal lumen might affect their success. The rectal mucosa seems to be less protective against HIV-1 than the vaginal mucosa. It consists of one layer of columnar epithelium, and the subepithelial lamina propria contains many cell types to which HIV-1 typically binds.[44] Furthermore, rectal lymphoid follicles contain specialised M cells (microfold cells), which have been shown to bind and present HIV-1 to underlying lymphoid tissue.[45,46] Finally, unlike the lumen of the vagina, which is ultimately circumscribed at one end, the rectal space, as part of the colon, is open-ended so that vulnerability above the rectal vault might require additional coverage.[4] Because of the differences in the vaginal and rectal lumens, and the high rates of rectal transmission of HIV with unprotected anal intercourse, certain microbicide compounds are currently being assessed for rectal use in addition to vaginal use.

The evolving conception of mucosal, submucosal, and lumenal vulnerability to HIV infection is informing a more targeted approach to microbicide development. Preclinical or non-clinical testing of microbicides before US Food and Drug Administration licensing now includes a battery of at least nine study types, which include: in-vitro assays; animal vaginal irritation tests; pharmacokinetic studies; genetic, general, and reproductive toxicity studies; safety pharmacology studies; carcinogenic studies; hypersensitivity/photosensitivity studies; and condom integrity studies.[4] Clinical testing includes phase I and phase II dosing, safety and acceptability studies, penile tolerance studies, and phase III trials for efficacy. Each preclinical and clinical phase of testing has its own set of limitations and it remains unclear which set of tests will best predict safety and effectiveness. In this context, we describe the five broad classes of microbicides and some of their most important representative agents.

Microbicide classes and key compounds

Surfactants/membrane disruptors

Surfactants are the earliest compounds to have been clinically evaluated as topical microbicides. These agents disrupt membranes non-specifically, offering contra ceptive properties and activity against a wide range of potential STI pathogens (table 1). Nonoxinol 9 (N-9; nonoxynol-9), an inexpensive and effective spermicide widely available in over-the-counter preparations, disrupts the HIV envelope and early in-vitro efficacy against HIV was initially quite promising.[58] N-9 was the first microbicide to be formally tested for efficacy in preventing HIV transmission.[38,47] One of the two blinded, randomised controlled efficacy trials of N-9, which was done among 1292 HIV-negative female sex workers in Cameroon, showed no difference in the rate of HIV infection, but a higher incidence of genital ulcers was associated with N-9 use compared with placebo use.[47] The second efficacy trial, in 892 female sex workers in four countries, showed an association between N-9 and increased HIV seroincidence when N-9 was used more than three times per day.[38] Toxicity to vaginal mucosal tissue at the higher doses was suggested as a possible cause for increased transmission among frequent users. These disappointing results ended the development of N-9 as an anti-HIV microbicide. The experience with N-9 also led to a greater scrutiny of safety studies before the commencement of larger clinical trials.

C31G (Savvy, Cellegy Pharmaceuticals, Quakertown, PA, USA), consisting of cetyl betaine and myristamine oxide, has shown in-vitro safety and broad-spectrum activity against bacteria, including *C trachomatis*, HSV, and especially HIV.[59–62] C31G had been tested clinically in at least three separate safety trials[63–65] and was to be assessed in two placebo-controlled, double-blind, phase III clinical trials in Africa (Ghana and Nigeria). The Ghanaian trial was halted in November, 2005, for futility: the HIV seroincidence rate in the study population was lower than anticipated, making the ability to observe statistically significant results highly improbable without doubling the sample size—a costly proposition declined by investigators.[48] The Nigerian trial was stopped for similar reasons in August, 2006. Analysis of the data from the 2153 participants with 12 months' follow-up found a trend toward higher HIV seroincidence in the C31G users compared with the placebo (hydroxyethylcellulose [HEC]) users, but this trend was not significant.[49]

Sodium lauryl sulfate (Invisible Condom, Université Laval, Quebec, Canada) is a third surfactant compound that has been shown to disrupt both non-enveloped and enveloped viruses.[66] This agent has been formulated to act as an "invisible condom" in that it can cover the vaginal wall as a liquid at room temperature, and then transform into a gel at body temperature. In this form it can block HIV-1 and STI transmission.[67,68] Safety of sodium lauryl sulfate has been shown in a rabbit model and in at least two phase I clinical trials.[68–70] Although results of a phase II study of 200 women in Cameroon are pending (table 1), interest in the development of agents with more virus-specific mechanisms of action continues to progress.

