HIV/AIDS – Research and Palliative Care

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REVIEW

Clinical use of vaginal or rectally applied microbicides in patients suffering from HIV/AIDS

This article was published in the following Dove Press journal:
HIV/AIDS – Research and Palliative Care
21 October 2013
Number of times this article has been viewed

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Abstract: Microbicides, primarily used as topical pre-exposure prophylaxis, have been proposed to prevent sexual transmission of HIV. This review covers the trends and challenges in the development of safe and effective microbicides to prevent sexual transmission of HIV. Initial phases of microbicide development used such surfactants as nonoxynol-9 (N-9), C13G, and sodium lauryl sulfate, aiming to inactivate the virus. Clinical trials of microbicides based on N-9 and C31G failed to inhibit sexual transmission of HIV. On the contrary, N-9 enhanced susceptibility to sexual transmission of HIV-1. Subsequently, microbicides based on polyanions and a variety of other compounds that inhibit the binding, fusion, or entry of virus to the host cells were evaluated for their efficacy in different clinical setups. Most of these trials failed to show either safety or efficacy for prevention of HIV transmission. The next phase of microbicide development involved antiretroviral drugs. Microbicide in the form of 1% tenofovir vaginal gel when tested in a Phase IIb trial (CAPRISA 004) in a coitally dependent manner revealed that tenofovir gel users were 39% less likely to become HIV-infected compared to placebo control. However, in another trial (VOICE MTN 003), tenofovir gel used once daily in a coitally independent mode failed to show any efficacy to prevent HIV infection. Tenofovir gel is currently in a Phase III safety and efficacy trial in South Africa (FACTS 001) employing a coitally dependent dosing regimen. Further, long-acting microbicide-delivery systems (vaginal ring) for slow release of such antiretroviral drugs as dapivirine are also undergoing clinical trials. Discovering new markers as correlates of protective efficacy, novel long-acting delivery systems with improved adherence in the use of microbicides, discovering new compounds effective against a broad spectrum of HIV strains, developing multipurpose technologies incorporating additional features of efficacy against other sexually transmitted infections, and contraception will help in moving the field of microbicide development forward.

Keywords: AIDS, microbicides, pre-exposure prophylaxis, sexual transmission of HIV, vaginal/rectal gel

Introduction

AIDS is an immunological disorder characterized by abnormalities of immunoregulation and opportunistic infections caused by HIV. At the end of 2010, there were an estimated 34 million people living with HIV infection across the world, and approximately 1.8 million people died from HIV/AIDS in the year 2010.[1] Approximately 2.6 million new infections were reported during the same year. In Africa, AIDS remains the main cause of death. Sub-Saharan Africa is most rigorously affected, with over 22.5 million people living with HIV/AIDS. In Asia, an estimated 4.9 million people were living with HIV infection in the year 2009. According to a Joint United Nations

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Program on HIV/AIDS 2011 update, the overall growth of the global AIDS epidemic appears to have stabilized, and the number of new infections has been falling. However, the overall level of new infections is still high, and with significant reduction in mortality, the number of people living with HIV infection worldwide has increased.[1]

In the majority of cases, HIV infection occurs through homo- or heterosexual routes; however, the transmission of HIV from infected mother to child and through blood transfusion is also on the rise. "Safe sex" has been proposed as one of the major approaches to prevent sexual transmission of HIV. The use of male condoms greatly reduces the chances of acquiring sexually transmitted infections (STIs), including HIV infection, and in addition provides protection against conception. However, the usage of condoms by males is very low, as its use is perceived to reduce sexual pleasure. Female condoms have been developed to overcome this problem. Due to objections from the male partners and higher cost, their use is many times lower than male condoms.[2] Currently, highly active antiretroviral (HAART) drugs belonging to four general categories – nucleoside/nucleotide viral reverse-transcriptase (RT) inhibitors (NRTIs), nonnucleoside RT inhibitors (NNRTIs), protease inhibitors, and fusion (or entry) inhibitors – are the mainstream drugs to prevent and cure HIV infection.[3] Usage of HAART drugs as a treatment option for HIV infection has expanded during the past few years.[4] Treatment with a HAART triple-drug cocktail of two nucleoside inhibitors and one protease inhibitor can reduce the blood virus load below the detectable level (<50 copies of viral RNA/mL of plasma) in HIV-infected patients.[5] Pre-exposure prophylaxis (PrEP) using a combination of two drugs, namely emtricitabine and tenofovir (TFV) disoproxil fumarate (TDF), showed an estimated efficacy of 44% against HIV infection in men who had sex with men, which is quite encouraging.[6] Long-term usage of available antiretroviral drugs leads to the issue of drug resistance and severe side effects, such as diarrhea, nausea, lipodystrophy, hyperglycemia, liver toxicity, pancreatitis, and neuropathy.[4,7,8]

