Microbicides for the Prevention of HIV Infection

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Abstract

Preventive interventions beyond behavior modification and physical barrier methods are needed to alter the natural course of the global HIV epidemic. Women need HIV-prevention tools that they can control to safeguard their health and that of their families and communities. This review focuses on one of the most promising prevention tools currently under development, namely microbicides. Microbicides are vaginally applied chemical products designed to prevent the sexual transmission of HIV. Microbicides work in one of the following ways: they assist the natural defences in the vagina to inactivate the virus, they inactivate the virus while it is in the vagina, they prevent the virus from attaching to and fusing with the host cells in the genital tract, or they prevent the virus from replicating if it should succeed in entering genital tract host cells. The potential public health benefits of microbicides will be enormous and even a low efficacy microbicde could prevent millions of HIV infections among women, men and children in the developing world.

Introduction

Worldwide there are an estimated 42 million people living with HIV/AIDS and more than 20 million people have already died of AIDS[1]. More than 95% of all new HIV infections are in developing countries and about 87% were acquired through heterosexual transmission. Further, of the 4.8 million new infections and 2.9 million AIDS deaths in 2003, over 85% were in persons who acquired HIV infection heterosexually. Heterosexual transmission of HIV is an important mechanism of transmission beyond Sub-Saharan Africa to most countries around the world and particularly in south and south-east Asia where a quarter of the new infections in 2003 occurred. Not only is heterosexual transmission important in driving the current major epidemic in Sub-Saharan Africa but is also a major factor driving the emerging epidemics in India and China.

Heterosexual transmission is influenced by several key epidemiological factors such as age, gender, mobility and the presence of other sexually transmitted infections. A striking characteristic of heterosexual transmission in sub-Saharan Africa is the disproportionate burden of HIV infection in women compared to men[2]. Biologically, women are two to four times more vulnerable than men to acquire sexually transmitted HIV infection[3]. Their vulnerability increases due to their lack of economic and social power in many societies, where women often cannot control sexual encounters or insist on protective measures such as condoms or mutual monogamy. In the context of mass labor migration, a feature of the southern African economy, many women who get infected with HIV have only one partner, their husbands, who have other partners or use the services of sex workers when they are away from home for long periods.

In the countries worst affected by the HIV epidemic, women acquire HIV infection at a younger age, at least 5–10 years earlier than men. In these settings, boys under 20 years have a low prevalence of HIV while teenage girls are already close to peak prevalence[4-5]. In most African countries where heterosexual transmission is the major mode of transmission, a key factor in the epidemic is the high incidence rates in young women between the ages of 14 and 24 years as a result of sexual coupling with older men[6]. This epidemiological characteristic of the southern African HIV epidemic is important.
not only for the risk to women but also because young women have the highest pregnancy rates and hence the additional burden of mother-to-child transmission of HIV.

Notwithstanding the greater vulnerability of women, current options to reduce transmission and acquisition of HIV infection remain limited for women. There is a clear need for new technologies to prevent the sexual transmission of HIV in women. Correct and consistent use of male condoms has been shown to prevent HIV transmission[12], but women often are unable to negotiate use of condoms by their male partners[13-15]. The female condom has been marketed as an alternative barrier method, but this device is relatively costly and requires a certain level of skill, and, importantly, it also requires acceptance by the male partner. There is thus a clear need for new technologies that women can use and control, to reduce their risk of sexual acquisition of HIV.

Topical microbicides, which will be self administered prophylactic agents that could be applied to the vagina or rectum in various formulations, is one of the most promising technologies under development to reduce their risk of sexual acquisition of HIV. This review describes how microbicides work, provides a brief history of the development of microbicides, discusses the current state of clinical development and highlights some of the obstacles to the development of microbicides.

