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Advances in the Development of Microbicides for the Prevention of HIV Infection

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Abstract

Microbicides are products that can be applied to vaginal or rectal mucosa with the intent of preventing, or at least significantly reducing, the transmission of sexually transmitted infections, including HIV-1. The past 2 or 3 years of microbicide research have generated several disappointments. Large, phase 2B/3 studies failed to demonstrate product efficacy, were stopped prematurely for futility, and in the worst-case scenario possibly demonstrated microbicide-induced harm. The most recently completed efficacy study (HPTN-035) did not reach statistical significance, but did show that use of PRO-2000 was associated with a 30% reduction in HIV acquisition. Current research focuses on much more potent targeted therapy, including reverse transcriptase inhibitors and CCR5 antagonists. Ongoing challenges include optimizing the identification of safety signals in phase 1/2 studies, defining a rationale for advancing products into efficacy studies, and identifying populations with adequate HIV seroincidence rates for these studies.

Keywords

Microbicide; HIV prevention; Drug development

Introduction

The past decade saw a movement away from the development of broad-spectrum microbicide products with relatively nonspecific mechanisms of action (eg, surfactants) to antiretroviral microbicides that target specific steps in the viral life cycle. Other recent innovations include the development of slow-release delivery systems (eg, vaginal rings impregnated with antiretroviral drugs), improved preclinical evaluation of candidate microbicides, sophisticated multicompartment pharmacokinetic characterization of product distribution, and the use of tissue explant systems to provide preliminary data on product efficacy. Despite these technological advances, fundamental questions remain unanswered. These include defining the criteria to move products from preclinical to clinical studies, the optimal phase 1 evaluation of candidate microbicides, and the use of nonhuman primate efficacy studies in the development pathway. The effectiveness phase of drug evaluation also remains problematic. In the absence of a robust surrogate for HIV infection, phase 2B/3 microbicide studies require thousands of participants from populations with a high annual seroincidence of new HIV infections. The contemporary design of phase 2B/3 studies necessitates inclusion of a comprehensive portfolio of HIV prevention measures including safer sex counseling, diagnosis

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and treatment of sexually transmitted infections, provision of male and female condoms, and potentially offering circumcision to male partners. These interventions all lower the risk of acquiring HIV infection and increase the difficulty of demonstrating microbicide efficacy. More recent challenges include the potential risk of resistance associated with the use of antiretroviral microbicides and the provision of study product once studies have been completed.

Mucosal Transmission of HIV Infection

Sexual transmission of HIV-1 is initiated when semen containing cell-free or cell-associated virus is deposited in the vagina or rectum or when virus passes from these compartments to the insertive partner. The exact mechanism of viral transmission remains uncertain and may well involve multiple pathways. The vaginal and rectal epithelia do not possess a traditional receptor for HIV-1, but in both cases the subepithelial tissue contains multiple viral targets. These include Langerhans cells (dendritic cells expressing the HIV-1 CD4 receptor and the CCR5 co-receptor), T cells, and macrophages. Passage of virus from the lumen to the cellular targets may be facilitated by binding of virus to dendritic cell projections that extend into the epithelial compartment with subsequent presentation to subepithelial target cells [1]. A more mundane but equally likely explanation is that virus accesses the subepithelial tissue through epithelial breaks caused by local trauma and/or sexually transmitted infections. After initial infection, local viral replication is followed by dissemination of virus to the regional lymph nodes, at which point systemic infection is established. Studies using animal models suggest that initial infection can occur within 1 h of exposure and dissemination can occur within 24 h [2].

In a rhesus macaque model of simian immunodeficiency virus (SIV) infection, Li et al. [3•] recently demonstrated that initial infection was associated with increased mucosal expression of a range of chemokines (including macrophage inflammatory protein [MIP]-3 α) that occurred almost immediately after viral challenge. This phenomenon led to increased recruitment of CD4⁺ target cells and amplification of SIV infection. Interestingly, the authors demonstrated that intravaginal administration of glycerol monolaurate resulted in significant inhibition of SIV infection, and postulated that this protection might have occurred through inhibition of local MIP-3 α -induced target cell recruitment [3•].

Microbicide Pipeline

Currently, about 50 candidate microbicides are in development. A complete listing can be obtained at the Alliance for Microbicide Development website (<http://www.microbicide.org>). It is unlikely that most of these candidates will progress to clinical studies. Many products will fail to demonstrate an adequate preclinical safety and efficacy profile or will prove refractory to attempts to formulate the product. Unfortunately, many development teams are simply unable to generate sufficient funds to develop good manufacturing practice-grade clinical trial material and/or conduct the necessary preclinical toxicology required to undertake subsequent human phase 1 studies.

