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Review Article

Theme: Emerging Drug Delivery Technologies
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Vaginal Drug Delivery Systems for HIV Prevention

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Abstract. Microbicides have become a principal focus for HIV prevention strategies. The successful design of drug delivery systems for vaginal microbicide drug candidates brings with it a multitude of challenges. It is imperative that the chemical and physical characteristics of the drug candidate and its mechanism of action be clearly understood and considered to successfully deliver and target drug candidates efficiently. In addition, an understanding of the dynamic nature of the vaginal environment, the tissue and innate barriers present, as well as patient preferences are critical considerations in the design of effective microbicide products. Although the majority of drug candidates clinically evaluated to date have been delivered using conventional semisolid aqueous-based gel dosage forms, drug delivery system design has recently been extended to include advanced delivery systems such as vaginal rings, quick-dissolve films, and tablets. Ultimately, it may be necessary to develop multiple dosage platforms for a single active agent to provide users with options that can be used within the constraints of their social environment, personal choice, and environmental conditions.

KEY WORDS: drug delivery; HIV prevention; microbicides; STI prevention; vaginal drug delivery

INTRODUCTION

Sexually transmitted infections (STIs) are a significant health problem worldwide. STIs affect more than 13 million men and women in the USA each year, and the World Health Organization estimated 340 million new cases of curable STIs worldwide annually. HIV infection rates have reached pandemic levels worldwide with the number of people living with HIV in 2007 estimated at 33.2 million (1). In 2007, 2.5 million people became infected with the HIV virus, nearly half of whom were people aged 15–24 (1). Women now account for nearly 50% of the people worldwide who are infected with HIV (1). Unprotected heterosexual vaginal intercourse has become a predominant route of infection for this disease. Women are disproportionately affected by HIV. In heterosexually acquired cases of HIV, women are more susceptible to infection than men. The basis of this increased susceptibility of HIV infection in women is due to several factors: women are physiologically more vulnerable to acquisition of HIV; women face social, economical, and legal disadvantages which limit their ability to protect themselves; and in many cases, women are unable to negotiate condom use with their male partners (2). Although great effort has been put toward a vaccine for HIV prevention, it is estimated that no vaccine will be available within the next decade (3). For these reasons, it is imperative that female-controlled HIV prevention strategies become available.

Microbicides are recognized as a promising method for the prevention of HIV. A microbicide is a chemical entity that can prevent or reduce transmission of sexually transmitted infections. Topical microbicides offer a mechanism to prophylactically inhibit the transmission of STIs including HIV-1 (4–6). A microbicide product would inactivate pathogens deposited into the vagina during sexual intercourse. One important facet of such a product is that it would provide a female-controlled method of prevention.

PHYSIOLOGY OF THE VAGINA AND BARRIERS TO DRUG DELIVERY

The appropriate formulation of microbicide agents is essential for the development of a safe, stable, effective, and acceptable pharmaceutical product. There are a multitude of considerations for development of a successful microbicide product. Physiologically, the vagina provides several obstacles for drug delivery.

The vagina is a thin-walled, collapsed fibromuscular tube (7 to 10 cm), extending from the body exterior to the uterus. The lower genital tract in women involves four distinct anatomical regions: (1) the introitus, which is covered by a keratinized, stratified squamous epithelium resembling skin; (2) the vaginal epithelium, which is covered by a nonkeratinized stratified squamous epithelium; (3) the ectocervix, which is covered by a mucosal layer histologically similar to that of the

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vagina; and (4) the endocervix, which consists of a simple columnar epithelium with numerous glands (7–9).

