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REVIEW ARTICLE

A HISTORY OF MICROBICIDES AND THEIR CURRENT DEVELOPMENT PIPELINE

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CONTENTS

SUMMARY

The idea for microbicides to prevent the transmission of sexually transmitted infections came from a discovery in the 1980s that over-the-counter spermicides showed antiviral activity in vitro. Since the advent of the HIV epidemic, microbicides have been tested as a possible tool for HIV prevention. The field of HIV microbicides has expanded to include five classes of mechanisms: surfactants, buffering agents, viral entry inhibitors, reverse transcriptase inhibitors and agents with an unknown mechanism. The most successful class to date has been the reverse transcriptase inhibitors, specifically tenofovir gel. Despite numerous setbacks in clinical studies (such as issues of adherence, interference with vaginal defenses and insufficient sample size to determine efficacy), the future of microbicide research is promising, with numerous agents in ongoing clinical studies.

INTRODUCTION

Worldwide, approximately 2-5 million individuals become infected with HIV and another 2 million will die of the disease each year (1). While the incidence has remained level due to intense global prevention campaigns, the prevalence of HIV is increasing (1). This requires continually escalating resources for the prevention of transmission, with a larger potentially infectious population. Since the early days of the HIV epidemic over three decades ago, understanding of viral transmission mechanisms and disease progression has advanced tremendously, although strategies to prevent new infections are only recently showing efficacy. Efforts towards a vaccine have met with limited success (particularly the recent RV144 vaccine trial in Thailand) (2). The use of antiretroviral therapy for pre- and post-exposure prophylaxis has shown modest effectiveness, but the cost is prohibitive for the majority of infected individuals (3). The most effective prevention strategy thus far is the use of treatment as prevention (3, 4), but in order to have substantial impact, HIV surveillance and access to care must be greatly increased. Other highly efficacious interventions include condom use, male circumcision and needle exchange programs (1, 5). However, the fastest-growing group of new infections worldwide occurs among women who acquire the virus through heterosexual transmission (1). Prevention options that enable women to protect themselves are desperately needed, particularly in instances of disparate power relationships where negotiation of condom use is not possible (1, 6-8). Microbicides have become an important step towards creating this type of intervention. In this article, we review the trials and errors in the development of microbicides in the hope that it will provide guidance for moving forward with research in the battle against the HIV epidemic (1).

We begin by providing a brief history of the search for microbicides, defining the development of alternative formulations and vehicles for their administration and their rationales. We then present the types of microbicides in more detail, with specific examples of clinical studies in each category. Finally, we discuss current efforts to find a safe, effective and acceptable microbicide. This review is not intended as an exhaustive compilation of all preclinical and clinical development, but it serves to highlight some key events in the advancements and setbacks of microbicides within the last few decades and to provide guidance for future areas of research.

A brief history of microbicides

The concept of microbicides was put forth before the beginning of the HIV epidemic, as spermicides were found to have anti-infective properties against other sexually transmitted infections (9). The use of microbicides for HIV prevention was first suggested in 1990 by Zena Stein (6). Since that time, researchers and organizations have been working tirelessly on developing an anti-HIV microbicide (7). Microbicides are topical formulations developed for application to the genital and rectal epithelia to prevent or lessen the risk of infection by pathogens (8). They do this by creating chemical, biological or physical barriers, which block or inactivate pathogens at mucosal surfaces (10). These are applied directly to the vaginal or rectal mucosa, generally prior to sexual contact, and can work in multiple ways —disruption of the structure of the HIV virus, interference with

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its ability to infect target cells or blockade of a stage in its life cycle. These different types are discussed in more detail below.

The main advantage of a microbicide over other forms of HIV prevention is the possibility for empowerment of women or men who have sex with men (MSM) who could use a microbicide without their partner's permission and/or knowledge (10). Receptive sexual partners are at much higher risk of HIV infection than penetrative partners (11), and thus the development of either a vaginal or rectal microbicide could benefit both MSMs and heterosexual women. There may be additional benefit in that a microbicide acts locally rather than systemically, potentially avoiding systemic toxicity and the emergence of drug resistance (8, 12, 13).

