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Progress and Perspectives on HIV-1 microbicide development

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ABSTRACT

The majority of HIV-1 infections occur via sexual intercourse. Women are the most affected by the epidemic, particularly in developing countries, due to their socio-economic dependence on men and the fact that they are often victims of gender based sexual violence. Despite significant efforts that resulted in the reduction of infection rates in some countries, there is still need for effective prevention methods against the virus. One of these methods for preventing sexual transmission in women is the use of microbicides. In this review we provide a summary of the progress made toward the discovery of affordable and effective HIV-1 microbicides and suggest future directions. We show that there is a wide range of compounds that have been proposed as potential microbicides. Although most of them have so far failed to show protection in humans, there are many promising ones currently in pre-clinical studies and in clinical trials.

Contents

1. Introduction

The World Health Organization estimated that over 30 million

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people are living with HIV/AIDS around the world and that the developing world, Africa in particular, remains the epicentre of the epidemic (http://www.who.int/gho/hiv/en/). In developing countries HIV-1 epidemic is characterized by a high proportion of infected women compared to men. This can be explained by women socio-economic dependence on men, domestic violence, high incidence of rape, cultural norms and women inability to negotiate safe sex practices, such as the use of condom, with their male partners (Elias and Coggins, 1996). These factors are among the driving forces behind the search for a female-controlled HIV-1 prevention method such as the use of microbicides, which are cervico-vaginally applied products that can prevent male to female transmission of the virus.

Many agents have been proposed as microbicide candidates to prevent HIV-1 infection at the site of mucosal transmission (Lederman et al., 2006). Some of these agents are specific inhibitors of HIV-1 infection while others are non-specific. The non-specific inhibitors include compounds that modify the cervico-vaginal environment (vaginal milieu protectors) such as buffering agents, those that interact with the positively charged viral envelope known as polyanionic polymers, and envelope inhibitors such as lectins. The specific inhibitors include antibodies and reverse transcriptase inhibitors such as tenofovir. Some HIV-1 microbicide candidates have already been tested in clinical trials while most are still in developmental stages. Here we review these compounds together with their potential modes of delivery.

2. Constitution of the female genital tract and male-to-female transmission of HIV-1

The female genital tract comprises the vaginal tract and the cervix; and the latter is divided into ectocervix and endocervix. Both the vaginal tract and cervix are covered by a stratified squamous epithelial cell lining starting in the vagina and ending at the ectocervix (Coombs et al., 2003; Shattock and Moore, 2003). This lining is made of five zones namely; the cornified, the condensation, the clear, the parabasal and the basal zone (Fig. 1). In contrast, the epithelial cell lining in the endocervical canal has only one layer, a structure that makes it vulnerable to disruptions such as those caused by sexual intercourse induced microtrauma (Miller and Shattock, 2003; Norvell et al., 1984). This being said, the epithelial cell lining in the vaginal tract and ectocervix is ~15 times larger than that found in the endocervix, thus, offering a larger surface area and more entry points for the virus (Hladik and McElrath, 2008).

In the vagina and cervix many HIV-1 target cells are found in and under the epithelial cell lining (Fig. 2A). These include Langerhans cells (LC), located within the lining and believed to play an important role in mediating the virus crossing of the genital mucosa (Shattock and Moore, 2003). Studies showed that after exposure intraepithelial LCs can internalize HIV-1 in their cytoplasmic compartments and migrate out of the epithelium, through the basal zone, to spread it to other sites (Ballweber et al., 2011; Hladik et al., 2007). Virus internalization by these cells appears not to be mediated by langerin as it is the case for epidermal LCs. The bypassing of this receptor during HIV-1 endocytosis may promote survival since langerin mediates the virus degradation in the Birbeck granules (de Witte et al., 2007; Savina et al., 2006). In addition to endocytosis LCs can be productively infected by the virus, although, the extent by which this occurs in the genital mucosa may be minimal (Ballweber et al., 2011; Kawamura et al., 2008). Significant populations of macrophages, known to be HIV-1 reservoirs (Abbas et al., 2015), are found in the sub-epithelium and lamina propria (Lederman et al., 2006; Shattock and Moore, 2003). Studies using human explants showed that macrophages are the main target of R5-tropic HIV-1 in the genital mucosa (Cummins et al., 2007; Greenhead et al., 2000). Furthermore, macrophages can trap the virus using the syndecans receptor or internalize it by micropinocytosis (Marechal et al., 2001). The sub-epithelium and lamina propria also host $CD4^+$ T cells that are as well found within the epithelial cell lining (Edwards and Morris, 1985; Johansson et al., 1999). As shown by McElrath et al. these cells are key HIV-1 targets in the outer epithelial cell lining (McElrath et al., 2010). Although within the genital mucosa $CD4^+$ T cells are located in close proximity to LCs studies showed that their initial infection is not dependent of these cells (Hladik et al., 2007). Most $CD4^+$ T cells in this tissue are memory T cells that express high level of the CCR5 receptor (Hladik et al., 1999b, 2007; Prakash et al., 2001). Lastly, dendritic cells (DCs) are also located in the sub-epithelium and lamina propria (Lederman et al., 2006; Miller and Shattock, 2003; Shattock and Moore, 2003). Genital mucosa DCs belong to the same family as LCs. However, HIV-1 entry in these two cell types may be mediated by different pathways since DCs express the CXCR4 and DC-SIGN receptors while LCs do not (Geijtenbeek et al., 2000; Hladik et al., 1999a; Jameson et al., 2002; Prakash et al., 2004). DCs can bind and internalize HIV-1 via the DC-SIGN receptor (Pohlmann et al., 2001a, 2001b) or they can be productively infected by the virus as studies in macaques showed (Hu et al., 2000, 1998; Spira et al., 1996). This was supported by observations that tissue biopsies of vaginal stroma from HIV-positive asymptomatic women contained infected DCs (Bhoopat et al., 2001). These cells disseminate the virus via transfer to $CD4^+$ T