Normal cervical stratified squamous epithelium consists of several layers of epithelial cells. The layers can be subdivided into different classes according to the stage of maturation: basal, parabasal, intermediate, and superficial. During maturation, cells move from the basal layer to the superficial layer, becoming flatter, with smaller nuclei and a larger cell volume. Tight junctions are predominant in the superficial layers of the cervix closer to the vaginal lumen. The thickness of cervical squamous epithelium varies (0.2 to 0.5 mm). Age is a main parameter which determines epithelial thickness. After menopause, the volume of the cytoplasm decreases, and the epithelium becomes atrophic. During perimenopausal (3 to 5 years preceding menopause), there is a reduction in the estrogen levels, which contributes to a reduction of epithelium thickness. However to date, no quantitative data have been reported comparing the thickness of pre- and postmenopausal women (10). An understanding of the epithelium of the target is essential for the delivery of both microbicide drug candidates intended for topical application which have activity either in the vaginal lumen or the upper epithelium without becoming systemically available, as well as those intended for systemic delivery. The permeability of microbicide drug candidates across these tissues will impact their efficacy and toxicity profiles.

Vaginal fluid can provide both a target and a barrier to drug delivery. It covers the vaginal epithelium and is composed of secretions from the cervical vestibular glands, plasma transudate, and endometrial and oviductal fluids. The fluid covering the vaginal mucosa protects against entry of pathogens into deeper tissues. Periodic sloughing of mucus and underlying cells removes adherent microbes. Cervical mucus has similar functions and in addition facilitates sperm penetration by changing its viscoelastic properties during ovulation. Properties of the mucus layer can either facilitate or impede the efficacy of a drug product. The presence of physiological fluids may alter the characteristics of a vaginal product, which can reduce the overall efficacy of the drug substance, increase leakage, and decrease drug residence time at the target tissue (11). Vaginal fluids including cervical mucus impacts drug delivery in the vagina in various ways. These fluids result in product dilution and can alter drug dissolution, ultimately playing a role in the success of getting the drug to its target site. Components of vaginal fluids have the ability to interfere with drug substance activity either by destabilization or nonspecific binding. Finally, the presence of vaginal fluid affects product residence time and bioadhesion.

The vagina is kept moist predominantly by the transudation of fluid through the vaginal epithelium and by cervical secretion. The amount, consistency, and characteristics of the fluid that accumulates in the vagina are difficult to describe because these parameters change with the menstrual cycle and with reproductive age (12,13). Vaginal fluid may include contributions from vaginal transudate, Bartholin's and Skene's glands, exfoliated epithelial cells, residual urine, and fluids from the upper reproductive tract such as cervical mucus and endometrial and tubal fluids (14,15). The contribution of cervical mucus to the vaginal fluid has never been fully quantified. The major component of cervical mucus is water, but it also contains mucin, glycoproteins, plasma proteins, enzymes, amino acids, cholesterol, lipids, and a range of inorganic ions (16–18). Vaginal fluids play a dual role in preventing HIV infection. On one hand, the vaginal fluids inhibit HIV transmission by acting as a natural mechanism of protection. Several antimicrobial peptides have been detected in the reproductive tissues including lysozyme, human beta-defensin, human defensin, and secretory leukocyte peptidase inhibitor (19). On the other hand, the physical presence of a fluid layer at the epithelium as well as enzymatic activity in the vaginal fluid has been identified as a major barrier for the delivery and absorption of drugs from mucosal sites.

Enzymatic activity of vaginal fluids has still not been fully characterized. Aminopeptidases are the most abundant type of enzymes present in human vaginal mucosa and a major contributor to protein and peptide degradation. The aminopeptidase activity is located mainly in the cytoplasm, and the concentration/activity in the vaginal mucosa ranges from 0.5 to 1.4 μmol substrate hydrolyzed per minute per milligram protein (20). The identification and quantification of enzymes present are not very well established, but lysozyme has also been identified in human cervical mucus (21). Other enzymes, are present in both vaginal and cervical secretions, and their activity may also vary with the menstrual cycle (22,23). Recently, with the advances of proteomics analysis, determination of proteins and enzymes present in human cervical-vaginal fluids have been conducted. In one study, most of the proteins identified in vaginal fluid were plasma components, such as: albumin, transferrin, apolipoprotein I, calgranulins A and B, and alpha-1-acid glycoprotein 1 (24). Other studies have found similar results (24–28). One of the caveats of all these studies is that the collected fluid was frozen before being analyzed. The freezing process may have compromised the enzymatic activity of the fluid. In addition, the type and amount of proteins present in the fluid after freezing may not reflect that in fresh fluid.