Microbicides were initially developed to have a broad range of activities and showed in vitro activity against several pathogens besides HIV (9). Gradually, evidence accumulated that these nonspecific microbicides actually damaged the milieu of the vaginal epithelium, which resulted in enhanced rates of sexually transmitted infections. This led to the development of microbicides that promoted or preserved the protection by natural flora and epithelial defenses. Additionally, in response to early microbicide trials, a universal placebo was developed during the HPTN 035 trial (14, 15).

Over the past two decades, attitudes towards microbicides have been appropriately skeptical; while the possibility that a successful microbicide could slow the HIV epidemic gained acceptance due to strong activism in the 1990s (16, 17), the failures of early surfactant trials tarnished their reputation (18, 19). Researchers continued to develop microbicide candidates, ranging from acidifying agents to antiretroviral compounds, but funding was scarce (19, 20). Not until recently has funding increased again as clinical studies have shown more promising results (19).

Microbicide formulations

Microbicides were first developed as gels, some of which were already in use as spermicides and were found to have antiretroviral activity (e.g., nonoxynol 9). However, the library of formulations has burgeoned as acceptability studies have revealed the importance of maintaining consistent use of the product (adherence) in evaluating overall efficacy. Additionally, advances in drug delivery methods have led to the development of new options, such as drug-eluting vaginal rings. Table I lists several of the product options in development, as well as some of their advantages and disadvantages revealed through clinical testing (21). However, individual preferences for types of microbicides have been shown to vary by country due to regional differences in cultural norms and perceptions (22, 23). As such, no single formulation will likely find universal acceptability and multiple formulations should be offered for each effective agent.

Some of the challenges in developing formulations have been identifying products that are not too messy because women participating in clinical studies often compensate by using less of the product, thereby reducing its efficacy. Regional sexual practices must be considered to anticipate whether particular formulations interfere with desired characteristics, such as "wet" versus "dry" sex. Additionally, the ability to use microbicides without the partner's knowledge may

Table I. *Advantages and disadvantages of the microbicide formulations in development.*

Formulation	Advantages	Disadvantages
Topical vaginal gels/creams	• Can be incorporated into lubricants/spermicides • Easy to use with applicators • Immediate efficacy (21) • May enhance sexual pleasure (22)	• Too wet/messy/sticky (22, 24) • Leakage and staining concerns • Low adherence in population studies due to frequent reapplication requirements • Coital dependency (21)
Foams	• Good uniformity of product distribution (21)	• Messy • Problems with dissolvability • Consistently disfavored over other methods (23, 24)
Films	• No leakage, discrete (22) • Preferred over gels, foams, tablets and suppositories (24, 115)	• Hesitancy among users about administration and efficacy (22, 24, 115)
Vaginal rings	• Sustained release allows for weekly or monthly application reducing failures from imperfect adherence • High acceptability among contraception users • Coitally independent (21)	• May be less discrete (21)
Tablets/suppositories/ovules	• Accurate dosing • Easy to use • Good stability • No leakage • Inexpensive to manufacture (21)	• Difficult to establish uniform content, optimal disintegration times, bioadhesion and distribution • Concerns about discharge of product residue (22)

be important in disparate power relationships, although this has generally been shown to be less important in monogamous relationships than initially anticipated. Part of the rationale may be that certain formulations have been reported to enhance sexual pleasure (21, 24). Other challenges in microbicide research have been high pregnancy rates and/or low HIV incidence, limiting assessments of efficacy (9).

MICROBICIDE CLASSES BY MECHANISM

Microbicides have generally been classified into five categories: surfactants/membrane disruptors, vaginal milieu protectors/buffering agents, entry inhibitors/anionic polymers, reverse transcriptase inhibitors and those with unknown mechanisms. These categories are defined, their mechanism explained and examples of each given in the following section. For a more complete list of agents within each category, see Table II.