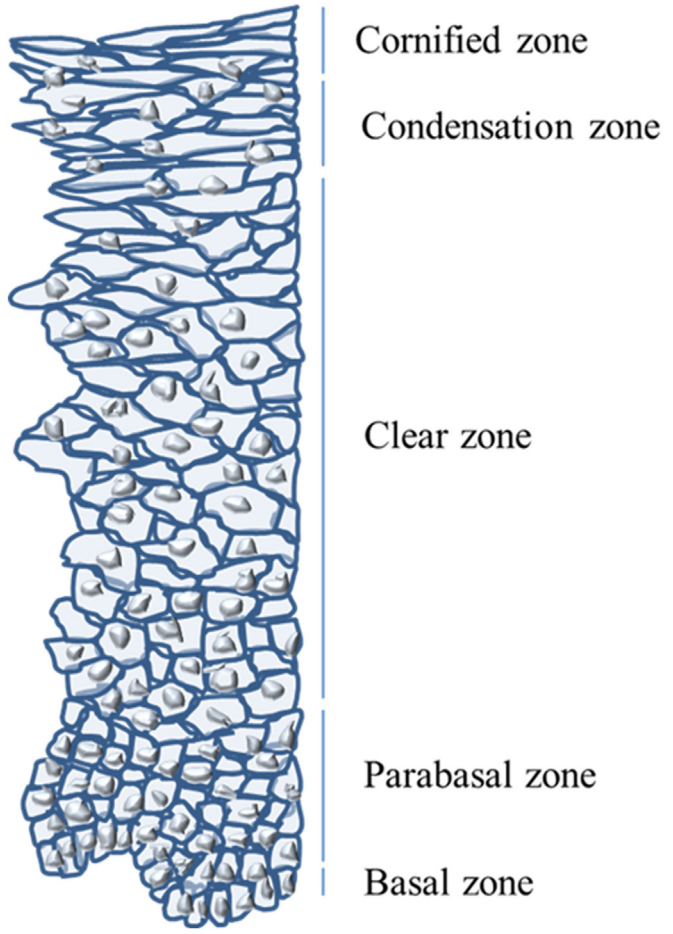

Fig. 1. Different zones of the stratified epithelial cell lining. The five zones of the epithelial cell lining found in the vaginal tract and ectocervix. Cells in the two upper-most zones have a flattened appearance. An intact epithelial cell lining does not allow particles the size of an HIV-1 virus to pass through.

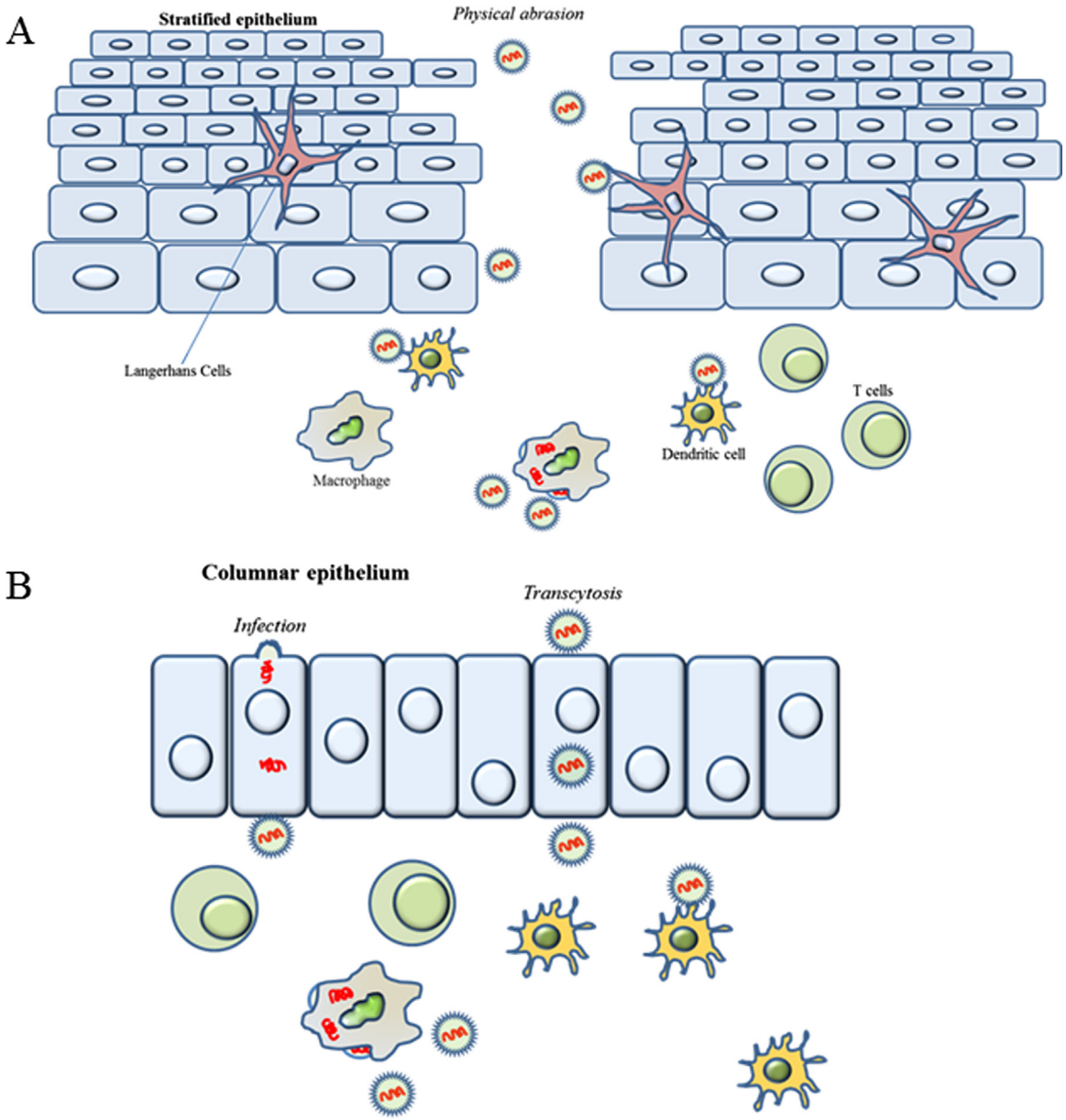

Fig. 2. HIV-1 transmission through the epithelial cell lining. (A) HIV-1 transmission through physical abrasion such as those caused by sexual intercourse induced microtrauma. (B) Mechanism of transmission by direct infection and transcytosis. Once in the sub-epithelium the virus can infect macrophages, $CD4^+$ T cells or bind dendritic cells that facilitate its systemic dissemination.