New Preclinical Microbicides

Cyanovirin-N (CV-N) is an 11-kD protein originally extracted from cyanobacterium (*Nostoc ellipsosporum*) and found to have antiretroviral properties [4]. CV-N works by binding to HIV envelope glycoproteins and preventing fusion with host cell membranes [5,6], and is active in the nanomolar range in vitro. The efficacy of CV-N was demonstrated in simian-human immunodeficiency virus (SHIV)_{89.6P} challenge models and in human cervical explant studies [7,8]. Development of lactobacilli that can produce CV-N [9] and transgenic plants that

synthesize CV-N may provide an economically viable way to produce CV-N for microbicide development [10,11].

Griffithsin (GRFT) is another product that targets HIV envelope glycoproteins. Derived from red algae, GRFT has extremely potent inhibitory activity with a median effective concentration of 40 pM, activity in the cervical explant model, and against multiple viral clades. Importantly, GRFT does not appear to induce cell activation or proliferation, a common feature of lectins. Similar to CV-N, GRFT has been manufactured using transgenic plant technology [12].

PSC-RANTES is a synthetic CCR5 antagonist. (PSC refers to the chemical structure thioproline [Pro + S = PS] at position 2 and cyclohexylglycine [C] at position 3 in the molecule. RANTES refers to regulated on activation normal T expressed and secreted protein.) Because these molecules target the host co-receptor and not the HIV-1 envelope, it is hoped that they will retain activity against all HIV clades, as confirmed by in vitro studies using peripheral blood mononuclear cell targets [13]. Recently, studies in the rhesus vaginal challenge model demonstrated that blocking CCR5 by PSC-RANTES provided high-level protection against vaginal challenge with the SHIV_{162P4} isolate [14]. Concern that manufacturing costs might limit the utility of PSC-RANTES in the developing world led to the production of two PSC-RANTES derivatives (5P12-RANTES and 6P4-RANTES) that appear stable, lack the ability to induce cell proliferation [15•], and are active in the macaque challenge model [16].

Microbicides in Clinical Development

Currently, 13 ongoing clinical trials involve administration of topical microbicides. Some also include randomization to arms with oral antiretroviral therapy. As shown in Table 1, the vast majority of microbicides in clinical development are reverse transcriptase (RT) inhibitors that act by inhibiting HIV-1 viral RT, a critical enzyme needed to convert viral RNA into DNA before integration into the host genome. Whether administered topically or orally, RT inhibitors work by delivering sufficient mucosal concentrations to abort nascent viral infection. In the case of UC781, it was also suggested that the drug can act as a “tight binder,” which might allow viral inactivation in the lumen before infection [17,18].

VivaGel (Starpharma Holdings, Melbourne, Australia) is one of two non-RT inhibitors in clinical development; it is a lysine dendrimer that showed HIV activity in preclinical studies, including a macaque challenge study [19–21]. The product was acceptable in a penile tolerance study [22], and three phase 1 vaginal studies are completed or ongoing. Such products may be important for women with HIV infection who would not be able to use an RT microbicide but who wish to reduce the risk of HIV transmission to an HIV-seronegative partner.

Advances in Microbicide Formulation

Microbicides in late-stage clinical development are all gel formulations. However, this delivery system is often associated with leakage and messiness, which limits patient acceptability. Consequently, other platforms (ie, foams, suppositories, films, and vaginal rings) are under active development [23]. Vaginal rings have been used to deliver contraception and estrogen-replacement therapy and are being developed to provide slow release of intravaginal RT inhibitors (eg, TMC-102) [24]. Vaginal rings that could be left in situ for weeks or months would certainly increase patient adherence. Jay et al. [25] described a pH-sensitive hydrogel that undergoes reversible conformational change at varying levels of pH. In an elegant experiment, they demonstrated significant retardation in the movement of HIV or nanoparticles in a gel as the pH moved from 4.3 to 4.8 [25]. It is hoped that the increase in vaginal pH associated with the presence of semen would be sufficient to induce these changes. It is unlikely that a pH-dependent gel would be sufficient to prevent HIV transmission, but it could provide the basis for a combination product with agents such as RT inhibitors or CCR5 antagonists.

