AAPS PharmSciTech, Vol. 9, No. 2, June 2008 (© 2008)
DOI: 10.1208/s12249-008-9073-5

Review Article

A Review of Current Intravaginal Drug Delivery Approaches Employed for the Prophylaxis of HIV/AIDS and Prevention of Sexually Transmitted Infections

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Received 21 November 2007; accepted 4 February 2008; published online 2 April 2008

Abstract. The objective of this review is to describe the current status of several intravaginal anti-HIV microbicidal delivery systems these delivery systems and microbicidal compounds in the context of their stage within clinical trials and their potential cervicovaginal defence successes. The global Human Immuno-Deficiency Virus (HIV) pandemic continues to spread at a rate of more than 15,000 new infections daily and sexually transmitted infections (STIs) can predispose people to acquiring HIV infection. Male-to-female transmission is eight times more likely to occur than female-to-male transmission due to the anatomical structure of the vagina as well as socio-economic factors and the disempowerment of women that renders them unable to refuse unsafe sexual practices in some communities. The increased incidence of HIV in women has identified the urgent need for efficacious and safe intravaginal delivery of anti-HIV agents that can be used and controlled by women. To meet this challenge, several intravaginal anti-HIV microbicidal delivery systems are in the process of been developed. The outcomes of three main categories are discussed in this review: namely, dual-function polymeric systems, non-polymeric systems and nanotechnology-based systems. These delivery systems include formulations that modify the genital environment (e.g. polyacrylic acid gels and *lactobacillus* gels), surfactants (e.g. sodium lauryl sulfate), polyanionic therapeutic polymers (e.g. carageenan and carbomer/lactic acid gels), proteins (e.g. cyanovirin-N, monoclonal antibodies and thromspondin-1 peptides), protease inhibitors and other molecules (e.g. dendrimer based-gels and the molecular condom). Intravaginal microbicide delivery systems are providing a new option for preventing the transmission of STIs and HIV.

KEY WORDS: HIV/STIs; intravaginal drug delivery systems; microbicides; nanostructures; polymers; prophylaxis.

INTRODUCTION

Infection with HIV remains an incurable condition (1,2). The highest rate of HIV transmission is through the exposure of the vaginal mucosal surface to HIV during sexual intercourse. Until January 2006, the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) estimated that AIDS had caused the mortality of more than 25 million people since it was first recognized, making it one of the most destructive epidemics in recorded history (3,4). The majority of deaths have occurred in sub-Saharan Africa where the prevalence rate for sexually active adults is greater than 35 (5–7). This culminates in causing the death of economically active adults with the accompanied macro-economic challenges. At present, the current use of antiretroviral treatment reduces both the mortality and morbidity associated with HIV infection, but routine access to antiretroviral medication is not available in all countries. Sexually transmitted infections (STIs) also predispose people to acquiring HIV/AIDS through the interference of the integrity of the vaginal epithelium. The presence of untreated STIs enhances both the acquisition and transmission of HIV by a factor of up to 10. Thus, effective STI treatment is an important HIV prevention strategy (8–11).

Several therapeutic agents for curing HIV/AIDS have been developed, but, to date none has been proven to be successful. A Reproductive Health Research Unit study by Pettifor *et al.* (12) showed an extremely high percentage of respondents (93%), identified condom use as being an effective preventative measure but 67% did not use condoms consistently and 31% had never used condoms. In the wake of this other effective female controlled measures that can

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 1530-9932/08/0200-0505/0 © 2008 American Association of Pharmaceutical Scientists

protect both parties from exposure to HIV/AIDS are needed. The most compelling solution to HIV/AIDS is an effective vaccine. However, after 25 years of research, development of an effective vaccine has remained unsuccessful due to various obstacles including inadequate resources, regulatory capacity concerns, intellectual property issues and mainly the scientific challenges (8,13,14). Therefore, it is likely that the greatest potential for prevention of STIs and HIV/AIDS will lie in the development of effective intravaginal microbicidal delivery systems (Table I).

Studies have shown that the vagina is a suitable site for local and systemic delivery of drugs (14–16). Traditionally, the intravaginal route has been used for the delivery of locally acting drugs such as antibacterials, antifungals, antiprotozoals, antivirals, labor-inducing and spermicidal agents, prostaglandins and steroids. The large surface area, permeability and rich blood supply of the mucous membrane of the vagina, provide significant potential for the delivery of a wide range of compounds, including peptides and proteins, and offers an alternative to the parenteral route of administration for numerous bioactive substances. However, despite all these advantages, the intravaginal route has not been extensively explored as a mode for drug delivery due to menstrual cycle variations (14). Intravaginal drug delivery systems have traditionally been used to deliver contraceptives and drugs to treat vaginal infections. Formulations have included pessaries and tablets designed after the advent of rectal suppositories. The first intravaginal controlled drug delivery system was developed in 1970, using a vaginal ring for the delivery of medroxyprogesterone acetate for contraception (14). However, tablets, creams, and suppositories are now the most conventional formulations in vaginal drug delivery while vaginal rings are more commonly employed for long-term drug delivery.

Recent advances have been made in the area of bioadhesive gels, microparticles and tablets, which show great promise for use as controlled intravaginal microbicide delivery systems (17–19). Numerous hydrophilic polymers and hydrogels have been used in a number of vaginal products (20–22,23,24) that violate the HIV life-cycle at multiple steps, have increased efficacy, limited cross-resistance and minimize microbicide-induced host toxicity (25,26). The objective of this review is therefore to describe the recent developments in intravaginal delivery systems for microbicides employed for the prophylaxis of HIV/AIDS and prevention of STIs.

Table I. Desirable Criteria for Ideal Intravaginal Microbicidal Delivery Systems

Criteria
Simple to manufacture, cost effective and easy to apply thus facilitating patient compliance
Non-irritative and free from producing any physical discomfort
Provide immediate and sustained protection by releasing the microbicide in a controlled manner over a prolonged period of time
Have suitable vaginal retention and distribution
Be versatile against various pathogens encompassing STIs and HIV

METHODS

Given the devastating effects of the HIV/AIDS epidemic and the continuing difficulties in developing an effective HIV vaccine, there is a clear scientific rationale for developing alternative methods to prevent STIs and the transmission of HIV infection. Microbicides circumvent many of the immunological difficulties associated with HIV vaccine development and make topical formulations a more realistic goal, especially in the short term (27,28). The most promising strategy currently being pursued is the utilization of intravaginal delivery systems for microbicides (13,26,29–34). Microbicides are chemical substances that when inserted intravaginally prior to sexual intercourse, have the potential to either prevent or reduce the risk of STIs and HIV transmission once released from a delivery system (24,30,31). The potential for developing controlled release formulations for long-term intravaginal delivery of microbicides has recently gained momentum and may overcome challenges relating to compliance, acceptability and efficacy associated with current gel-based formulations (30,35). Furthermore, the emergence of resistant HIV strains and toxic side-effects of current antiretroviral drugs (ARVs) require intravaginal delivery systems with superior safety profiles and potential for improved patient compliance.