cells and this can be achieved through infectious synapses (Arrighi et al., 2004; Jolly and Sattentau, 2004, 2007; McDonald et al., 2003). DCs can also transfer the virus to $CD4^+$ T cells via contacts between the tips of these cells nanotubes (Hope, 2007; Sherer et al., 2007).

Most HIV-1 infections occur through the female genital tract that contributes ~12.6 million of cases worldwide (Hladik and McElrath, 2008). However, the probability of infection per exposure in this location is less than in other sites such as the rectum or upper gastrointestinal tract (Hladik and McElrath, 2008). The female genital tract has various defence mechanisms that can inactivate the virus or provide physical and chemical barriers against it. Amongst these are: (i) its acidic pH due to the presence of lactic acid produced by resident *lactobacilli* (Aldunate et al., 2013; O'Hanlon et al., 2013); (ii) the stratified structure of its epithelial cell lining (Coombs et al., 2003; Miller and Shattock, 2003), which is impenetrable to particles the size of HIV-1 (Marx et al., 1996; Shattock and Moore, 2003; Smith et al., 2000); and (iii) the mucus secreted by cervicovaginal epithelial cells that can prevent the virus from interacting with its target cells (Shukair et al., 2013) and contains anti-HIV-1 proteins such as the secretory leukocyte protease inhibitors, lactoferrin, human-β-defensin (HBD-1) and lysozyme (Cohen et al., 1987; Schumacher et al., 1977; Turpin, 2002). Lastly, endocervical cells express the CXCR4 ligand SDF-1 that is inhibitory to X4 HIV-1 viruses (Agace et al., 2000).

In females, heterosexual transmission of HIV-1 begins when cell-free or cell-associated viruses are released into the genital tract and infect target cells (Lederman et al., 2006; Stein, 2003). HIV-1 can be transmitted via both the cervix and vaginal tract. This is supported by the fact that cervical ectopy, caused by damage in the epithelial cell lining, has been associated with increased risk of infection (Miller et al., 1993); and the report of virus transmission in a woman suffering from the Meyer-Rokitansky-Küster-Hauser syndrome, a congenital disorder that results in the lack of a uterus (Kell et al., 1992). Moreover, there were observations that the inoculation of cell-free simian immunodeficiency virus (SIV) into the

vaginal tract of rhesus macaques resulted in a systemic infection (Miller et al., 1994). However, it is possible that HIV-1 infection predominantly occurs in the vaginal tract since a clinical trial showed that women who blocked the virus exposure to the cervix by use of a diaphragm did not have a significant reduction in transmission (Padian et al., 2007).

Different mechanisms have been proposed to explain how viruses released into the vaginal tract cross the epithelial cell lining to infect cells in the lamina propria and sub-epithelium. Among these, the breach of the genital mucosa during sexual penetration is widely accepted as the main route of infection (Fig. 2A) (Coombs et al., 2003; Miller and Shattock, 2003; Shattock and Moore, 2003). When breached, intraepithelial LCs get exposed to HIV-1 and migrate to the sub-epithelium and lymph nodes, to disseminate the virus (Miller and Shattock, 2003). The rupture of this lining can also directly expose sub-epithelial DCs to the virus resulting in subsequent dissemination (Piguet and Sattentau, 2004; Pohlmann et al., 2001a, 2001b). Other proposed mechanisms include the direct infection of epithelial cells and transcytosis, the passing of the virus through a cell without infecting it (Fig. 2B) (Shattock and Moore, 2003). HIV-1 has been reported to migrate through narrow gaps between epithelial cells to reach cells constituting the basal layer that are more susceptible to transcytosis (Maher et al., 2005). Among receptors that may promote the virus attachment and subsequent transcytosis are the two surface glycosphingolipids, sulphated lactosylceramide, found on vaginal epithelial cells, and galactosylceramide, expressed by ectocervical epithelial cells (Bomsel, 1997; Yeaman et al., 2004). However, it should be noted that transcytosis has been shown in cell lines and primary cells only but not conclusively in intact tissues (Bobardt et al., 2007; Bomsel, 1997). Furthermore, some studies suggested that primary cervical and vaginal epithelial cells do not get infected by HIV or transcytosis (Dezzutti et al., 2001; Greenhead et al., 2000).

3. HIV-1 microbicide candidates

3.1. Vaginal milieu protectors

During sexual intercourse the release of semen increases the pH in the female genital tract, which neutralizes vaginal acidity and renders the environment favourable for HIV-1 infection (Tevi-Benissan et al., 1997). Thus, buffering agents are designed to counteract this process. BufferGel™ or Carbopol 974 (BufferGel, ReProtect, Baltimore, MD, USA) is a carbopol polymer formulated with a buffering agent that has the ability to buffer twice its volume in semen to pH ≤ 5. This is a microbicide candidate designed to maintain the acidic pH of the vaginal tract. Although, clinical trials in India, Thailand, Malawi and Zimbabwe showed that BufferGel™ destroys bacteria that cause vaginosis (Turpin, 2002), this formulation has no effect on resident bacterial populations such as *lactobacilli*. BufferGel™ has already passed two phase I safety trials (Mayer et al., 2001). It also proved to be safe during a penile tolerance study with HIV-1 infected and uninfected men (Tabet et al., 2003). However, BufferGel was unable to prevent HIV-1 transmission during a clinical trial conducted in Southern Africa and USA (Abdool Karim et al., 2011). Like BufferGel™, Acidform™ is a buffering agent that was originally designed for use as a sexual lubricant (Cutler and Justman, 2008; Ndesendo et al., 2008). Acidform™ passed two phase I safety trials and one penile tolerance trial (Amaral et al., 1999, 2006; Schwartz et al., 2005). Currently there is a phase III trial underway with this buffering agent (Bayer and Jensen, 2014).

