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The importance of the vaginal delivery route for antiretrovirals in HIV prevention

Lindsay M Ferguson^{1,2} and Lisa Cencia Rohan^{1,2,3,*}

¹Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA, USA

²Magee-Womens Research Institute, 204 Craft Avenue, Pittsburgh, PA 15213, USA

³Department of Obstetrics, Gynecology, and Reproductive Sciences, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

Abstract

The HIV/AIDS pandemic continues to be a global health priority, with high rates of new HIV-1 infections persisting in young women. One HIV prevention strategy is topical pre-exposure prophylactics or microbicides, which are applied vaginally or rectally to protect the user from HIV and possibly other sexually transmitted infections. Vaginal microbicide delivery will be the focus of this review. Multiple nonspecific and specific antiretroviral microbicide products have been clinically evaluated, and many are in preclinical development. The events of HIV mucosal transmission and dynamics of the cervicovaginal environment should be considered for successful vaginal microbicide delivery. Beyond conventional vaginal formulations, intravaginal rings, tablets and films are employed as platforms in the hope to increase the likelihood of microbicide use. Furthermore, combining multiple antiretrovirals within a given formulation, combining a microbicide product with a vaginal device and integrating novel drug-delivery strategies within a microbicide product are approaches to successful vaginal-microbicide delivery.

HIV affects over 33 million people worldwide. Close to 3 million new HIV infections and 2 million AIDS-related deaths occur each year. Leading the world in HIV incidence rates, sub-Saharan Africa accounted for 71% of new infections in 2008 [1]. Sexual transmission remains a leading cause of HIV infection and females, especially African women and girls, are disproportionately affected by this disease accounting for approximately 60% infections in sub-Saharan Africa. This feminization of the HIV/AIDS pandemic is fueled by the fact that females are at a greater infection risk than men, with young females at the most risk. Physiological susceptibility and social, legal and economic disadvantages make women more likely to become infected [1]. Prevention of this life-threatening disease is critical in order to change the course of this pandemic. Abstinence, reduction of the number of sexual partners and concurrent sexual relationships, and correct, consistent condom use are highly effective against HIV acquisition but have proven to be insufficient to combat this incurable

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*Author for correspondence: Tel.: +1 412 641 6108 Fax: +1 412 641 6170 rohanlc@upmc.edu.

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disease [2–5]. Development of safe, effective and acceptable female controlled-prevention methods, specifically those applied vaginally, will play a major role in reducing the incidence of HIV-1 transmission.

Mucosal transmission of HIV

Male-to-female sexual transmission accounts for the greatest percentage of HIV-1 infection, yet HIV is not easily acquired via this route with an estimated transmission incidence between discordant couples to be 0.0005–0.0050 for a sexual act [6]. Several factors drive the susceptibility and likelihood of vaginal transmission including the duration of viral shedding, genital health, a high frequency of sexual intercourse and compromise to the mucosal lining. Genital abrasions, lesions, ulcerations and inflammation caused by various sexually transmitted infections (STIs) and/or other vaginal infections or conditions may increase the risk of HIV-1 transmission. Additionally, hormonal status, nutrient levels or the use of certain vaginal preparations may increase a female's vulnerability to infection [7]. Determining the true male-to-female transmission risk is not achievable due to the multitude of possible variables involved. Therefore, as stated by Shattock *et al.*, any HIV prevention strategy must assume that each sexual contact has the ability to transmit viruses [7].

Upon deposition of HIV-containing seminal fluid within the vagina, infected cells or free viruses may become trapped within the vaginal fluid, specifically within the cervical mucus [8,9]. This entrapment may allow longer contact time of infected cells or free virus with the mucosa. Conversely, the mucus entrapment may halt transmission by the cells and viruses and further increase the likelihood of attack by innate antiviral substances [10].

Male-to-female sexual transmission of HIV-1 may occur via several mechanisms. Uncertainty remains as to whether these pathways collectively or individually explain transmission [7,10,11]. HIV can be transmitted through both the multilayered squamous epithelium of the vagina and ectocervix and the single layer columnar epithelium of the endocervix (Figure 1) [6,12].

Despite stratified mucosal epithelial cells being CD4 negative, Langerhans cells and T lymphocytes, which reside in this area are CD4 positive, marking themselves as targets for HIV-1 [7,11,13]. The virus can penetrate this mucosa via thin gaps between the squamous epithelial cells [10]. This route may result in the virus coming into direct contact with Langerhans cells and T cells. Breaches in the epithelium may allow the virus to contact the deeper, basal epithelial cells that are vulnerable to viral binding, endocytosis and transcytosis. These breaches may also allow the virus access to subepithelial targets within the stroma [7]. The stromal tissues are densely populated with dendritic cells (DCs), macrophages and T cells that express CD4 and chemokine co-receptors, CCR5 or CXCR4 [14,15]. DCs also express C-type lectin receptors, including DC-SIGN [16]. Each is vulnerable to HIV-1 infection. Once HIV-1 gains contact with these target cells, establishment of infection is possible [7]. Additionally, inflammation may facilitate HIV-1 infection by thinning and disrupting the cellular lining, recruiting a pool of target cells for local HIV-1 expansion, and interfering with innate antimicrobial activity [17]. After HIV expands locally, dissemination of infection occurs. Movement of virus to lymph nodes and secondary lymphoid organs generates a systemic infection. Given the role of heterosexual transmission in the spread of HIV, the susceptibility of the cervicovaginal mucosa, and the fact that women have a higher vulnerability to infection than men, the vagina provides an optimal route for HIV-1 prevention therapy.

Vaginal anatomy & physiology

The vagina is situated between the rectum, urethra and bladder, and is the gateway from the vulva, the outer genitalia, to the cervix, the opening of the uterus, which is a central organ of the reproduction in a female (Figure 1) [18]. The vagina provides several important functions within the female body. These include facilitating the entrance of seminal fluid, providing an outlet for menstrual fluids during menstruation, providing structure for the lower portion of the birth canal and providing an opening for internal examination of the female reproductive system [19].