To prevent HIV infections, attempts are also being made to develop vaccines, which are at different stages of development and clinical trials. Several vaccines using a variety of vectors, such as canarypox, adenovirus serotype 5 (Ad5), adeno-associated virus, and modified vaccinia virus Ankara strain, incorporating varieties of HIV proteins, aiming to generate either neutralizing antibodies or HIV-1-specific $CD8^+$ T cells, have been evaluated in humans without significant success.[9–13] DNA vaccines against HIV have also been proposed, primarily as a prime-boost strategy, whereby priming is done with DNA vaccine followed by a vector-based vaccine. An alternate prime-boost strategy also comprises priming with live vector-based vaccine followed by booster with recombinant protein subunit vaccine. The RV144 vaccine, a combination of two genetically engineered vaccines (a "prime" vaccine called ALVAC-HIV [CCP1521] with a boost of the AIDSVAX gp120 vaccine, an envelope-protein segment from HIV subtypes B and E), showed protective efficacy of 31.2% when tested on more than 16,000 human volunteers in Thailand.[14] The recent discontinuation of HVTN505 clinical trials by the US National Institute of Allergy and Infectious Diseases is a setback to the area of vaccine development. This vaccine comprised of priming with DNA vaccine followed by booster with Ad5-expressing surface and structural proteins of three major HIV clades. The vaccine regimen neither prevented HIV infection compared to the placebo group nor reduced the viral load. Since neutralizing antibodies have provided the best correlate of vaccine efficacy, it is imperative to elicit broadly neutralizing antibodies. As estimated, 10%–25% of HIV-infected individuals develop broadly neutralizing antibodies and monoclonal antibodies from these subjects have been successfully developed.[15] Attempts to develop vaccines incorporating multiple epitopes recognized by broadly neutralizing antibodies are under way, with the aim to have better efficacy against HIV infection.[16,17] Keeping in view that a vaccine for prevention of HIV infection with proven efficacy is still awaited, it is imperative to explore alternate prophylactic and therapeutic options.

Microbicides for prevention of HIV

To prevent homo- or heterosexual transmission of HIV, microbicides have been proposed. Microbicides are formulations that can be applied topically to the vagina or rectum for prevention of sexual transmission of HIV or other pathogens.[18] Microbicides can be formulated as semisolid gel, cream, vaginal film, or tablet. Topical microbicides can provide excellent potential for a female-controlled, preventive option, which would not require negotiation, consent, or even knowledge of the partner. Both women and men would benefit, as these can be bidirectional.[19] Microbicides will also be very useful for prevention of HIV infection in those cases that have multiple sex partners.

An ideal microbicide should effectively inhibit transmission of pathogens causing STIs while resulting in minimal disruption to the structural integrity and function of the healthy cervicovaginal epithelium without inhibiting vaginal *Lactobacillus*, the most prevalent component of the reproductive tract's dynamic ecosystem. These beneficial

bacteria help protect the vagina from pathogenic microbes.[20] An ideal microbicide should be:

- Safe: it should preserve the natural anatomy of the female reproductive tract (does not lead to lesion and aberration in epithelial layer), produce no proinflammatory response, and protect the natural vaginal microecological system, including lactobacilli
- Acceptable: applicable hours before sex; not messy or 'leaky'; rapid and even-spreading properties; long-acting; not smelly and taste OK
- Effective against HIV and a wide range of pathogens causing STIs, eg, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *Chlamydia trachomatis*, and herpes simplex virus (HSV).

Such a microbicide would lead to the empowerment of susceptible receptive partners to adopt an independent and effective measure for their own protection without the other partner's consent or knowledge compared to the usage of condoms. In the direction of multipurpose technologies (MPTs), attempts are also being made to develop microbicides with an added component of contraceptive efficacy. This review provides an update on clinical usage of vaginal or rectally applied microbicides and identifies the critical challenges to their progress.

Mechanism of action of microbicides

Advances made in our understanding of the basic biology of HIV and its transmission has led to the development of microbicides aiming to interfere at different stages of the virus life cycle. HIV infects vital cells of the human immune system, such as T helper cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells. HIV entry into the host cell is initiated by the binding of the envelope protein gp120 to a set of molecules present over the host cell surface, comprising the primary receptor CD4 and a coreceptor, usually either CCR5 or CXCR4.[21–23] HIV preferentially uses CCR5 during the acute phase of infection, but switches to CXCR4 later as the disease progresses in approximately 50% of patients.[24] After the initial binding of gp120 with the CD4 receptor present on the target cells, it is further stabilized by the heparan sulfate proteoglycans present on the host cell surface. This binding induces a conformational change in the gp120, exposing sites that interact with the chemokine receptor (CCR5 or CXCR4). The virus-fusion protein (gp41) then gets uncovered and undergoes a conformational change. Glycoprotein gp41 inserts itself into the membrane of the host cell to initiate the fusion of the two bilayers.

Viral RNA released into the cytoplasm undergoes reverse transcription with the help of the RT enzyme and is converted into DNA. This viral DNA enters the host cell nucleus, where it integrates in the host genetic material by the integrase enzyme. The integrated genome of HIV may lay dormant, until cellular transcription factors enhance transcription of the viral genome and trigger the production of viral proteins. HIV gene transcription from the integrated viral DNA (known as provirus) requires several host and viral proteins. The assembly and release (known as budding) of HIV from the host occurs in a series of organized steps that are driven by the viral gag protein.[25] Once the immature and noninfectious virus particles are released from the cell, further processing of gag polyproteins by HIV protease leads to the generation of mature infectious virus particles. Microbicides have been developed aiming to interfere at various stages of HIV-1 virus life cycle, as shown in Figure 1.