The human female genital tract contains all the essential elements for an effective immune response against genital pathogens (29). It is important to understand this structure when developing microbicide drug delivery systems given that these immune cells are the primary target for HIV as well as potentially the target for some microbicide drug candidates based on their mechanism of action. Studies using immunohistochemistry and flow cytometry have shown that T cells and antigen-presenting cells are present throughout the human cervical and vaginal mucosa (30–33); however, the precise localization and quantity of these cells is still under investigation (34). It has been estimated that leucocytes represent 6% to 20% of the total number of cells in fallopian tubes, endometrium, cervix, and vaginal mucosa. T cells account for about 50% of the leucocytes, with $CD8^+$ cells predominant over $CD4^+$ cells. A study conducted by Johansson *et al.* showed a band of $CD4^+$ and $CD8^+$ cells under the epithelium of vaginal and ecto-cervical tissues (29,35). In addition, Langerhans cells have been shown to be present within the epithelial mucosa (36–38).

The normal vaginal flora plays a significant role in defining the environment to which drugs must be delivered for microbicide products. The microorganisms present are dependent on the physiological conditions of the vagina. Age, menarche, time in the menstrual cycle, pregnancy, menopause, infection, and douching practices all can influence the

vagina microflora. The vaginal microflora consist of both gram-positive and gram-negative species for both cocci and bacilli classes. The organisms present are both anaerobic and aerobic. However, normal healthy tissue is well oxygenated and thus not generally conducive to the survival of anaerobic organisms. They are generally present in relatively low numbers. If necrosis or tissue damage occurs, the oxidation/reduction potential of the tissue decreases, and anaerobic bacteria proliferate, which may lead to infection.

Lactobacilli are a major component of the vaginal microflora in premenopausal women. Lactobacilli are gram-positive bacteria which may or may not produce H_2O_2 dependent on strain. The pH of the vagina is maintained by these bacteria which convert glycogen from exfoliated cells to lactic acid. Glycogen is metabolized by epithelial cells to glucose which is further converted by the *Lactobacillus* to lactic acid. Glycogen deposition by the vaginal epithelium is dependent on the presence of estrogen. Depletion of glycogen is observed in postmenopausal women who are not on estrogen replacement therapy. Any drug delivery system inserted into the vagina must take into account the presence of these bacteria and their contribution to creation of barriers to drug delivery. In addition, given that the commensal bacteria are required to maintain a healthy vaginal environment, care must be taken that drug delivery systems must not be toxic to nor disturb them. An interesting utilization of the innate microflora system with respect to microbicides is their application as a vehicle for sustained release of drug candidates. Use of genetically modified innate vaginal bacteria which secrete anti-HIV agents as microbicide drug candidates is in early development (39,40). However, their incorporation into product formulations has been challenging.

There are unique properties of vaginal drug delivery that must be considered when developing a dosage form. The vaginal pH is normally between 3.5 and 5.0, but it can be altered in some disease states (15). The low pH of the vagina provides innate protection from pathogens. In addition to pH, other physiological factors may impact the absorption and efficacy of vaginally delivered drugs. Cyclical changes, associates to age, phase of the menstrual cycle, and pregnancy are known to modify the epithelial thickness and composition of vaginal fluids (41–47). Certainly, barriers to successful drug delivery and targeting such as a changing epithelial barrier, presence of biological fluids at the surface of the tissue, enzymatic activity of vaginal fluid, and hydrogen peroxide as well as other constituents produced by normal microflora have the potential to reduce biological activity of microbicide candidates if not considered during product development (48).