The vagina is a fibromuscular tube that exists in a relaxed state, with the anterior and posterior walls being slack and remaining in contact with each other, yet it is easily distensible. The lateral walls of the vagina are fairly rigid, and the vagina is characterized as a potential space but not an actual open space. In a woman standing upright, the vagina is similar in shape to a convex curve, with the upper portion of the vagina being almost at a right angle to the lower portion [18–20].

The dimensions of the vagina vary greatly from woman-to-woman, depending on sexual arousal and age. In a reproductive aged woman, the anterior vaginal wall averages a length of 6–8 cm. The posterior wall can reach a length of 14 cm, with the length of the cervix included [21]. The vaginal diameter ranges from 2.4–6.5 cm, and the largest diameter is achieved at a vaginal depth of 2 to 5 cm from the introitus (vaginal opening) [21]. The surface area of the vagina ranges from 65.73 to 107.07 cm², with a mean of 87.46 cm² [22]. These surface area data do not include the area of the vaginal rugal folds, therefore, representing an underestimation of true surface area.

The shape of the vagina also varies from woman to woman and can be categorized as parallel sides, conical, heart, slug and pumpkin seed [21,23]. When compared among races, the pumpkin seed shape was found to be specific to African-American women [23]. These varying vaginal dimensions, sizes and shapes may be reflected in the acceptability of certain vaginal products, such as gels and tablets, and may build justification for the need of multiple vaginal dosage forms for HIV prevention products. Furthermore, given the variability of the vagina among women, design of products which attempt to cover the entire vaginal surface by the particular formulation, may be challenging. Clearly, a formulation that covers the largest surface area will also cover the smallest, but the opposite is not true [22]. It is yet to be determined if full vaginal/cervical coverage by a microbicide is necessary.

The vagina and ectocervix, the portion of the cervix that is exposed to the vaginal environment, are lined by a nonkeratinized, stratified squamous epithelium (Figure 1). The vaginal mucosal layer is composed of multiple rugal folds, increasing the surface area. The squamous epithelium is constantly renewed and desquamated during the premenopausal years and contains three cellular zones: germinal or basal cell layer; midzone or stratum spinosum, the dominant portion of the epithelium; and superficial zone, consisting of the most mature cell population [18]. The superficial cells help to protect the underlying epithelial cells and subepithelial vasculature from trauma and infection. The squamous cells feature gap junction nexuses that represent an open channel system between adjacent cells through which certain molecules and electrolytes can transverse [18].

The endocervix is lined by a single layer of columnar epithelium and invaginates the underlying stroma (Figure 1). The transformation zone is located on the exposed portion of the cervix and is found in most women of reproductive age [18]. This area is the region where the columnar endocervical epithelium meets the squamous epithelium of the

ectocervix. The transformation zone is thought to be a highly immunologically active region thought to be important in HIV infection.

The connective tissue of the cervical stroma may be divided into two zones. One zone has a superficial, subepithelial location and is rich in interstitial fluid. The second is a deep, dense collagen layer. The superficial endocervical stroma also contains many capillaries, particularly abundant beneath the endocervical epithelial lining. These blood vessels do not have direct contact with the overlying squamous or columnar epithelial membranes. Therefore, metabolic exchanges including oxygen supply of the cervical epithelium must occur via diffusion [18].

The thickness of the vaginal epithelium varies as a result of changes in estrogen levels during the menstrual cycle. This estrogenization has important consequences for drug permeation through the tissue and may influence targeting and the pharmacokinetics (PK) of vaginally delivered drugs [20,24].

In addition to the epithelium and stroma (submucosa), two more layers, the muscularis and tunica adventitia constitute the walls of the vagina. The muscularis layer is made up of smooth muscle, collagen and elastic fibers that together allows for substantial stretching without tearing during child birth [20]. The tunica adventitia is a thin layer of dense connective tissue that helps to anchor the vagina to the lateral and anterior walls of the pelvis. Blood vessels, lymphatic vessels and nerves are located in the tunica adventitia.

The fluid that collects within the vagina originates from endometrial or salpingeal mucous membranes and from serum transudate. The transudate originates from blood vessels surrounding this area, transverse the vaginal wall, and mixes with vulval secretions from sebaceous and sweat glands and from the Skene's glands. Endometrial and oviductal fluids may also contribute to the vaginal fluid. This fluid may contain macrophages, lymphocytes, plasma cells, Langerhan's cells, eosinophils and mast cells. Incorporated into this fluid are cervical mucus (a major component), sloughed vaginal epithelial cells and elements associated with the innate microflora. This mixture can also contain various components such as amino acids, proteins, carbohydrates, enzymes, enzyme inhibitors, ions and lipids [18].

Major contributions to cervical mucus consist of water and a matrix of mucins, which are high-molecular weight glycoproteins. Sources of mucus secretions include the goblet cells within the columnar epithelium of the endocervix and the Bartholin's glands. The squamous epithelium of the vagina and ectocervix does not play a role in mucus production. The physical characteristics, composition and volume of mucus secretions of the endocervical epithelium are dependent on the menstrual cycle, which deems this secretion production as estrogen dependent. At the time of ovulation, the amount of cervical secretions increases, resulting in an increase in the overall volume of vaginal fluid. An increase in pH and mucin content and a decrease in viscosity are also evident. Cervical mucus has a complex net-like structure resembling interlacing microfibers. Orientation and pore size are influenced by circulating hormones. Under estrogen stimulation (during ovulation), mucus pore size is larger than when under progesterone stimulation. Additionally, during ovulation mucus fibers run parallel to each other and are long and thick, making the mucus less viscoelastic. Such organization is suitable for ease of sperm entry. Conversely, the nonovulatory mucus structure becomes dense and compact, which is unfavorable for sperm entry. Mucus-penetrating nanoparticles have been recently utilized to assess the pore size of human cervicovaginal mucus. This assessment found the average pore size to be 340 ± 70 nm [25]. These dynamic changes in fluid volume and physical makeup can modify the drug-release

profiles from intra-vaginal formulations and alter drug targeting *in vivo*. Changes in mucus network pore size also have the ability to affect drug targeting.