3.2. Surfactants

Surfactants solubilise bacterial and viral membranes, therefore, rendering them inactive. C31G or SAVVY® (Cellegy Pharmaceuticals, Quakertown, PA, U.S.A), made of cetyl betaine and myristamine oxide, is one of the well-known surfactants (Cutler and Justman, 2008). This compound most important characteristic is the ability to dissolve and spread quickly through the genital mucosa (Turpin, 2002). Besides its anti-viral activities, C31G is also a spermicide and this may limit its use in women who want to conceive or communities that stand against contraception (Calis et al., 1992; Krebs et al., 1999). Two phase III trials with C31G in Ghana and Nigeria were abandoned because of lower than expected rate of HIV-1 seroincidence in the selected populations (Cutler and Justman, 2008). Nonoxynol-9 (N-9) is also a well-known surfactant studied for use as HIV-1 microbicide. Two phase III trials were conducted with N-9 in Africa. One trial amongst sex workers showed no difference in the rate of HIV-1 infection between the study and the placebo group. However, this study found an association between N-9 and a higher incidence of genital ulcers among trial participants (Roddy et al., 1998). The other trial tested the use of 52.5 mg of gel-formulated N-9 that had to be applied before and after sexual intercourse. Contrary to the previous study this latter found a 50% increase in the risk of contracting HIV-1 in the study group (Statement on results of products containing N-9 from the CDC, 2000). The risk was even higher in women who reported a reduced use of condom. This was believed to be due to N-9 induced superficial de-epithelialisation, changes in genital microflora, high rate of petechial haemorrhage and erythemia. These findings were supported by Van Damme et al. randomised placebo controlled triple-blinded trial conducted in four African countries and Thailand (Van Damme et al., 2002). This study reported that enhanced use of N-9 increased the breach of genital epithelial cell lining as well as the risk of HIV transmission. This work, thus, gave the final verdict on the demerit of N-9 as a HIV-1 microbicide candidate. Another surfactant studied as microbicide is sodium lauryl sulphate, which is liquid at room temperature and converts into a gel that coats the vaginal tract at body temperature (Cutler and Justman, 2008; Piret et al., 2000). The results of a phase II trial conducted in Cameroon showed that this microbicide candidate could be used for extended periods without significant adverse effects (Mbopi-Keou et al., 2009). This was supported by another extended phase II safety trial, also in Cameroon, that found sodium lauryl sulphate safe and acceptable when used intravaginally twice daily for two months (Mbopi-Keou et al., 2010).

3.3. Polyanionic polymers

Polyanionic polymers inhibit infection by interacting with the positively charged V3 loop of HIV-1 gp120 (Doms, 2000; Doms and Trono, 2000; Turpin, 2002). The binding of these compounds to the virus is enhanced by the conformational changes that unmask charged regions of gp120 during entry into target cells (Doms and Trono, 2000). Naphthalene sulfonate (PRO2000; Indevus Pharmaceuticals, Lexington, MA, USA) and the seaweed derived sulphated polysaccharide carrageenan (Carraguard/515, Population Council, New York, NY, USA) are the most studied polyanionic polymers (Cutler and Justman, 2008; Turpin, 2002). PRO2000 passed a phase I safety trial (Van Damme et al., 2000) but failed a phase III efficacy trial in Southern Africa and the United States (Abdool Karim et al., 2011; McCormack et al., 2010; Ramjee, 2010). Carraguard showed anti-HIV activities in a mouse model and proved to be safe in a phase I trial conducted with both HIV-positive and negative individuals (Bollen et al., 2008; Coggins et al., 2000; Perotti et al., 2003; van de Wijgert et al., 2007).

However, like PRO2000, it failed a phase III trial (Skoler-Karpoff et al., 2008). It was speculated that poor adherence to carraguard by trial participants might have contributed to the observed lack of efficacy. Cellulose sulphate (Ushercell, Polydex Pharmaceuticals, Toronto, ON, Canada and Topical Prevention of Conception and Disease [TOPCAD], Chicago, IL, USA) is also among polyanionic polymers studied as potential HIV-1 microbicides due to its strong anti-viral activities against both X4 and R5 HIV-1 viruses (Scordi-Bello et al., 2005). However, although a phase I trial showed cellulose sulphate to be safe for use in humans (Malonza et al., 2005) the results of a phase III trial indicated that there was a slightly higher incidence of infection in the cellulose sulphate group compared to placebo (Van Damme et al., 2008); and this led to it being abandoned as a microbicide candidate. Cellulose acetate phthalate (CAP) is a polyanionic polymer that binds HIV-1 gp120 resulting in the formation of the hybrid six helix conformation, an intermediate in the virus entry (Melikyan et al., 2000). However, this six-helix bundle is premature and non-functional. CAP also has a unique ability to synergize with soluble CD4 (sCD4) to inhibit HIV-1 infection (Neurath et al., 2002). These compounds where shown to enhance each other's binding to the viral envelope as well as to induce the six-helix bundle by cooperativity. A major limitation of CAP is that it also increase infection of HIV-1 R5 strains *in vitro* (Tao et al., 2008). In addition, many women in the CAP gel acceptability study complained of heavy discharge (Microbicides 2008 Conference, New Delhi, India, Abstract No. 527).