Table II. *Examples of products within each microbicide category.*

Microbicide category	Examples in clinical study
Surfactants/membrane disruptors	• Nonoxynol 9 (N-9) • Glyminox (C31G; Savvy®) • Sodium dodecyl sulfate (SDS; invisible condom)
Vaginal milieu protectors/ buffering agents	• BufferGel® • Acidform™ (Amphora™) • Recombinant lactic acid bacteria • Genetically engineered probiotics
Viral entry inhibitors	Anionic polymers: • PRO-2000 • PC-515 (Carraguard®) • Ushercell • Cellulose acetate phthalate (CAP) • SPL-7013 (VivaGel®) Chemokine CCR5 receptor antagonists: • PSC-RANTES • CMPD-167 Fusion inhibitors: • Cyanovirin N
Reverse transcriptase inhibitors	NRTIs: • Tenofovir NNRTIs: • Dapivirine (TMC-120) • MC-1220
Unknown mechanism	• Praneem

NRTI, nucleotide reverse transcriptase inhibitor; NNRTI, non-NRTI.

Surfactants/membrane disruptors

The first class of compounds to be developed for use as microbicides were surfactants. They act by nonspecifically disrupting the HIV envelope, thereby directly inactivating the virus. The first microbicide tested in large-scale clinical efficacy studies was the nonionic surfactant nonoxynol 9 (N-9). Assessment of the use of N-9 in HIV-negative female sex workers in Cameroon revealed no effect on the rate of HIV infection. However, there was an increased rate of genital ulcers among N-9 users (25). A subsequent trial that took place in four countries showed no efficacy at low-frequency use but an increase in HIV incidence in women who used N-9 more than three times per day (26).

Because its surfactant properties are not virus-specific, high concentrations of N-9 disrupt phospholipid membranes of vaginal epithelial cells. The direct cell damage and resulting irritation cause breaks in the mucosa that increase permeability of the epithelial barrier (27). As a result of inflammation, production of proinflammatory cytokines such as interleukin-1 (IL-1) can induce nuclear factor NF-κB activation, which subsequently upregulates expression of prostaglandin G/H synthase 2 (cyclooxygenase-2, COX-2) and prostaglandin E_2 (PGE_2). These inflammatory mediators increase recruitment of macrophages, as well as their receptiveness to viral replication. These cells can then transport the virus across the epithelium, as demonstrated in vitro (28, 29). N-9 was also shown to alter the native and protective vaginal microbial flora (28-30). These mechanisms of barrier compromise are particularly relevant when used in conjunction with hormonal contraception, which results in thinning of the epithelium and enhanced vulnerability to damage. Furthermore, N-9-induced injury of the epithelial barrier increases the likelihood of infection by other sexually transmitted pathogens, which are independent risk factors for HIV transmission. Finally, N-9 was known to be toxic to lymphocytes and the columnar cells of the endocervix in animal studies, but these data were considered insignificant because N-9 had been "safely" used as a spermicide for decades (27). These disconcerting results ended the trials of N-9 and resulted in stricter safety regulations for clinical studies.

Glyminox (C31G; Savvy®) is another surfactant microbicide that functions through a mechanism of membrane disruption. Its two active components, cetyl betaine and myristamine oxide, demonstrated promising in vitro safety and efficacy against HIV (31). Two phase III trials were conducted in Nigeria and Ghana. Both were discontinued prematurely due to a lower than anticipated incidence of HIV seroconversion, such that the studies would be unable to demonstrate statistical significance (32, 33). In the Nigerian trial, data analysis after 12 months of follow-up revealed a higher rate of HIV seroincidence among C31G users, but the difference did not reach significance. However, reasons for this trend are attributed to similar inflammatory mechanisms as occurred with N-9 (32).