Mucus has the ability to maintain an unstirred layer adjacent to epithelial surfaces despite the shearing action that occurs during vaginal intercourse. The depth of the unstirred layer is determined by the balance between the rate of secretion and the rate of shedding/degradation. Drugs delivered vaginally, which have targets within the mucosa must move through and penetrate the unstirred layer before it is shed or degraded [26].

Normal vaginal fluid of an adult premenopausal woman has a pH value between 4 and 4.5. This value is maintained by the commensal *Lactobacillus* sp., which produces lactic acid from glycogen in the sloughed epithelial cells of the mucosa. In addition to the acidic environment, the production of hydrogen peroxide by some *Lactobacillus* sp. offers protection from overgrowth of various pathogens, including those causing bacterial vaginosis [27]. Furthermore, a high pH can increase risk or be indicative of an infection. The pH value of vaginal fluid in adult women rises during menstruation and after frequent acts of vaginal intercourse, since both vaginal transudate and ejaculate are alkaline. These alterations in pH can manipulate the release profile of pH-sensitive drugs from vaginal-delivery formulations [20].

The vaginal microflora is dynamic and may consist of both Gram-positive and Gram-negative bacteria species from both cocci and bacilli classes [28,29]. Anaerobic microorganisms are usually present in small quantities. The menstrual cycle, bacterial species present and vaginal infections caused by various pathogenic microorganisms have the ability to alter the constituents present and the enzymatic composition of the vagina and vaginal fluid. The enzymatic activity within the vaginal lumen/fluid and within the epithelium can affect drug transport and drug stability [20].

Furthermore, the act of coitus has the ability to affect the volume and composition of vaginal fluids due to tension-induced changes. These alterations have the ability to affect the drug-release profile within the vaginal compartment [20].

Despite the multitude of challenges to drug delivery presented by the anatomy and physiology of the vagina, the vaginal route of drug delivery is used for the administration of both locally and systemically acting agents.

Vaginal drug delivery

The vaginal route of drug delivery has been recognized since ancient Egyptian times, with the use of various substances as vaginal contraceptives. Agents commonly delivered vaginally include antimicrobials, spermicides and agents used for contraception, hormone replacement, cervical ripening/labor induction and pregnancy termination. Commonly utilized dosage formulations for these indications include solid dosage forms such as suppositories and tablets and semi-solid forms such as creams and gels. Intravaginal rings (IVRs), vaginal films, and foams are also utilized. Woolfson *et al.* also expressed that choice of vaginal delivery platform depends upon multiple variables, spanning from drug properties to clinical requirements to user acceptability [20].

Several advantages of the vaginal drug-delivery route have been defined [19,20,24,30]. The vagina can provide an accessible route of delivery, due to ease of self-insertion. Vaginal delivery represents a non-invasive route of delivery, by avoiding pain, tissue damage and possible infections associated with the parenteral route. Drugs delivered vaginally are able to avoid hepatic first-pass metabolism, and the vagina has a well-developed blood supply for drugs needing systemic delivery. Furthermore, the vagina provides great permeability for

drugs with certain physicochemical characteristics. Drug absorption depends on the physicochemical properties of the agent in question, specifically molecular weight, dissolution characteristics, and ionization properties. Vaginal drug absorption may occur by diffusion and/or active transport. This delivery route also allows for avoidance of the incidence and severity of various side effects, such as gastrointestinal and hepatic effects, associated with oral and parental drug delivery.

The lower genital tract is the initial site of insult for HIV infection during male-to-female sexual transmission. The advantages of vaginal delivery also hold true for HIV prevention strategies, such as the delivery of antiretrovirals. Furthermore, by locally delivering anti-HIV products to this area, systemic exposure can be limited as compared with the oral or parental routes of drug delivery. Limiting systemic exposure by local vaginal delivery has the ability to limit the occurrence and severity of side effects associated with systemic exposure to the anti-HIV products. Besides providing an adequate route of delivery for the HIV preventative product, a vaginal dosage formulation may provide additional benefits to the female user. With regard to vaginal gels, once inserted the gel can provide a physical barrier to HIV-1 epithelial penetration. Additionally, a gel may provide lubrication, which may decrease the likelihood of genital abrasions induced by the act of vaginal intercourse and may increase sexual pleasure experienced by both female and male partner. Upon disintegration, the same can be said for both vaginal tablets and quick-dissolving polymeric films; however, these cases do not hold true for oral preparations. Although threshold tissue or cervicovaginal fluid drug concentrations for the prevention of HIV acquisition has yet to be determined, a vaginal microbicide formulation may have the ability to elicit a PK benefit by creating a higher drug concentration within the female genital tract, in comparison to an oral formulation. In fact, Karim *et al.* highlight that after vaginal tenofovir 1% gel use, tenofovir diphosphate concentrations are approximately 1000-fold higher in vaginal tissue samples than after oral tenofovir disoproxil fumarate/emtricitabine (Truvada) use [31]. Additionally, these authors examined the concentration of tenofovir in undiluted cervicovaginal fluid after vaginal tenofovir 1% gel use. The women with tenofovir concentrations greater than 1000 ng/ml had a significantly lower HIV incidence rate, in comparison with placebo gel users. This concentration greatly exceeds the undiluted cervicovaginal fluid concentration seen after oral Truvada use [31]. Similar trends were found in cervicovaginal tissue samples from the MTN 001 study [201]. Therefore, vaginal delivery of antiretrovirals may prove advantageous, given that the female lower genital tract is the initial site of male-to-female HIV acquisition.

Microbicide dosage formulation considerations

Topical pre-exposure prophylactics (PrEP) or microbicides are products that can be applied vaginally or rectally to protect the user from HIV transmission and, possibly, other STIs. The effectiveness of any given microbicide product is dependent on both the anti-HIV activity (efficacy of the product) and the user's willingness and ability to use the product as instructed (acceptability and adherence). Furthermore, Morrow *et al.* shows that the success of microbicide products depends on both drug-related variables and HIV 'dose'-related variables [32]. The importance of product formulation in the development of a successful microbicide is evident. The appropriate drug-delivery strategy for each microbicide drug candidate will depend upon aspects such as the physicochemical characteristics of the candidate and its mechanism of action against HIV-1 transmission [33]. For example, if the drug binds to the receptor or co-receptor of the target host cells to prevent HIV-1 infection, then the method of delivery must be able to get the drug to the site of action within the tissue. Considering these concepts early within the microbicide product-development process would prove beneficial.