Preclinical Development of Microbicides

Clinical development of candidate microbicides is expensive and time consuming, and it is critical that the products moving from the preclinical to the clinical phase of evaluation are safe, effective, and economically viable. Unfortunately, the current preclinical process is imperfect and efforts are underway to develop new safety biomarkers and efficacy models [26]. It is also now recognized that product evaluation should include exposing candidate microbicides to relevant biologic matrices (eg, semen or cervicovaginal fluid), physiologically relevant pH, and the types of bacterial flora found in the vaginal or rectal compartment because all these parameters have the potential to significantly reduce product efficacy [27]. Mesquita et al. [28••] described an enhanced approach to the preclinical evaluation of candidate microbicides. The components include a dual-chamber transwell model to evaluate microbicide-induced epithelial toxicity combined with a broad range of cell-based assays. Key end points include reduced transepithelial resistance, enhanced infection of target cells, damage to cellular tight junctions, cytokine release, and induction of nuclear factor- κ B [28••]. Using this approach, they demonstrated that both N-9 and cellulose sulfate reduced transepithelial resistance, increased passage of HIV across the epithelial membrane, and caused down-regulation of junctional proteins (eg, desmoglein and E-cadherin). Interestingly, both cellulose sulfate and PRO-2000 (Endo Pharmaceuticals, Chadds Ford, PA) increased production of the proinflammatory cytokine IL-6. In contrast, tenofovir did not induce any of these changes.

Animal models play an important role in the evaluation of microbicide safety and efficacy. Recent advances include the development of humanized murine models that allow vaginal and rectal HIV efficacy challenge studies [29]. The development of a low-dose, repeat-challenge macaque model with SHIV moves the system closer to what may happen in human HIV infection and was used successfully to screen a range of RT inhibitors, including tenofovir gel [30••]. Currently, the macaque challenge model is not regarded as part of the critical path toward human studies. It is suggested that incorporation of a macaque challenge model as a gatekeeper would increase the likelihood of success in phase 2B/3 efficacy studies [31]. Others argue that the macaque model is too variable, is not validated, and uses SIV or a pathogenic chimeric SHIV rather than HIV. This area remains highly controversial.

The explant challenge model provides a less contentious and important bridge between the preclinical and clinical phases of microbicide evaluation. Tissue explants (cervical, rectal, foreskin, or tonsil) collected during elective surgery, or via endoscopy for rectal explants, are exposed in vitro to combinations of drug and HIV to determine whether the candidate microbicide has activity in the relevant tissue. Explant production of HIV-1 p24 is monitored over 2 to 3 weeks for evidence of viral inhibition. Elliott et al. [32] incorporated this approach into the design of phase 1 rectal microbicide trials. The study participants insert the test product rectally and endoscopic biopsies are collected after single or multiple doses of the drug. Harvested tissue is then exposed to virus in vitro [32]. Importantly, this technique has the potential to provide preliminary evidence of efficacy in phase 1 studies before progressing to extremely expensive phase 2B/3 efficacy studies. Using this approach, the UCLA team was able to demonstrate the efficacy of UC781. A recent multisite study demonstrated that different laboratories can provide consistent measurements of anti-HIV efficacy in the explant model when 1) standardized end points are used, 2) drugs, reagents, and virus are centrally sourced, and 3) similar explant tissue techniques are used [33].

Clinical Development of Microbicides

Phase 1/2 microbicide studies are used to generate pharmacokinetic and clinical safety data and, as mentioned earlier, may provide preliminary efficacy data. In the absence of a specific safety biomarker, phase 1/2 studies use clinical symptoms and signs to try to identify harm.

Unfortunately, in the case of N-9 and cellulose sulfate, this approach was inadequate and the design of these studies was expanded to include biomarkers (eg, cytokines) [26]. It was also argued that the size and duration of current phase 1/2 studies may be inadequate to identify conventional clinical safety signals [34].

The success or failure of a microbicide is likely to be determined by the complex interaction between product pharmacokinetics, viral kinetics, and possible product-induced toxicity [35]. With regard to antiretroviral drugs, considerable variability exists in genital tract concentration after oral administration [36]. As one example, the cervicovaginal fluid concentration of the CCR5 antagonist maraviroc is almost twofold higher than the blood plasma level [37]. These data emphasize the importance of developing compartmental pharmacokinetic profiles for microbicide candidates that encompass plasma and tissue levels. These assays are technically demanding but are beginning to be included in phase 1 studies.