An intravaginal microbicidal delivery system combining several mechanisms of preventing the transmission of STIs and HIV would need to possess added effectiveness and less side-effects than a delivery system having a single mechanism (26) (Fig. 1). The challenge is to design a delivery system that is able to provide high concentrations of the microbicidal compound in the vagina over a prolonged period of time (36). Studies have indicated that dosages in first-generation microbicides remain effective only for a few hours and therefore necessitate administration shortly before coitus (37). For controlled, zero-order release sustained over prolonged periods (days extending to months), solid polymeric systems may be the most suitable biocompatible delivery system with the physicochemical nature of the microbicide or drug to be delivered.

Current Intravaginal Microbicide Delivery Methods for Preventing the Transmission of STIs and HIV

The majority of microbicidal delivery systems for intravaginal administration that have been developed and evaluated in ongoing clinical trials are conventional semi-solid aqueous gels and vaginal ring formulations. These are designed to provide a single dose of a microbicidal agent (38–42). Intravaginal delivery systems for microbicides have been formulated and traditionally include a large variety of pharmaceutical dosage forms such as semi-solids, tablets, capsules, pessaries, liquid preparations, vaginal films, vaginal rings, foams, and tampons. Currently, the maximum duration of drug release for intravaginal microbicidal delivery systems is as follows: (1) Vaginal gels (6 h) (42,43), (2) vaginal tablets (8 h) (44,45), and (3) vaginal rings (71 days) (8,46,69). Vaginal gels and tablets have rapid release rates which, for effective use, ultimately require administration several times a day. Vaginal rings have adequate release rates but have only been formulated for preventing the transmission of HIV and as a

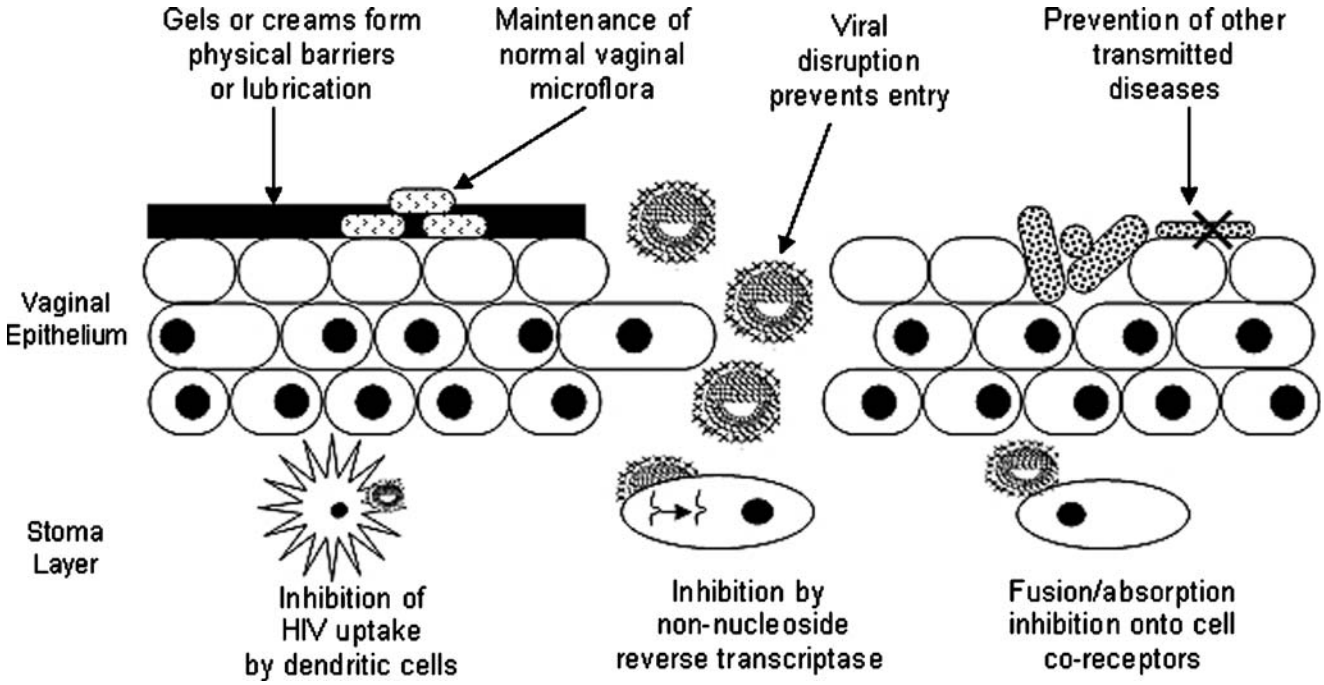


Fig. 1. Schematic depicting the various mechanisms of preventing the transmission of sexually transmitted infections and HIV by employing microbicide delivery systems (adapted: Stone *et al.* (38))

contraceptive. The most widely used semi-solid preparations for vaginal drug delivery include creams, ointments, and gels (14,26,37).

Over the past decade, there have been major advances in the field of microbicidal delivery with diverse types of delivery systems in various stages of development (47). The ultimate success of an intravaginal microbicide delivery system requires consideration of variables including the microbicidal agent, vaginal physiology and the design of the delivery system. Furthermore, bioavailability is affected by numerous physiological factors, and the ability of the formulation to effectively deliver the microbicide may vary with the menstrual cycle, pH variations and the presence of co-pathogens (48,49).

Targeting HIV entry into the body is a favored preventative approach as it is the initial step in the process of infection. Several readily available anionic polymers interfere with the entry processes of HIV, and hence these polymers qualify as primary candidates for designing various microbicidal delivery formulations (36). However, few of these formulations have been tested in Phase I/II clinical trials, in HIV-uninfected, and HIV-infected populations (50). Current multi-center Phase I/II safety and Phase II/III efficacy studies are being conducted or planned in different geographical locations, by various groups which aim to design systems for rapid clinical applications.