Several dosage form strategies are under development and investigation in the microbicide field [34–36]. Gels, or semi-solid dosage forms, are historically the most common vaginal drug-delivery formulation for microbicide products [37–60]. Typically, gels are applied as a single daily dose or a single dose before and/or after vaginal intercourse; the latter being defined as coitally dependent. Advantages of vaginal gels include familiarity of gel dosage form for this route of delivery, resulting lubrication effect (enhanced sexual pleasure) and the relative ease of manufacture (inexpensive). Despite vaginal gel utilization within trials, reports of leakage or messiness are extremely common [37–41]. Furthermore, gel and/or gel-applicator acceptability within an early generation Phase III microbicide trial might have been a factor in the disconnect between the subjective and objective measures of gel use [42].

Unlike other dosage formulation, IVRs offer a unique delivery method. Most other dosage formulations are designed to be inserted on a daily basis or in a coitally dependent fashion. These dosing frequencies might be unfavorable or unachievable for some women. IVRs offer a sustained release (coitally-independent release) of microbicide within the female lower genital tract. IVR types under investigation include matrix, reservoir and coated pod systems [36]. IVRs are to be placed within the upper third of the vagina, and the drug-release rate depends on excipients and IVR design and composition. Additionally, for the coated pod-ring system, drug release also depends on quantity of pods and size of the delivery window.

The stresses of one's social environment, environmental conditions and personal choice factor into the acceptability and adherence of a microbicide product. Based not only on the physicochemical properties of a given microbicide, but also on the varied characteristics of potential consumers, multiple options for microbicide dosage forms are necessary. Vaginal quick-dissolving polymeric thin films and tablets may aid in fulfilling these diverse needs by providing another platform for vaginal drug delivery. This type of delivery formulation quickly disintegrates and dissolves upon contact with fluids. The acceptability of the vaginal contraceptive film (VCF) containing nonoxynol-9 (N-9) has been investigated. The VCF was found to be the most favorable vaginal product when compared with other vaginal products including tablet, suppository, and gels [61–63]. The women favoring the VCF reported that it was easy to use, comfortable, discreet and less messy (than gels) [61,63]. Other women liked the tight-fitting feeling that the film provided within the vagina [62]. Conversely, other studies have shown the vaginal film to be the least favored among various vaginal products [64,65]. Concerns of vaginal film use that surfaced during these studies included fears that the film would not dissolve and would accumulate in the body [64]. Additionally, some women disliked the plastic appearance of the film and did not trust its efficacy [65].

There is not a single vaginal microbicide dosage formulation that will fit the product preferences of every potential female user. Morrow *et al.* acknowledges that acceptability data from previous clinical microbicide trials has been gained [32]. However, acceptability assessments that focus on product-related factors may be lacking important evaluations, for example, that pertain to HIV risk perception, which may factor into the acceptability/adherence of a vaginal microbicide product. Ideally, product-related, user-related and contextual factors should be integrated into acceptability/adherence assessments of microbicide products. Furthermore, these types of assessments may best be utilized within the preclinical and early clinical settings before moving microbicide products forward into advanced clinical safety and efficacy trials [32].

The development of a vaginally applied microbicide has been the major focus of microbicide research over the past two decades, yet women around the world, in both

developed and developing countries, practice receptive anal intercourse (RAI) [66,67]. In fact, a US national survey found that 36% of female responders aged 25–44 had ever had anal sex with an opposite-sex partner [66]. Although the absolute frequency of RAI may be low, the increased risk per sexual act is such that unprotected RAI may play an important role in spreading HIV infection in women. Personal lubricants are used in multiple fashions, including both vaginal and rectal application to decrease friction and to enhance the sexual experience. Therefore, there is no guarantee that a woman, who engages in both vaginal and anal sexual intercourse, will solely use a vaginal microbicide product for the indicated route, even with clearly written instructions/labeling and verbal counseling. Development of a microbicide that is effective and safe in both mucosal compartments should be considered [68].

History of clinically evaluated microbicides

Early generation microbicides

The products first developed as vaginal microbicides contained agents that do not directly affect the life cycle of the virus and may be effective against other pathogens. These ‘first generation’ or nonspecific antiretroviral microbicides include surfactants/detergents, acidifying agents and anionic polyanions. Surfactants disrupt membranes of cells, viruses and bacteria. Three surfactants tested clinically were products containing N-9 (gel, sponge and film), SAVVY® or C31G gel, and sodium lauryl sulfate (SLS) gel. Results of Phase II/III trials concluded that N-9 formulations were not effective at reducing the rate of male-to-female vaginal HIV-1 transmission [43,69,70]. In fact, the gel product was found to increase HIV infection risk in women with frequent use (use of more than 3.5 vaginal applications per day) and in those who had high incidences of vaginal lesions [43]. Another surfactant that was assessed clinically is the SAVVY or C31G gel [44,45]. However, these Phase III studies were terminated early due to futility [44]. Overall, it could not be concluded whether this gel was effective at preventing HIV infection. The third surfactant studied in the clinic was SLS. SLS is the chemical component in one gel formulation of the Invisible Condom® gel product. Both Phase I safety and acceptability and Phase II extended safety trials have found the Invisible Condom formulations to be safe, well-tolerated, and acceptable [46–48]. To date, no clinical efficacy trials have been published pertaining to this product. A proof-of-concept trial testing the Invisible Condom gel formulations for prevention of HIV-1 transmission during vaginal sexual intercourse is planned [48].

Acidifying agents help to restore or maintain the protective acidic pH of the vagina. Acidifying agents clinically assessed for safety and effectiveness against male-to-female HIV-1 transmission include BufferGel and Acidform. Both BufferGel and Acidform are acid-buffering bioadhesive vaginal gels. Acidform and BufferGel are formulated to pH values of approximately 3.55 and 3.9, respectively. BufferGel was found to be well tolerated, but not effective for the prevention of HIV-1 vaginal transmission in a Phase II/Ib trial [49]. Acidform vaginal gel was found to have favorable formulation properties [50]. However, no clinical effectiveness trials pertaining to the prevention of male-to-female vaginal transmission of HIV-1 have been reported for Acidform.