A third surfactant tested for use as a potential microbicide was sodium lauryl sulfate (SLS), also known as sodium dodecyl sulfate (SDS). SLS demonstrated in vitro and in vivo activity against HIV, herpes simplex virus (HSV) and human papillomavirus (HPV), and it was found to have lower cytotoxicity than N-9 and C31G (31, 34). In addition to acting as a detergent, its negatively charged sulfate groups enhance its activity by denaturing membrane proteins (34). More specifically, SLS interacts with HIV envelope glycoproteins, compromising fusion of the virus with its target cells and inducing viral inactivation (35). SLS has been uniquely formulated to act as a thermoreversible "invisible condom". Using an applicator, it can be applied to the vaginal wall as a liquid at room temperature that subsequently converts to a gel at body temperature, acting both as a physical and chemical barrier (36). A series of phase I/II trials in

Canada and Cameroon have demonstrated encouraging safety and acceptability results, warranting further investigation into the product's efficacy (37-39). However, it remains to be determined whether its surfactant properties will induce the same type of inflammatory reaction and enhancement of HIV infection as other surfactants in long-term use. In general, microbicide development efforts have been shifting away from these nonspecific surfactants to find more targeted means of preventing HIV infections.

Vaginal milieu protectors/buffering agents

The mucosa of the vagina contains a host of natural defenses against infection. For example, normally the vagina is rather acidic at a pH of 4-5, and normal bacterial flora help maintain an environment hostile to potential pathogens. The vaginal epithelia can be damaged from traumatic or dry sex or from the presence of infection, and the existence of ulcerations, tears or other disruptions of the vaginal mucosa greatly increases the risk of HIV infection (40). For these reasons, some microbicide developers have sought to enhance naturally occurring defense mechanisms in the hope that intact mucosal defenses can lessen the risk of HIV infection. The two main types of microbicides in this class are pH-lowering agents or buffers and vaginal flora enhancers.

During intercourse with ejaculation, the acidic vaginal pH is neutralized by the basic properties of semen (40, 41). The rationale behind buffering microbicides is that vaginal mucosal defenses presumably function most effectively in the acidic vaginal environment. Following this reasoning, prevention of neutralization following ejaculation could avoid compromise of the vaginal defenses. Because HIV has been shown to be inactivated at low pH in vitro, it was hypothesized that microbicide buffers could reduce the risk of HIV infection in women (40, 41).

Two examples of pH-lowering microbicides are Acidform™ (Amphora™) and BufferGel® (10). Acidform™ was initially studied in conjunction with N-9 because it was thought that it could counteract some of the surfactant's side effects (42, 43). Although this was not the case, the study demonstrated that it could still be an appropriate vehicle or base for other active microbicides. It has also been tested in men and is tolerated as well as K-Y® jelly, a currently marketed lubricant (44). More recently, Acidform™ was tested in conjunction with a diaphragm for tolerability. Although there were no statistically significant differences observed in the two study arms (diaphragm + Acidform™ vs. diaphragm + K-Y® jelly), the authors note that there were "slightly more safety events" in the Acidform™ arm (45). In theory, any gel formulation could also serve as a physical barrier in conjunction with a microbicide to contribute to protection, but no efficacy studies have been published. Acidform™ has been described as a "vaginal flora helper" and is currently being tested alone in a phase I trial (see Fig. 1) (43).

BufferGel®, an osmotically balanced, detergent-free aqueous gel made of carbopol 974P, prevents neutralization of pH by semen in vivo, is well tolerated and has a safer toxicity profile than surfactant microbicides (46, 47). However, in a large-scale, randomized, con-

