Review

The future of HIV microbicides: challenges and opportunities

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HIV microbicides are topical, self-administered products aimed at preventing or reducing HIV infection in women and may represent the most promising strategy for combating the HIV/AIDS epidemic at the present time. Although a safe and effective microbicide has yet to be identified, all products tested in Phase III trials to date have been vaginal gels containing non-specific compounds with modest potency that had to be applied close to the time of sexual intercourse. Issues regarding these early generation products were further complicated by widely publicized cases of halted efficacy trials. However, as a result of each of these challenges, new information and essential lessons have emerged for the field. These lessons have resulted in a meaningful 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.

Introduction

The HIV/AIDS epidemic continues to be an enormous public health crisis worldwide [1]. There are an estimated 33 million people living with HIV, of which an estimated 2.7 million became infected in 2007 [2]. Although there have been significant advances in the quality and availability of antiretroviral (ARV) treatment, the majority of HIV-infected individuals across the world do not have access to these therapeutics [3]. The epidemic continues to grow and it is widely recognized that prevention is key to curbing this growth. Available HIV prevention options are few. Research and experience have shown that the ‘ABC’ method (abstinence, being faithful and condom use) may not be a viable option for certain at-risk populations, namely women in some developing-world settings [4]. Many women, especially when married, cannot negotiate the use of condoms or other barrier methods and are not in control of their partner’s sexual activity outside the relationship [5]. HIV vaccines are a potential prevention method, but success in this challenging class of products has also been elusive [6]. Recent clinical trial results from HIV vaccine efficacy and proof-of-concept studies have prompted the vaccine field to refocus more efforts towards gaining a better understanding of the basic immune factors involved in HIV transmission and infection [7]. Male circumcision has been demonstrated to reduce transmission of HIV, but the benefit is skewed toward males [8] and is dependent upon their willingness to undergo the procedure.

One option that presents a promising hope for women in the current landscape of HIV prevention strategies is microbicides. Microbicides are self-administered prophylactic agents designed to impede the sexual transmission of HIV and/or other sexually transmitted pathogens [9]. A successful microbicide would provide women with an affordable and feasible option for HIV prevention that is self-initiated and self-managed. This is extremely important given the increasing HIV incidence rates in women in several countries [2]. Although microbicides aimed at preventing HIV have been in development for over a decade, a safe and efficacious product has yet to be developed. However, all products tested in Phase III efficacy trials, so far, have been gels containing non-specific compounds with modest potency that had to be applied close to the time of sexual intercourse. More recently, product development has turned to microbicides containing highly potent and HIV-specific ARVs that have characteristics amenable to alternative formulation or delivery methods and combination products.
Mechanisms of action

The first microbicide to be tested clinically contained the surfactant nonoxynol-9. In a clinical study involving 892 female sex workers in four countries, the participants using nonoxynol-9 several times a day (a mean of ≥3.5 uses/day) had a rate of infection almost twice that of the placebo group [10]. Subsequent studies have demonstrated that frequent vaginal administration of nonoxynol-9 can cause adverse effects such as inflammation, irritation, tissue infiltration by immune cells and changes to the vaginal flora, all of which might contribute to an increase in HIV transmission [11]. Independent of the surfactant class of compounds, polyanionic compounds, which function by electrostatically associating with the virus thereby interfering with the ability of the virus to attach to target cells in the vagina, were formulated and evaluated [12]. Two non-specific polyanionic candidates of this class of microbicides, Carraguard® and cellulose sulfate, have been evaluated in Phase III clinical studies; however, they were unsuccessful in demonstrating efficacy [13,14]. A third polyanion-based microbicide gel (PRO 2000) as well as a vaginal pH-buffering formulation (BufferGel®) are currently being tested for efficacy, and data are expected to be available in 2009 [15]. Other similar products that are at an earlier stage of clinical evaluation include VivaGel®, a dendrimer-based entry inhibitor, and ACIDFORM™, another buffering agent [16,17]. Interestingly, all of these products were designed to be used at or near the time of intercourse. Unfortunately, the microbicide field does not currently have reliable methods available to confirm product compliance during a study. Typically, studies have included some type of participant self-reporting mechanism, which has demonstrated to be problematic and unreliable [18]. This raises questions regarding the potential relationship between product compliance and demonstration of efficacy in these past studies. An alternative and objective method to measure product compliance needs to be identified or developed for use in further studies.