Vaginal milieu protectors

The second broad class of microbicides in development, vaginal milieu protectors, works to maintain, restore, or enhance the natural protective mechanisms within the vaginal canal—the acidic pH maintained by lactobacilli (table 1). A pH between 4·0 and 5·8 has been shown to inactivate HIV.[71–73] However, a variety of situations, including the presence of semen or bacterial vaginosis, neutralise the baseline acidity of the vagina. The microbicide compounds in this class either operate as direct acidifying agents or as enhancers of lactobacilli production.

Carbopol 974P (BufferGel, ReProtect, Baltimore, MD, USA) is a polyacrylic acid that buffers twice its volume of semen to a pH of 5 or less.[74] BufferGel has been shown to be spermicidal,

[74] virucidal in vitro to HIV[72] and HSV,26 and protective in mouse vaginal models against HSV and *C trachomatis*.[75] The gel also inhibits human papillomavirus (HPV) in animal models.[76] BufferGel was found to be safe in two phase I trials.[77,78] One important ancillary finding of phase I testing was a decline in the prevalence of bacterial vaginosis, reported in 27 (30%) of 90 participants at enrolment and five (6%) of 90 participants after the first week of product use.[77] BufferGel was safe and acceptable among men in a penile tolerance study in HIV-infected and uninfected men.[79] A phase II/IIb trial, HPTN 035, is assessing the safety and effectiveness of BufferGel compared with a placebo gel and with condoms and has completed enrolment of 3101 participants in five countries (Malawi, South Africa, USA, Zambia, and Zimbabwe). HPTN 035 will also assess the safety and effectiveness of PRO2000, an HIV entry inhibitor.

Acidform (Amphora, Instead Inc, Dallas, TX, USA) is currently approved as a sexual lubricant gel, but its acid-buffering and bioadhesive properties make it appealing for development as a candidate microbicide. Acidform has undergone two phase I safety studies, as well as a male penile tolerance study.[80–82] The first phase I study assessed Acidform alone and in combination with N-9. Acidform was well tolerated when used alone, but produced vaginal irritation when combined with N-9.[80] A second study, done in Brazil, assessed the safety of Acidform used 30 min or 8–10 h before intercourse in 20 women. Mild to moderate vulvar irritation was reported in five colposcopies completed 3 h after intercourse.[81] 36 men participated in a penile tolerance study of Acidform versus K-Y Jelly (Personal Product Co, Skillman, NJ, USA) lubricant.[82] The Acidform group had fewer genital symptoms (including itching, tingling, burning, and dryness) and both groups had similar rates of genital examination findings (including erythema, ulceration, and vesicles; 8% for each group), which were considered mild. An efficacy trial in Madagascar, testing Acidform's ability to prevent *Neisseria gonorrheae* and *C trachomatis*, is currently being planned.

A more recent "probiotic" strategy being developed to protect the vaginal milieu is the use of exogenous lactobacilli for colonisation since lactobacillus colonisation has been shown to correlate with decreased HIV proliferation.[28,29] Colonisation of macaque vaginal canals was safely achieved with *Lactobacillus crispatus* in one study and a pilot investigation of nine women also showed a 60% colonisation rate.[83,84] Bioengineered lactobacilli (or "live microbicides") are also being developed to express proteins that bind to HIV and block either viral—host cell fusion or viral entry into host cells. Three proteins expressed through this type of system are CD4,[85] a derivative of gp41,[86] and cyanovirin.[87] These live microbicides are all in preclinical development. Finally, in certain societies, naturally occurring acidic compounds such as lime juice have been applied with limited effect.[4] Recent clinical trials evaluating lime juice have shown toxicity.[88]

Entry inhibitors

Viral entry inhibitors form a third broad class of microbicide agents and bind sequences that block either the attachment of HIV-1 to host cells, the fusion of virus and host-cell membranes, or the entry of HIV-1 into host cells (table 1).

Anionic polymers—The first group of viral entry inhibitors to be investigated were anionic polymers.[89] Through their negative charge, anionic polymers interact with HIV's viral envelope proteins and interfere with the attachment of HIV to CD4+ cells.[90,91] The greater net positive charge on the gp120 protein of CXCR4-tropic viruses makes them particularly vulnerable to these compounds, but this is not always as reliably the case for CCR5-tropic viruses. For example, dextrin sulfate reduced in-vitro cell infectivity of a CXCR4 virus (HIV-1 HSBc2) by 77%, but did not reduce infectivity of an CCR5 virus (HIV-1 JRCSF).[92]