Betacyclodextrin (BCD) is a polyanionic polymer that depletes cholesterol from lipid rafts found in the cell membrane and HIV-1 envelope (Graham et al., 2003; Kilsdonk et al., 1995). Given that the CD4 receptor is located in these rafts, an interference with their integrity inhibits viral entry (Liao et al., 2001; Popik et al., 2002). The main advantages of using BCD as a microbicide include its low cost and the fact that it is already used as a carrier for other FDA-approved drugs (Bibby et al., 2000). A study in rhesus macaques showed that BCD is protective against SIV challenge (Ambrose et al., 2008). However, this compound failed to protect the same animals when challenged for the second time.

Dendrimers are the newest members of the polyanionic polymer family. These compounds are able to bind multiple locations on different cells at the same time (Bourne et al., 2000). Dendrimers are made of a core, interior branches and terminal surface groups for selective interaction with specific targets (Bourne et al., 2000). SPL7013 is a dendrimer gel (Vivagel, Starpharma Holdings Ltd., Melbourne, Australia) that showed protection against the simian/human immunodeficiency virus (SHIV) chimera in a macaque model (Jiang et al., 2005). A 3% formulation of this gel also showed safety in a phase I trial in USA (McGowan et al., 2011). G3-S16 and G2-NF16 are sulphated and naphthylsulfonated functionalized carbosilane dendrimers that are as well being investigated for potential use as microbicides (Vacas-Cordoba et al., 2016; Vacas Cordoba et al., 2013). These relatively new dendrimers act by inhibiting HIV-1 binding and fusion with target cells. They also inhibit cell-to-cell transmission of the virus.

3.4. Reverse transcriptase inhibitors

Reverse transcriptase inhibitors (RTI) interfere with the conversion of the viral RNA into DNA. This group of drugs include nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI). Tenofovir is a NRTI that mimics an adenosine nucleotide (De Clercq, 2007) and once incorporated into the nascent HIV-1 cDNA prematurely terminates chain elongation. This drug has a cellular half-life of 9–50 h depending on the cell type and can be effective in non-dividing cells such as macrophages that have limited phosphorylation ability (Aquaro et al., 2002; Balzarini et al., 1991; Robbins et al., 1998). A study using macaques showed that pre- and post-exposure prophylaxis with tenofovir protects against intravenous challenge with SIV (Tsai et al., 1995). This report was among the first to provide evidence that tenofovir could be used for HIV-1 prevention. Since then tenofovir has become one of the leading *anti*retrovirals in HIV-1 microbicide research. A clinical trial conducted in South Africa, using 1% tenofovir gel within 12 h before and after sex (CAPRISA 004 trial), showed protection against heterosexual transmission of the virus (Abdool Karim et al., 2010). More precisely, after 30 months of trial the gel prevented transmission by 39%. The study also revealed that women with higher adherence to the gel were better protected compared to those with low adherence. HIV-1 RNA genotyping of women who got infected during the trial did not reveal the presence of tenofovir resistance mutations suggesting that vaginal application of the gel did not give rise to resistant viruses (Abdool Karim et al., 2010). However, it is not yet known what will result after a longer term use of the drug. The VOICE trial using the same formulation of tenofovir concluded that it did not protect against the sexual transmission of HIV-1 although this trial also reported poor adherence among participants (Marrazzo et al., 2015; Quinones-Mateu and Vanham, 2012). Lastly, the FACTS 001 trial in South Africa, enrolling 2, 900 women between the ages of 18 and 40 years old (www.facts-consortium.co.za), aimed at confirming the CAPRISA 004 results also reached the conclusion that tenofovir did not prevent HIV-1 transmission (Helen Rees presentation at CROI 2015: Tenofovir Vaginal Gel Not Effective Overall Against HIV). However, similar to CAPRISA 004, this study reported an association between higher adherence and stronger protection against the virus. Nonetheless, a recent study by Hladik et al. (2015) reported potential toxic effects associated with the use of 1% tenofovir microbicide gel. Using vaginal and rectal mucosal tissues, these researchers showed that the gel suppressed anti-inflammatory mediators, increased T lymphocytes infiltration of the mucosa, and induced mitochondrial dysfunction. In addition, tenofovir promoted the expression of genes associated with growth of cancer cells, KIAA0101 and UBD (Hladik et al., 2015; Jain et al., 2011). The toxic effects were observed only with high concentrations of the drug and not with low ones such that are taken orally.

The thiocarboxanilide derivative UC-781 is a NNRTI characterized by high affinity for HIV-1 reverse transcriptase. Cells treated with UC-781 *in vitro* showed long term or “memory” protection against HIV-1 (Balzarini et al., 1998; Barnard et al., 1997; Borkow et al., 1997). Also UC781 is very hydrophobic, thus, able to easily penetrate the viral envelope and enter the capsid to bind reverse transcriptase even before entry into target cells (Borkow et al., 1997; Zussman et al., 2003). A phase I trial evaluating a once-daily dose of this compound for six days proved that it was safe for use in humans (Schwartz et al., 2008). Recently, McConville et al. investigated the use of a vaginal ring for controlled release of UC-781 (McConville et al., 2015). The ring was able to release enough UC-781 capable of preventing the sexual transmission of HIV-1. Moreover, their study in pigtailed macaques showed no toxicity associated with this drug. Other NNRTI microbicide candidates include SJ-3366 and TMC120 (Dapivirine). SJ-3366 is a homocyclic pyrimidinedione that has a dual mode of action against HIV-1, it inhibits both viral entry and reverse transcription (Turpin, 2002). Dapivirine is the only microbicide candidate thus far to be tested in humans as a vaginal ring (Gupta et al., 2008; Nel et al., 2016; Spence et al., 2016; Woolfson et al., 2006). Phase 1 and 2 trials with once monthly dapivirine vaginal ring application proved to be safe and genital tissue biopsies of women using this drug showed protection against *ex-vivo* challenge with HIV-1 (Chen et al., 2015; Devlin et al., 2013; Nel et al., 2014). More recently, a phase III clinical trial with dapivirine vaginal ring, conducted in Uganda, Malawi, Zimbabwe and South Africa (MTN-020-ASPIRE