Mechanisms of action of microbicides

The principal target of microbicides is to reduce male-to-female HIV transmission though, they could potentially, prevent both male-to-female and female-to-male transmission. Some microbicides are also contraceptive and prevent transmission of other sexually transmitted pathogens[13-16]. Several currently marketed chemical spermicides, which have shown some activity against HIV and STI pathogens in vitro, have been evaluated as topical microbicides.

In order for a microbicide to prevent HIV infection it must be able to do one or more of the following[17]:

• assist the natural defences in the vagina to inactivate the virus
• inactivate the virus while it is in the vagina
• prevent the virus from attaching to and fusing with the host cells in the genital tract
• prevent the virus from replicating if it should succeed in entering host cells[13].

Assist the natural defences in the vagina to inactivate the virus

A microbicide could be used to supplement or enhance the natural immune defences of the vagina. Combinations of microbiological, chemical, and physical barriers act to protect the vagina naturally from infection. Lactobacilli, for example, occur naturally in the vagina and release a variety of anti-microbial compounds such as lactic acid, hydrogen peroxide, bacteriocins, and biosurfactants. A disruption of the natural balance of the vaginal ecosystem enhances the risk of HIV infection. Furthermore, the squamous epithelial cells lining the vagina are the first line of defense against pathogens. Epithelial cells are capable of synthesizing anti-microbial peptides that inactivate or recruit key immune cells. In addition they stimulate the secretion of cytokines which support the survival of lymphocytes. Antibodies such as IgA and IgG are also abundant in the secretions in the vagina.

The vagina is usually maintained at a low pH of about 4. This low pH is achieved through the secretion of lactic acid by the lactobacilli that colonise the vagina. These lactobacilli are sometimes destroyed by intercurrent vaginal infections e.g. bacterial vaginosis. Microbicides have been developed to maintain the colonization of the vagina by lactobacilli or to recolonise the vagina with lactobacilli when these commensal organisms have been adversely affected, for example, by the use of antibiotics or genital tract infections.

The naturally low pH of the vagina is affected substantially by semen which is alkaline. This effect of semen on the vaginal pH results in the loss of this barrier to pathogens. Microbicides have been developed to assist the vagina maintain a low pH even in the presence of semen. One such microbicide, known as Buffergel, acts as a pH buffer. This buffering capacity enables the vagina to maintain a low pH by buffering the alkali in semen.
Inactivate the virus while it is in the vagina

The “first generation” microbicide candidates were surfactants. These products had detergent-like properties to disrupt cell membranes or, in some instances, changed the cell’s membrane structure to make it more porous and thereby more liable to disruption. These products impacted on all cells; host, commensal and pathogen. Hence these products have a wide spectrum of activity against several microbes, spermatozoa and cell membranes. The most notable of these products was nonoxynol-9 (N-9)[18-20], which has been widely available as a spermicide for many years. N-9 has been tested in various doses and formulations, but has shown to be ineffective in preventing HIV and possibly harmful[21-22].

Prevent the virus from attaching to and fusing with the host cells in the genital tract

The “second generation” microbicides are the polymers, which have a more limited spectrum of activity. Polymers act against viruses, predominantly by interfering with attachment to host cells. The envelope of HIV, particularly the gp41 component, which enables fusion with the cell membrane is a critical target for a potentially successful microbicide. Polymers act by blocking viral entry into susceptible cells by blocking CD4 attachment and/or co-receptor attachment. Several efficacy trials with different polymers such as PRO 2000, Carraguard, Cellulose Sulfate and Dextrin-2-Sulfate, are already underway.

Several other entry inhibitors are currently in pre-clinical stages of development among animal models and could soon enter the clinical trial pipeline in humans. These include

• the use of broadly neutralizing monoclonal antibodies, which have also shown promise in preventing simian-human immunodeficiency (SHIV) infection in macaques[23]; and

• the use of genetically engineered naturally occurring bacteria which is capable of protecting against HIV infection by secreting compounds that interfere with viral attachment, fusion, entry or replication [24-25].