Current focus in the microbicide field has shifted toward the development of HIV-specific ARVs. The infection cycle of HIV presents several opportunities for the specific blocking of infection (Figure 1) and microbicide developers are rapidly advancing candidate ARVs with various mechanisms of action. It is generally accepted that a microbicide should act prior to the integration of proviral DNA into the host cell DNA; consequently, the two major classes of ARV microbicides are entry inhibitors and reverse transcriptase (RT) inhibitors [9]. Other classes of compounds may also be suitable for use as microbicides, but, to date, these have received less development attention.

Entry inhibitors

During HIV infection, the HIV surface glycoprotein gp120 binds to the CD4 receptor on the target cell [19]. There are several microbicides under development that work to block this specific interaction. A novel protein candidate, Cyanovirin-N, derived from cyanobacteria, has a strong binding affinity for the manose groups on the gp120 protein. Once bound to gp120, infection is effectively inhibited [20]. Another compound that blocks entry of HIV by interacting with gp120 and is being developed as a microbicide is BMS 599,793, which is structurally related to BMS 378,806 [21]. BMS 599,793 is highly potent and undergoing preclinical and formulation development [22].

In addition to binding CD4, HIV must bind to either the CCR5 or the CXCR4 chemokine receptor in order to infect target cells. Blockade of these coreceptors, therefore, presents another opportunity for intervention by a microbicide to prevent infection. Sexual transmission of HIV occurs primarily via the CCR5 receptor [23]. Several CCR5 antagonists are under development as microbicides including maraviroc (Pfizer, Inc., New York, NY, USA), which was approved in 2007 by the US Food and Drug Administration (FDA) as the first CCR5 antagonist for treatment of HIV infection in treatment-experienced adults [12,24]. Other CCR5 antagonists under development for a microbicide indication include regulated on activation normal T-cell

Figure 1. HIV infection cycle in a target cell

Free virus
Attachment
Fusion
Replication (reverse transcriptase)
Protein synthesis and assembly
Budding
Maturation
expressed and secreted analogues [25] and L-860,167 (CMPD167) [26].

Following viral attachment, HIV fuses with the target cell via gp41, a viral protein complexed to gp120. In 2003, the FDA approved the first fusion inhibitor peptide, enfuvirtide (Trimeris, Inc., Durham, NC, USA), to treat adults and children with advanced HIV infection [27]. This drug blocks a conformational change in gp41 that is required for the fusion of the lipid envelope of HIV with the membrane of CD4+ T-cells, thus preventing viral entry [28]. A similar peptide, T1249, has been shown to block vaginal transmission of HIV in the macaque model system [29]. Furthermore, another potent fusion inhibitor peptide, L’644, has been recently licensed for development as a microbicide [30].

Reverse transcriptase inhibitors
RT is a crucial HIV enzyme responsible for transcription or conversion of viral RNA to proviral DNA [31]. Similar to CCR5 blockers and fusion entry inhibitors, the RT inhibitors have demonstrated efficacy as HIV therapeutics [32]. These molecules act by either mimicking endogenous nucleotides (nucleoside RT inhibitors [NRTIs] or nucleotide RT inhibitors), thereby terminating the viral DNA chain extension in the target cell, or by allosterically disrupting the binding of substrates to the active site of the enzyme (non-NRTIs [NNRTIs]). The NNRTIs have been demonstrated to bind irreversibly to RT, which explains their high potency and long half-lives [9]. Examples of NNRTIs in preclinical and clinical evaluation as microbicides include dapivirine (TMC120), MIV 150, UC 781 and S-DABO [9]. The lead NRTI microbicide candidate is tenofovir (Gilead Sciences, Inc., Foster City, CA, USA), which is currently in Phase IIb testing as a vaginal gel [33,34]. The prodrug of tenofovir, tenofovir disoproxil fumarate, is an approved HIV therapeutic [35]; therefore, extensive information is available on the safety and efficacy of tenofovir.