The last class of nonspecific microbicide candidates includes the anionic polyanions Carraguard® gel, cellulose sulfate gel, cellulose acetate phthalate gel, VivaGel® and PRO2000 gel. These agents carry a negative charge, which results in a charge interaction with viral envelope proteins interfering with attachment of virus to CD4 positive cells. A Phase III study did not find Carraguard to be effective against the vaginal transmission of HIV; however, the gel was found to be safe [42]. Cellulose sulfate gel effectiveness was tested in two Phase III trials [51,52]. Both studies were stopped early due to increased HIV infection at an interim analysis [52]. Cellulose acetate phthalate gel safety and acceptability

was assessed in a Phase I trial [41]. This trial was terminated early, only enrolling five women, due to complaints of wetness and leakage after vaginal application. Upon conducting further osmolarity and rheologic assessments, it was found that the gel was hyperosmolar in comparison with vaginal fluid and became hypoviscous upon vaginal application, which explains the unacceptable events experienced by the participants [41]. Several Phase I trials have been performed to assess the vaginal safety of VivaGel [53–55]. Two studies found that genitourinary adverse events were more common among women using VivaGel [54,55]. However, another study found VivaGel to be safe and well tolerated, with no systemic absorption [53].

PRO2000 gel was assessed in two large-scale effectiveness trials [49,56]. The Phase IIb trial found that 0.5% PRO2000 gel was safe and reduced the risk of HIV infection by 30%. However, the results of this study were not statistically significant [49]. To provide further insight into the effectiveness of PRO2000 gel for the prevention of male-to-female HIV-1 transmission, a Phase III trial was designed to evaluate the efficacy of 0.5 and 2% PRO2000 gel [56]. The 2% PRO2000 gel was terminated early due to futility. At the end of the study, the 0.5% PRO2000 gel was found to not reduce the risk of male-to-female HIV-1 transmission [56].

Replication inhibitors: nucleotide reverse transcriptase inhibitors

Due to less than desirable *in vivo* results obtained for nonspecific microbicide candidates, clinical focus in the field has now shifted to microbicide candidates that directly and specifically act against the HIV life cycle, namely specific antiretroviral products. Most of the antiretrovirals under current clinical investigation as microbicide candidates are reverse transcriptase inhibitors (RTIs). Upon HIV entering a cell, the viral enzyme, reverse transcriptase, converts the viral RNA into DNA, which can then be integrated into the host cell's DNA. An RTI prevents this step in the HIV life cycle from occurring. Tenofovir is the most clinically studied microbicide candidate that is a RTI. Specifically, tenofovir is a nucleotide RTI (NRTI).

A Phase I safety trial testing two concentrations of tenofovir gel (0.3 and 1%) at two dosing frequencies (once daily and twice daily) in HIV-positive and -negative sexually active and sexually abstinent women found the gel to be safe and well tolerated [57]. A total of 14 out of 25 women had a detectable, yet low, plasma tenofovir level at least once throughout the study. None of the HIV-positive women who had detectable plasma or cervicovaginal HIV RNA developed mutations associated with tenofovir resistance. Furthermore, in terms of safety, Keller *et al.* observed no increase in pro-inflammatory cytokines or chemokines or loss in protective immune mediators or endogenous antimicrobial activity with short term use of tenofovir 1% vaginal gel in a Phase I study [58]. Additionally, two Phase I PK trials assessing tenofovir 1% gel (NCT00561496) [59] and one Phase II extended safety study have been completed (NCT00111943).

A Phase IIb trial assessing the safety and effectiveness of tenofovir 1% gel in the prevention of male-to-female HIV transmission was performed in South Africa [71]. This trial is known as CAPRISA 004. Women were instructed to insert gel within 12 hours before and after vaginal intercourse. The results of this trial showed that tenofovir gel use was associated with an overall 39% decrease in HIV-1 acquisition, which is statistically significant. Additionally, among women with high gel adherence, the tenofovir gel reduced HIV infection by 54% in comparison to placebo gel [71]. Other exciting results from this study include: no significant renal, hepatic, hematologic, bone, pregnancy or genital adverse event concerns were associated with gel use; and 97.4% of participants found gel use to be acceptable, with 97.9% stating that they would use the gel in the future if it did prevent HIV [71]. For those women in the tenofovir gel group that did seroconvert, no tenofovir

resistance manifested. The results of CAPRISA 004 have paved the way for future vaginal microbicide trials by providing proof-of-concept for antiretrovirals as microbicides for HIV prevention.

In addition to being investigated as a topical microbicide, tenofovir is being studied as an agent for oral PrEP in both women and men. To directly investigate and compare the acceptability, adherence and PK profiles of daily regimens of oral and vaginal tenofovir formulations, a multicentered Phase II clinical study was performed in sexually active HIV-negative women around the world (MTN 001). Acceptability of the two tenofovir products differed among groups of women. For example, African women favored both the vaginal gel (42%) and oral tablet (40%); whereas, the US women favored the tablet (72%) over the gel (14%). Outcomes of PK assessments showed that daily use of the vaginal gel resulted in a more than 100-times greater concentration of tenofovir diphosphate drug in vaginal tissue in comparison to the oral tablet. Additionally, daily use of the oral tablet achieved a 20-times higher concentration of tenofovir diphosphate in the blood in comparison to the vaginal gel [201]. The VOICE trial is a Phase IIb five-group study examining the safety and efficacy of daily oral tenofovir disoproxil fumarate, oral Truvada, oral placebo, tenofovir 1% vaginal gel and placebo vaginal gel in HIV-negative women in Malawi, South Africa, Uganda and Zimbabwe (also called MTN 003). This daily-use regimen of a vaginal microbicide (topical PrEP) and oral PrEP differs from the coitally dependent frequency utilized in CAPRISA 004 and earlier microbicide efficacy trials. As of June 2011, the VOICE trial has achieved its enrollment goal of 5,000 participants and the information obtained from this trial aims to complement that of the CAPRISA trial and MTN 001. In September 2011, it was recommended that the VOICE participants randomized to oral tenofovir be discontinued from this group due to futility.