MICROBICIDE DRUG CANDIDATES

There are over 50 drug candidates currently in preclinical development for their utility as a microbicide for HIV prevention and 12 microbicides candidates in various stages of clinical development (http://www.microbicide.org/, consulted on 09/2/2008). These drugs encompass different mechanisms of action which require different drug targeting strategies for successful delivery (49–55). The mechanism of action of each microbicide agent will define the appropriate vaginal drug delivery system for those products. If a microbicide agent acts by blocking the receptor or co-receptor in the target host cells (macrophages, T cells, and dendritic cells), it is imperative that the delivery system is able to deliver the drug to the site of action. Conversely, a microbicide agent that disrupts the viral membrane before attachment of the virus to the host cell can be delivered to the vaginal lumen without deeper penetration in the vaginal mucosa. Microbicide candidates currently being investigated target the virus via different mechanisms of action and are classified as virucides, entry/fusion inhibitors, reverse-transcriptase inhibitors, integrase inhibitors, and protease inhibitors.

MICROBICIDE DRUG DELIVERY SYSTEMS

The vagina has traditionally been the site for delivery of local-acting drugs. However, it also has great potential for systemic delivery due to its large surface area and great blood supply. A major advantage of this delivery route is that the first pass metabolism is avoided by vaginal delivery as it is for rectal delivery. Its use in systemic delivery is increasing. New controlled-release products currently being researched generally target the mucus-covered cervix, which can serve as a reservoir for such systems. Vaginal drug delivery has been used for a number of therapeutic agents such as antibacterial, antifungal, spermicidal, and steroids (56,57). The most common dosage forms utilized in vaginal delivery are also being currently investigated as microbicides (58–62). More recently, new dosage forms have been investigated such as vaginal rings and polymeric films (63). The choice of the dosage form is directly dependent on the physical and chemical characteristics of the drug candidate to be delivered, the target for the drug candidate, and patient acceptability.

Current Drug Delivery Strategies for Microbicides Being Evaluated Clinically

Several dosage forms have been clinically investigated for use as drug delivery systems for microbicide products. Among these are gels and suppositories (58–60,64) and, more recently, vaginal rings. The preponderance of microbicide dosage forms evaluated to date have been water-based gels. The consistency of this dosage form is the most desirable attribute to justify its use for the vaginal route of administration (65). Semisolid dosage forms are currently the most common dosage form used for vaginal delivery. However, this dosage form is largely associated with messiness and leakage of the product. In addition, the physical and chemical characteristics of each microbicide drug candidate will define the most suitable vehicle and dosage form selected. Early-generation microbicides studied used common pharmaceutical product excipients that had shown contraceptive activity and were effective against HIV-1 *in vitro*. Later-generation microbicides either combined the active excipient with a microbicide drug candidate or incorporated a microbicide drug candidate into an inert vehicle.

An important parameter for a semisolid vaginal product is its viscoelastic properties. These properties contribute to several product attributes including its stability, spreadability, and retention. It is essential that a thorough viscoelastic characterization of any semisolid microbicide be conducted. In addition, given that microbicide products will be used in

tropical climates, it is necessary to characterize viscosity profiles for semisolid dosage forms at these conditions.

Semisolids—Ointment

Terameprocol, tetra-*o*-methyl nordihydroguaiaretic acid, has been shown to have activity against HIV and herpes simplex virus (HSV) (66, 67). It was found that terameprocol formulated at 1% and 2% (*w*/*w*) in a white petrolatum ointment for vaginal administration was safe and well tolerated by healthy women enrolled in a clinical safety study (68,69). Expanded safety and efficacy studies have been planned. However, when the ideal characteristics of a microbicide are taken into consideration from several studies conducted on women's preferences for microbicide products (70–75), a clear aqueous base product will most likely prevail over a white ointment.

Semisolids—Hydrogels

ACIDFORM, an acid-buffering vaginal gel formulation, was formulated to have the properties of maintaining the acidic vaginal pH after semen deposition: being bioadhesive to ensure retention in the vagina for prolonged periods of time; forming a protective layer over the vaginal and cervical epithelia; and maintaining substantial viscosity upon dilution with semen or vaginal fluids, reducing leakage as much as possible (76). A recent vaginal safety study conducted compared ACIDFORM to the "universal placebo" gel. The universal placebo gel is a hydroxyethylcellulose-based gel designed specifically for use as a placebo in microbicide clinical trials (77). This study showed ACIDFORM to be safe; however, several participants complained about irritation, burning, and itching, and those complaints were similar to those described in previous safety studies of ACIDFORM (78).