Microbicide combinations
The use of combinations of highly active antiviral drugs (known as ‘highly active antiretroviral therapy’) is well established as the standard of care for patients with HIV/AIDS [36] because it has been proven to be much more effective than monotherapy [37]. Similarly, it would be expected that combination microbicides would have greater efficacy than microbicides consisting of only one active component.

The main benefit with a combination microbicide would be the increased barrier to infection, particularly if each active ingredient targets a different stage in the HIV lifecycle. For example, a microbicide containing only a CCR5-blocking agent may be ineffective against CXCR4-tropic virus, but a combination product containing a CCR5 blocker and an NNRTI would be more likely to be effective. Similarly, a virus containing resistance mutations is much less likely to be resistant to two drugs of different classes than it is to one [38] and viruses with resistance mutations are often less fit for transmission than wild-type viruses [39], which further reduces the likelihood of infection.

An additional potential benefit of combination microbicides is the possibility of reducing the amount of each drug present in the product if the drugs act additively or synergistically. This would theoretically reduce the potential for any toxicity associated with each component.

Formulation and delivery
Although potent antiviral activity is an essential attribute of a microbicide candidate, other attributes are equally important. For example, a candidate must be first formulated in a dosage form that is safe, acceptable and effective. Fortunately, there are multiple vaginal dosage forms available for microbicide development, including single dose forms (gels, tablets, films and ovules) as well as sustained release delivery options.
(vaginal rings) [40]. Figure 2 depicts prototypes of each of these dosage forms.

Formulating an antiviral drug in a particular dosage form requires physical–chemical compatibility between the drug compound and excipients. For example, drugs that are not stable in aqueous solutions may not be compatible with gel formulations because they routinely involve large amounts of water. Such drugs may be more readily formulated in solid dosage forms. Other drugs may not be stable at the higher temperatures typically required for the moulding processes associated with vaginal ring fabrication. Therefore, it is necessary to conduct appropriate pre-formulation compatibility and stability studies in order to appropriately identify formulation options for specific vaginal microbicides.

The intricacies and complexities of microbicide formulation are further compounded when combination formulations are desired. In addition to the compatibilities required between the individual drugs and the excipients, the drugs being combined in the formulation must also be compatible with each other. Potential additive, synergistic or antagonistic interactions of drug combinations can be investigated using in vitro virology studies. Physical–chemical interactions can be determined using standard analytical techniques and the potentiation of toxicities because of drug–drug interactions should be evaluated in combination toxicity studies.

Each of the dosage forms described above could potentially be employed to deliver combination formulations. Tablets, for example, can be fabricated to contain multiple antiviral drugs for oral dosing [41]. Similar potential exists for film-based formulations. Different drugs can be loaded into different configurations of vaginal rings as well. An example is the reservoir configuration of a ring, which involves a central core containing the active drug surrounded by an unmedicated outer ring. It is possible to have individual core segments inserted into such rings, allowing for combination products (Figure 3). Importantly, the reservoir configurations of vaginal rings can be designed to deliver drug for at least 90 days [42].

A crucial component of microbicide formulation is acceptability. Despite extremely high potency, a formulation of a drug that is not acceptable to the target population will not be used and will therefore offer no more protection than a weakly potent drug. It is clear from the contraceptive field that the vaginal dosage forms described above are all acceptable to varying degrees [43–45]. In the case of combination microbicides, acceptability should not be altered by the presence of more than one drug since the dosage form is not altered.