Design of Intravaginal Microbicide Delivery Systems

Intravaginal delivery may be designed for administration of microbicides by using an applicator or specifically designed systems for intravaginal administration. In general, based on the delivery system or the microbicide used, drug absorption, distribution and residence time in the vagina may vary. Early studies by Johnson and Masters (51) showed that the microbicide distribution in the vaginal tissue varies considerably with the nature of the delivery system. Solutions, suspensions and foams displayed greater superiority over tablet dosage forms. Ideally, a vaginal delivery system that is intended for localized microbicidal delivery should distribute uniformly throughout the vaginal cavity. Thus, for a local effect to occur in the vagina, semi-solid, or fast dissolving solid systems are required. Bioadhesive delivery systems or intravaginal ring systems are more suited for topical effects. Currently there is no data available regarding the bioavailability of most intravaginal microbicide delivery systems after extended vaginal exposure (50,52). Engineering and materials science may provide critical and new information to the process of designing and developing superior intravaginal microbicide delivery systems. First-generation microbicidal compounds that are currently under investigation are expected to be available within a few years. These systems may only be 50–60% effective in delivering microbicides, but even with this efficacy if used (37) by only 20% of women, in 73 low-income countries, it may still lead to the prevention of 2.5 million new infections during a 3-year period (26,37).

Creams and Gels

To date the greatest number of intravaginal drug delivery systems for microbicides, by far, is in the form of creams or gels. Although commonly used for the topical intravaginal delivery of microbicides, these systems are messy, uncomfortable and may not provide an exact dose due to non-uniform distribution and leakage (53). To evaluate the efficacy of a 3-day course of clindamycin vaginal cream in the treatment of bacterial vaginosis, Lamont *et al.* (54) performed a randomized, placebo controlled trial in pregnant women and found

that the clindamycin cream was well tolerated and more efficacious than placebo. During the past few years, considerable work has been done on the development of hydrogel controlled release microbicide delivery systems (14–19,55,95). For example, a 3% alginate gel of nonoxynol-9 has been investigated for intravaginal spermicidal activity (55). The study found that the spermicidal activity and the diffusion of the agent changed with the pH and osmolarity of the formulation. Recently, a gel microemulsion-based spermicide formulation, phenyl phosphate derivative of zidovudine, with anti-HIV effect, has been developed (56). Multiple intravaginal applications of this drug as a microemulsion gel formulation did not cause any damage in the vaginal epithelium in the rabbit model (57–59).

Tablets and Suppositories

A large number of intravaginal delivery systems are also available in the form of tablets or suppositories. Some authors use the terms pessaries and suppositories interchangeably and consider vaginal tablets as a separate dosage form. These formulations are designed to melt in the vaginal cavity and release the microbicide over several hours. Suppositories are most commonly used to administer drugs for cervical ripening prior to child birth and for local delivery of various drugs. Vaginal tablets may contain binders, disintegrants and other excipients that are used to prepare conventional oral tablets. Mucoadhesive polymers are sometimes used in tablet formulations to increase the vaginal residence time of the microbicide been delivered. Other vaginal tablet-like formulations are extrapolations of silicone-based vaginal rings. Research groups have studied the release of microbicides from silicone matrices (60,61). Release studies were performed *in vitro* for up to 1 year and *in vivo* in rabbits for up to 52 days. Both *in vitro* and *in vivo* studies showed consistent release profiles over time, showing that microbicide delivery is controlled by diffusion from the silicone delivery device and was not limited by absorption through the vaginal epithelium.

Vaginal Rings

Vaginal rings are circular ring-type drug delivery devices designed to release microbicides in a controlled manner after insertion (8,31,62). The advantages of such a device are that it can be controlled by the user; does not interfere with coitus and allows for the continuous delivery of microbicidal compounds. In simple vaginal rings, the microbicide is homogeneously dispersed within a polymeric ring with the surface of the ring releasing the microbicide faster than the inner layers. The key challenge in development of these systems is finding the optimum dose that will deliver the least amount of microbicide necessary to ensure protection. Advances have been made on the original two-layer ring system by adding a third, outer, rate controlling drug-free elastomer layer to minimize the drug concentration spike (63). Much of the methods in vaginal ring literature relates to the commonly used polymer, poly(dimethylsiloxane) or silicone devices, although other elastomeric polymers such as ethylene vinyl acetate and styrene butandiene block copolymers have been tested in recent years (46,64). Most women judged the ring easy to insert and remove, and no side-effects are experienced (65–68).

Bioadhesive Intravaginal Systems

Most conventional intravaginal formulations however are associated with disadvantages of low retention to the vaginal epithelium, leakage and messiness, thereby causing poor patient compliance. To circumvent these challenges, bioadhesive microbicidal delivery systems are being propagated (67). Bioadhesive polymers that have been used for intravaginal formulations include polycarbophil, hydroxypropylcellulose and polyacrylic acid (70). The first bioadhesive systems for vaginal drug delivery were in the form of tablets for the delivery of bleomycin, an anti-caner agent (70–75). Attempts have also been made to delivery of microbicides using bioadhesive microparticulate vaginal systems (73–78,99). These systems may be a multi-phase liquid or a semi-solid, but have been designed so as not to seep from the vagina like pessary formulations. Table II lists the numerous intravaginal delivery systems that have been identified.

RESULTS AND DISCUSSION

Dual-Function Polymers Employed in the Design of Intravaginal Microbicide Delivery Systems: As Excipients with Potential Therapeutic Activity

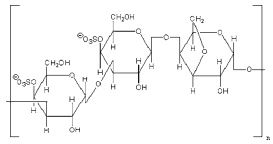
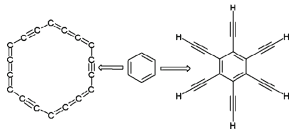
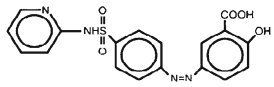
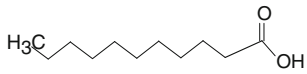
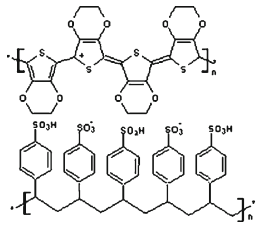
A Carageenan Vaginal Gel Formulation for HIV and Human Papilloma Virus Inhibition

Carraguard™ is a gel-like delivery system comprising carageenan that acts as an absorption inhibitor by coating the vagina. Carageenan is an excipient that is used as a gelling agent and presents as one of the most promising class of potential microbicidal compounds (77–82,86) (Table III). Carraguard™ has several advantages over other vaginal microbicide delivery systems such as a higher bioavailability, safety, versatility, and economic saving (83). There appears to be no effect on sperm motility (84–86).