The establishment of infection at the portal of entry and timing of dissemination might also be affected by the number and types of cells that are initially infected. During male-to-female sexual transmission of HIV, following ejaculation, HIV is believed to remain infectious in semen for several hours, although the precise duration is not known.[26] During this time, diffusion is likely to be a principle mechanism of HIV transport from semen to vaginal epithelial surfaces. HIV-infected cells present in the semen can also cross the epithelial barrier, leading to transmission of HIV infection (transmigration). The available data suggest that susceptibility to HIV can be enhanced in the presence of an ulcerative STI, either through mucosal disruption or through increased number or activation of cells susceptible to HIV.[27] Nonulcerative infections have also been linked to increased susceptibility to HIV infection by triggering the proinflammatory responses that enhance viral replication or by proliferation of HIV-susceptible cells.[28]

Based on their active properties, microbicides can be broadly classified into four categories: (1) inactivation of the virus; (2) inhibition of virus binding, fusion, or entry to the susceptible host cell; (3) inhibition of HIV replication in the host cells; and (4) maintenance or enhancement of the vaginal defense (Figure 1). Table 1 summarizes some of the microbicides that have undergone clinical trials in humans and the outcome of their efficacy to inhibit sexual transmission of HIV-1.

Inactivation of the virus

This class of inhibitors leads to disruption of the outer viral lipid membrane or acts by a tight and irreversible binding to the HIV envelope and hence inactivates the virus. Therefore, complete or even incomplete inactivation of cell-free or cell-associated HIV, or both, in semen by appropriate micro-

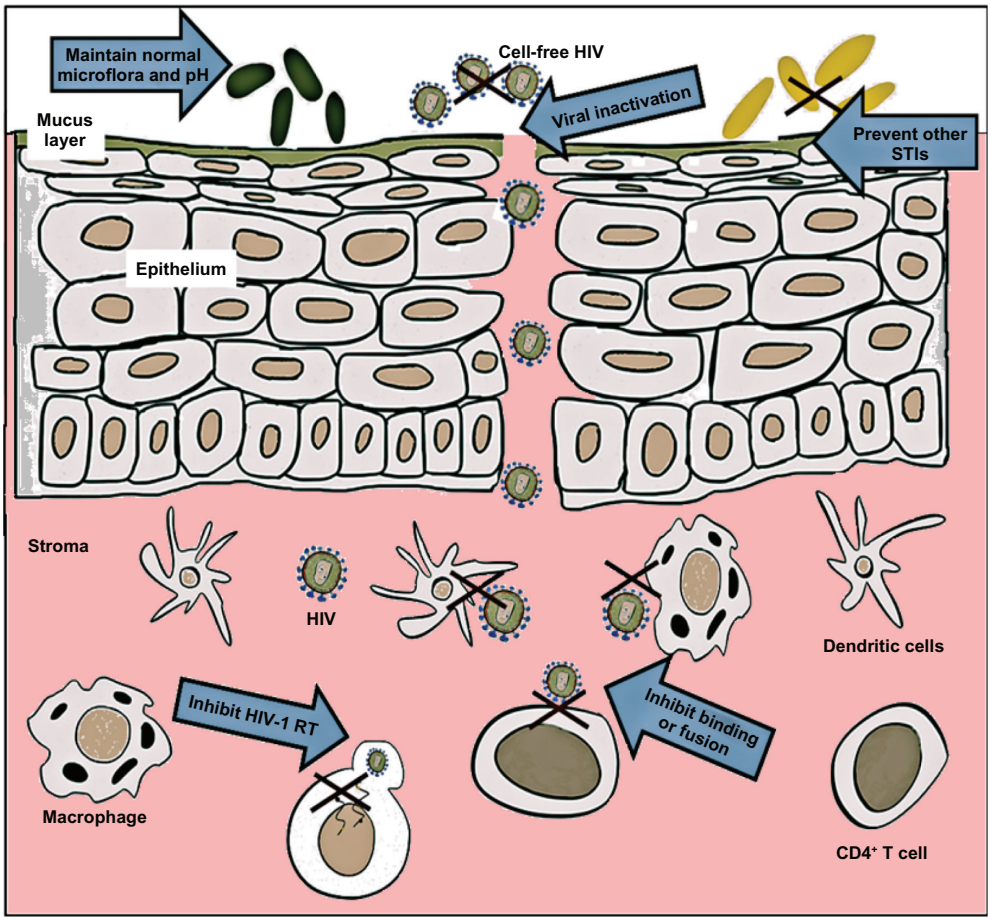

Figure 1 Targeted modes of action of vaginally administered microbicides. To prevent HIV-1 infection, microbicides enabling vaginal milieu protection, such as lactobacilli or agents maintaining acidic pH of cervicovaginal fluid, have been developed. Microbicides based on surfactants are virucidal and inactivate cell-free virus. Microbicides can also be developed based on compounds that prevent binding, fusion or entry of HIV-1 to the host cells, such as $CD4^+$ T cells, dendritic cells, and macrophages. Among the more target-specific microbicides are those based on antiviral drugs, including inhibitors of HIV-1 reverse transcriptase (RT).
Abbreviation: STI, sexually transmitted infection.

Table 1 Outcome of clinical trials in humans of some selected microbicides acting at different stages of the HIV life cycle

Name of the compound/product	Mechanism of action	Outcome of clinical trial
Nonoxynol-9 (N-9)	Surfactant	Failed to prevent HIV transmission in commercial sex workers; increased HIV transmission in microbicide-treated group
C31G (Savvy)	Surfactant	No protection against HIV infection; adverse events associated with reproductive tract
Sodium lauryl sulfate (invisible condom)	Surfactant	Well tolerated and accepted by women
Pro 2000 (naphthalene sulfonate)	Polyanion	Not effective in preventing HIV infection
Carraguard gel	Polyanion	No difference in HIV incidence between users of Carraguard gel and placebo group
Ushercell (cellulose sulfate)	Polyanion	No beneficial effect in curtailing the risk of HIV transmission
Cellulose acetate phthalate (CAP)	Blocks gp120- and gp41-binding sites	Heavy vaginal discharge, clinical trials stopped
SPL7013	Fusion inhibitor	Safe and well tolerated
Tenofovir (TFV) 1% gel	Reverse-transcriptase inhibitor	CAPRISA 004 trial showed moderate efficacy to prevent sexual transmission of HIV-1; VOICE study failed to show prevention of HIV transmission
Buffer gel	Buffers the pH of vaginal fluid	Failed to prevent HIV infection
Lersivirine (UK-453061)	Nonnucleoside reverse-transcriptase inhibitor	Safe in Phase IIb clinical trial