Naphthalene sulfonate (PRO2000; Indevus Pharmaceuticals, Lexington, MA, USA), is a sulfonated polymer with in-vitro activity against HIV, *C trachomatis*, *N gonorrhoeae*, and HSV.[93,94] Phase I clinical trials in Europe,[95] the USA, and South Africa[96] showed that PRO2000 was generally well tolerated; however, at the highest concentrations tested (4%), it was associated with a slightly higher incidence of intermenstrual bleeding compared with placebo.[97] Clinical investigation continues with both a phase II/IIb safety and efficacy study of 3101 participants (HPTN 035), and a phase III efficacy trial (MDP-301). The HPTN 035 trial randomised participants to one of four arms: 0·5% PRO2000, BufferGel, a placebo gel, or a condom; results are expected in early 2009. The 2% PRO2000 arm in the MDP-301 trial was closed early in 2008 because interim results indicated futility; the 0·5% arm continues and will be evaluated for efficacy versus placebo. Target recruitment for the MDP-301 study is 9673 women; as of July, 2008, 9395 women had been enrolled and the trial will be completed in late 2009.

Carrageenan (Carraguard/R515, Population Council, New York, NY, USA) is a sulfated polysaccharide derived from a seaweed extract. In addition to blocking HIV-1 transmission by binding the HIV-1 envelope, Carraguard has been found to prevent HIV-infected mononuclear cells from migrating across vaginal epithelia to pelvic lymph nodes in mouse models.[98] Phase I safety trials of Carraguard and similar carageenan-based formulations in 1999,[99] and more recently in 2006,[100] showed safety in HIV-negative men and women. An additional phase I trial in South Africa showed that Carraguard was safe in HIV-positive men and women.[101] Two phase II studies involving 565 women in South Africa and Thailand also demonstrated safety.[102,103] A placebo-controlled phase III study of 6202 HIV-negative, non-pregnant women enrolled at three sites in South Africa completed data collection in March, 2007. Results released in February, 2008, found that although Carraguard gel was safe when used over a 2-year period, incident HIV infections occurred at a similar rate in the Carraguard and placebo groups (134 new infections for an incidence of 3·3 infections per 100 woman-years in the Carraguard group and 151 new infections for an incidence of 3·7 per 100 woman-years in the placebo group).[51,104] Although there was a trend towards fewer HIV infections in the Carraguard group, an applicator dye test[105] indicated that gel was used in less than 50% of sex acts,[51] raising major questions about whether poor adherence contributed to the lack of efficacy found in the trial.

Cellulose sulfate (Ushercell, Polydex Pharmaceuticals, Toronto, ON, Canada and Topical Prevention of Conception and Disease [TOPCAD], Chicago, IL, USA) is a compound that has shown in-vitro activity against *N gonorrhoeae*, *C trachomatis*, HPV, and *Gardnerella vaginalis*.[106–109] Cellulose sulfate acts by binding the V3 loop of the gp120 HIV-1 envelope, and it can inhibit both CXCR4 and CCR5-tropic virus types.[110] Phase I safety studies of cellulose sulfate, which involved at least 518 women and 48 men in the cellulose sulfate arms, found the gel to be safe.[111–116] Two phase III efficacy trials of cellulose sulfate versus placebo were initiated in Africa and India, but both studies were halted in 2007 after interim analysis in one of the studies showed a higher HIV seroincidence than expected in the cellulose sulfate arm. Of 1425 women enrolled (717 in the cellulose sulfate arm and 708 in the placebo arm), there were 25 seroconversions in the cellulose sulfate arm compared with 16 seroconversions in the placebo arm.[52] The second phase III study was halted as a precaution because of safety concerns arising from the first trial, despite the fact that an interim analysis had indicated no effect on the risk of HIV transmission.[53]

An additional anionic polymer under investigation as a microbicide is cellulose acetate phthalate (CAP). This compound, which blocks gp120 binding sites, has shown in-vitro activity against HIV-1 and HSV (types 1 and 2).[117] Like cellulose sulfate, CAP has the ability in tissue explant and animal models to block CXCR4 and CCR5-tropic virus types,[118,119] and its preclinical evaluation to date shows minimal induction of inflammatory change.[120] CAP is

being developed as both a film and a micronised gel. In addition to blocking gp120 binding sites, the micronised form of CAP provides an acidic environment, which was shown in one study to cause disintegration and loss of infectivity of HIV-1.[121] A recent phase I CAP trial of a 13% gel was halted because of the occurrence of heavy vaginal discharge in all five participants, a side-effect attributed to the hyperosmolarity of the glycerol-based formulation.[54]

Panel 1: **Key epidemiological issues in microbicide development**

Seroincidence

Phase III efficacy trials need to be held in settings where HIV seroincidence is high (typically at least 2% per year)

- Even with an HIV seroincidence rate of 2% per year, the study group must be large —eg, over 2000 participants per study arm
- The cost of large trials can be prohibitive, with the estimated cost ranging from US$46 to 70 million for a phase III microbicide trial[164,165]
- "Hawthorne" effect: participation in a clinical trial itself, regardless of the study product, might affect the outcome.[166] In the context of HIV prevention trials, attention from study staff, HIV prevention counselling, and access to free condoms decreases HIV seroincidence among participants. The study group must therefore be even larger to detect a significant difference between study product and control