Table II. Classification of the Numerous Intravaginal Compounds Delivered Intravaginally

Classification	Compounds
Polymeric	Carageenan, Monocaprin, Polyacrylic Acid, Lactobacillus, Cellulose Acetate Phthalate, Cellulose Sulfate, Polystyrene Sulfonate, Naphthalene Sulfonate, Sulfated Polyvinyl Alcohol and Lactic Acid
Non-Polymeric	Cetyl Betaine, Myristamine Oxide, Stampidine, Cyanovirin-N, Monoclonal Antibodies, Lyposomes, Thrombospondin-1, Lime Juice, Yoghurt, Tenofovir and Zidovudine
Nano-Structured	Dendrimers, Thiourea, Silver, Polystyrene and Sodium Lauryl Sulfate

Table III. Chemical Structures of Polymeric Microbicide Compounds

Compound	Structure	Key Function	Reference
Kappa Carageenan		Absorption Inhibitor	[80]
Carbomer		Interruption of HIV cell binding	[109]
Cellulose Acetate Phthalate		Inactivates HIV and HSV	[115]
Capric Acid		Inactivates HIV and HSV	[118]
Polystyrene Sulfonate		Activity against HIV and HSV	[74]

A Combinatory Gel of Carageenan and a Non-Nucleoside Reverse Transcriptase Inhibitor

PC-815 gel is an intravaginal delivery system combining a microbicide containing carageenan with MIV-150. MIV-150 is an NNRTI which prevents HIV-infected cells from replicating (85,87). Pre-clinical tests of MIV-150 have shown a significant increase of activity against HIV-1 primary isolates with no toxic effects. However, it has a low oral bioavailability which makes it an ideal compound for intravaginal delivery, since the chances of causing systemic side-effects become diminished (85). *In vitro* pharmacological studies have indicated that the PC-815 delivery system has significantly higher activity against HIV than that of the Carraguard™ system (85).

A Topical Non-Contraceptive Carageenan Gel Formulation

PC-515 gel is a topical gel formulation containing 3% *w/w* carageenan. It is under development as a non-contraceptive microbicidal delivery system that may offer HIV protection while allowing women to conceive. Zacharopoulos and Phillips (88) showed that PC-515 protected against HSV with an effect superior to many microbicidal delivery systems. The protective effect was seen across a wide range of pH levels and lasted up to 18 h (88). PC-515 has undergone developmental trials in humans to ascertain the overall performance of the formulation (89).

A Polyacrylic Acid-Based Gel Formulation

A polyacrylic acid polymeric gel (BufferGel™, ReProtect, LLC, Baltimore, MD, USA), that is currently under Phase II clinical trials for contraception and the prevention of HIV (8). Its maintains the natural acidity of the vagina to ensure survival of lactobacilli that are able to produce lactic acid and hydrogen peroxide which inactivate many pathogens that cause STIs (82,90). BufferGel™ acidifies semen, thereby destroying sperm cells, HIV and a wide range of STI pathogens during sexual intercourse and thus creates a physical barrier that inhibits the passage of pathogens into the vaginal and cervical epithelium (90–93) (Fig. 2). A similar

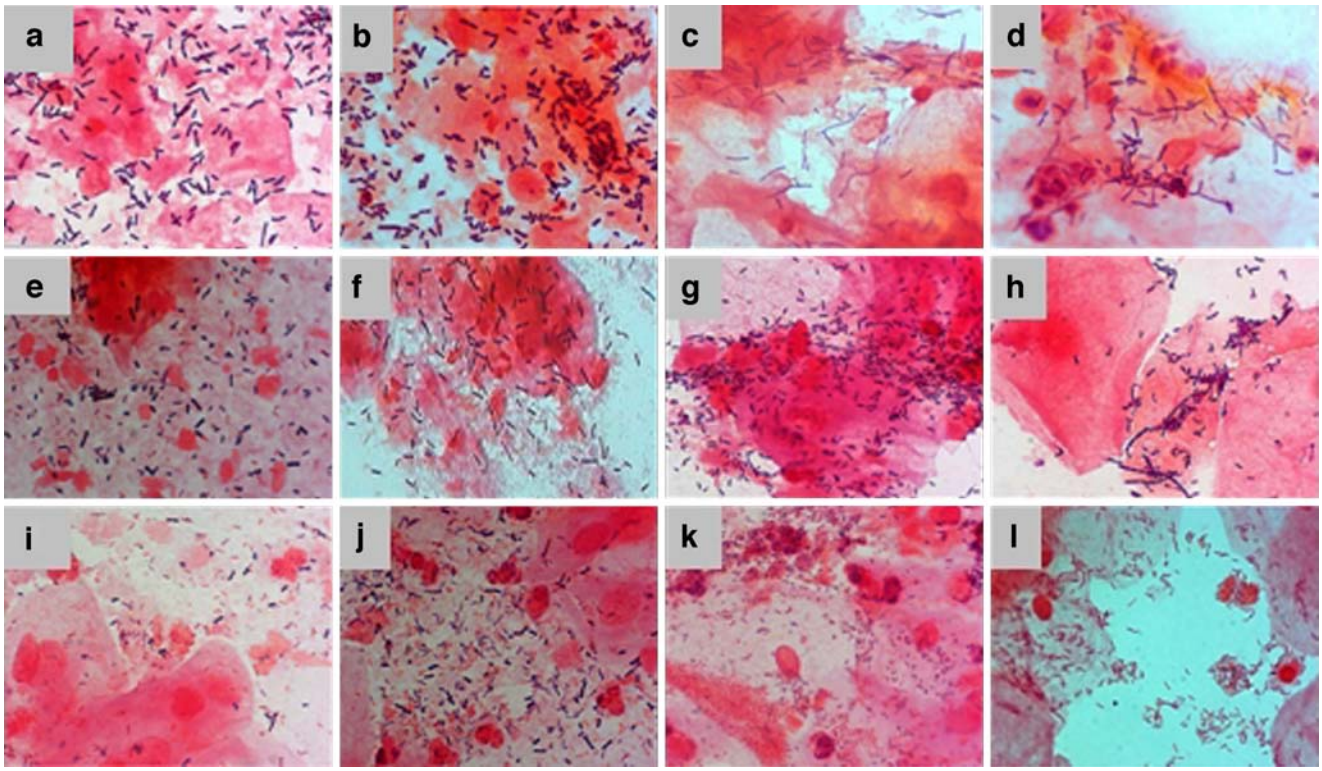


Fig. 2. Smears of vaginal microflora cells. **a** and **b** *L. crispatus*; **c** and **d** non-*L. crispatus*; **e** and **f** *L. crispatus* and non-*L. crispatus*; **g** and **h**, Gram+ve rods; **i** and **j** Lactobacillus and vaginosis bacteria; **k** and **l** bacterial vaginosis (Verhelst *et al.* (91))

system includes Acidform™ gel, an anti-HIV vaginal gel formulation which is currently under Phase III clinical trials (94).