Another buffering vaginal microbicide gel, BufferGel®, is a strongly buffered (pH 4) aqueous carbopol-based gel that has been shown to be spermicidal, active against HIV and herpes simplex virus type 2 (HSV 2), protective against HSV and *Chlamydia trachomatis* in mouse vaginal models, and safe in healthy women volunteers (79). BufferGel® has been incorporated in a novel applicator device (BufferGel® Duet™), which acts as a cervical barrier and a delivery system for the gel. Studies in healthy women volunteers showed that BufferGel® Duet™ was safe for short-term use and acceptable by the volunteers (80).

Carraguard, a sulfated polysaccharide hydrogel formulation containing 3% carrageenan, was formulated to achieve a viscosity of 33,000 to 34,000 centipoise (cp) (81). The product was tested for safety and efficacy *in vitro* in a cell-based model using HIV-1_{IIIB} (CXCR4 virus) (82) and an explant tissue-based model using HIV-1_{BaL} (CCR5 virus) (83). Human phase 1 studies for this product have shown that the product is safe to healthy women at low risk for HIV who were sexually abstinent (58), safe and acceptable in HIV-positive women who were sexually abstinent (84), and acceptable by both partners (85).

Sodium cellulose sulfate (Ushercell) was formulated in an aqueous gel at the 6% (*w*/*w*) level. The product has a viscosity of 15,800 cps and includes additional excipients such as glycerol, methylparaben, and propylparaben (86). Originally investigated as a contraceptive (87), Ushercell product's application has been expanded as a vaginal microbicide since *in vitro* studies had shown efficacy against HIV-1 (86). The product was shown to be safe in humans (88); however, lack of effectiveness for the prevention of vaginal transmission of HIV in humans created a setback in the microbicide field to re-evaluate *in vitro* and animal models as well as clinical trial design (89–91).

Dapivirine, a next-generation nonnucleoside reverse-transcriptase inhibitor, was initially developed as an HIV therapeutic drug; however, due to poor gastro-intestinal absorption, it is now in development as a vaginal microbicide. Aqueous gel products as well as a vaginal ring system have been evaluated for the delivery of dapivirine. The vaginal gel products evaluated for delivery of this promising drug candidate are hydrophilic based (92). Several clinical studies have been conducted to determine safety, tolerability, and pharmacokinetics for the dapivirine vaginal microbicide gel product in healthy women volunteers. Study results have been presented at both the Microbicides 2008 Conference and the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy. The conclusions from these studies showed that the gel product was safe and well tolerated by women, and the pharmacokinetic data obtained supported use of Dapivirine gel as a once-daily microbicide.

PRO 2000, naphthalene sulfonate polymer, is another topical vaginal microbicide under development. Aqueous gel formulations of 4.0%, 2.0%, and 0.5% (*w*/*w*) PRO 2000 have been evaluated. It has been demonstrated that PRO2000 is capable of protecting against HSV-2 in the mouse model (93), In these studies, the vaginal gel formulations used contained PRO 2000, Carbopol 1382, 0.05% lactic acid, and trolamine to adjust the pH to 4.5 (93). In a phase 1 study, the formulation was shown to be safe and tolerable to healthy volunteer women (94) and HIV-1 infected women (54). Although in a study comparing 2% and 4% formulations it was found that women exposed to the 4% gel product twice daily had more mild adverse events related to the genital tract than those women who were exposed to placebo or 2% formulation (54), PRO 2000 is currently being investigated in phase 2 and 3 clinical trials (95).