Study populations

Most microbicide trials enrol healthy HIV-uninfected women over 18 years of age who stop using study product if they become pregnant

- HIV-infected women, pregnant women, breastfeeding women, and adolescents all need to be included in study populations, since all are likely to use vaginal microbicides, either intentionally or inadvertently
- Trials in such groups should occur after initial trials show efficacy but before widespread product availability, which poses a challenge
- Any microbicide approved for vaginal use will likely be used rectally by both men and women. It remains to be seen what, if any, are the key safety and efficacy differences between vaginal and rectal use of microbicides

Covert use of microbicides

Vaginal microbicides might offer women control over HIV prevention, including through covert use.[167] Such covert use raises ethical and pragmatic challenges

- Covert use could bring increased mistrust and even physical danger
- Covert use would expose male partners to microbicide products without knowledge or consent
- Some challenge the very idea that covert use would be empowering for women, or the likelihood that women would use microbicides covertly at all[168]

The newest category of anionic polymers are dendrimers. These macromolecules contain a central core, interior branches, and terminal surface groups adapted to specific targets. Because of their size and multiple terminal surface groups, they possess the ability to bind to multiple locations on multiple cells. The first dendrimer to be formulated as a microbicide gel and tested clinically, SPL7013 (Vivagel, Starpharma Holdings Ltd, Melbourne, Australia), provided

protection from chimeric simian/human immuno deficiency virus (SHIV) in a macaque model and from HSV2 in two different animal models.[55,56] SPL7013 has been tested for male tolerance in one Australian phase I safety trial,[57] and a 3% formulation is undergoing further phase I trials in Kenya and the USA.

CCR5 inhibitors—A second set of entry inhibitors under investigation as topical microbicides for the prevention of HIV transmission are CCR5 inhibitors (table 2). CCR5 is the most important co-receptor for macrophage-tropic viral strains, which can predominate in the early stages of viral transmission.[126] PSC-RANTES, a potent synthetic inhibitor of the CCR5 co-receptor, exhibits in-vitro antiviral activity against all HIV clades and inhibits HIV-1 infection of Langerhans cells—crucial cells for HIV-1 transmission across the vaginal epithelium.[127–129] Complete protection from SHIV SF162 was seen in macaques who received the highest dose of PSC-RANTES (1 mmol) tested, with no evidence of systemic absorption or toxicity.[122]

A second CCR5 receptor antagonist, CMPD167, a cyclopentane-based compound formulated as a 5 mmol vaginal gel, provided protection from vaginal SHIV challenge in eight of ten macaques.[123] CMPD167 has been assessed in combination with two peptides that block the viral—host cell interaction at different loci, BMS-378806 and C52-L. BMS-378806 binds viral gp120 and prevents attachment to the CD4 and CCR5 receptors,[130,131] whereas C52-L, a modified version of enfuvirtide, inhibits gp41-mediated viral—cell fusion.[123,132] Using two of these agents in combination protected 16 out of 20 macaques from SHIV, and using all three inhibitors together protected all animals tested. There was no evidence of genital irritation or inflammation from these three compounds by colposcopy or biopsy.[123] Although these animal studies evaluating combinations of compounds with different mechanisms are promising, it is not yet clear whether they will correlate with protection from HIV in human trials. Additionally, study investigators noted that the concentrations of all three compounds necessary for consistent protection (1–10 mmol) were substantially higher than the in-vitro inhibitory concentrations that were required (1–10 nmol). One reason for the need for higher in-vivo concentrations could be the difficulty in shielding all exposed vaginal surfaces from a high titre viral inoculum, which would be particularly important during sexual encounters with partners who have primary infection.[123]

An important challenge in considering the CCR5 inhibitors for use as topical microbicides is their inability to block the entry of CXCR4-tropic virus. Although this latter pathway is less important in sexual transmission, it might still have a role. Another concern is the pressure that CCR5-inhibiting compounds might place on HIV-1 to shift toward the use of non-CCR5 pathways/ co-receptors to gain entry into cells. A clinically effective microbicide most likely will need to block all modes of receptor-mediated entry.