A Lactobacillus Crispatus Soft-Gel Capsule Formulation

A *Lactobacillus crispatus (LC)* microbicidal agent (Lactin Vaginal Capsules™, Gynelogix, Louisville, CO, USA) which acts by re-colonizing the vagina with lactobacillus once released from the soft-gel capsule has been developed (47). *LC* assists in keeping the vagina free from infection by producing hydrogen-peroxide. Normally, upon disturbing the ecology of the vagina either through infection, douching, or poor hygiene when the vaginal pH rises above 4.5, a loss of lactobacilli occurs, resulting in the overgrowth of other bacteria and bacterial vaginosis which is a significant risk for pre-term labor, amnionitis, PID and HIV acquisition (82,90).

Thermosensitive Gel Formulations

Thermosensitive gels are systems that alter the physical characteristics of the gel with exposure to environmental changes within the vagina usually in the range of 25–37°C (96–98). Commonly used thermosensitive polymers for intravaginal microbicide delivery include the polysaccharides, polyacrylamides, poloxamers, polyoxides, polyesters, and a few liposome-based systems. Poloxamer hydrogels represent the most extensively studied thermosensitive polymeric systems, while polysaccharides usually demonstrate good biocompatibility and/or biodegradability (96). Gels, which present mucoadhesive behaviour, are prepared with mixtures of poloxamers and polycarbophil (96,98,100).

Long-Chain Sulfated Polysaccharides and Sulfonated Polymeric Formulations

A poly(sodium 4-styrene sulfonate) (T-PSS) and cellulose sulfate (Ushercell™, Polydex Pharmaceuticals, Toronto, Canada) microbicidal delivery systems have reached Phase I and III clinical trials respectively (Table III). Both are known to inhibit multiplication or activity of HIV, and various other pathogens (101–106). Gel formulations tested contained 5–10% *w/w* T-PSS, hydroxyethylcellulose and propylene glycol (107). T-PSS has been proven safe in animal studies (103, 106). As a result, the US FDA approved an Investigational New Drug (IND) application for both products. However, the Contraceptive Research and Development Program reported that interim data, from a trial where women received Ushercell™, the prevalence of HIV infection was greater in these women, receiving the delivery system then those receiving the placebo gel (108–111). Thus, these clinical trials have been halted pending the outcome of these investigations.

A Carbomer, Lactic Acid and Naphthalene Sulfonate Gel Formulation

A gel comprising a synthetic carbomer, a lactate buffer system (Table III) and naphthalene sulfonate as an antiviral agent (PRO 2000™ Gel, Indevus Pharmaceuticals, Inc. Lexington, MA, USA) is currently under development for the prevention of STIs and HIV (112,113). PRO 2000™ Gel disrupts any interaction of the virus with target cells. Results from *in vivo* animal studies have shown that PRO 2000™ gel is safe and well tolerated (114–116). Currently it is under Phase III clinical trials where it has displayed promising

results (117). PRO 2000™ gels are also compatible with the use of condoms and may even provide more benefits for women worldwide (25).

A Micronized Cellulose Acetate Phthalate Gel Formulation

Cellulose Acetate Phthalate (CAP) has been used for several decades in the pharmaceutical industry for enteric coating of oral tablets and capsules (Table III). Micronized CAP has shown to adsorb and inactivate HIV-1, HSV and other STIs (118). Earlier studies indicate that a gel formulation of micronized CAP has the potential to be used for topical intravaginal delivery of microbicides for prevention of STIs and HIV (119). It is converted into a gel and therefore does not have to be removed following its application (119). CAP is one of the potential anti-HIV vaginal gel formulations that are under Phase II clinical trials (120).

A Monocaprin-Loaded Hydrogel Formulation

Monocaprin hydrogel formulations possess potent microbicidal activity against HIV, HSV, *Chlamydia trachomatis* and *Neisseria gonorrhea* (121,122) (Table III). *In vitro* studies have shown that monocaprin gels formulated using sodium carboxymethylcellulose and polyvinylpyrrolidone or carbomer and hydroxypropylmethylcellulose, are virucidal to HSV-1 and less cytotoxic than nonoxynol-9 (123). *In vivo* studies in mice showed that the gels were non-irritant and non-toxic in the vagina (122). The formulation could be further pursued as intravaginal microbicide delivery systems for the prevention of STIs and HIV.

Polystyrene Sulfonate Vaginal Tablets

Polystyrene Sulfonate (PSS) is also a microbicide that has been developed as an intravaginal tablet formulation (77) (Table III). When PSS intravaginal tablets were used as a microbicide delivery system it was shown that PSS provided superior antimicrobial activity against HIV and HSV (77). This formulation did not immobilize sperm, was not cytotoxic and did not inhibit normal vaginal microflora. This demonstrates its potential as a safe and effective vaginal microbicide delivery system for the prevention STIs and HIV (77,124).

Non-Polymeric Intravaginal Microbicide Delivery Systems for Preventing the Transmission of STIs and HIV

A Cetyl Betaine and Myristamine Oxide Combinatory Cream Formulation

C31G cream (Savvy™, Cellegy Pharmaceuticals, Inc, San Francisco, CA, USA) comprises a broad-spectrum antimicrobial and spermicidal agent namely cetyl betaine and myristamine oxide that displays activity against bacteria, fungi, yeasts, and enveloped viruses (125) (Table IV). These effects occur once the microbicidal agent diffuses through the cream, which is formulated with hydroxyethylcellulose that also acts as a surface-active microbicide. In Phase III clinical trials, the delivery system has shown the ability to prevent HIV-1 and HIV-2 transmission and also has a desirable contraceptive activity (49,50).