trial), reported a 27% reduction in the sexual transmission of the virus (Baeten et al., 2016). Similar to CAPRISA 004 and FACTS trials (Abdool Karim et al., 2010) (Helen Rees presentation at CROI 2015: Tenofovir Vaginal Gel Not Effective Overall Against HIV), higher adherence corresponded to higher protection against viral transmission. Moreover, the MTN-020-ASPIRE trial reported a significant difference in protection and adherence between age groups with a lack of protection and low adherence observed for women in the 18-21 years old age group while a 56% protection and adherence of over 70% were recorded for those older than 21 years of age. A second, dapivirine vaginal ring phase III trial is currently underway (Baeten et al., 2016).

3.5. Proteins

Different proteins are being investigated for use as HIV-1 microbicides (Lederman et al., 2006). These are essentially entry inhibitors with varying modes of action against the virus. Some of these proteins bind glycosylated and non-glycosylated regions of the viral envelope while others interact with receptors on target cells that are critical for infection. Griffithsin (GRFT) is a lectin, carbohydrate binding protein, isolated from the red alga *Griffithsia sp*. (Mori et al., 2005). This lectin is studied for use as a microbicide due to its potent and broad activity against the virus (Alexandre et al., 2010). GRFT is non-toxic to human cells and can be produced in large quantities in plants (Kouokam et al., 2011; O'Keefe et al., 2009). In addition, GRFT has the ability to synergize with other HIV-1 inhibitors such as tenofovir (Alexandre et al., 2011; Ferir et al., 2012, 2011) suggesting that it can be combined with these compounds in a single more potent microbicide formulation. Due to GRFT potency and efficacy there are on-going discussions about testing it in human clinical trials. Cyanovirin-N (CV-N) and scytovirin (SVN) are also lectins, and like GRFT have shown potent and broad inhibitory activities against HIV-1 (Alexandre et al., 2010). Studies by Tsai et al. showed that a CV-N gel was effective in protecting pigtailed macaques against SHIV 86.9P after vaginal and rectal challenges (Tsai et al., 2004, 2003). However, there have been conflicting reports on CV-N toxicity to human cells. Some studies reported it to be toxic while others did not (Alexandre et al., 2010; Balzarini et al., 2006). Large scale production of CV-N in plants has been recently demonstrated, thus, promoting its use as a microbicide (Vamvaka et al., 2016). Thus far, SVN studies have been conducted exclusively *in vitro*.

PSC-RANTES potently binds the CCR5 co-receptor to inhibit HIV-1 entry (Lobritz et al., 2013). This makes it an attractive microbicide candidate given that the sexual transmission of HIV-1 is almost always initiated by R5 viruses. *In vitro*, this compound inhibits all subtypes of the virus (Torre et al., 2000) and *in vivo* a high dose of PSC-RANTES was found to protect macaques against SHIV SF162 challenge (Lederman et al., 2004). Another potential protein-based microbicide is the recombinant tetravalent construct CD4-IgG2 or PRO-542 that is designed to bind the CD4 binding site on gp120 and inhibit its interaction with the CD4 receptor (O'Hara and Olson, 2002). Protein microbicide candidates also include the chimeric protein syndecan-Fc, an inhibitor of HIV-1 binding to the syndecan-1 receptor expressed on macrophages (Bobardt et al., 2010); thrombospondin type I (TSP), a plasma protein that blocks viral entry; and the pokeweed antiviral protein (PAP) that inhibits HIV-1 replication.

Some monoclonal antibodies isolated from HIV-positive patients have demonstrated potent and broad anti-HIV-1 activities that led to their inclusion among microbicide candidates. These include b12, 4E10, VRC01, 2F5 and 2G12 (Veazey et al., 2003; Veselinovic et al., 2012). Some of these antibodies have been successfully produced in plants and shown to retain their anti-viral activities (Ramessar et al., 2008; Rosenberg et al., 2013). This is important since a suitable microbicide needs to be produced in large quantity and at a low cost.

Finally, PIE-12, C52L, RC-101, T20 and T1249 are peptides specific to the viral gp41 glycoprotein. These compounds have potent anti-HIV-1 fusion activities and their suitability as microbicides is being investigated (Pozzetto et al., 2012; Sassi et al., 2011; Veazey et al., 2005; Welch et al., 2010).

3.6. Small molecules

Small molecules that are being studied for use as HIV-1 microbicide include CMPD167, a CCR5 inhibitor that showed strong protective effects against SHIV in macaques (Veazey et al., 2005). No irritation of the genital tract or toxicity was observed with the use of this compound. In addition, the combination of CMPD167, BMS-378806 that binds gp120 and C52L, a gp41 binder, resulted in a synergistic inhibition of SHIV infection of macaques. Maraviroc is a potent inhibitor of CCR5 interaction with HIV-1 (Maeda et al., 2012). Its formulation as an aqueous gel or vaginal ring protected macaques against SHIV acquisition (Malcolm et al., 2013, 2012). Moreover, Maraviroc combination with a T-cell based vaccine significantly reduced both SHIV-SF162P3 infection and post-infection viral load (Barouch et al., 2012). A potential drawback with maraviroc use as a microbicide is that it is already used clinically to treat HIV-1 infection. Its application as a microbicide in infected individuals may give rise to resistant strains.

3.7. Nucleic acids

Aptamers are synthetic, single-stranded DNA and RNA molecules that fold into unique 3-D structures to allow high affinity binding to target molecules (Ellington and Szostak, 1990; Proske et al., 2005; Temme and Krauss, 2015; Zhang et al., 2004). These oligonucleotides are synthesized through systematic evolution of ligands by exponential enrichment (SELEX) to virtually recognize any class of targets (Bunka and Stockley, 2006; Ellington and Szostak, 1990; James, 2001; Jayasena, 1999; Proske et al., 2005; Tuerk and Gold, 1990; Zhang et al., 2004). This makes aptamers attractive as potential HIV-1 microbicides given that they can be made to bind any site on the virus.