Fusion inhibitors—In addition to C52-L, which inhibits gp41-mediated viral—cell fusion,[123,132] another fusion inhibitor that has undergone early clinical testing as a topical microbicide is cyanovirin-N, a lectin compound purified from cyanobacterium. This compound prevents viral—host cell fusion by binding high mannose residues in the HIV envelope.[133,134] Cyanovirin-N blocks transmission of SHIV 89.6P both vaginally and rectally in a macaque model, and the compound has also demonstrated efficacy in human cervical explants.[135,136] However, some lectins have shown unwanted side-effects, such as human red blood cell agglutination, mitogenic stimulation of peripheral blood mononuclear cells, inflammatory activity, and cellular toxicity.[137] Various formulations of cyanovirin-N, including those expressed by lactobacilli, are under development.[138]

Reverse transcriptase inhibitors

With the success of antiretroviral therapy in the treatment of HIV disease, as well as in the prevention of mother-to-child HIV transmission, interest has grown in using these more targeted drugs for prevention of the sexual transmission of HIV. Relying on compounds that interact with specific viral or cellular receptors, such as the CCR5 inhibitors and fusion inhibitors previously described, offers a more tailored approach than earlier microbicide formulations, with the promise of less toxicity and greater efficacy. The use of such targeted topical compounds has also been suggested as an adjunct strategy in preventing mother-to-child transmission of HIV.[139]

Reverse transcriptase inhibitors bind the HIV-1 reverse transcriptase enzyme and block the conversion of viral RNA into DNA—effectively halting viral replication (table 2). The nucleotide reverse transcriptase inhibitor tenofovir was the first antiretroviral drug to safely demonstrate in animal models both pre-exposure and post-exposure prophylaxis as proof-of-concept against the sexual transmission of HIV.[140] Unlike nucleoside analogues, tenofovir is active as a diphosphate, rather than a triphosphate, and does not act via HIV DNA chain termination. Both of these reasons, coupled with the limited phosporylation ability of macrophages, explain why the drug might be effective in macrophages and other non-dividing cells.[141,142] Formulated as a diphosphate, tenofovir has a prolonged intracellular half-life of 9–50 h, depending on cell type.[143] In 1995, Tsai and colleagues[140] gave tenofovir to macaques before or after intravenous simian immunodeficiency virus (SIV) inoculation and continued treatment for 4 weeks after exposure. None of the treated animals became infected, whereas all ten controls did. Additional studies using vaginal inoculation of macaques with HIV-2 showed transient HIV-2 RNA in early cervicovaginal lavage specimens from five of 12 animals who received tenofovir from 12 to 72 h after the last viral inoculation.[144] Breakthrough systemic infection was noted in one of four animals initially treated 72 h after HIV-2 inoculation. Tsai and colleagues'[140] initial finding of protection against infection in animals treated with tenofovir up to 48 h before inoculation with SIV suggested that pre-exposure prophylaxis with tenofovir in human beings might successfully prevent HIV infection.

Panel 2: **Key biological issues in microbicide development**

Placebo gels

It is challenging to find a placebo gel identical in appearance, consistency, and odour to the microbicide under investigation

- At least five different gels* have been used in trials, some more as a comparator product than as placebo
- HEC, with no known anti-HIV effect and no buffering properties, has been proposed as a "universal" placebo for vaginal microbicide gel studies to facilitate comparison across studies.[169,170] The HPTN 035 trial may establish whether HEC is an inert placebo
- Even an inert gel will form a physical barrier over the vaginal mucosa, and the added lubrication makes it less likely that microabrasions will occur during coitus. Both of these properties might reduce HIV transmission

Condom-only arms

Some researchers and regulatory officials endorse a second control arm consisting of no treatment—ie, condom-use only

- Since there is no gel involved in a condom-only arm, it is necessarily unblinded
- Retention of participants in the condom-only arm could be more challenging

- The no-treatment group might be more adherent to condom use than the other study groups

Delivery of microbicides

Microbicides are being formulated in a variety of delivery mechanisms, including gels/creams, rings, tablets, foams, and films.[171] Mode of delivery will affect adherence. Considerations in the choice of delivery mode include:

- Leakage, ease of use, potential for covert application, general acceptability, and cost
- Changes in the physiology of the cervix or vagina with age, as well as menstruation
- The physical properties of the microbicide itself

Drug resistance

ART resistance mutations could occur and be passed on by women not aware of their HIV-seropositive status

- The low concentrations of systemic tenofovir seen in the HPTN 050 and HPTN 059 trials suggest that the development of viral resistance is plausible[124,147]
- It is unclear whether low levels of systemic absorption are necessary for efficacy and whether the systemic levels seen would induce mutations
- More detailed attention to drug resistance and systemic absorption, both of parent drug and metabolites, is needed in future trials[4]

Combinations of prevention strategies

Combinations of microbicide agents with different mechanisms of action may be the best approach to the use of microbicides for HIV prevention

- Combining agents might increase activity across viral subtypes, reduce the development of HIV resistance, and prevent other STIs
- In human beings, safety and efficacy trials of microbicide combinations will require the demonstration of safety and efficacy of each single agent first
- As with HIV therapeutic trials, concurrent trials assessing combinations of agents will be necessary to advance this field of research

ART=antiretroviral therapy. HEC=hydroxyethylcellulose. STIs=sexually transmitted infections. *K-Y Jelly (Personal Product Co, Skillman, NJ, USA), Conceptrol (Personal Products Co, Skillman, NJ, USA), RePlens (LDS Consumer Products, Cedar Rapids, IA, USA), HEC, and methylcellulose.