Other clinical trials have been implemented to examine oral pre-exposure prophylaxis, all of which include tenofovir as an investigational agent [72,202]. Recently, the iPrex study was completed. This Phase III randomized, placebo -controlled, double-blind study examined daily oral Truvada for the prevention of anal transmission of HIV in HIV-negative homosexual men, transgender women and other men who have sex with men [73]. Results of the study indicate that daily oral tenofovir disoproxil fumarate/emtricitabine reduced the risk of HIV anal infection by 43.8%.

Replication inhibitors: non-nucleoside RTIs

Tenofovir is not the only antiretroviral that is being clinically tested. Many other antiretroviral agents are also under development as potential microbicide candidates. However, these agents are not as far along in the development process. Most are in early safety and acceptability studies (Phase I and II) or preclinical development.

Gel formulations of UC781 and dapivirine, both non-nucleoside RTI (NNRTIs), have been clinically evaluated. Early-phase trials of these NNRTI formulations looked to assess short term safety, tolerability, acceptability, and PK of these products [37–40,60]. Although currently there are no plans for UC781 vaginal gel efficacy trials, dapivirine vaginal gel efficacy studies are planned. Please refer to Table 1 for an overview of the early-phase clinical trials evaluating UC781 and dapivirine vaginal gels.

Early-phase clinical trials assessing IVRs as potential microbicide delivery platforms are also under evaluation. This type of drug-delivery system offers sustained release of drug over an extended period of time, for example, ideally 4 weeks or 1 month. It has been reported that reservoir-type silicone IVRs containing 200 or 25 mg of dapivirine were found to be safe and well tolerated over the time span of 7 days [74]. During this study, mean dapivirine plasma concentrations throughout the 7 days never reached above 50 pg/ml [74].

Additionally, mean dapivirine concentrations in both cervicovaginal fluids and tissues, at all sample sites and time points, were greater than 1000-times the EC₅₀ reported *in vitro* [75], indicating successful distribution throughout the lower genital tract. Another Phase I safety and PK study looked to compare two types of dapivirine (25 mg) IVRs over 28 days [76]. The adverse event pro-file was similar across all groups, including the placebo group. Tissue samples showed that successful distribution with adequate concentration of dapivirine was achieved throughout the lower genital tract. The dapivirine matrix IVR achieved greater values for both maximum concentration and area under the concentration versus time curve than the reservoir IVR. Mean dapivirine plasma concentration across both IVRs was less than 2 ng/ml [76].

To examine the effects of drug distribution with differing vaginal microbicide formulations, studies of dapivirine gels and IVR can be compared [40,74,76]. The dapivirine gels contained 1.25 mg/dose and were administered vaginally once daily for 11 days (administered on day 1, single dose, and then on days 5–14, continuous dosing). The dosing levels and length of the studies for the dapivirine IVRs have been previously stated. Over the course of the vaginal gel and IVR studies, the dapivirine plasma concentration never exceeded the single digit nanogram/milliliter range [40,74,76].

Cervicovaginal dapivirine concentrations, for the gel study, reach their maximum level around day 11 (day 7 of continuous daily dosing) and remained elevated for over 24 h after the last dose [40]. These maximal cervicovaginal fluid levels and the levels achieved during the course of the dapivirine IVR studies are all above *in vitro* inhibitory concentrations [74,76]. Tissue dapivirine concentrations cannot be compared across formulations (gel vs IVR) because they were not quantified during the dapivirine gel study. Interestingly, PK parameters for the two dapivirine gels varied to some extent over the course of the study and may call into question the effects of excipients on drug release and these parameters [40].

To date, early-phase clinical trials have found RTIs to be generally safe and well tolerated. Phase I and II clinical trials involving both dapivirine vaginal gel and IVR are ongoing [201,202]. Additionally, a Phase I safety and PK trial of a dapivirine/maraviroc IVR is planned (MTN-013/IPM 026) [201]. Maraviroc is a CCR5 co-receptor antagonist. One of these dapivirine IVR studies will be the first trial in Africa assessing an IVR that contains an antiretroviral agent. Furthermore, an expanded safety and acceptability trial of a placebo (non-medicated) IVR is currently enrolling participants (MTN-005) [201]. Preclinical trials and assessments involving the delivery of specific antiretroviral agents in vaginal polymeric quick-dissolving films are currently ongoing as well.

Other specific antiretroviral agents

Multiple additional antiretrovirals are in pre-clinical testing as topical pre-exposure prophylactics for HIV-1. These include agents that target cell membrane receptors, including agents that target the CD4 receptor, CXCR4 and CCR5 co-receptors, and C-type lectin receptor. Additionally, agents that bind to HIV envelope glycoproteins are under pre-clinical investigation. These include gp120 and gp41 binding agents. Various NRTIs and NNRTIs besides tenofovir and dapivirine are under investigation as potential microbicides. Viral integrase and protease inhibitors are also under preclinical evaluation as HIV prevention agents. Agents span small molecules to peptides to proteins including monoclonal antibodies [33–34,77].

Further opportunities for HIV prevention with vaginal drug-delivery strategies

Combination vaginal microbicides

Antiretroviral treatment for HIV-1 infected individuals (antiretroviral-naïve patients) includes the use of combination therapy, namely one NNRTI and two NRTIs or ritonavir-boosted/unboosted protease inhibitor and two NRTIs [78]. Combining more than one antiretroviral agent with different mechanisms of action within a single vaginal microbicide formulation may be a viable option for HIV prevention. Combining active pharmaceutical ingredients (APIs) in a single formulation may increase activity across viral subtypes, reduce the development of HIV resistance, and prevent other STIs [79]. Microbicide drug candidate combinations are currently under development and evaluation [80,201].