A critical step in the HIV life cycle is the binding of HIV to chemokine co-receptors such as CCR5 or CXCR4 on the cell surface. Thus, molecules that are capable of attaching to the co-receptors thereby preventing them from attaching to the cell surface may also be potentially effective vaginal microbicides. This is the mechanism of action of “fourth generation” microbicides, for example, the co-receptor blocker, PSC-RANTES, which has been shown to provide protection in vaginal challenge studies in rhesus macaques without causing detectable toxicity or histological changes[26]. However, this requires high doses of PSC-RANTES, which are expensive using current technology. In addition, resistant isolate to some CCR5 inhibitors have already been described[27]. The most valuable type of inhibitor microbicides will be those that are capable of acting against diverse strains of HIV. One such molecule, C34, a 34 residue peptide of gp 41, is a promising candidate and it is a broad spectrum, highly potent inhibitor of ENV-mediated cell fusion over the entire panel of HIV-1 and SIV envelope glycoproteins which suggested that C34 may be a promising therapeutic against diverse or resistant strains of HIV-1 [28].

Inhibit a virus from replicating

Several antiretroviral drugs, which were originally developed as HIV therapeutics, are now being tested as microbicides because of their capability of inhibiting the replication process - these are the “third generation” microbicides. These antiretroviral agents e.g. tenofovir, UC781 and TMC120, act locally in the reproductive tract mucosa at specific steps in the HIV replication cycle and therefore have a narrow spectrum of activity against HIV only. One such product, Tenofovir gel (PMPA) developed by Gilead Sciences, is entering Phase II trials and is likely to reach efficacy trials within the next three years [29-33]. However, since viral escape from these types of inhibitors is expected, a combined strategy that targets multiple components of HIV infection, will likely be necessary to maximize the effectiveness of a successful microbicide.

An overview providing examples of microbicides in each of these categories is provided in Table 1.
Microbicides

Brief historical overview of microbicide development

Microbicide research targeting the development of a chemical product for vaginal use to prevent HIV infection began in earnest in the early 1990s at about the time of the publication of a seminal paper in the American Journal of Public Health by Dr Zena Stein on “HIV prevention: the need for methods women can use” [34]. The concept of a microbicide for HIV prevention gained substantial ground within the research community with the first commitment of funding from the US National Institutes of Health in 1993. Since then the microbicide field has grown substantially.

The microbicide research and development pipeline in the mid-1990s was primarily focused on a large number of surface active agents. One of the first trials to assess the effectiveness of a microbicide against HIV infection was a trial of the N-9 sponge in sex workers in Kenya, which showed no protection but an increase in genital tract lesions[35]. These controversial findings highlighted the importance of the dosing strategy and the vehicle of delivery since the sponge in the Kenyan trial contained a relatively high dose of N-9 and the sponge did not lead to adequate spread of the product in the vagina. A subsequent trial of N-9 film in Cameroon showed no harm or benefit and once again raised questions on the spread of the product in the vagina[22]. After the release of the COL-1492 results at the International AIDS Conference in Durban in 2000, the development of this generation of microbicides rapidly fell into disfavour. However, not all surfactants were excluded from the development pipeline and three agents in this class are still in clinical testing.

Clinical trials of polymer products, the second generation microbicides, began in humans in the late 1990s and this class of products dominates the late stage portion of the current pipeline, with several products (including PRO2000, Carraguard, and Cellulose Sulfate) being tested in phase IIb or phase III trials.

The antiretroviral agents are starting to enter clinical testing with the number of such products likely to expand in the near future. The future microbicide development pipeline is likely to focus on coreceptor blockers (Figure 1).

