Microbicides for HIV

Microbicides are chemical entities that can be incorporated in gels, films, tablets or rings for application to the vagina or rectum to prevent the transmission of HIV and other sexually transmitted infections. Leading Australian microbicide efforts include the development of a dendrimer nanoparticle with broad-spectrum activity against HIV, HSV and HPV, and a natural factor produced by lactobacilli in the healthy female genital tract. Clinical trials have revealed that nonspecific agents such as nonoxynol-9 and moderately specific linear polyanions lack efficacy in preventing male to female HIV transmission. In contrast, the CAPRISA 004 study demonstrates that a gel containing an antiretroviral agent, 1% tenofovir, provides women with 39% protection against HIV infection. While this proof of concept study is arguably one of the most encouraging results seen in the HIV prevention field, efficacy and adherence could be improved by developing combination microbicides and coitaly independent dosing strategies, respectively.

The HIV pandemic

Human immunodeficiency virus (HIV) is a major public health problem with an estimated 33.2 million people infected worldwide (UNAIDS 2007). Fifty per cent of individuals infected with HIV are women, with greater than 60% infected in Sub-Saharan Africa. While male condoms prevent HIV transmission, negotiating their use can be difficult for women. Accordingly, vaginally applied microbicides have been pursued as female-initiated strategies to prevent HIV and other sexually transmitted infections (STIs). STIs such as herpes simplex virus (HSV) and bacterial vaginosis (BV) are associated with an increased risk of HIV acquisition providing a rationale for targeting by microbicides. In addition, rectal microbicides are being developed for protection against HIV acquired by anal receptive sex in women and men who have sex with men. Other HIV prevention strategies include pre-exposure prophylaxis (PrEP) with antiretroviral agents. This review focuses on the development of topical vaginal microbicides.

Essential characteristics of a microbicide

Microbicides should have broad-spectrum activity against HIV clades circulating in different geographical regions. They must also inhibit cell-free and cell-to-cell HIV transmission. Microbicides must retain HIV inhibitory activity in the presence of cervicovaginal secretions and semen. It is critical that the microbicide does not disrupt tight junctions formed by epithelial cells of the genital mucosa nor cause inflammation, which promote HIV infection. Microbicides should not inhibit the growth of lactobacilli sp. found in the healthy female genital tract (FGT) and should be stable in the presence of low pH. They also need to be stable at high temperatures, preferably odourless, colourless, tasteless and compatible for use with latex. Microbicides should ideally be low cost and acceptable for use by women and their partners. The contraceptive potential of a microbicide should also be known.

Non-specific microbicides

The first microbicides to be developed were nonspecific agents that include surfactants, detergents and acid buffering agents that directly inactivate viral and bacterial STIs. The term ‘microbicide’ has now become ingrained in the field, despite the development of compounds that are moderately or highly specific for blocking HIV infection but do not necessarily involve direct inactivation of pathogens.

The nonionic surfactant, nonoxynol-9 (used as an over-the-counter spermicide) and the detergent, sodium dodecyl sulphate (SAVVY), are non-specific agents that dissolve the lipid envelope and denature membrane proteins of pathogens, respectively. However, these agents have a low therapeutic window and cause inflammation and disruption of the vaginal epithelium. A clinical trial demonstrated that sex workers who applied nonoxynol-9 several times a day were at an increased risk of HIV acquisition compared to women using placebo gel. Clinical trials to evaluate the efficacy of SAVVY in preventing HIV transmission were ceased due to futility. Thus there is no longer enthusiasm for the development of these agents as microbicides. The acid buffering agent BufferGel® is designed to maintain the low pH of the vagina (pH 3.5–4.5) in the presence of alkaline semen, thus preventing the window of opportunity for acid labile pathogens such as HIV to infect the mucosa. BufferGel® was found to be safe, although it failed to prevent HIV transmission in women.