To date, phase 2B/3 efficacy studies have been conducted on six microbicides (N-9, C31G, Carraguard [Population Council, Inc., New York, NY], cellulose sulfate, BufferGel [ReProtect, Inc., Baltimore, MD], and PRO-2000) without evidence of a reduction in HIV incidence [38–43]. Indeed, the use of N-9 and cellulose sulfate may have increased the risk of HIV acquisition. These very public failures have encouraged some to question the direction of microbicide research [31]. It is clear that we need to improve the preclinical and phase 1/2 evaluation of candidate microbicides to prevent unsafe products moving into phase 2B/3 evaluation. In addition, it is important to determine whether the four current RT microbicides (tenofovir, UC781, TMC-120, and MIV-150) have sufficiently different profiles to warrant moving them all into phase 2B/3 evaluation.

It is likely that an HIV-infected individual repeatedly exposed to an antiretroviral microbicide will develop resistance to that product, and so use of this class of microbicide must be linked to prospective voluntary counseling and HIV testing. The virologic and clinical sequelae of an individual becoming infected while using an antiretroviral microbicide are far less clear, as are the ramifications at a community level. Data on seroconverters are almost nonexistent but are being collected in ongoing oral and topical antiretroviral HIV prevention trials. Combination therapy is routinely used in the management of HIV infection to prevent treatment failure and the development of antiviral resistance. Combinations of antiretroviral agents have been evaluated in the macaque model [44,45] and in colorectal explants [46]. Dual and triple combinations appear to be more potent than single agents, but it is unclear if combinations would reduce the development of resistance if individuals became infected while using multiple agents. Conversely, Parikh et al. [30••] have shown that tenofovir gel alone protected macaques from 20 exposures to SHIV_{SF162p3}.

Phase 2B/3 evaluation of microbicide candidates is extremely demanding. In the absence of a biomarker for HIV prevention, it is necessary to use changes in incidence rates of HIV infection to determine whether a product is efficacious. This method requires identifying clinical trial populations with annual seroincidence rates greater than 3%, enrolling several thousand participants, and finding more than \$50 million to pay for the study. A new complication is the fact that topical microbicide studies are not conducted in isolation. Other phase 2B/3 prevention trials (including oral pre-exposure prophylaxis and vaccines) are also being run in parallel. Should one of these trials demonstrate a significant efficacy signal, repercussions would be immediate for ongoing and future HIV prevention trials. The key issue would be whether it remained ethical to conduct placebo-controlled studies. This question is more complex than might be imagined, and depends on the level of efficacy demonstrated in a phase 2B/3 study. A nonsignificant 30% reduction in HIV incidence, as seen with PRO-2000 in HPTN-035, would not affect ongoing studies, but a statistically significant 30% reduction, as might be seen in the much larger and recently completed United Kingdom Medical Research

Council trial (MDP-301), could have different implications. A further wrinkle is whether a regulatory agency would license a product with this level of activity or whether a donor nation or other sponsor would pay to manufacture the product. As might be predicted, these issues constitute an area of ongoing debate between the various stakeholder communities in microbicide development.

The somewhat artificial division between oral and topical antiretroviral prophylaxis studies is steadily eroding. In September 2009, the US National Institutes of Health–sponsored Microbicide Trials Network started screening for the Vaginal and Oral Interventions to Control the Epidemic study in sub-Saharan Africa (<http://www.mtnstopsHIV.org>). This study will enroll about 5000 women who will be randomly assigned to receive tenofovir gel, oral tenofovir, tenofovir disoproxil fumarate (Truvada; Gilead, Foster City, CA), or placebo, and will provide unique data on the differential safety, acceptability, and efficacy of oral versus topical antiretroviral prevention.

Rectal Microbicide Development

The primary focus of microbicide research has been the development of a safe and effective vaginal microbicide. Although this goal should remain a key scientific priority, emerging epidemiologic data provide a rationale for a parallel program to develop rectal microbicides. Since the beginning of the HIV pandemic, men who have sex with men (MSM) have been the main focus of HIV infection in the developed world. Unprotected receptive anal intercourse (URAI) is the primary risk factor for HIV acquisition in MSM. The unique vulnerability of the intestinal mucosa to HIV transmission results in a per act exposure risk about 20-fold greater than unprotected vaginal intercourse. Increasingly, it is apparent that women in the developed and developing worlds practice URAI [47,48]. Although the absolute frequency of URAI in women may be low, the increased risk per act is such that URAI may play an important role in propagating HIV infection in women as well as in MSM. Another recent important development is the recognition of sexually active MSM in sub-Saharan Africa [49]. These men have a high prevalence of HIV infection, often have male and female partners, and may play an important bridging role in disseminating HIV infection. Even with these limited epidemiologic data, a need clearly exists for rectal and vaginal microbicides and, even better, a product that is safe and effective in both compartments. In contrast to vaginal microbicide development, the field of rectal microbicide development is relatively new. In some respects, its novelty is advantageous because the field has the opportunity to incorporate lessons learned from vaginal microbicide development into the preclinical and clinical development of rectal microbicides [50].