Table 1 Categories of microbicides by mechanism of action

<table>
<thead>
<tr>
<th>Category</th>
<th>Action</th>
<th>Microbicide examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffers</td>
<td>Maintenance or mobilisation of normal vaginal defences</td>
<td>Buffergel, Engineered lactobacillus, Hydrogen peroxide/peroxidases</td>
</tr>
<tr>
<td>Surfactants</td>
<td>Destroying surface active pathogens by disrupting membranes</td>
<td>Nonoxynol-9/Octoxynol-9, Benzalkonium chloride, C31G - Savvy, Chlorhexidine zinc gel</td>
</tr>
<tr>
<td>Blockers</td>
<td>Inhibiting pathogen entry into mucosal cells</td>
<td>Carraguard®/PC-515, PRO2000 gel, Emmelle/Dextrin-2-Sulfate</td>
</tr>
<tr>
<td>Co-receptor blockers</td>
<td>Preventing fusion between the membranes of the pathogen and mucosal cells</td>
<td>CCR5 inhibitors, Soluble CD4</td>
</tr>
<tr>
<td>Antiretroviral agents</td>
<td>Inhibiting post-fusion replication (these are often poorly absorbed antiretroviral drugs)</td>
<td>Nucleoside reverse transcriptase inhibitors (e.g., Tenofovir), NRTIs (UC-781), Protease inhibitors (WHI-07)</td>
</tr>
</tbody>
</table>

Note: this table is by no means exhaustive as the microbicide pipeline is continually evolving
Nonoxynol-9 (N-9) is a surfactant that works as a spermicide by disaggregating the lipid membrane of spermatozoa. A phase III trial of nonoxynol-9 (COL-1492), among 892 female sex workers from four African countries, showed that the use of the product was associated with a significant increase in the incidence of HIV infection [20]. This was particularly so for women who used the product more than 3.5 times a day (Table 2).

Microbicides are ultimately intended for prolonged, repeated use and must be shown to be safe with repeated use. The COL-1492 study has shown us that compounds like N-9 that are designed to disintegrate the lipid membrane of a pathogen also act on a broad range of cells and hence, can damage host cell lining of the vagina, possibly facilitating access to HIV to gain entry into the sub-epithelial layer. An alternative plausible explanation for the outcome of the COL-1492 trial is that multiple use of

Table 2  Summary of COL-1492 trial results

<table>
<thead>
<tr>
<th>Sexually Transmitted Infection</th>
<th>Nonoxynol-9</th>
<th>Placebo</th>
</tr>
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<tbody>
<tr>
<td><em>Trichomonas vaginalis</em></td>
<td>14 (4%)</td>
<td>13 (3%)</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>55 (15%)</td>
<td>55 (14%)</td>
</tr>
<tr>
<td><em>Syphylis</em></td>
<td>39 (10%)</td>
<td>47 (12%)</td>
</tr>
<tr>
<td><em>Chlamydia trachomatis</em></td>
<td>18 (5%)</td>
<td>16 (4%)</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>17 (5%)</td>
<td>22 (6%)</td>
</tr>
</tbody>
</table>

HIV outcome

<table>
<thead>
<tr>
<th></th>
<th>Nonoxynol-9</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (women years)</td>
<td>376</td>
<td>389</td>
</tr>
<tr>
<td>Cumulative incidence</td>
<td>59/376 (16%)</td>
<td>45/389 (12%)</td>
</tr>
<tr>
<td>Incidence rate</td>
<td>14.7</td>
<td>10.3</td>
</tr>
<tr>
<td>RR=1.5 (CI:1.0-2.2; p=0.047)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
the placebo gel, which is widely used vaginal lubricant, may be protective. Since the placebo in the COL-1492 trial was not likely to be inert, various alternatives have been proposed to the study design to deal with this problem. One approach has been the inclusion of a no gel arm; however, this approach is severely limited as it is an un-blinded arm and even small differences in safer sex behaviors between the arms may confound any association between the gel and risk of HIV infection. The other has been the development of new generation placebos which have lower levels of preservatives and fewer lubricating characteristics.