Abbreviations: CAPRISA, Centre for the AIDS Program of Research in South Africa; VOICE, Vaginal and Oral Interventions to Control the Epidemic.

bicides would prevent or markedly decrease the probability of virus transmission. Detergents or surfactants that destroy the integrity of the viral envelope by solubilizing membrane lipids or by denaturing viral proteins have been categorized under this class. Nonoxynol-9 (N-9), an anionic surfactant initially developed in the 1960s as a spermicide, was the first vaginal microbicide to be studied.[29] It has virucidal activity by disrupting the viral envelope. In a macaque vaginal challenge model, administration of N-9 led to reduction in transmission of simian immunodeficiency virus.[30] However, a Phase III multicentric randomized placebo-controlled trial (COL1492) in commercial sex workers showed that N-9 failed to prevent HIV transmission.[31] In fact, the transmission rate was marginally higher in the N-9 group compared to placebo control group. This may have been due to the development of lesions in the female reproductive tract as a consequence of its use.[32]

C31G (Savvy; Cellegy Pharmaceutical, Quakertown, PA, USA), an equimolar mixture of two surface-active amphoteric agents (cetyl betaine and myristamine oxide) buffered with citric acid, has shown in vitro safety and broad-spectrum activity against different bacteria and viruses, including *C. trachomatis*, HSV, and HIV.[33,34] However, Phase III clinical studies in three countries revealed that C31G failed to demonstrate any protection against HIV-1 transmission.[35,36] Further, its safety was a concern, as several adverse events associated with reproductive tract were reported.[35,36] Sodium lauryl sulfate (Invisible Condom; Université Laval, Quebec, Canada), another surfactant has been shown to disrupt both nonenveloped and enveloped viruses.[37] A randomized, double blind, placebo-controlled Phase II study in Cameroonian women revealed that the Invisible Condom gel formulation was well tolerated and acceptable.[38] Further phases of clinical development of Invisible Condom as a potential microbicide to prevent sexual transmission of HIV are awaited. Following the disappointment of microbicides based on nonspecific surfactant, attempts have been made to develop microbicides incorporating compounds that may interfere with HIV binding, fusion, or entry into the host cells and their subsequent replication.

Inhibition of virus binding, fusion, or entry to the susceptible host cell

Another broad class of microbicide agents are the fusion or entry inhibitors that block either attachment of HIV-1 to the host cells, the fusion of virus and host cell membranes, or the entry of HIV-1 into the host cells. Through their negative charge, a variety of anionic polymers inhibit the HIV adsorption and fusion process, and hence further infection. Pro 2000 (naphthalene sulfonate; Endo Health Solutions, Malvern, PA, USA) is a sulfonated polymer that interacts not only with viral gp120 but also with CD4 and CXCR4 receptors on the cell surface, and hence interferes with virus attachment or fusion with $CD4^+$ T cells.[39] It possesses in vitro activity against both X4 and R5 strains of HIV, *C. trachomatis*, *N. gonorrhoeae*, and HSV.[40] However, the MDP-301 trials demonstrated conclusively that Pro 2000 was not effective in preventing HIV infection.[41] The Population Council (www.popcouncil.org) has developed Carraguard™, a sulfated polysaccharide formulation, which is basically derived from red seaweed (*Gigartina skottsbergii*).[42] It blocks HIV-1 infection of cervical epithelial cells and trafficking of HIV-infected macrophages from the vagina to lymph nodes by binding to the HIV-1 envelope.[43] A large study in South Africa sponsored by the Population Council showed that the Carraguard gel was safe. Further Phase III trials revealed no difference in HIV incidence between users of Carraguard gel and placebo groups.[44] Another polyanion, Ushercell (cellulose sulfate; Polydex Pharmaceuticals, Toronto, ON, Canada), a contraceptive product possessing anti-HIV activity by binding to the V3 loop of gp120 of the HIV-1 envelope can inhibit the entry of both CXCR4 and CCR5-tropic virus.[45] However, different clinical trials indicated that it has no beneficial effect in curtailing the risk of HIV transmission and its use may increase the risk of HIV infection, possibly owing to toxicity of the active ingredient or the hyperosmolar gel vehicle (iso-osmolar placebo).[46,47]

Cellulose acetate phthalate (CAP) blocks gp120- and gp41-binding sites and has shown virucidal activity against HIV-1, HSV-1, and HSV-2.[48] CAP blocks infection of both cell-free and cell-associated HIV as well as blocks CXCR4 and CCR5-tropic virus types in tissue explants.[49] Its preclinical evaluation to date has shown neither any increase in the production of proinflammatory mediators during or after exposure, nor did it modify the epithelial resistance to leukocytes.[50] The micronized form of CAP (~1 μm diameter) leads to disintegration and loss of infectivity of HIV-1, and its lack of systemic absorption increases its bioavailability to the topical surface.[51] However, due to heavy vaginal discharge in all the recipients of the CAP-based microbicide, the clinical trials were halted.[52]