Based on these animal studies, and with an appreciation for tenofovir's relatively high barrier to resistance compared with other reverse transcriptase inhibitors,[145] the compound became the first antiretroviral drug to be assessed as a vaginal microbicide in a clinical trial. In a phase I study (HPTN 050), 0·3% and 1% vaginal tenofovir gel, formulated as a diphosphate, was used once or twice daily for 14 days by HIV-infected and uninfected women. The gel was found to be safe, well tolerated, and acceptable to participants.[124] A pharmacokinetic substudy found 14 (56%) of 25 women had low but detectable serum tenofovir levels, with a median C_{max} of 3.4 ng/mL (range 3·0–25.8 ng/mL) and no clear dose-concentration relation. By contrast, the tenofovir concentration associated with the median steady-state 24 h post-dose blood concentration following an oral 300 mg tenofovir dose is approximately 47 ng/mL.[146] Study investigators also assessed the tenofovir gel's capacity to induce resistance mutations. Plasma and cervicovaginal lavage specimens were obtained from 22 of the HIV-positive

women in this study. Genotyping from all specimens with sufficient quantities of HIV RNA showed that none of the samples contained either the K65R or 69SS mutations. Low-level tenofovir resistance mutations (M41L, L210M, and T215I/Y) were detected in the plasma of three women at baseline who were taking nucleoside reverse transcriptase inhibitors at enrolment, and these mutations were unchanged after 2 weeks of tenofovir exposure.[124] It is not known whether low levels of absorption may be necessary for protection from HIV transmission nor whether longer periods of exposure would result in the induction of resistance mutations.

The preliminary results of a larger, phase II expanded safety trial of tenofovir vaginal gel, done in India and the USA among 200 sexually active HIV-negative women (HPTN 059) found the gel to be safe when applied daily or before each act of sex over a 6-month period.[147] Adherence to gel use between the daily-use and pre-coital groups was found to be similar (83% of 99 women in the daily-use arm reported study gel use in the previous 24 h compared with 80% of 101 women in the pre-coital arm). The two most commonly cited reasons women gave for not using gel were menstruation (41%) and forgetting (23%). Preliminary pharmacokinetic data again indicated low levels of systemic tenofovir absorption in many of the women. An additional pharmacokinetic study by Schwartz and colleagues[148] found tenofovir concentrations in vaginal tissue and vaginal fluid to be higher than blood plasma concentrations after a single 4 g dose, suggesting that tenofovir gel can be administered well in advance of coitus. A phase IIb trial of 1% tenofovir gel in South Africa (CAPRISA 004) with a planned enrolment of 980 women is ongoing, and an efficacy trial currently in development (the Vaginal and Oral Interventions to Control the Epidemic, or VOICE study) will compare oral pre-exposure prophylaxis (tenofovir or a combination of tenofovir plus emtricitabine) with topical pre-exposure prophylaxis (tenofovir gel).

Two non-nucleoside reverse transcriptase inhibitors (NNRTIs), TMC120 and UC781, have proceeded to preclinical or clinical testing as potential topical microbicides and have several features in common: unlike first-generation NNRTIs that only require one mutation before viral resistance occurs, TMC120 and UC781 usually require at least two mutations.[149–151] Both compounds show minimal systemic absorption, and both had good safety profiles in animal studies.[152,153] In vitro, TMC120 and UC781 prevent cell-free and cell-associated virus from infecting co-cultures of monocyte-derived dendritic cells and T cells.[154–156]

TMC120 (4-[{4-[(2,4,6-trimethylphenyl)amino]pyrimidi n-2-yl}amino]benzenecarbonitrile), a diarylpyrimidine, was the first NNRTI shown to have in-vivo effectiveness as a topical microbicide. By use of a severe combined immunodeficient mouse model, Di Fabio and colleagues[153] showed that the most viscous TMC120 gel formulation provided 70–80% protection, while a less viscous formulation protected 100% of mice, whether they were inoculated with CCR5-tropic or CXCR4-tropic viral strains. It was concluded that the thicker gel might have been unevenly distributed across the vaginal mucosa. TMC120 is now being tested in several phase I and II trials. One formulation employs a slow release vaginal ring, which would allow for once monthly, non-coitally dependent dosing.