To date, there have been specific attempts to formulate diverse chemical classes of compounds as vaginal dosage forms using all of the formulation strategies noted above. For example, gels have been used to formulate microbicides such as PRO2000 [46] and Savvy (a surface active compound that functions by disruption of the HIV envelope) [47]; both compounds have been evaluated in Phase IIb/III trials [48,49]. Small antiretroviral compounds such as UC781 [50], tenofovir [34] and dapivirine [51–53] have been evaluated in early Phase clinical
trials in gel formulations. These studies have shown these formulations to be safe and well tolerated.

The only microbicide-containing intravaginal ring to be evaluated clinically to date is one containing dapivirine [42,54]. In these studies it was shown that both solid matrix configurations (rings with dapivirine dispersed completely throughout the structure) and reservoir core-type rings (with dapivirine cores) were safe and well tolerated. The successful formulation of dapivirine into the reservoir ring suggests the possibility that independent segmented cores, each containing a different drug, could be configured into this system to achieve combination delivery. The combination of dapivirine with other ARVs that share the same physical–chemical properties as dapivirine (small, hydrophobic molecules with melting points >200°C) may be a viable option if the other ARVs are compatible with the other materials in the ring (for example, silicone and ethylvinyl acetate).

The successful development of ovules and films for vaginal delivery of single-agent drugs for other indications, for example, ovule delivery of antifungals (MONISTAT® 1; McNeil-PPC, Skillman, NJ, USA) and film delivery of contraceptive spermicide (Vaginal Contraceptive Film®; APOTHECUS, Oyster Bay, NY, USA), suggests that these technologies can be used in vaginal microbicide formulation and combination microbicide formulation. The successful formulation of the microbicide tablet Praneem [55], currently in clinical trials, suggests that vaginal tablets are another viable technology for single agent and combination formulations.

In summary, the potential utility of highly potent ARVs as vaginal microbicides, as both single agent and combination formulations, is strongly supported by multiple formulation technologies. Although these technologies have not yet been fully evaluated for combination microbicides, they have been used to successfully formulate several single-agent candidate microbicides, contraceptive products and other vaginal products. Moreover, they have been (or could be) used to formulate combinations of drugs for other non-vaginal dosing indications. Importantly, these and newer technologies (for example, multicompartment liposomes, biodegradable microcapsules and biodegradable implants) have been used for systemic delivery and can potentially be adapted for the local intravaginal delivery of microbicides [56–58]. Consequently, formulation technologies are available to help drive microbicide development through the next generation of combination product development.

Clinical evaluation of microbicides

The development of safe and effective microbicides, although similar in many ways to traditional pharmaceutical development, does present a number of unique challenges from the clinical perspective that are not faced in the development of therapeutic drugs. Similar to therapeutic clinical studies, participant safety is rigorously evaluated at regular time points throughout a microbicide study. Examples of standard safety tests include organ function assessments, pharmacology tests, virology tests and genital infection tests. One unique safety parameter that must be closely monitored for microbicides is the potential to increase the likelihood of HIV infection [10]. As there is currently no validated surrogate marker that can signal increased vulnerability to HIV infection, the issue of detecting increased infection rates in microbicide studies remains an important and unresolved issue.

Another key challenge is establishing the clinical efficacy of candidate microbicides. In therapeutic drug development, the efficacy of a drug can be evaluated and the dose optimized in a Phase II dose-range-finding study. However, the ethical requirements for the conduct of HIV prevention trials and the absence of validated surrogate endpoints mean that thousands of participants must be enrolled from communities with relatively high HIV incidence rates and receive treatment for long periods of time in order to show a potential reduction in new HIV infections. In reality, this only can be achieved in Phase III trials [18]. In addition, adherence to the microbicide regimen is a relevant factor in clinical trial outcome and inconsistent or lower levels of product adherence could result in failure of an otherwise effective product to be proven so in a trial.

For these and other reasons, the microbicide field has looked to preclinical animal models to potentially provide some measure of efficacy prior to Phase III. The animal models used have primarily been the hu-SCID mouse [59], the BLT mouse [60] and the macaque [26,61]. Although these models may have some utility in establishing whether a microbicide can prevent HIV infection in vivo, their limitations are considerable (see Box 1).