The CCR5 inhibitor PSC-RANTES (recombinant chemokine analogs), exhibits in vitro antiviral activity against most of the HIV clades and inhibits HIV-1 infection of Langerhans cells.[53] CCR5 inhibitors fully protect against simian-human immunodeficiency virus (SHIV) infection

in the rhesus vaginal challenge model and are amenable to low-cost production, representing promising new additions to the microbicide pipeline.[54] TAK-779, a nonpeptide compound, binds specifically to the CCR5 coreceptor, and thereby selectively inhibits R5 HIV-1 entry and replication in peripheral blood mononuclear cells.[55] CMPD167, a cyclopentane-based compound, has been recently shown to protect macaques from vaginal challenge of CCR5-using SHIV162P3 and act synergistically or additively with other cell-entry inhibitors.[56] Maraviroc, a small-molecule drug that binds the CCR5 coreceptor and impedes HIV-1 entry into cells, has also been evaluated as a vaginal microbicide, and provided a dose-dependent protection against CCR5-using virus in rhesus macaques.[57] The bicyclam AMD3100 binds selectively to the CXCR4 coreceptor and inhibits entry of T-tropic HIV-1 and HIV-2.[58] TAK-779 and AMP3100, which block CCR5 and CXCR4 coreceptors, respectively, may provide incomplete protection, as infection by migratory dendritic cells may still take place. However, inclusion of monoclonal antibody (mAb) b12 and CD4-immunoglobulin G_2, both of which target gp120, reduced infection of T cells and migratory dendritic cells by more than 95% in activated cervical explant tissues.[59,60] Hence, simultaneously blocking the pathways that lead to localized infection as well as viral dissemination represents better prevention from HIV-1 infection.

Carbohydrate-binding agents (CBAs) that bind to the HIV glycoprotein (gp120), facilitate the neutralization of a broad variety of HIV clades, including HIV-2 strains.[61] Examples of such CBAs are cyanobacterial cyanovirin-N (CV-N) purified from *Nostoc ellipsosporum* and several other plant lectins, including BanLec isolated from *Musa acuminata* (banana).[62,63] CV-N demonstrated potent in vitro activity in the low nanomolar range against free as well as cell-associated HIV-1.[64] Additionally, it also inhibited infection of ectocervical explants by HIV-1 as well as its dissemination by tissue-migratory cells.[64] Evaluation of CV-N gel (either 1% or 2%) as a topical rectal microbicide in male macaques (*Macaca fascicularis*) showed complete protection when challenged with SHIV89.6P.[65] Further, CV-N gel also showed protection in female macaques when challenged with SHIV89.6P, suggesting that it may be a suitable candidate for development of both rectal and vaginal microbicides.[66] Further, human vaginal commensal bacteria like *Lactobacillus jensenii* have been engineered to produce CV-N, and potent anti-HIV activity was exhibited by *Lactobacillus*-derived CV-N.[67] *L. jensenii* bacteria have also been engineered to secrete the anti-HIV-1 chemokine RANTES as well as C1C5 RANTES as a proof of concept for the use of *L. jensenii*-produced C1C5 RANTES to block HIV-1 infection of $CD4^+$ T cells and macrophages, hence moving towards the development of a live anti-HIV-1 microbicide.[68] Since most cells of human origin have glycoproteins expressed on their surface, highly selective CBA should be designed, and side effects of CBA should be evaluated carefully.[69]

Interaction with either CCR5 or CXCR4 coreceptors triggers a rearrangement of the transmembrane subunit of the envelope glycoprotein gp41, which leads to fusion between the virus and cell membrane, and hence inhibiting gp41-mediated virus–cell fusion is also a promising approach. A proof of concept for this approach has been established with the use of enfuvirtide (T-20 peptide), which blocks virus entry at the stage of HIV-envelope fusion with the cell membrane by targeting gp41.[70] It was the first antiretroviral agent to act by inhibiting the fusion of HIV-1 with $CD4^+$ T cells to be approved by the US FDA, in 2003. It possesses potent antiviral activity, but has two critical drawbacks: high cost of production and short in vivo half-life.[71] C52L, a peptide that also inhibits gp41-mediated virus–cell fusion, is another potent and broad inhibitor of viral infection that remains fully active against T-20-resistant HIV-1.[72]

Dendrimers are highly branched macromolecules synthesized from a polyfunctional core, with interior branches and terminal surface groups adapted to specific targets. The first dendrimer formulated and tested as a microbicide gel clinically was SPL7013 (VivaGel; Starpharma, Melbourne, Australia). SPL7013, a lysine-based dendrimer with naphthalene disulfonic acid surface groups, was optimized and found potent against HIV and HSV.[73] In a Phase I clinical trial, it was found to be safe and well tolerated in healthy women, with no evidence of systemic toxicity or absorption.[74]

Neutralizing mAbs to gp120 (surface glycoprotein of HIV envelope) and gp41 (the fusion transmembrane glycoprotein) have broad neutralizing activity against primary HIV-1 isolates. Moreover, researchers have recently shown that a combination of different mAbs (b12, 2G12, 2F5, and 4E10) showed neutralization synergy.[75]