**Current state of clinical development of microbicides**

Effective vaginal microbicides will probably be delivered in many forms, such as gels, creams, suppositories, films, sponges and vaginal rings. Many microbicidal products are in various stages of development but testing the efficacy and safety of microbicides involves many thousands of women over several years (Figure 2). If the polymer products are shown to have a protective effect against HIV, an effective microbicide is scientifically possible within the next five years.

Currently, several second generation products like PRO2000, cellulose sulfate and Carraguard®, are in advanced stages of efficacy testing. While some of the trials are large scale trials aiming to show efficacy at such low levels of 30%, most have been designed with a target efficacy of 50%. Microbicides currently in various stages of development are summarized in Table 3.

Importantly, the pipeline of new products is growing rapidly and more than 60 products are progressing from the pre-clinical stage into human safety trials. Among these are antiretroviral agents and co-receptor blockers, which have a high degree of anti-HIV specificity and are therefore more likely to have a better safety profile. This high degree of specificity may also result in these generation products not being contraceptive.

Microbicides for men, in the form of penile wipes[36] with antibacterial and possibly antiviral properties, for use before and after sex, have been developed and are currently being assessed in clinical trials. In Malawi and Kenya these products have been widely accepted among both circumcised and uncircumcised men[37].

![Figure 2: Current Microbicide pipeline with HIV as primary endpoint.](image-url)
Rectal microbicides

The mucosal surfaces in the rectum are vulnerable to physical damage during sex and potentially increases the risk of HIV infection. It is a common misconception that anal intercourse is an exclusively homosexual male practice[38]. Several surveys indicate that heterosexual anal intercourse is far more common than generally acknowledged [39-42]. These surveys indicate that an estimated 10 to 30% of women and their male consorts engage in anal sex with some regularity. There is a possibility that vaginal microbicide products may also be beneficial if used rectally in both men and women. However, there are distinct structural differences between the vagina and rectum and little is known about the necessary rectal mucous membrane coating required to prevent HIV. Clinical trials evaluating the safety and effectiveness of rectal microbicides are underway.

Obstacles to microbicide development

Several obstacles have slowed the development of a safe and effective microbicide.

- *Lack of a validated animal model*: Several animal models are used in pre-clinical microbicide testing e.g. the mouse HSV-2 model, the rabbit vaginal irritation index, and the macaque SIV model. The predominant model being used is the SHIV challenge in macaques, but it is unknown whether this has any relevance to microbicide effects in humans due to the substantial differences in the human and primate vagina e.g. the primate vagina is neutral pH while the human vagina has a low pH. The absence of a validated animal model is a major obstacle as it means that costly and time consuming human studies are required to assess any effect of a microbicide.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Microbicide</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>UC-781</td>
</tr>
<tr>
<td></td>
<td>TMC120</td>
</tr>
<tr>
<td></td>
<td>SPL7013 / Viva gel</td>
</tr>
<tr>
<td></td>
<td>Polystyrene sulfonate</td>
</tr>
<tr>
<td></td>
<td>PMPA - topical formulation</td>
</tr>
<tr>
<td></td>
<td>PMPA - oral formulation</td>
</tr>
<tr>
<td></td>
<td>Acidform</td>
</tr>
<tr>
<td></td>
<td>Lactin vaginal capsule</td>
</tr>
<tr>
<td></td>
<td>Human Monoclonal antibodies</td>
</tr>
<tr>
<td></td>
<td>Cellulose acetate phthalate (CAP)</td>
</tr>
<tr>
<td></td>
<td>Benzalkonium Chloride (BZK)</td>
</tr>
<tr>
<td>I / II</td>
<td>Invisible condom</td>
</tr>
<tr>
<td>II</td>
<td>Praneen polyherbal formulations</td>
</tr>
<tr>
<td></td>
<td>Lactocacillus suppository</td>
</tr>
<tr>
<td></td>
<td>Emmelle (dextrin-2 sulphate)</td>
</tr>
<tr>
<td>II / Ib</td>
<td>PRO2000 and Buffergel</td>
</tr>
<tr>
<td>Ib</td>
<td>PRO2000 and Tenofovir</td>
</tr>
<tr>
<td>II / III</td>
<td>C3IG (SAVY)</td>
</tr>
<tr>
<td></td>
<td>Buffergel (for contraception)</td>
</tr>
<tr>
<td>III</td>
<td>Cellulose sulfate</td>
</tr>
<tr>
<td></td>
<td>Carraguard®</td>
</tr>
<tr>
<td></td>
<td>C3IG (SAVY)</td>
</tr>
<tr>
<td></td>
<td>PRO2000</td>
</tr>
</tbody>
</table>
Microbicides