An alternative approach to obtaining a measure of efficacy is pharmacokinetic–pharmacodynamic modelling in women. This is a means of correlating the pharmacokinetics of a drug (for example, drug concentrations in cervical or vaginal tissue collected by biopsy and/or cervico-vaginal fluid) with the ability to prevent infection of biopsied tissues following viral challenge ex vivo or demonstrate inhibition of in vitro infection in tissue culture assays with the cervico-vaginal fluid.

The potential benefits of this technique are the use of human tissue that has been exposed to drug in vivo, the use of HIV rather than an alternative virus and the ability to use sufficient samples to achieve statistical significance.

However, it is also recognized that there are a number of limitations associated with pharmacokinetic–pharmacodynamic modelling for microbicide studies. For example, the actual viral challenges are of a single viral strain and are conducted ex vivo in culture-based...
systems that are not representative of viral exposure in vivo. The biopsy procedure may cause traumatic changes that influence the susceptibility of the tissue to infection. The effect of intercourse is not evaluated in this system. Women may be at different stages of the menstrual cycle, which may influence infection. Furthermore, ex vivo work with explants suggests that there is a high level of variability associated with this technique, possibly because of varying density of target cells in a given biopsy sample. Finally, as is the case for animals, the assays have not been standardized or validated.

Although there are currently no published data on the successful use of this technique vaginally, the technique has been used successfully for rectal tissue obtained by biopsy after rectal administration of microbicides in men and women [5], as well as for cervical tissue obtained surgically and treated with drugs ex vivo [62]. In addition, the protective effect of cervico-vaginal fluid from women using microbicides has been successfully demonstrated in clinical trials [63], and methods have been established for determining drug concentrations in cervical and vaginal tissues and cervico-vaginal fluid [64–67]. Thus, with the advent of ARV-based microbicides, it is clear that new and innovative means of assessing activity in vivo are needed and pharmacodynamic assessments in women represent such an option.

Pharmacokinetic evaluations are also important in microbicide studies for establishing that there is adequate distribution of drug throughout the genital tract. Ensuring uniform spread of the drug in the vagina and measuring it accurately is a challenge. Relating this to potential efficacy is complicated further because the precise events associated with HIV transmission and the cells involved in this process are unknown. This is further increased in complexity when the events of intercourse and its effects on product distribution are considered. However, determining the distribution of drug to potential sites of infection and establishing the concentrations achieved and their change with time is important in informing product design and establishing the appropriate dose regimen. In addition to chemistry-based analytical methods, various imaging techniques such as magnetic resonance imaging, fibre-optic probe and scintigraphy can be used to assess product distribution [68–71].

Conclusions

The future of HIV microbicides will benefit significantly from the lessons learned from the earlier generation of candidates. The field continues to move toward careful selection of potent, highly characterized ARVs, both alone and in combination. The availability of compounds for microbicide development that are already licensed for HIV therapy, such as tenofovir and maraviroc, have the benefit of established safety profiles in humans as well as demonstrated therapeutic effects against HIV. The trend away from coitally-dependent products toward once-a-day dosing regimens or sustained release delivery configurations has the potential to reduce the challenges associated with user adherence. The intravaginal ring has been demonstrated to deliver drug locally for 28 days [53], potentially significantly reducing the burden of compliance for women using the product. The value of HIV prevention animal efficacy models continues to be hotly debated [72], new model development continues [60] and microbicide developers are beginning to explore the utility of human pharmacokinetic and pharmacodynamic investigations in Phase I trials as potential predictors of clinical efficacy.

Despite the many technical challenges associated with developing a safe and effective microbicide for the prevention or reduction of HIV infection in women, it is
clear that the possibility of success requires the field to continue to forge ahead with focused efforts and science-driven strategies. By building upon past lessons learned and refining development strategies, the field is becoming increasingly equipped to overcome the challenges that exist in the complex pathway of achieving a successful HIV microbicide.

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