Inhibition of HIV replication in the host cells

Once in the intracellular environment, HIV infection can only be stopped through inhibition of the virus-encoded RT or integrase. TDF is one of the important compounds being used in HAART drug regimens. TFV 1% gel either alone or in combination with emtricitabine (5% gel) showed its efficacy to prevent sexual transmission of SHIV in pigtailed

macaques.[76,77] After careful pharmacokinetic and safety studies, TFV 1% gel was evaluated in Phase IIb studies in women for its efficacy to prevent sexual transmission of HIV.[78] The Centre for the AIDS Program of Research in South Africa (CAPRISA) 004 study for the first time indicated that PrEP with TFV was successful in prevention against HIV infection.[79] The dosing regimen comprised using gel within 12 hours before coitus and a second dose as soon as possible after coitus but not exceeding 24 hours of the first dose. The success of this study has buoyed the microbicide field, providing the first proof of principle that vaginal microbicide gels can successfully function in a clinical trial setting to reduce the rate of HIV transmission. The TFV gel users were 39% less likely to become infected with HIV than other women, who received a placebo gel. TFV gel also reduced the rate of new genital herpes infections.[80]

The Microbicide Trials Network (MTN) conducted another Phase IIb safety and efficacy trial – the Vaginal and Oral Interventions to Control the Epidemic (VOICE) study – that tested two different HIV-prevention methods, oral PrEP (Viread and Truvada®) and a vaginal microbicide gel (1% TFV), with both pills and gel used daily regardless of sexual activity. Both the oral Viread and Truvada arms failed to show any effective decrease in HIV transmission. Further, HIV incidence was almost identical in women using TFV gel and the ones using placebo gel. As a result, both the TFV-gel and placebo-gel arms have now been stopped. The reasons for the differential efficacy of TFV 1% gel to prevent HIV transmission in the CAPRISA 004 and VOICE studies are not clear. One of the possible reasons may be the adherence towards usage of microbicides as advised.[81] Prior to the announcement of VOICE results, another Phase III efficacy trial of TFV 1% gel was initiated in South Africa – Follow-on African Consortium for Tenofovir Studies (FACTS) 001 – using the same dosing regimen as in the CAPRISA 004 trial. The study is likely to be completed in 2014 (Table 2).

HIV-1-specific NNRTIs compared to NRTIs have the advantage of a very high therapeutic index and acting directly (without metabolization) against the virus replication. Two NNRTI-based microbicides, TMC120 (dapivirine) and UC781, are the most advanced in clinical trials as potential topical microbicides, and usually require at least two mutations before viral resistance occurs.[82,83] These small molecules with low solubility in water or physiological fluids have the potential to form a long-lasting "depot" at sites susceptible to cervicovaginal HIV infection. This could allow application of the microbicide well before sexual intercourse.[84] However, the extremely poor water solubility of UC781 leads to a great challenge for its formulation development. A beta-cyclodextran-based drug-delivery system is being developed to enhance the aqueous solubility of UC781.[85] Recently, an ongoing, multicenter, randomized, double-blind, Phase IIb clinical trial concluded that lersivirine (UK-453,061), a next generation NNRTI, is safe and displayed comparable efficacy to efavirenz in the treatment of naïve HIV-1-infected patients.[86]

Among the integrase inhibitors, raltegravir was the first to be approved by the FDA, in 2007, to overcome the problem of multidrug resistance in AIDS patients. In August 2012, elvitegravir was also approved by the FDA as the second integrase inhibitor.[87] Subsequently, Stribild®, a single daily tablet comprising elvitegravir, cobicistat (pharmaco-enhancing agent), emtricitabine (NRTI), and TDF (NRTI), was formulated by Gilead Sciences for treatment of naïve HIV-infected human

Table 2 Microbicides in the pipeline undergoing Phase III clinical trial for prevention of sexual transmission of HIV-1

Nature of formulation	Mode of action	Clinical trial
Vaginal ring with a combination of dapivirine/maraviroc	Nonnucleotide reverse-transcriptase inhibitor + entry inhibitor	MTN 013/IPM 26
Vaginal ring with dapivirine	Nonnucleotide reverse-transcriptase inhibitor	ASPIRE (MTN 20): to recruit 3,500 women in several sub-Saharan countries, completion expected in 2015 Ring Study (IPM 27): to recruit 1,650 women, expected completion in 2015
1% tenofovir vaginal gel	Nucleotide reverse-transcriptase inhibitor	CAPRISA 008 FACTS 001: to recruit 2,900 women, results expected in 2014
1% tenofovir rectal formulation	Nucleotide reverse-transcriptase inhibitor	MTN 017
Stribild – a coformulation of elvitegravir/cobicistat/emtricitabine/tenofovir disk	Integrase inhibitor + pharmacokinetic enhancer + nucleotide reverse-transcriptase inhibitor	Approved by FDA[123]
VivaGel (SPL-7013)	Virucidal, blocks viral entry	Start of antiviral efficacy of VivaGel ahead of Phase III studies[124]

Abbreviations: MTN, Microbicide Trials Network; ASPIRE, A Study to Prevent Infection with a Ring for Extended Use; CAPRISA, Centre for the AIDS Program of Research in South Africa; FACTS, Follow-on African Consortium for Tenofovir Studies; IPM, International Partnership for Microbicides.

subjects.[88] A Phase III trial revealed that Stribild is effective in decreasing the viral load (HIV-1 RNA < 50 copies/mL blood) in naïve HIV-infected subjects.[89]