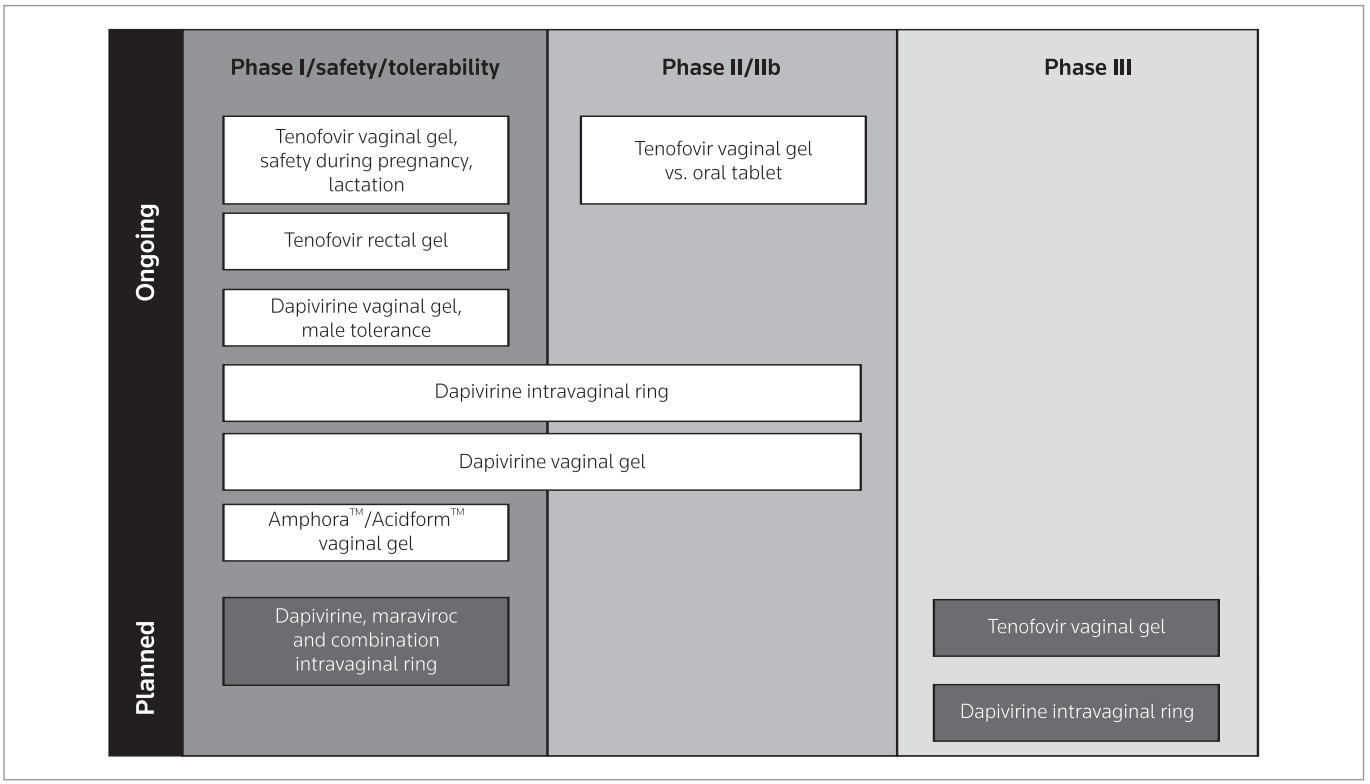

Figure 1. Overview of current and planned microbicide research (20, 87, 97).

trolled trial, HPTN 035, which examined efficacy of both BufferGel® and PRO-2000 (see next section), the BufferGel®, while well tolerated, did not reduce the incidence of HIV infection (48).

In addition to buffers and acidifying agents, a more recent trend in vaginal milieu protection is the development of probiotic supplements containing lactobacilli, i.e., bacteria that coat the vaginal mucosa and produce hydrogen peroxide and lactic acid (49-52). The presence of lactobacilli has been shown to decrease the incidence of bacterial vaginosis and sexually transmitted infections, and repopulating the vagina with lactobacilli also maintains an acidic pH (53-56). It is thus conceivable that buffering and vaginal flora-enhancing microbicides could act symbiotically, since lactobacilli help maintain an acidic pH and normal vaginal flora thrive at acidic pH. Additionally, recent research has focused on engineering lactobacilli that produce antiviral peptides; this formulation has not yet advanced to studies in humans but prevented simian/HIV infection in macaques (51).

Entry inhibitors/anionic polymers

A more specific class of microbicides, the viral entry inhibitors, acts through three types of mechanisms. Anionic polymers, chemokine CCR5 receptor antagonists and fusion inhibitors each block an aspect of viral adsorption, fusion or entry. The only type of entry inhibitors that has yet been studied in phase III trials are the anionic polymers. Additional entry inhibitors under investigation include the anionic polymers cellulose acetate phthalate (CAP) (57) and SPL-7013 (VivaGel®) (58), the chemokine CCR5 receptor antagonists PSC-RANTES (59) and CMPD-167 (60), and the fusion inhibitor cyanovirin N (61).