A Non-Nucleoside Reverse Transcriptase Inhibitor-Loaded Gel Formulation

UC781 is a NNRTI that is incorporated into gel formulations (Table IV). Short pre-treatment both of isolated cells and human cervical tissue explants, with low concentrations of UC781 provided a strong barrier to subsequent virus infection by cell-free or cell-associated HIV-1 (126,127). UC781 is readily formulated as an appropriate carrier for vaginal application in the form of Replens® Gel (Anglian Pharma, Hertfordshire, UK). It also has a favorable toxicity profile (126). UC781 warrants further clinical assessment for its use as a topical intravaginal delivery system.

A Nucleotide Analogue-Loaded Gel Formulation

PMPA Gel (Tenofovir™, Gilead Sciences, Foster City, CA, USA) is a microbicide delivery system that blocks HIV replication (128). Tenofovir™ could be absorbed by the vaginal epithelium to prohibit the replication and penetration of the virus in the outer cells of the vaginal wall (Table IV). Preliminary results from studies of Tenofovir™ are encouraging and indicate that it may be a new microbicide delivery system to consider as part of a potent anti-HIV regimen. Tenofovir™ may require less frequent dosing than currently available therapies and appears to be active against resistant strains of HIV (83).

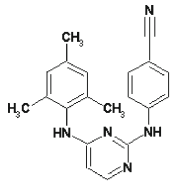
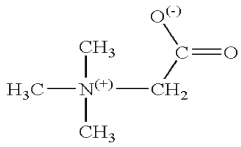
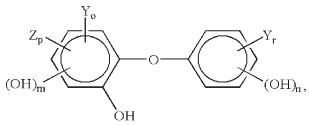
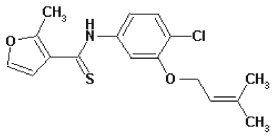
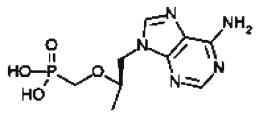
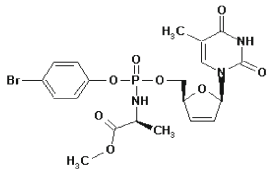
A Novel Synthetically Derived Aryl Phosphate-Loaded Gel Formulation

Stampidine is a derivative of stavudine (Table IV) that is a potent, broad-spectrum anti-HIV agent with potential to be used as a newer class of non-spermicidal microbicides (129). A vaginal gel formulation of stampidine was tested for its potential to cause vaginal mucosal toxicity in New Zealand white rabbits (129,130). Application of 0.5–2.0% *w/w* of a stampidine formulation, produced minimal-to-mild vaginal irritation, which is in an acceptable range for a clinical trial (130). Thus, the favorable toxicity profile of intravaginally administered stampidine-containing gel may provide the foundation for its clinical development as a safe and effective broad-spectrum anti-HIV microbicide delivery system.

A Cyanovirin-N Gel Formulation for Chemotherapeutic and Immunoprophylactic Prevention of HIV

Cyanovirin-N (CV-N) is a novel protein that has generated interest for the chemotherapy of HIV infection (131,132). A possible combined chemotherapeutic and immunoprophylactic approach for preventing HIV may be based upon the concept that continuous exposure of HIV to gp120-binding agents from a gel formulation may diminish glycosylation sites, thus triggering the production of specific neutralizing antibodies to previously hidden gp120 epitopes (133–136). CV-N inhibits the fusion of HIV-infected cells as well as cell-to-cell transmission of HIV-1 infection. CV-N is a promising candidate as a microbicide gel formulation for intravaginal delivery (137). In cell cultures it is non-toxic and resistant to degradation with a shelf-life of at least 6 months (131).

Table IV. Chemical Structures of Non-polymeric Microbicide Compounds

Compound	Structure	Key Function	Reference
Cetyl Betaine		Broad-spectrum antimicrobial	[122]
Myristamine		Spermicidal agent	[122]
UC781		Tight binding NNRTI	[123]
Tenofovir		Blocks HIV replication	[125]
Stampidine		Broad-spectrum anti-HIV agent	[126]
Dapirivine		Potent NNRTI	[139]

Sodium Lauryl Sulfate as an Invisible Condom™ Gel-Like Formulation

Sodium lauryl sulfate (SLS) is currently under Phase II clinical trials as an entry fusion inhibitor (9) (Invisible Condom™, Laval University, Infectious Diseases Research Center, Quebec City, QC, Canada). Ward and Ashley (138) were demonstrated that SLS at low concentrations is a potent inactivator of rotavirus and poliovirus. Previous *in vitro* studies have demonstrated the ability of SLS to inhibit the infective capacity of different enveloped viruses such as HSV-1, HSV-2, and HIV-1 (139–141). This suggested that SLS could be a candidate for use as an intravaginal microbicide gel formulation to prevent the sexual transmission of STIs and HIV (139).

Dapivirine Anti-HIV Vaginal Rings

While most research on microbicides has focused on single-dose and semi-solid topical gels, ring-based delivery systems could reduce the burden of patient compliance. These can provide long-term, controlled release of microbicides that may confer continuous protection against STIs and heterosexually transmitted HIV, and rule out the need for application of the intravaginal microbicidal delivery system near the moment of sexual intercourse (63, 142). Dapivirine, which is also known as TMC120, is a potent non-nucleoside reverse transcriptase inhibitor that is the only vaginal ring system used as an intravaginal microbicide delivery system for preventing the transmission of STIs and HIV (9,142,143) (Table IV).

Polyherbal Anti-HIV Praneem-Loaded Vaginal Tablets

Praneem has been developed as an anti-HIV vaginal tablet formulation. Praneem contains purified extracts of *Azadirachta indica* also known as the Neem tree. Praneem has preventative activity against STIs *in vitro* and therefore it has been developed as a possible intravaginal microbicide delivery system. Phase I safety studies on Praneem tablets,

revealed that the formulation is safe for once daily intravaginal use, for 14 consecutive days, in sexually active uninfected women (113).

Nanotechnology-Based Intravaginal Microbicide Delivery Systems

A Dendrimer-Based Microbicide Formulation

SPL2008 (VivaGel™, Starpharma Ltd., Melbourne, Victoria, Australia) is a dendrimer-based microbicide delivery system in which the dendrimer is not a carrier but an active ingredient (107,144). SPL7013 emerged as the most promising dendrimer after pre-clinical studies, and has been formulated as a gel that is undergoing clinical trials (145). Bernstein *et al.* (146) showed that a 5% *w/w* Carbopol gel can be a suitable vehicle for the intravaginal administration of SPL7013 (147). SPL7013 binds and blocks HIV-1 thereby preventing STIs, including HIV and genital herpes (108,144,141). Phase I trials have shown that VivaGel™ is safe and well-tolerated. Further trials are being conducted to test its efficacy against genital herpes and HIV (108,144).