Conclusions

The developing world desperately needs an HIV vaccine. However, despite more than 25 years of research and a current annual research budget of more than \$800 million, there is no evidence to suggest that any of the current candidate vaccines are likely to provide sterilizing immunity against HIV. In this setting, microbicides have the potential to be a critical component of the biomedical HIV-1 prevention portfolio. Despite recent disappointing experience with efficacy trials of the first generation of vaginal microbicides, the microbicide research portfolio has grown and matured. The development pipeline now has breadth and depth. It is hoped that more sophisticated preclinical and phase 1/2 evaluation will prevent the movement of inferior products into efficacy studies. New formulation platforms offer the possibility of enhanced acceptability and adherence. Potent antiretroviral microbicides are being evaluated in effectiveness trials, and it is hoped that these studies will finally demonstrate significant efficacy against HIV-1. Rectal microbicide development is now recognized as a priority within the biomedical prevention research agenda, and multiple phase 1 studies are ongoing or

planned. The critical challenge for the next decade is to find sufficient financial resources to accelerate microbicide development. To quote the late Senator Edward Kennedy, “The work goes on, the cause endures, the hope still lives, and the dream shall never die.” Certainly, the need for effective interventions to prevent HIV infection is greater than ever.

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Table 1

Current clinical microbicide trials

Phase	Drug(s)	Route	Study title	N	Countries	Comment
2B	Tenofovir gel Oral tenofovir Truvada	V/O	MTN-003 (VOICE)	5000	Malawi, South Africa, Zimbabwe, Zambia, Uganda,	Safety and effectiveness study with daily dosing of study product
2B	Tenofovir gel	V	CAPRISA-004	980	South Africa	Safety and effectiveness study: participants will be asked to apply study product within 12 h before coitus and within 12 h after coitus
2	Tenofovir gel Oral tenofovir	V/O	MTN-001	144	South Africa, USA, Uganda	Safety, acceptability, and pharmacokinetic study (blood, cervicovaginal, and tissue levels)
1	TMC-120 gel (4759 and 4789)	V	IPM020	180	USA	Safety, acceptability, and pharmacokinetic study (blood, cervicovaginal, and tissue levels)
1	TMC-120 gel (4759)	V	IPM014A	320	Kenya, Malawi, Rwanda, South Africa, Tanzania	Safety and acceptability study with evaluation of daily monitored adherence
1	TMC-120 gel (4789)	V	IPM014B	320	Kenya, Malawi, Rwanda, South Africa, Tanzania	Safety and acceptability study with evaluation of daily monitored adherence
1	TMC-120 ring	V	IPM024	16	Belgium	Safety, acceptability, and pharmacokinetic study (blood and cervicovaginal levels)
1	VivaGel	V	MTN-004	61	USA	Safety and acceptability study
1	Tenofovir gel	V	MTN-002	16	USA	Single-dose pharmacokinetics study in healthy, term gravidas with collection of blood, placental, cord, and cervicovaginal levels
1	Tenofovir gel	V	NIAID, DAIDS, AECOM	24	USA	Changes in cytokines, chemokines, and other mediators of innate immunity through examination of cervicovaginal secretions
1	Tenofovir gel Oral tenofovir	R/O	RMP-02, MTN-006	18	USA	Placebo-controlled rectal microbicide safety, acceptability, and pharmacokinetic study including explant challenge studies
1	Tenofovir gel	R	MTN-007	60	USA	Placebo-controlled rectal safety and acceptability study including N-9 comparator arm
1	Acidform gel	V	AECOM, AF020	36	USA	Safety and acceptability study

AECOM Albert Einstein College of Medicine, CAPRISA Centre for the AIDS Programme of Research in South Africa, DAIDS Division of Acquired Immunodeficiency Syndrome, IPM International Partnership for Microbicides, MTN Microbicide Trials Network, NIAID National Institute of Allergy and Infectious Diseases, O oral, R rectal, V vaginal, VOICE Vaginal and Oral Interventions to Control the Epidemic study