Moderately specific microbicides

Moderately specific microbicides mainly comprise macromolecular linear anionic polymers including cellulose sulphate (UsherCell), carrageenan (Carraguard) and PRO 2000. Linear polyanions are active against HIV, HSV and in some cases demonstrate inhibitory activity against human papillomavirus.
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(HPV), *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. They block viral entry into cells through electrostatic interactions with the viral envelope proteins and, in some cases, host cell receptors14,15. Members of this class tend to be more potent inhibitors of HIV strains that use the CXCR4 chemokine receptor compared to those using CCR516, the latter being more relevant in HIV transmission.

Evaluation of linear polyanion-based microbicides in efficacy trials has led to disappointing results. Cellulose sulphate demonstrated a trend towards increased HIV acquisition and Carraguard lacked efficacy17,18. While PRO 2000 demonstrated 30% efficacy in a phase IIb trial19 (which did not reach significance), it failed to prevent HIV transmission in a larger phase III trial20. The lack of potency of linear polyanions for HIV and the ability of semen to further abrogate anti-HIV activity, may in part explain the lack of *in vivo* efficacy of these microbicides1. In addition, adherence to product use and transmission via anal receptive sex, which would not be prevented by a vaginally applied microbicide, are other considerations3.

Specific microbicides

The third major microbicide class comprises HIV-specific agents. Potentially, any agent that inhibits a critical step in the HIV replication cycle could be developed as a topical microbicide, although initial emphasis was focused on agents that act early in the virus life cycle. Early acting agents currently under preclinical development include entry inhibitors that target the HIV gp120 and gp41 envelope proteins (for example, griffithsin, fuzeon), agents that target host cell receptors required for HIV entry (for example, maraviroc, 5P12-RANTES), reverse transcriptase inhibitors (for example, dapivirine) and inhibitors of HIV integrase (for example, raltegravir)7,20. Inhibitors that block HIV protease, which act late in the virus life cycle to produce non-infectious virions, are also under preclinical development20. While specific agents are generally more potent HIV inhibitors than members of the other microbicide classes, considerations regarding their use include systemic toxicity due to absorption through the vagina and the emergence of drug-resistant strains. Combination microbicides, comprising agents that act by different mechanisms, are being pursued and are likely to delay the development of drug resistance. Additionally, they would be expected to be more potent compared to microbicides containing a single active pharmaceutical agent7,20.

The landmark CAPRISA 004 phase IIb study demonstrates for the first time that a gel containing 1% tenofovir (a reverse transcriptase inhibitor) prevents HIV acquisition in women with 39% efficacy compared to placebo gel21. While these findings need to be confirmed in a larger phase III clinical trial, it provides proof-of-concept that a topical microbicide gel can be effective in preventing HIV transmission. Sustained mucosal delivery of the reverse transcriptase inhibitor dapivirine via an intravaginal ring represents a coitally independent strategy, which can be exploited for the delivery of microbicides3.

Leading Australian microbicide efforts

In collaboration with the Australian biotechnology company Starpharma Pty Ltd, we have been undertaking the preclinical evaluation of dendrimer microbicides for the inhibition of HIV, HSV ad HPV22. Dendrimers (dendri- = tree, -mer = branching) are a relatively new class of macromolecule recognised as a key building block of nanotechnology. The controlled synthesis of dendrimers allows the assembly of highly defined structures that radiate out in branches from a central core. The type of core and branching units can be altered to generate dendrimers of varying size and shape (Figure 1). Dendrimer branches can be capped with different surface groups that impart distinct biological and pharmacological properties. We have performed structure activity relationship studies analysing dendrimers of different sizes and surface groups for their ability to inhibit HIV and HSV22. SPL7013, a fourth-generation dendrimer comprising a benzylhydryl amide core, lysine branches and naphthalene disulphonic acid surface groups (Figure 2) was the most potent in blocking HIV and HSV in cell culture assays. SPL7013 has broad-spectrum activity against different HIV-1 clades and HIV-2 by inhibiting viral attachment and entry (Figure 5) and is equally potent against CXCR4 and CCR5 using strains21. SPL7013 is active against HIV in cell culture assays in the presence of bodily fluids22 and inhibits cell-to-cell transmission and replication of HIV-1 in colorectal and cervical explants1,22.