Additionally, the combination of a chemical barrier (API) with a physical/mechanical barrier (e.g., a barrier contraceptive method) may be another approach to HIV prevention. Furthermore, this combination might provide female-controlled protection against both pregnancy and STIs. The safety and acceptability of Acidform gel and BufferGel, both used with a diaphragm, have been tested in a Phase I trial across three sites in the USA [81]. Approximately 80% of the women either liked the diaphragm and gel, or had no opinion. Furthermore, over 93% of the study participants stated that the gel and diaphragm were comfortable. Additionally, less than 25% of women reported any problems (e.g., insertion and removal) with the combination product [81]. The acceptability of using Acidform with a diaphragm for prevention of STIs has also been assessed in Madagascar [82]. The overwhelming majority of women who were randomized to the diaphragm plus gel group found that the diaphragm was easy to insert and to remove [82].

Another combination vaginal microbicide is evident in the BufferGel Duet™. The BufferGel Duet is a diaphragm-like vaginal device that is preloaded with BufferGel. This gel/device provides three layers of protection: BufferGel at cervix, physical barrier and BufferGel within vaginal lumen. In this Phase I study, the BufferGel Duet was found to be safe and acceptable by both participating women and their male partners [83]. Additionally, the majority of participants preferred the BufferGel Duet over male condoms, were satisfied or felt neutral about the product, and would purchase the product if found to be effective for HIV prevention [83].

This favorable acceptability data warrants further investigation of chemical/device combinations in the vaginal microbicide research field, namely specific antiretroviral products and vaginal device combinations.

Nanocarriers

In addition to the vaginal dosage formulations discussed above, more advanced types of drug delivery have been suggested as strategies to enhance the vaginal delivery of microbicides. Several nanocarriers are available such as liposomes, polymeric nanoparticles, solid lipid nanoparticles, metal nanoparticles, complexes, dendrimers and nanocrystals. Some of the advantages presented by antiretroviral-loaded nanocarriers for microbicide formulation are the ability to modulate drug release [84], the capacity to penetrate epithelial linings [85] and the possibility of providing specific drug targeting [86,87] to HIV-target cells. Furthermore, nanotechnology offers a novel approach to formulate microbicide agents potentially leading to uniform epithelial delivery [88]. Delivery through cervicovaginal mucus may be possible by controlling nanoparticle size and surface characteristics [88]. Mallipeddi *et al.* present a thorough review on the utilization of nanotechnology in antiretroviral drug delivery [89].

Nanoparticles are one such strategy that may improve microbicide characteristics and targeted delivery. Polymeric nanoparticles can be made with such polymers as poly(lactic-co-glycolic acid), poly(sebacic acid), poly(lactic acid), poly(alkyl)cynoacrylates, poly(ethylene glycol-co-(lactic-glycolic acid)), poly(caprolactone) and poly(methyl methacrylate) [88,89]. Recent efforts have been taken to utilize nanotechnology in order to encapsulate and characterize the microbicide candidates PSC-RANTES, tenofovir and dapivirine [90–92].

Conventional nanoparticles are mucoadhesive [93,94]. Mucus is negatively charged due to carboxyl and sulfate groups located on mucin and has high densities of hydrophobic domains, which entrap materials by engaging polyvalent, low-affinity, adhesive interactions [26]. Many polymers employed for nanoparticles are hydrophobic or have a net charge; therefore, these interactions may create a delivery challenge. Furthermore, many microbicide drugs in development need to reach target cells within the epithelium and stroma, and these drugs may also be held back by the mucus. If encapsulated, these drug-loaded nanoparticles must diffuse readily through the unstirred adherent layer of mucus if they are to efficiently contact and/or permeate the epithelial cells [26].

Designing nanoparticles to be non-mucoadhesive would appear advantageous for microbicides that are not lumenally active and need to bypass the mucus to reach their cellular targets. Due to knowledge as to how viruses avoid mucoadhesion, Lai *et al.* were able to develop mucus-penetrating nanoparticles by modifying the surface of the particle with a high density of low-molecular-weight polyethylene glycol (PEG) [94]. This PEG surface modification allows for minimization of hydrophobic and electrostatic adhesive interactions [26,95]. Furthermore, this group showed that larger nanoparticles (200 and 500 nm in diameter) densely coated with PEG could diffuse through the cervicovaginal mucus [94]. Furthermore, Wang *et al.* examined the effects of coating density and PEG molecular weight and found that results suggested that both low-molecular-weight PEG and dense surface coverage are required for the coated particles to transverse the cervicovaginal mucus [93]. In addition to enhancing mucus penetration, PEGylation has been suggested to improve the stability of nanoparticles in mucus [96–98].

Application of advanced drug-delivery strategies, such as nanocarriers, provides the opportunity to design products with greater efficacy, greater stability, improved targeting or extended/sustained release. The aspect of sustained release would allow the user to apply the product independent of coitus, which may increase acceptability of a microbicide product.

Future perspective

HIV prevention indication considerations

The US FDA and other regulatory bodies usually require results from two or more adequate and well-controlled clinical trials in order to approve a pharmaceutical product for a new prevention or treatment indication [99]. When a pharmaceutical product is indicated for use by HIV-1 uninfected, healthy individuals, the safety and efficacy data must be very compelling. Various large-scale clinical-efficacy trials are underway examining both oral and topical PrEP in differing high-risk populations and/or settings [72]. Recently, interim-efficacy data for the Partner PrEP study heralded cessation of the oral placebo arm of the trial, and VOICE interim-efficacy data, as previously stated, called for termination of the oral tenofovir arm. Although data from multiple populations and studies is preferred, it has yet to be determined if and how changes might be made to the conduct of continuing clinical efficacy trials if compelling efficacy is found in one trial. An adequate risk:benefit ratio may differ widely among those with different routes of HIV exposure and levels of HIV infection risk. More generalizable efficacy and safety data will allow an HIV prevention product to be

recommended or prescribed to a broader patient/consumer population, not specifically limiting the indication to a specific route of exposure or level of risk.

Placebo-controlled trials

The CAPRISA 004 trial showed that tenofovir gel use was associated with an overall 39% decrease in HIV-1 acquisition [71]. This result was found to be statistically significant. If results of the VOICE trial also indicate a compelling efficacy in the reduction of HIV-1 acquisition with use of vaginal tenofovir 1% gel, then the use of placebo-controlled trials within the vaginal microbicides field might become obsolete. It would no longer be ethically valid to perform a placebo-controlled vaginal microbicide trials if the tenofovir 1% gel was to be considered a superior agent compared with the placebo gel [100]. This tenofovir 1% gel would then, in essence, become a 'standard of care' in vaginal HIV-1 prevention toolbox, along with HIV prevention counseling and condoms.