The negative charges of anionic polymers interfere with the binding of HIV surface protein gp120 to cells expressing T-cell surface glycoprotein CD4 ($CD4^+$) cells by neutralizing the electrostatic potential of gp120 (62, 63). The compounds vary by degree and distribution of negative charges, which are commonly supplied by sulfate and sulfonate groups. The anionic compounds are particularly effective against chemokine CXCR4 receptor-tropic viruses due to a more highly positive V3 region on the gp120 molecule (64).

One promising microbicide, the naphthalene sulfonate PRO-2000, was developed as an anionic microbicidal polymer gel. Its mechanism of action is interference with CD4 and CXCR4 cell receptors in addition to gp120 inhibition. It was also shown to cause minimal inflammatory changes (65). PRO-2000 demonstrated early in vitro efficacy (66) and was found to be safe and well tolerated in clinical studies (67). However, it was not effective at preventing HIV transmission at various concentrations assessed in phase III trials (48, 68). The reason for its failure was not a lack of bioavailability or inactivity against HIV (69); rather, it was attributed to unanticipated competition from the mucosal host factors human defensin-5 and -6, which counteract the activity of the polyanion microbicides (70), as well as decreased activity due to semen and other postcoital effects (71).

Another significant polyanionic entry inhibitor to enter phase III testing was the carrageenan gel PC-515 (Carraguard®). Carraguard® is a sulfated polysaccharide that is derived from red seaweed. In addition to its interaction with gp120, it also prevents HIV-infected mononuclear cells from migrating across the vaginal epithelium (72). Although safety was demonstrated in multiple phase I and II trials (73-75), the difference in HIV seroincidence versus placebo was not significant. Furthermore, biomarkers indicated only 43% adherence by users of the product (76). This lack of adherence was considered an important barrier to efficacy in the study.

A similar story occurred with the cellulose sulfate polymer Ushercell, which did not demonstrate a difference compared to placebo in phase III trials (77, 78). The reason for this failure remains unknown, but it has been suggested that it may have been due to inflammatory reactions, drug-induced immune dysfunction or disruption of the vaginal epithelium (13, 79, 80).

The entry inhibitor and dendrimer SPL-7013 (VivaGel®) has recently come under clinical investigation as another potential anionic polymer candidate. Early phase I studies demonstrated good safety results by colposcopy and pathology when used daily over the course of a week (81). Additionally, the product maintained 70% efficacy for inhibition of HIV infection 24 hours after administration, with > 90% efficacy within 3 hours (58). However, subsequent phase I trials demonstrated mild epithelial irritation and inflammation when used over 14 days (82, 83). Two studies noted a change in vaginal microflora as a result of product use (81, 83). Although this change was considered insignificant in terms of adverse safety outcomes, its relevance may become apparent during subsequent efficacy studies. There is clearly much that remains to be understood in the development of microbicides and the translation from in vitro systems to in vivo use.

Reverse transcriptase inhibitors

The most recent and, to date, the most promising trend in microbicide research has been the formulation of microbicides from agents that target various stages in viral replication. Two reverse transcriptase inhibitors (RTIs) have been formulated and studied independently as microbicides: the nucleotide RTI (NRTI) tenofovir, which has been used in HIV therapy, to prevent transmission from mother to child and in pre- and post-exposure prophylaxis, and the non-NRTI (NNRTI) dapivirine (40, 84, 85). HIV reverse transcriptase is an enzyme that enables production of a DNA copy of the viral RNA genome, an essential step in its life cycle, without which its replication is halted. Hence, the success of these RTI microbicides is their highly specific anti-HIV activity and their relative potency as HIV inhibitors as compared with other agents.