Figure 1. Model representation of dendrimer structure showing the central core, branches in black, red and green for first (G1), second (G2) and third (G3) generations and surface groups denoted as blue round spheres. The image was obtained from Tyssen et al. 2010 PLoS ONE 5:e12309.
VivaGel® (SPL7013 Gel) is a formulated vaginal microbicide containing 3% w/w SPL7013 (3% w/w) in a mucoadhesive Carbopol®-based aqueous gel at pH 4.5. SPL7013 Gel demonstrates protection in animal models against SHIV and HSV and is not toxic in vivo. SPL7013 represents a promising microbicide candidate that could be combined with specific antiretroviral agents such as tenofovir, providing protection in the lumen and upper epithelial layers of the FGT while tenofovir, which requires intracellular activation, provides protection inside HIV target cells. While a microbicide should ideally be low cost, there is now a shift towards using specific antiretrovirals, which are likely to be more expensive to produce than the earlier non-specific microbicides. In this regard, a continuous supply of a dendrimer microbicide to developing countries would be facilitated by partnership with a pharmaceutical company that has the capacity to produce the antiviral in large quantities and with organisations such as WHO, UNAIDS or the GATES Foundation to subsidise the cost of the microbicide.

Other approaches we are pursuing include determining the potential for lactic acid, produced by lactobacilli sp. in the FGT, as a topical vaginal microbicide. Studies on the ability of lactic acid to inactivate microbes associated with bacterial vaginosis, HIV and HSV are in progress, together with assessing its potential to be combined with specific antiretrovirals. Lactic acid provides an alternative to the development of lactobacilli sp. as probiotics for delivery in the FGT where the ability to achieve adequate levels of lactic acid may be problematic since vaginal colonisation is adversely affected by vaginal intercourse and the presence of lactobacilli of the same species.

Figure 2. Chemical structure of SPL7013, a fourth-generation dendrimer comprising a benzylhydryl amide core, lysine branches and naphthalene disulphonic acid surface groups. The formula weight of SPL7013 is 16,582. The image was obtained from Tyssen et al. 2010 PLoS ONE 5:e12309.

Figure 3. Cartoon of dendrimers binding to the trimeric gp120 spikes on HIV that prevent infection of the target cell (in green). The image was provided by Starpharma Pty Ltd.
Future directions

Microbicides show considerable promise as one strategy in the HIV prevention toolbox that can empower women to protect themselves against HIV infection. While it is important that new agents are identified and progress through the microbicide development pipeline, there is a need for improved selection criteria for microbicides to enter the clinical pathway. Different delivery strategies and formulations such as gels, intravaginal rings, films and tablets need to be pursued to give women options for microbicide application. Adherence remains one of the main challenges for coitaly-dependent gels that may be overcome with the development of intravaginal rings. While antiretroviral agents show promise, the development of combination microbicides that act by different mechanisms which are either additive or synergistic in the inhibition of HIV, and that also have broad-spectrum activity against HSV and other STIs (which increase HIV transmission) would potentially be more potent and delay the emergence of drug-resistant HIV compared to a microbicide comprising a single agent. Development of novel antiretrovirals for use as microbicides that are not currently used in the treatment of HIV-infected individuals and that have different drug resistance profiles to the current therapeutic agents should be pursued to prevent transmission of drug-resistant HIV. Acceptability studies with different microbicides formulations should be performed as a first step in making available microbicides to women in the Asia-Pacific Region such as Papua New Guinea, where male to female HIV transmission is predominant. This is an exciting time in the HIV prevention field with many challenges ahead.

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References

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Movie link: http://www.starpharma.com/data/VivaGel08095_5282x240.wmv

Biography

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