Microbicide vaginal formulations

Future vaginal microbicide preclinical and clinical studies will involve more exploration into the PK and pharmacodynamics of anti-retroviral agents and vaginal formulations during vaginal delivery. These studies will assist in elucidating a dosing strategy pertaining to a specific API and/or vaginal formulation. For example, more knowledge will be gained in order to specify the amount of drug within a given formulation and frequency of use for a female human user. Additionally, future studies will also involve the investigation of sustained and controlled release vaginal microbicide formulations and various drug-targeting strategies. For example, a specific targeting strategy for a vaginally delivered antiretroviral may involve transport to specific tissues or cells composing or surrounding the vaginal compartment or neighboring area, such as lymph nodes.

Key Terms

Vaginal microbicide formulation

Vaginal semisolids(gels),tablets, films and rings are under investigation and evaluation, in both preclinical and clinical settings, as potential vaginal microbicide dosage formulations. This variety of dosage formulations may offer the user different dosing regimens, including coitally dependent (prior to sexual intercourse) or independent (daily and monthly use) application

Topical pre-exposure prophylactic or microbicide

Product that when applied either vaginally or rectally is intended to prevent, or significantly reduce, HIV-1 acquisition via sexual transmission at the respective mucosal sites

Nonspecific antiretroviral microbicide

Microbicide product that contains an active pharmaceutical ingredient that has activity against not only HIV-1, but also against other pathogens. The action of the active pharmaceutical ingredient is not specific to the HIV life cycle. Nonspecific antiretrovirals that have been investigated in microbicide products include surfactants/detergents, acidifying agents and anionic polyanions

Specific antiretroviral product

Microbicide product containing an active pharmaceutical ingredient that has pharmacological action that is specific to the HIV life cycle. Classes of specific antiretrovirals that are being investigated for HIV prevention include inhibitors of viral binding/fusion, reverse transcription, integration and assembly

**Combination
vaginal
microbicide**

Vaginal microbicide product that contains two active pharmaceutical ingredients (APIs), API(s) and vaginal device (e.g., diaphragm-like device) or API(s) with an additional drug-delivery technique (e.g., nanoparticles)

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Executive summary

- HIV prevention strategies are of great importance worldwide.
- The results of the CAPRISA 004 trial have brought hope to the microbicides field that success of a vaginal microbicide for prevention of male-to-female sexual transmission of HIV-1 is achievable.
- Appreciating the processes that occur during mucosal transmission of HIV-1 assists in the development of more effective vaginal prevention strategies.
- Understanding the anatomy and physiology of the vagina and its associated components is critical to development, improvement and evaluation of vaginal microbicide products.
- Furthermore, integrating combination strategies and newly identified drug-delivery strategies within microbicide dosage forms may prove to be beneficial toward the effort to battle the HIV pandemic.

Figure 1. Female genital tract anatomy

(A) Represents a side view of the gross anatomy of the female genital tract with respect to other organs. (B) Represents tissue types and areas of the upper vagina and cervix. (C) Display of cells composing the vaginal and ectocervical tissue and display of cells composing the endocervical tissue.

Table 1

Clinical trials of non-nucleoside reverse transcriptase inhibitor-based vaginal gel microbicide products.

Microbicide vaginal gel candidate	Clinical trial phase	Study objective(s)	Results/conclusions	Ref.
UC781	Phase I	Assessed safety and acceptability of 0.1 and 0.25% UC781 gel (NCT 00441909 – single vaginal use for varying exposure length times; NCT00132444 – 14 days of twice-daily vaginal use)	Not reported	
	Phase I	Assessed safety and acceptability of 0.1, 0.25 and 1.0% UC781 gel over 6 days of once-daily vaginal use	Vaginal health and genital irritation was similar among all groups including placebo. 2/12 women using 1.0% UC781 gel had detectable but not quantifiable UC781 plasma levels. 92% of participants reported slight to moderate gel leakage	[38]
Dapivirine (TMC120)	Phase I	Assessed safety, tolerability, and systemic exposure of 25, 50 and 150 µM dapivirine gel over 7 days of twice daily vaginal use	All concentrations were well tolerated and safe. Dapivirine plasma levels were quantifiable in 13% of users on day 1 and in 75% of users on day 7. Women in the lowest concentration group did not have detectable levels on day 1, and only 3/14 had detectable levels upon day 7. Most women reported that the gel was easy to insert. 96% of participants reported a concern of gel leakage after insertion	[37]
	Phase I and II	Assessed safety and tolerability of 42 days of twice-daily vagina use (Belgium – dapivirine 0.02% gel; African sites – dapivirine 0.001, 0.002 and 0.005% gels)	Safety data similar across all groups. Mean dapivirine plasma level was dose dependent (max. mean concentration of 474 pg/ml occurred on day 28 in the 0.02% group). Two weeks after study cessation, the mean plasma concentration across all groups dropped to <5 pg/ml. Most women were very willing or willing to use the gel in the future if it were to be effective as HIV prevention. Approximately 83% of the women reported that their male partners liked or had neutral feelings toward the gel. Gel leakage was reported by all Belgian participants and 52% of African participants	[39]
	Phase I	Assessed pharmacokinetics of 10-day vaginal use of dapivirine vaginal gel (0.001, 0.005 and 0.02%)	The dapivirine plasma concentration did increase (greatest on day 10). Dapivirine was detected in the cervicovaginal fluid of each participant on days 1 and 10 (concentrations highly variable, but always greater than plasma concentrations). Absorption of dapivirine was found in all sampled regions (vagina near vulva, vagina near cervix and cervix) in all dose groups at each time point	[60]
	Phase I	Assessed the safety and pharmacokinetics of 11 days of once-daily vaginal use for two differing dapivirine gel formulations (both containing 0.05% dapivirine).	Both dapivirine formulations were well tolerated and safe. Adverse event data was similar across all groups. Gel leakage after insertion was a major complaint across all groups	[40]