The thiocarboxanilide UC781 (N-[4-chloro-3-(3-methyl-2-butenyloxy)phyenyl]-2-methyl-3-furancarbothio-amide), an NNRTI with poor oral bioavailability, showed promise as a vaginal microbicide in rabbit safety studies[152] and in its ability to block cell-free and cell-associated HIV-1 transmission in a human cervical tissue-based organ culture.[157] The latter findings were recently corroborated in a cervical explant model.[154] UC781 has shown decreased activity against NNRTI-resistant HIV-1 virus at some concentrations, although its activity against wildtype and NNRTI-resistant virus at low concentrations (below current microbicide formulations of 25 μmol) has been similar.[158] A recent phase I trial of UC781 demonstrated safety after 6 days of once-daily dosing.[125] Additional phase I trials are underway.

Search strategy and selection criteria

We undertook a search for English-language preclinical and clinical trials of microbicides, with a focus on recent clinical trials. We used Medline, OVID, PubMed, the Cochrane Library, and systematic reviews to identify trials from 1966 to 2008 and reviewed abstracts from the major meetings in infectious diseases and microbicides during 2004–08. In addition to "microbicides", we used other terms (eg, "HIV", "topical", "vaginal", "rectal", as well as specific compound names) to identify sources. 118 studies were identified: 73 preclinical and 45 clinical. Meta-analysis was not done.

Unknown mechanism agents

Several microbicides in development have shown anti-HIV-1 activity, but their mechanism of action remains unknown. The most clinically advanced of these compounds is Praneem (Panacea Biotech Ltd, New Delhi, India)—a combination of extracts originally developed as a spermicide from the Indian neem tree *(Azadirachta indica*), saponins from *Sapindus mukorossi* trees, and menthe citrate oil.[159] Praneem has shown wide-spectrum antimicrobial activity against reproductive tract infections, and has also shown antiretroviral properties.[159] Praneem has undergone phase I and II safety and acceptability studies.[160–162]

Challenges in microbicide development

This year, the Institute of Medicine issued its first comprehensive report addressing methodological challenges in non-vaccine biomedical HIV prevention trials.[163] The key epidemiological and biological issues in microbicide development are shown in panel 1 and panel 2. As with HIV therapeutic trials, it is likely that combinations of microbicide agents with different mechanisms of action will be more successful than single agents, and this strategy is gaining momentum. Efficacy trials, however, with HIV seroincidence as an endpoint, need to be large and are expensive to fund. The effect of concomitant sexually transmitted diseases, such as HSV and other ulcerative lesions, on a microbicide's ability to effectively prevent HIV also remains unknown. Despite these challenges, more than 20 000 HIV-uninfected sexually active women are scheduled to participate in phase II and phase III microbicide studies from 2007 through 2009.

Conclusions

The increasingly specific targets used to develop topical microbicides to prevent HIV infection is a reflection of the advances that have been made in understanding the pathophysiology of HIV sexual transmission. New classes of targeted therapeutic agents, such as integrase inhibitors, are also beginning to move into preclinical investigation.[172] It is clear that the development of a topical microbicide to prevent the sexual transmission of HIV is scientifically, ethically, and culturally complicated. However, the benefit in lives saved may far exceed those risks seen and, as yet, unforeseen.