Maintenance or enhancement of the vaginal defense

The other broad class of microbicides under development is vaginal milieu protectors that work to maintain, restore, or enhance the natural protective mechanisms within the vagina. The pH in cervicovaginal fluid is acidic (3.5–4.5), and is important for innate immunity against STI-causing pathogens, including HIV. The alkaline nature of semen (pH 7.1–8.0), after coming in contact with the vagina, leads to an increase in the pH, thereby diminishing the natural defense mechanisms. Hence, the compounds that preserve the acidic pH in the vaginal environment, thereby increasing the instability of the virus particles, are the main compounds in this category. To re-acidify the vaginal environment, *Lactobacillus* suppositories or "probiotics" that maintain the acidic pH, primarily due to the production of lactic acid and H_2O_2, can be used. A pH between 4.0 and 5.8 has been shown to inactivate HIV. Colonization of exogenous lactobacilli has been shown to correlate with decreased HIV proliferation.[20,90]

A polyacrylic acid, Carbopol® 974P (BufferGel®; ReProtect, Baltimore, MD, USA), which buffers twice its volume of semen to a pH of 5.0 or less, has been shown to be spermicidal and virucidal to HIV, HSV, *C. trachomatis*, and human papillomavirus. However, during clinical trials, BufferGel was found to have no effect on preventing HIV infection.[91] Acidform (Amphora; Evofem, San Diego, CA, USA) is currently approved as a sexual lubricant gel. Its acid-buffering and bioadhesive properties make it a suitable candidate for microbicide development. A Phase I study revealed that Acidform was well tolerated when used alone, but produced vaginal irritation when combined with N-9.[92] Naturally occurring acidic compounds, such as lime juice, have also been found to be effective against HIV infection, but clinical trials of formulations based on lime juice have shown toxicity.[93]

Use of microbicides: acceptability and adherence

Acceptability studies in a range of countries, such as Brazil, India, South Africa, Thailand, the US, and Zimbabwe, have revealed that women by and large show a positive attitude towards the use of microbicides for prevention of sexually transmitted HIV infection. Further, men are also supportive of the idea of use of microbicide.[94] Vaginal microbicides have been found to be acceptable to adolescent girls, women, and heterosexual men, and rectal microbicides were acceptable to men who have sex with men.[95] The biggest advantage of microbicides is their ease of use, hence providing privacy. Consumer product-preference studies conducted on 526 sexually active women in Burkina Faso, Tanzania, and Zambia revealed that 80% of women liked using all three forms of products, namely vaginal tablets, film, and soft-gel capsules.[96] However, compared to vaginal tablets, film and soft-gel capsules were preferred, and was not associated with age of the participants, socioeconomic status, or marital status.[96] A Phase II, randomized, double-blind, placebo-controlled trial in Pune, India of a vaginal polyherbal tablet microbicide used in a coitally dependent manner revealed that 70.5% of participants showed 100% adherence.[97] Undesirable sexual experience and odor of the product were major barriers to its adherence. In another study done in couples in Mexico, it was suggested that use of microbicides by the female partner could imply mistrust/infidelity in the mind of the male partner within their intimate relationship.[98]

Consideration of microbicides for prevention of HIV transmission should take into account intimate relationship dynamics, which may be a potential barrier to their acceptability and adherence; therefore, the involvement of male partners and promoting risk-communication skills are important for better compliance in the use of microbicides.[98] One of the reasons for low adherence in women of PrEP vaginal microbicides may be the perception that they do not see themselves at risk of HIV infection. The use of topical PrEP vaginal microbicide in a coitally dependent manner may be easier for women to adhere to compared to coitally independent once-daily dosing. To improve acceptability and adherence of topical PrEP microbicide, either long-acting microbicides or MPTs have been proposed.

Long-acting microbicide

Intravaginal rings (IVRs) incorporating antiretroviral drugs have been designed with the premise that the drug will be slowly released over a long period of time, thereby increasing compliance/adherence.[99,100] An IVR for contraception, namely NuvaRing (Merck, Whitehouse Station, NJ, USA), has been well accepted by women.[101] The majority of the participants were willing to use IVRs for HIV-1 prevention in a study from Kenya involving men and at-risk women. However, the risk of its covert use by women and discovery by the male partner may have an impact on its use.[102] IVRs based on hydrophilic polyurethanes have been developed for long-term release (over 90 days) of polar drugs such as TFV

or TDF.[103,104] An IVR based on DPV, a potent NNRTI, has also been designed.[105] An IVR incorporating DPV is currently undergoing two different Phase III clinical trials: ASPIRE (A Study to Prevent Infection with a Ring for Extended Use), conducted by the MTN in several sub-Saharan countries, and the Ring Study (IPM 027), the outcome of which will be known in 2015. Additionally, IVR incorporating DPV and the CCR5 inhibitor maraviroc has also been formulated, which is undergoing Phase I safety and pharmacokinetic studies (MTN 013).[106]

Multipurpose technologies

To enhance the acceptability and adherence of vaginal/rectal microbicides, MPTs are being developed that aim simultaneously to inhibit HIV-1 infections and other STIs. In this direction, the Population Council is developing MPTs that may be effective against HIV-1, human papilloma virus, and HSV-2 using a combination of MIV-150, carrageenan, and zinc acetate.[107,108] Various combinations of MIV-150, carrageenan, and zinc acetate have shown efficacy in preventing both vaginal and rectal transmission of RT-SHIV and HSV-2.[109,110] Further, MPTs that are capable of preventing sexual transmission of HIV-1 and also have contraceptive efficacy are being developed, and will have greater acceptability among women.[111,112]