Silver Nanoparticles Employed as Microbicide Delivery Systems

Various studies are currently investigating the production of a novel class of nanomaterials called, protein-conjugated noble metal nanoparticles (147–149). The production involves direct conjugation of noble-metal nanoparticles with proteins that may be used in numerous forms including antiviral and antibacterial delivery systems (148,150). Under certain pH conditions, disulfide bonds of the protein are available for direct bonding with the noble metal nanoparticles. The polypeptide backbone of the protein remains intact and the method does not affect the functional groups of the constituent amino acid residues. Silver nanoparticles take advantage of the microbicide properties of silver with different materials to produce effective microbicide delivery systems for preventing STIs and HIV transmission (147,148,151).

Polystyrene Nanospheres as Microbicide Delivery Systems

Mucosal secretory IgA may have an important role in the prevention of HIV-1 transmission during sexual intercourse. Therefore, substances that induce HIV-1-specific IgA antibodies have shown promise for use as prophylactic vaccines against HIV-1 infection. It has been reported that Concanavalin A-immobilized Polystyrene Nanospheres (Con A-NS) could efficiently capture HIV-1 particles on the surface and that intravaginal immunization with the nanospheres induces vaginal anti-HIV-1 IgA antibodies in mice (152). Thus, application of Con A-NS by intravaginal delivery is a practical approach to promote an effective immunization approach. Likewise, lectin-immobilized polystyrene nanospheres have been synthesized and examined for HIV-1 capturing abilities (153,154).

A Gel-Like Molecular Condom Formulation as a Barrier for STIs and HIV Transmission

The molecular condom is a recently developed anti-HIV vaginal gel (38). The term "molecular condom" arises from the concept that the polymer construct is liquid at room temperature and, when applied intravaginally, spreads and converts to a gel that effectively coats the vaginal wall (38). The gel is designed to release the anti-HIV bioactives upon contact with semen during sexual intercourse. It is a hydrogel sensitive to body temperature and pH, and serves as a "smart semen-triggered vaginal microbicide delivery vehicle." The system is nano-enabled and explores the use of bioresponsive drug delivery by tailoring the physiological and mechanical requirements essential for intravaginal application. The ultimate hope for this technology is to protect women and unborn or nursing children from HIV (38).

Other Microbicidal Compounds Currently Under Investigation

Thiophen-Thiourea: A Non-Nucleoside Reverse Transcriptase Inhibitor

PHI-443 is a rationally designed novel thiophen–thiourea NNRTI with potent activity against HIV-1 isolates (94) (Table V). Exposure of human sperm to PHI-443 at doses 30,000 times greater than those that yield effective concentrations against HIV has shown that PHI-443 has no effects on sperm motility, kinematics, cervical mucus penetrability, or the viability of vaginal and cervical epithelial cells. Repeated intravaginal administration of 0.5–2% *w/w* of PHI-443 as a gel has been found to be safe in rabbits (94). Thus, PHI-443 has potential as a prophylactic broad-spectrum anti-HIV microbicide without contraceptive activity.

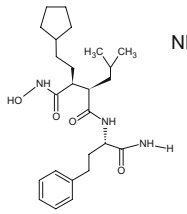
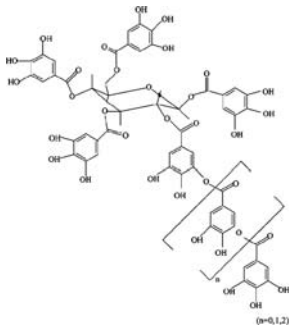
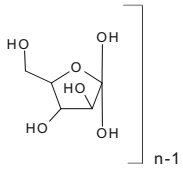
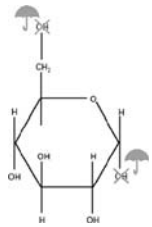
A Secretory Leukocyte Protease Inhibitor

Secretory leukocyte protease inhibitor (SLPI) is an endogenous antimicrobial agent found in mucosal tissue and saliva, most prominently in mucosal sites open to the external environment. Its original function is believed to be an anti-protease defense against neutrophils at the mucosal surfaces (155). *In vitro* laboratory studies have shown that SLPI can inhibit HIV entry into $CD4^+$ cells by interacting with targeted cells blocking access as a barrier function. This may provide a reason as to the low rate of HIV infection through oral sex due to the presence of SLPI in saliva. Data from studies of various mucosal tissues such as the mouth, gut, rectum and endocervix, suggest an inverse relationship between infectious HIV and the presence of SLPI (155). Further studies of SLPI as "a natural endogenous product" need to be performed since it has been shown to have the potential to function as an effective microbicide (155–157).

Monoclonal Antibodies as Microbicides

Monoclonal antibodies are molecules synthesized by the immune system as 'mirror images' of foreign substances (158). A long-range goal in vaginal protection is to develop monoclonal human antibodies in a microbicidal gel for

Table V. Chemical Structures of Other Novel Microbicide Compounds

Compound	Structure	Key Function	Reference
Thiophen-Thiourea		NNRTI against drug-resistant HIV	[91]
Monoclonal		Antibodies Passive immunization	[155]
Lime Juice		Retards HIV transmission	[169]
Yoghurt		Inhibits transmission of HIV	[170]

protecting genital skin and epithelia against infections by topical passive immunization. Mucus secretions contain large quantities of antibodies that are highly specific and potent agents for preventing the infectious entry of pathogens (Table V). Monoclonal synthetic antibodies against sperm, HIV, and other STI pathogens can be applied directly to genital skin and epithelia for protection (159,160). This may closely mimic the normal function of antibodies in the mucosal immune system, e.g., antibodies in breast milk help protect the surfaces of the mouth, nose, eyes, and digestive tract of the baby. To date, the results of studies performed indicate that monoclonal antibodies delivered to the vagina may help prevent pregnancy as well as sexual transmission of genital herpes and HIV (161–164).

Thrombospondin-1 as a Barrier to Mucosal Transmission of HIV-1

During an investigation of the physiology of HIV inhibition by human saliva, laboratory studies identified thrombospondin-1 (TSP1) to have potential as a barrier agent against local mucosal transmission of HIV-1 (165–169) (Fig. 3). TSP1 is found in lower concentrations in plasma and most body fluids (170,172), but not in saliva (165). Since TSP1 is a high molecular mass compound, difficult to purify, and labile in the presence of tissue proteases, it is an impractical candidate to be formulated as a localized intravaginal delivery system. Characterization of alternative TSP1 peptide modifications currently under design may identify conformations that are more potent and stable and thus better for localized intravaginal therapy (166).