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Table 1

Selected non-specific microbicide agents

	Advantages	Disadvantages	Examples in class	Clinical trial status
Surfactants				
Non-specifi c disruption of cellular and microbial membranes	Active against wide range of pathogens; often spermicidal	Potentially toxic to host cells	Nonoxinol 9 (nonoxynol-9)	No current clinical trials for HIV prevention. Two phase III efficacy trials completed in 1996 and 2000, one of which showed increased HIV-1 seroincidence with nonoxinol 9 when used more than three times per day[38,47]
			C31G (Savvy)	Two phase III trials in Ghana (n=2142) and Nigeria (n=1800) halted in November, 2005, and August, 2006, because of low HIV seroincidence rate in the study population[48,49]
			Sodium lauryl sulfate (Invisible Condom)	Phase II safety trial in Cameroon completed. Results pending (clinicaltrials.gov identifier NCT00136643)
				Phase II/III trial assessing efficacy in high-risk women planned[50]
Vaginal milieu protectors/acidifying agents				
Restores protective acidic pH of vagina by buffering semen	Spermicidal; activity against HIV, HSV, *C trachomatis*	None known	Carbopol 974P (BufferGel) Acidform (Amphora)	Phase II/IIb trial (HPTN 035) ongoing: 3101 women in five countries (Malawi, South Africa, USA, Zambia, and Zimbabwe; clinicaltrials.gov NCT00074425) Phase III trial in Madagascar testing diaphragm with Acidform for prevention of *N gonorrhoeae* and *C trachomatis* is planned[50]
Entry inhibitors: anionic polymers				
Negative charge causes interaction with HIV's viral envelope proteins and interferes with attachment of HIV to CD4+ cells	Many have activity against other STI pathogens (including *C trachomatis*, *Neisseria gonorrhoeae*, and HSV)	Not all virus types respond equally well to negative charge properties of these compounds	Naphthalene sulfonate (PRO2000)	Phase II/IIb trial (HPTN 035) ongoing: 3101 women in five countries (Malawi, South Africa, USA, Zambia, and Zimbabwe; clinicaltrials.gov NCT00074425) Phase III (MDP-301, UK Medical Research Council): PRO2000 originally in two concentrations (0.5% *vs* 2.0%)*vs* placebo gel. 2.0% arm stopped in February, 2008. Enrolment of 9395 women completed in July, 2008, and trial to be completed in late 2009 (clinicaltrials.gov NCT00262106)
			Carrageenan (Carraguard/ PC-515)	Phase III trial completed in South Africa (n=6202). Results released in February, 2008, show gel to be safe with no difference in HIV incidence between study and placebo groups[51]
			Cellulose sulfate (Ushercell)	Two phase III trials in Africa and India halted in January, 2007, for increased HIV seroincidence during interim analysis of one trial[52,53]
			Cellulose acetate phthalate (CAP)	Phase I trial of 13% gel halted because of heavy vaginal discharge in multiple participants[54]
			Dendrimers: SPL7013 (Vivagel)	Showed protection from HIV in a macaque model and from HSV in two animal models.[55,56] Completed phase I male tolerance study.[57] Phase I safety trial completed in Kenya with results pending (clinicaltrials.gov NCT00331032). Phase I trial ongoing in the USA (clinicaltrials.gov NCT00442910)

HSV=herpes simplex virus. STI=sexually transmitted infection. More information about the ongoing clinical trials can be found on the clinicaltrials.gov website.

Table 2

Selected specific microbicide agents

	Advantages	Disadvantages	Examples in class	Clinical trial status
Entry inhibitors: CCR5 blockers				
Block CCR5 co-receptor and interfere with attachment of HIV to host cells	Targets specific ligand	No activity against other STI pathogens	PSC-RANTES	Protected macaques from SHIV (SF162) with no evidence of systemic absorption or toxicity[122]
			CMPD167	Full protection of macaques from SHIV (162P4) not achieved alone, but only with addition of BMS-378806 and C52-L, two peptides that block the viral—host cell interaction at diff erent loci (gp120 and gp41, respectively)[123]
Reverse transcriptase inhibitors				
Interfere with HIV reverse transcriptase enzyme	Tenofovir: active in multiple cell types. TMC-120 and UC781 (NNRTIs): delayed development of resistance compared with first-generation NNRTIs	No activity against other STI pathogens	Tenofovir (PMPA; nucleotide analogue)	Phase I safety trial testing 0.3% and 1% gel formulations in HIV-positive and HIV-negative sexually active and sexually abstinent women found gel to be safe and well tolerated[124] Two phase I pharmacokinetic trials and a third phase I trial evaluating the effect of tenofovir gel on mediators of mucosal immunity are ongoing (clinicaltrials.gov identifiers NCT00561496, NCT00540605, and NCT00594373) Phase II expanded safety trial in India and USA completed in 2007; results pending (clinicaltrials.gov NCT00111943) Phase IIb trial in South Africa ongoing (CAPRISA 004; clinicaltrials.gov NCT00441298) Phase II/IIb trial (MTN 003) in South Africa comparing two oral antiretroviral drugs (tenofovir and emtricitabine) *vs* 1% tenofovir gel is planned (clinicaltrials.gov NCT00705679)
			TMC120 (NNRTI)	Phase III efficacy study (IPM 009) and at least eight phase I/II safety trials planned[50] Phase I study completed, indicating safety after 6 days of daily dosing[125]
			UC781 (NNRTI)	Three phase I trials assessing safety and acceptability of 0.1% or 0.25% formulation applied vaginally are ongoing (clinicaltrials.gov NCT00441909, NCT00132444, and NCT00385554) Phase I trial assessing safety and acceptability with rectal use in HIV-negatives adults ongoing (clinicaltrials.gov NCT00408538) Male tolerance study ongoing (A06-104; clinicaltrials.gov NCT00385554)

SHIV=chimeric simian/human immunodeficiency virus. STI=sexually transmitted infection. NNRTI=non-nucleoside reverse transcriptase inhibitor. More information about the ongoing clinical trials can be found on the clinicaltrials.gov website.