Challenges in microbicide development

Several microbicide clinical trials based on the successful in vitro anti-HIV efficacy of the microbicide candidates, however, have failed to demonstrate in vivo efficacy, presenting (except the CAPRISA 004 trial) the discrepancies between the in vitro and in vivo data. A reevaluation of the current microbicide-development paradigm and a renewed approach for preclinical testing systems that can predict negative outcomes of microbicide clinical trials is necessary. There are at least two important issues: (1) the microbicide should not have any effect on vaginal mucosa, and (2) the microbicide should not generate any proinflammatory response. To determine preclinical efficacy as well as safety of the candidate topical microbicides, human cervical and colorectal explant cultures have been developed.[113,114] Further, a three-dimensional in vitro human vaginal epithelial cell model has been developed that mimics human stratified squamous epithelium with microvilli, tight junctions, microfolds, and mucus.[115] Using this model, N-9 treatment led to an increase in tumor necrosis factor-associated apoptosis as well as biomarkers of cervicovaginal inflammation.[115] There is a need to develop additional cell-based and explant-based models for discovering new biomarkers of cervicovaginal inflammation for assessment of microbicide safety before clinical evaluation can be initiated.[116]

For testing the safety and efficacy of the microbicide candidates, the absence of a validated animal model is a major obstacle. The animal models used currently (the mouse HSV-2 model, the rabbit vaginal irritation index, and the macaque SIV model) have substantial differences from humans. However, recent advances include the development of humanized murine models, which allow better vaginal and rectal HIV efficacy-challenge studies.[117] To evaluate proinflammatory response and disruption of mucosal integrity by a candidate microbicide that may facilitate transepithelial viral penetration as well as replication, a Th17-based mouse model has been developed for preclinical assessment.[118] The sheep cervicovaginal tract, which comprises stratified squamous epithelium similar to humans supplemented with optical coherence tomography, may also constitute one of the large animal models before testing the microbicides in either nonhuman primates or humans.[119] A lack of markers for the biological activity of the microbicides as well as correlates of protection also poses a big hindrance. Hence, the development of surrogate markers of the efficacy of microbicides will help in moving forward in this area.

A candidate microbicide's efficacy is underestimated if the placebo itself provides a certain degree of protection against HIV-1 infection. On the other hand, a placebo leading to epithelial toxicity that increases susceptibility towards HIV-1 infection may result in false efficacy to prevent HIV-1 infection of the microbicide undergoing clinical trial. Hence, the development of an inert universal placebo that is stable, safe, and acceptable is also an important issue while conducting clinical trials of microbicides. A hydroxyethyl cellulose placebo formulation has been developed that appeared to be safe and acceptable when used twice daily for 14 days.[120,121]

The VOICE clinical trials of PrEP of vaginal 1% TFV gel used once daily regardless of sexual activity revealed that adherence to the use of microbicide can also be an important issue. It is imperative to develop new quantitative measures to monitor adherence. Used applicators can be tested by staining methods or by ultraviolet light to know if they have come in contact with the vaginal surface.[122] The participation of multiple countries in microbicide clinical trials has its own unique challenges. Both ethical and regulatory approvals not only in the country of the trial sponsor but also in each country where the trial will be held need to be granted. Recruiting the

required number of women and ensuring their stay in the trial is also challenging; maintaining the tracing mechanisms and databases to keep track of the participants is also one of the major challenges. A difficult but important issue in HIV trials is the care of people who seroconvert during the study. The formation of a coordination body that would help facilitate harmonizing across a number of areas, including protocol design, monitoring, and decision-making for next-generation candidate microbicides, has been convened by the Bill and Melinda Gates Foundation and the Alliance for Microbicide Development. Table 2 summarizes the microbicides that are undergoing thorough Phase III clinical trials including stribild and VivaGel (SPL-7013).[123,124] The outcome of these trials is eagerly awaited, and will facilitate the next generation of microbicides.

New information and essential lessons have emerged in this field as a result of each of these challenges. These have also resulted in a momentous increase in microbicide-development efforts focusing on compounds with highly potent and HIV-specific mechanisms of action, combination products, novel formulations, and carefully designed pharmacokinetic and pharmacodynamic evaluations, all of which are reasons for renewed confidence that a safe and effective microbicide is achievable. The development of MPTs that can simultaneously address the issue of sexual transmission of HIV-1 and contraception may be acceptable with increased adherence.

Conclusion

To prevent sexual transmission of HIV-1, topical microbicides have been proposed. In spite of good anti-HIV-1 activity in various in vitro assays and challenge experiments in macaques, clinical trials of microbicides based on surfactants and polyanions failed to inhibit sexual transmission of HIV-1. Unfortunately, microbicides based on N-9 enhanced the susceptibility of sexual transmission of HIV-1. Microbicides based on antiretroviral drugs such as TFV (1% gel) showed moderate efficacy in the CAPRISA 004 clinical trial, but failed to inhibit sexual transmission of HIV-1 in the VOICE Phase IIb clinical trial. Though the reasons for these contradictory observations are not clear, pharmacodynamic studies suggest that poor adherence may be responsible for the lack of efficacy in the VOICE study. The ongoing efficacy trials of microbicides based on antiretroviral drugs, namely TFV and dapivirine, will help in establishing their utility or otherwise in preventing sexual transmission of HIV-1. The next generation of microbicides will comprise MPTs, which are effective not only against HIV-1 but also against other STIs. Development of long-acting (up to 90 days) delivery systems for the microbicides will act as an impetus for the development of this field.

Acknowledgments

We would like to acknowledge financial support from the Department of Biotechnology and the Indian Council of Medical Research, Government of India. The authors thank Ms Shruti Upadhyay for help in the preparation of the manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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