We previously isolated 2′-fluoro-substituted RNA aptamers against recombinant HIV-1_{BaL} gp120 and showed that they neutralized infection of group M (subtypes A, C, D E and F) and group O R5 HIV-1 (Khati et al., 2003). We also showed that derivatives of one of these aptamers, called UCLA1 and UCLA005, were potent inhibitors of the virus (Cohen et al., 2008; Dey et al., 2005; Mufhandu et al., 2012). In addition, UCLA1 exhibited synergism with other anti-HIV-1 compounds such as T20 and the monoclonal antibody b12 (Mufhandu et al., 2012). Some researchers have conjugated aptamers to different inhibitory molecules to improve activity. For example Wheeler and colleagues engineered CD4-aptamer-siRNA chimeras (CD4-AsiCs) that potently inhibit HIV-1 (Wheeler et al., 2011). The aptamer in CD4-AsiCs, binds the V4 domain of gp120, the CD4 portion blocks gp120 interaction with the CD4 receptor, while the siRNA inactivates Gag, Vif and CCR5 to block the expression of genes required for viral replication (Kraus et al., 1998; McNamara et al., 2006; Wheeler et al., 2011). CD4-AsiCs inhibited HIV replication in macrophages (MDMs) and could penetrate the vaginal epithelium to knock down viral gene expression in $CD4^+$ T cells (Wheeler et al., 2011). The topical use of this compound was also reported to protect humanized mice, NSG-BLT mice, against vaginal challenge with HIV-1 (Wheeler et al., 2013).

4. HIV-1 microbicides delivery systems

The vagina and cervix are made of various natural defences that need to be taken into consideration when designing a microbicide delivery system as they can compromise the efficiency of delivery. For example the secretion of vaginal fluids can lead to leakages that may decrease drug retention time; and the presence in the vaginal mucosa of enzymes such as aminopeptidase (Acarturk et al., 2001) can cause the degradation of peptides and proteins. Below are the main delivery systems studied to date.

4.1. Gels

Semisolid gels are the most commonly used HIV-1 microbicide delivery systems (Abdool Karim et al., 2010; Elias and Coggins, 1996; Van Damme et al., 2000). Often they are based on water soluble polymers such as the cellulose derivative hydroxyl ethyl cellulose or the polyacrylic acid derivative carbopol 974 (das Neves and Bahia, 2006; Jarrett et al., 2016). Gels have the advantage of being simple to use and show high consistency of drug delivery (das Neves and Bahia, 2006; Ndesendo et al., 2008) since they can spread easily. Also their viscosity and elasticity enhance drug stability and retention. However, one of their most important disadvantages is the tendency to be messy and leaky resulting in low adherence (Primrose et al., 2016). Gels are mainly designed for application before sexual intercourse, although, they can also be used after sex (Abdool Karim et al., 2010).

4.2. Vaginal rings

Vaginal rings, containing one or more anti-HIV-1 compounds, are used for controlled release of drugs into the genital tract over long periods of time (Rohan and Sassi, 2009). These rings can consist of polymers such as poly (dimethylsiloxane), silicon, ethylene vinyl acetate and styrene butadiene. One of the key advantages of vaginal rings is that they provide an alternative to users sustained adherence since once inserted into the genital tract they continuously release the drug for long durations without requiring intervention (Baeten et al., 2016). Furthermore, vaginal rings are already used to deliver hormones and contraceptives into the female genital tract (Thurman et al., 2013). However, the most significant limitations of this system are its cost, since vaginal rings are complex and expensive to manufacture, and the fact that some compounds have limited diffusion through the polymeric matrix (Malcolm et al., 2010).

4.3. Vaginal films

Perhaps a solution to the messy and leaky characteristics of gels is the use of vaginal films which are applied in much smaller quantities. Because of this, vaginal films have a higher likelihood of acceptability among women than gels (Elias and Coggins, 2001). Films dissolve very rapidly once in contact with vaginal fluids allowing for a faster release of drugs. In addition, their application does not require the use of an applicator, thus, making them inherently less expensive than gels. The drawback with this system, however, is the fact that vaginal films have low overall mass that is limiting for anti-viral agents requiring high dosage (Akil et al., 2011; Mahalingam et al., 2011; Sassi et al., 2011). Moreover, there is a potential for physical abrasion from films sharp corners and edges.

4.4. Tablets and suppositories

Tablets and suppositories are designed to release the microbicidal agent by melting in the genital tract. With these systems the microbicide can be delivered over several hours. Tablets are often formulated with mucoadhesive polymers to increase their retention time (Ndesendo et al., 2008). Recently, McConville et al. designed a multi-layered tablet capable of releasing, at independent rates, the antiretroviral dapivirine, the contraceptive hormone levonorgestrel and the herpes simplex virus 2 inhibitor acyclovir (McConville et al., 2016). The main advantage of using tablets and suppositories is that they are already employed to deliver intravaginal drugs. For example, cervical ripening prior to child birth is achieved by use of the misoprostol suppository and prostaglandin E2 tablet (Khoury et al., 2001; Taher et al., 2011). However, the disadvantage of using this system is the possibility of leaving granny residues in the vaginal tract following dissolution (Garg et al., 2003).