Short Interfering RNA as Potential Liposomal Microbicide Delivery Systems

Short interfering RNA (siRNA) is a type of microbicide based on RNA interference (RNAi), a natural selective process for turning off genes, which holds great potential in its ability to treat disease at the genetic level. RNAi is triggered by siRNA molecules that engage a group of cellular proteins, known as RNA-induced silencing complexes (RISC). RISC guides the siRNA to its target messenger

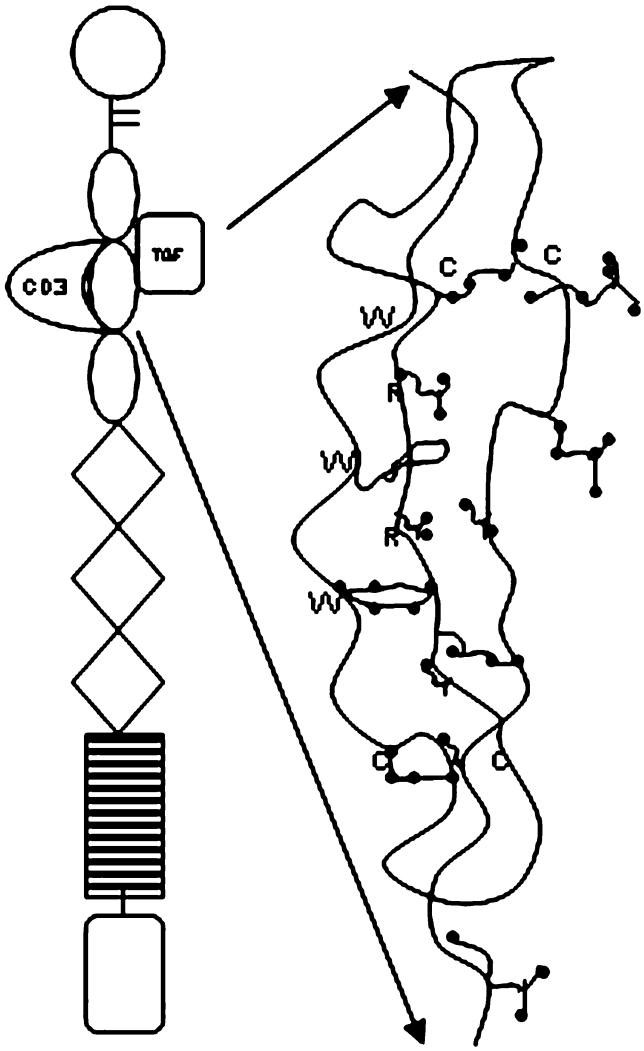


Fig. 3. Schematic of the trimeric-sulphated glycoprotein Thrombospondin-1 with five distinct extracellular matrix adhesion molecules (adapted: Mosher *et al.* (168))

RNA (mRNA) by complementary base pairing and splits it in a selective fashion, thus halting protein expression or viral replication (170). Currently, *in vitro* vaginal tissue culture systems have been investigated and work continues with *in vivo* vaginal xenograft models to determine the optimum dose required for maximum down-regulation of virus replication. *In vitro* tests utilizing fluorescent tagged siRNA in liposomal delivery systems indicate that siRNAs are absorbed throughout the vaginal tissue (34,171).

Lime Juice as a Microbicide

Lime juice has a long history as a contraceptive and vaginal douche (Table V). *In vitro* studies of lemon/lime juice demonstrate that it is an effective microbicide and *in vivo* primate studies, repeated use of lime juice did not damage the vagina (173). Recent studies have revealed that lime juice, at a concentration of up 20% *w/w* has a good safety profile for vaginal use. However, there is an urgent need for a systematic study to determine if the use of lime juice can retard HIV transmission. If it slows HIV transmission, lime juice could prove a life saving microbicide. If the effect is modest, lime juice might still prove to be a 'gold standard' microbicide with which artificial microbicides could be compared, short circuiting the need for time consuming expensive placebo controlled trials (173). Thus, further research is needed to determine the safety of lime juice at higher concentrations, and on the effects of lime juice in women that already use it, and on the efficacy of lime juice as an inexpensive and ubiquitous intravaginal microbicide/spermicide used in delivery systems.

Yoghurt as a Microbicide

Yoghurt may soon be enlisted in the battle against HIV/AIDS (Table V). Lactobacillus, a harmless bacterium that helps turn milk into yoghurt, has been engineered to make HIV-fighting microbicides. Consuming yoghurt containing Lactobacillus could provide a way for women to fend off HIV if no other means are available (174). As well as appearing in yoghurt, lactobacillus naturally inhabits the human vagina. Lactobacilli play a significant physiological role in the maintenance of the ecological balance mainly due to lactic acid production responsible for maintaining a low pH level in the vaginal tract. It survives the passage through the gut and can easily traverse the short distance from the anus and colonize the vagina. Once there, the bioengineered bacteria could produce compounds that inhibit transmission of HIV and maintain a normal vaginal pH, thus helping prevent HIV and STIs infection (174–176). Administration of preparations containing a well-characterized probiotic strains to humans could be used to prevent or treat bacterial vaginitis (177).

CONCLUSIONS

Intravaginal microbicide delivery systems are providing a new option for preventing the transmission of STIs and HIV and this could have considerable public health and economic impact especially in resource-poor countries. As described in this review, numerous safe and effective anti-HIV intravaginal microbicide delivery systems are currently being evaluated at various stages in clinical trials but to date none, which comprise mainly gel formulations, have been found to be fully effective at preventing the transmission of STIs and HIV. Several clinical trials have demonstrated that intravaginal microbicide delivery systems have the ability to protect against the transmission of STIs and HIV but numerous challenges still remain before these systems are used commercially. It is thus anticipated that future research will focus on the design and development of more effective intravaginal microbicidal delivery systems for preventing the transmission of STIs and HIV employing a wide variety of dosage forms with multiple mechanisms of action and microbicidal delivery.

ACKNOWLEDGEMENTS

Grants from the Norwegian Agency for Development Co-operation (NORAD) and the Faculty Research Committee of the University of the Witwatersrand, Johannesburg, South Africa are acknowledged.

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