Currently, the most safety and efficacy data exist for tenofovir vaginal gel, the first RTI to be formulated as a microbicide (84, 86). Tenofovir gel showed great promise in vitro and in vivo (87) and was effective in animal studies (88). In a phase I trial, 1% tenofovir gel was well tolerated in both HIV-negative and -positive sexually active and abstinent women (89). In a recent breakthrough for the microbicide research effort, the randomized, double-blind, controlled phase IIb trial CAPRISA 004 showed a 39% reduction in HIV acquisition when tenofovir gel was used before and after sex, according to a coitally dependent dosing regimen (86). Furthermore, there were indications that the protection afforded was as high as a 54% reduction in HIV infection among so-called "high adherers" (i.e., those who used gel as indicated more than 80% of the time), although this was not a statistically significant finding (86). Currently, tenofovir is being

tested in several trials (Fig. 1); note that the VOICE study initially involved testing tenofovir both as a gel and an oral formulation in order to assess the relative efficacy of the same drug as a microbicide and for pre-exposure prophylaxis (90). However, the oral tenofovir arm of the study was recently halted due to futility (91). The VOICE trial continues to assess the remaining study arms: tenofovir vaginal gel and the oral formulation of Truvada® (tenofovir disoproxil fumarate/emtricitabine) (91).

Another RTI that has advanced to efficacy testing is dapivirine, which has been developed both as a gel and as a silicone intravaginal ring (IVR) (92-96). In vivo and cervical explant studies showed dose-dependent efficacy against HIV, even with semen present (93). Additionally, dapivirine gel also offered protection against infection due to NNRTI-resistant strains of HIV (93). In a phase I/II trial in 119 women, dapivirine gel was safe and well tolerated, with low systemic absorption when administered twice daily for 42 days (95). The phase I studies IPM 001 and IPM 008 examined the safety of dapivirine IVRs at doses of 25 and 200 mg, respectively (96). Both trials showed that, after 7 days, dapivirine was evenly distributed through the genital tract and low plasma concentrations were observed, indicating that the IVR is an appropriate delivery vehicle for dapivirine as a microbicide (94, 96). Dapivirine is currently undergoing additional phase I/II testing for safety and efficacy both as gel and IVR formulations (Fig. 1).

Although reverse transcriptase inhibitors have demonstrated the greatest potential among microbicides under investigation, their long-term affordability and efficacy is complicated by their concomitant use in highly active antiretroviral therapy (HAART). The tremendous demand for HAART and continual development of new therapies to treat resistant strains has resulted in increased cost of antiretroviral medications. Therefore, it may be difficult to control the cost of microbicides that utilize these drugs. Furthermore, resistance is a major hurdle in the treatment of HIV. Because RTI microbicides have shown systemic absorption (89), this creates a setting of subtherapeutic monotherapy, which could promote drug resistance if infection occurs. Encouragingly, however, both the results of earlier safety trials and the recent CAPRISA 004 study indicate that the use of tenofovir gel did not result in resistance mutations (86, 89). Conversely, RTI microbicides may offer less protection against acquisition of drug-resistant viral strains. As RTIs are increasingly employed as microbicides, their effectiveness may decline as part of a HAART regimen. Thus, they may need to be restricted to prescription-only distribution to monitor adherence and dissemination. The consequences of such a restriction, however, may impair availability on a population level.

Microbicides with an unknown mechanism

The praneem vaginal tablet, formulated from *Azadirachta indica* (the neem tree) and other herbal ingredients, is another notable microbicide candidate (9, 50, 97, 98). The mechanism of this microbicide is unknown, but it has demonstrated in vivo activity against HIV and other sexually transmitted infections (99), as well as contraceptive activity (100). Phase I studies have yielded mixed results; while adherence was generally very high, many participants reported non-serious side effects such as itching, unpleasant odor and other vaginal irritation (97, 98, 101, 102). In light of the surfactant microbicide trials, praneem's developers have noted that more toxicity and preclinical data are needed before the microbicide advances to efficacy testing (102).

CHALLENGES AND FUTURE DIRECTIONS

The demand for microbicides has led to the fact that several phases of clinical studies of a single compound are run simultaneously to increase efficiency (8, 103). While this may speed up potential licensing and availability of a successful microbicide, there are also some drawbacks to this approach. The first few microbicides were arguably advanced to human testing too soon before adequate preclinical data were available (103). Other large-scale trials have been halted early due to safety concerns, and early in vitro and in vivo models have needed significant revision in order to more closely approximate human HIV infection. Additionally, as a result of the early surfactant trials that resulted in epithelial injury, most phase I trials have modified their approach to assessing safety by using colposcopy to examine changes in the vaginal epithelium (8) and by developing more sophisticated imaging techniques (104).