4.5. Nanoparticles

The nanoparticles delivery system involves attaching an organic particle or noble-metal such as silver to an anti-HIV-1 molecule to be delivered into the genital tract. One of this system advantages is the ability to protect the attached compound against biodegradation and to facilitate its penetration into HIV-1 susceptible sites. PSC-RANTES has been studied for delivery via nanoparticles (Ham et al., 2009). Using human ectocervical tissues Ham et al. showed that PSC-RANTES encapsulated into nanoparticles had a 4.8 times greater uptake than its nonencapsulated counterpart (Ham et al., 2009). Also Lakshmi et al. studied lactoferrin nanoparticles carrying the anti-HIV-1 drug efavirenz and the anti-microbial-spermicidal curcumin for use as microbicide (Lakshmi et al., 2016). This formulation did show inhibitory activities against HIV-1 and no toxicity to vaginal tissues and resident *Lactobacilli*.

4.6. Electrospun fibers

Electrospun fibers are a relatively new platform for delivery of HIV-1 microbicides (Ball et al., 2012; Blakney et al., 2013; Huang et al., 2012). These are polymers generated by electrospinning that can be engineered to incorporate multiple agents via composites and to facilitate controlled release of anti-viral drugs for periods ranging from 15 min to several days (Blakney et al., 2013). Moreover, electrospun fibers can be made in various forms and mechanical properties allowing for user centered designs. The activities of electrospun fibers loaded with tenofovir dipivoxil fumarate, etravirine, and maraviroc have been studied *in vitro* (Ball et al., 2016; Ball et al., 2012; Ball and Woodrow, 2014; Huang et al., 2012). However, the retention of these fibers in the vaginal vault and toxicity to the genital mucosa require further investigation.

5. Conclusion

There are many compounds targeting different stages of HIV-1 life cycle that are being studied for use as microbicides. However, the critical challenge with these studies is the progress from *in vitro* to human trials. The implementation of these trials is often slow; one of the main reasons being high costs. There are also socio-economic and cultural challenges that come with these studies (MacQueen et al., 2016; Mensch et al., 2016). All these challenges will inevitably have a negative impact on progress in HIV-1 microbicides research. Therefore, the onus is on governments, business leaders, non-governmental organizations, scientists, and community leaders to fund microbicide research and/or help educate populations where trials are to be conducted in order to accelerate their implementations. Here, studies similar to the one conducted by Primrose et al., on drivers of vaginal drug

delivery system acceptability, will be helpful (Primrose et al., 2016). Such studies should be conducted within each population targeted for trials in order to determine beforehand which microbicide vehicle and application routines are likely to have high acceptability.

In future microbicides research envelope inhibitors and other anti-HIV-1 compounds not used for the treatment of infection should be given more prominence. It is a genuine concern that the use of antiretrovirals as microbicides may in the long term give rise to resistant viruses. This threatens to render the primary use of these drugs ineffective. Lectins are among the most promising envelope inhibitors studied as potential HIV-1 microbicides. Many of these compounds have a broad spectrum of activity against the virus, showed no toxicity to human cells (Alexandre et al., 2010; Kouokam et al., 2011), have a high genetic barrier to resistance (Alexandre et al., 2010, 2013) and are cheap to produce (O'Keefe et al., 2009; Vamvaka et al., 2016). It is, therefore, important that there be more support and diligence in getting these molecules into animal and human trials.

Aptamers can inhibit their targets through interactions that are superior, in specificity and affinity, than many biologics and small molecules (Nimjee et al., 2005; Shum et al., 2013). They also do not have the toxicity and immunogenicity associated with some of these agents. However, research with these molecules as microbicides is not as advanced as it is for other compounds. Thus, more financial and human resources need to be channelled to studying these highly flexible and potent compounds for use as HIV-1 microbicides.

In future, more animal and human trials with microbicide formulations combining different classes of anti-HIV-1 molecules should be conducted. Such formulations may provide better protection against the virus than a single compound. This is supported by several *in vitro* studies that showed synergistic effects between some HIV-1 inhibitors (Ferir et al., 2011; Tremblay et al., 2000). For example, we previously reported that the microbicide candidate GRFT and the b12 antibody act synergistically to inhibit HIV-1 (Alexandre et al., 2011). Also a microbicide formulation containing maraviroc and reverse transcriptase inhibitors resulted in enhanced activities of maraviroc and inhibited viruses that are resistant to this drug in cellular and colorectal explant models (Herrera et al., 2016). Furthermore, given the high rate of mutations in the HIV-1 genome, whenever and wherever microbicides are rolled out, we will inevitably reach the stage where we are compelled to use more than one inhibitor in formulations to achieve effective prevention. Thus, it is better for combined microbicides formulations to be investigated in trials now than wait until the need for them arises.

Zirafi et al. reported that human semen can reduce the inhibitory activities of some anti-HIV-1 compounds (Zirafi et al., 2014). More precisely this study showed semen exposed viruses had 10–12 fold decrease in sensitivity to protease, integrase and reverse transcriptase inhibitors, including tenofovir. This may be one of the reasons why compounds that looked promising *in vitro*, or even in animal trials, failed to protect during human trials (Marrazzo et al., 2015; Tsai et al., 1995). It is, therefore, imperative that all microbicide pre-clinical studies incorporate testing in the presence of human semen.

To date, HIV-1 microbicides have been delivered mainly as gels. However, given the negative effects that poor adherence has had on many trial outcomes (Abdool Karim et al., 2010; Marrazzo et al., 2015) it is important that delivery by vaginal ring be more frequently used in clinical trials. These rings are the easiest and most effective solution to poor adherence (Baeten et al., 2016; Nel et al., 2016; van der Straten et al., 2016). Research on tablets, suppositories and vaginal films must also be accelerated since these systems can solve the problem of leakage, a key determining factor for acceptability and consequently adherence (Primrose et al., 2016). Ultimately the ideal would be for the same microbicide to be formulated in more than one delivery system to give the user a greater choice range. This is better illustrated by the availability of dapivirine gel, film and ring (Baeten et al., 2016; Bunge et al., 2016).

Regardless of challenges faced by HIV-1 microbicide research, advances already made and the fact that microbicides potentially offer the most effective alternative to the yet to be discovered HIV-1 vaccine, strongly support our continued and increased investment in this field.

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