- **No correlates of protection:** There are currently no markers for the biological activity of microbicides and there are no markers that have been established as correlates of protection. This obstacle presents a major impediment to rapid progress in the field as HIV infection in humans is the key marker of biological activity, safety and efficacy. This means that meaningful studies of safety and efficacy of a microbicide can only be designed with HIV infection as the primary endpoint.

- **Funding:** An estimated $775 million in product development costs are required over the next five years to develop safe and effective microbicide products, but only about $230 million has been committed thus far. A successful product will require extensive and sustained investment in research and development, as well as mechanisms for global access, especially in the developing world.

- **Ethical issues:** To show safety and efficacy, the product must be tested on large numbers of sexually active women. The trials also need to be conducted in populations that are likely to be at high risk of acquiring HIV infection. Clinical trials are therefore often carried out in developing countries that have high levels of infection, especially among sex workers. Counseling on use and provision of condoms as a proven HIV prevention method in addition to the experimental product is an ethical and moral pre-requisite in all HIV prevention trials, including microbicide trials. Under these conditions the trial can only measure whether microbicides improve upon the protection afforded by condom use. Other practical, ethical and scientific challenges include behaviours such as anal sex.

- **Intravaginal substance use:** In many societies throughout the world, women use substances intravaginally for hygiene purposes, medicinal purposes, pleasure, or “tightening” the vagina to give men more pleasure. The use of these products may interfere with the products under study and thereby impact on the results of trials in populations with high levels of intravaginal substance use.

- **Lack of a true placebo:** Since any placebo vehicle (gel, foam, etc) is likely to increase vaginal lubrication, this may lead to a decrease in the risk of HIV infection in the placebo arm. This adds a further complexity to the design of microbicide efficacy trials as this potential beneficial effect of a placebo undermines the trial’s ability to show a beneficial effect.

- **Lack of commitment from big pharmaceutical companies:** Microbicide research and development is being conducted mainly by small biotech companies. None of the major pharmaceutical companies have a substantive microbicide R&D portfolio and none are conducting human microbicide trials at present. Other concerns surrounding the development of microbicide include the potential hazards related to reproductive toxicity, and the increased risk of local toxicity from applying a product repeatedly to the same tissue that may enhance risk of infection.

**Conclusion**

There is a clear and urgent need for a women controlled method to prevent HIV infection. There has been substantial progress in the microbicide development field with several potential products entering phase III trials in the last 12 months.

Acceptability studies among men and women in several countries show that microbicides would be acceptable, and both men and women would be happy to use the product if it was available on the market. The first vaginal microbicides developed are not expected to provide complete protection against HIV and it is unclear if they will be contraceptive as well. However, a microbicide even with 30 to 40% efficacy in preventing HIV infection could have an enormous public health benefit.

The development of a microbicide as a protection mechanism for women remains hopeful and provides real potential to influence the course of the HIV epidemic. Even after a safe and effective vaccine is discovered, vaccines and microbicides will have different but complementary approaches towards a global HIV prevention strategy with the same urgent objective, i.e. abating global HIV pandemic.

**Acknowledgement**

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Microbicides


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**Useful websites**

Alliance for Microbicide development. Microbicide and Research Development Database. www.microbicide.org


The Pipeline Project HIV vaccines in development. chi.ucsf.edu/vaccines