One of the debates surrounding microbicides (as well as other HIV prevention methods such as pre-exposure prophylaxis) has been whether the availability of such methods would cause a decrease in condom use, or condom migration, and therefore indirectly result in an increase in transmission risk (16). However, mathematical models predict that a microbicide that only reduces the risk of HIV transmission by 50% would outweigh the risks of decreased condom use (16). Another model predicting hypothetical microbicide use in female sex workers demonstrated that the ability to use/adherence to a microbicide formulation will have more impact on overall efficacy than the HIV-inhibitory properties of the microbicide itself (17). This will have important implications for licensing, which depends on efficacy as one of its criteria. More recently, a model was developed which assumed the generalizability of the CAPRISA 004 results (39% reduction in risk), and predicted that the use of tenofovir gel could prevent 1.3 million new HIV infections in a 3-year time span (105). Taken together, these models indicate that the development of a microbicide that is acceptable to users and even modestly efficacious can substantially decrease the risk of HIV infection.

Adherence has been a problem in almost all microbicide trials to date, and over- or underuse threatens the efficacy and safety of these compounds. For example, during N-9 trials, the use of the microbicide three to five times daily (i.e., overuse) was associated with an increased risk of HIV infection (26). On the other hand, lower adherence (i.e., underuse) was associated with a higher risk of infection, although this finding was not statistically significant (48). In response, researchers have recently begun exploring the use of a vaginal ring or similar timed-release options for the controlled delivery of microbicides. For example, a dapivirine vaginal ring is currently in phase I/II trials (106). Another very exciting delivery method for microbicides is the use of pH-dependent nanoparticles, but this method is still in early preclinical stages of testing (103, 107).

Although a majority of this microbicide review (and indeed a majority of the microbicide literature) is focused on vaginal microbicides, it should be noted that rectal microbicides are also currently in development and being tested in clinical studies (Fig. 1). The development of rectal microbicides is paramount; unprotected receptive anal

intercourse places an individual at the highest risk of HIV acquisition, both within MSMs and heterosexual individuals (12, 108-110). Furthermore, the established use of lubricants during anal intercourse may render a microbicide gel more acceptable than an oral pre-exposure prophylaxis formulation (12). Ex vivo tests of several RTIs acting in combination on rectal tissue demonstrated potent anti-HIV activity (111). In macaque models, both MIV-150 (an NNRTI) in carrageenan gel and MC-220 (another RTI) prevented rectal transmission of simian immunodeficiency virus (SIV) (112, 113). Currently, tenofovir rectal gel is being tested in a phase I safety study (Fig. 1).

In order to minimize drug resistance and maximize protection, a combination of microbicidal agents may be warranted (103). In vivo tests indicate that a combination of antiretroviral agents retains anti-HIV activity even against drug-resistant HIV strains (114). In an ex vivo model testing different combinations of agents as a microbicide, it was found that a combination of three RTIs was optimal for protection against HIV (108). It has been demonstrated repeatedly that microbicides formulated with RTIs exhibit very low systemic absorption (87-89, 93-96), and the fear of fostering drug resistance as a consequence of microbicide use could therefore be allayed with the development of combinations rather than single agents (103).

CONCLUSION

While the history of microbicide development has been largely fraught with disappointment, new directions and novel applications have changed the landscape and opened doors of unexplored opportunity. Recent advances have reignited research efforts and elevated expectations. Future directions in HIV prevention cannot dismiss the potential impact that an acceptable and effective microbicide could have on curbing the spread of the HIV epidemic, as sexual transmission is still the leading cause of transmission worldwide. Whether this strategy emerges as the leading prevention tool will depend on the ability to learn from previous impediments, understand cultural expectations leading to adherence, develop innovative solutions, and sustain funding to support extensive clinical testing to determine efficacy.

DISCLOSURES

The authors state no conflicts of interest.

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