Tenofovir (PMPA) 9-[2-(phosphonomethoxy) propyl] adenine gel was the first vaginal topical gel to contain an antiretroviral drug currently used as an oral medication. The vaginal gel formulation contains 1% of tenofovir and excipients such as edetate disodium, citric acid, glycerin, methylparaben, and propylparaben formulated into a hydroxyethylcellulose gel base at a pH of 4.5 (96,97). The gel has been shown to be safe and tolerable in a phase 1 clinical study in healthy women (96).

UC781, a tight-binding nonnucleoside reverse-transcriptase inhibitor, is being developed as microbicide product. Water-based gel formulations containing 0.1%, 0.25%, and 1.0% UC781 were formulated with carbomer 974PNF, methylcellulose, glycerin, methylparaben, and propylparaben (98). The product is currently being investigated in human clinical trials for safety in both the vaginal and rectal compartments as well as pharmacokinetic parameters. In a study which evaluated repeated dosing of the UC781 gel formulation in healthy sexually abstinent women it was found that the product was well tolerated (99). There are currently five safety/pharmacokinetic studies of UC781 in progress (99).

In product acceptability studies for 6% cellulose sulfate (CS) vaginal gel and KY gel, 20% reported product leakage (100). In another CS gel study, 44% of the women in the

sexually abstinent group and 18% in the CS sexually active group reported that leakage would prevent them from using the gel in the future (101). In a separate study, the author noted that reformulating the gel to reduce leakage could lead to increased irritation (102). In BufferGel® trials, approximately 50% of participants reported that the product "felt too wet or drippy" (103). The PRO2000 study found 22% and 23% of the participants reporting at least some problems with the gel being "too wet or drippy" or "too sticky," respectively (104). For the microbicide C31G gel study, product acceptability was limited by heat or burning sensation, and 85% experienced product leakage. Only 55% of the participants had a greater than neutral overall experience (105). Studies of acceptability of polystyrene sulfonate (PSS) gels resulted in one to three women in each product group reporting problems from leakage, irritation, and nausea (106). In the studies with the application of gel microbicides, a marked number of participants reported messy applications, leakage, and overall negative experience (107, 108). In addition, 31% of women did not like cleaning and reusing applicators for the gels (103). Based on these studies, alternate drug dosage forms should be explored.

Advanced Drug Delivery Strategies for Microbicides

In addition to conventional hydrogel products, other dosage form options for the delivery of microbicide drug candidates have been investigated. Advanced drug delivery strategies may be required to achieve drug stability, drug targeting, long-term controlled release, enhance efficacy, or increase patient acceptability. Ultimately, it may be necessary to develop multiple dosage platforms for a single active agent to provide users with options without the constraints of their social environment, personal choice, and environmental conditions. For this reason, it is critical to consider various drug delivery platforms when designing a microbicide product which will be utilized by women in various parts of the world, in a range of social situations.

Vaginal Rings

Most of the vaginal microbicide products in clinical trials or in preclinical evaluations have been investigated for use as a single dose in a coitally dependent manner. Vaginal rings currently available in the US market as contraceptive products are being investigated as controlled-release products for vaginal microbicide application. These microbicide vaginal ring products would provide a long-term release of the drug which results in less frequent need for application and in the end improved patient compliance. The only clinical evaluations of ring-based microbicide products incorporate TMC 120 (dapivirine), a potent nonnucleoside reverse-transcriptase inhibitor. A dapivirine-loaded silicone-based, reservoir-type intravaginal ring was developed with the addition of polymers to modify controlled-release characteristics (109,110). A phase 1 safety study has been completed, and results reported at the XVI International AIDS Conference in Toronto, Canada showed the ring product to be safe and well tolerated and confirmed *in vivo* drug release.

Vaginal Films

Quick-dissolve film dosage forms offer a promising platform for delivery of microbicide drug candidates into the vagina. Compared to gels, films may be easier to apply and less messy, leading to increased user acceptability. Commercial application of films for vaginal delivery of pharmacologically active agents is limited and requires research to determine the optimal formulation conditions. It is also imperative that physiologically appropriate product assessment techniques be developed for vaginal film product development.

Polymeric films can address some product acceptability issues identified by women in studies of gels. The films are a solid dry drug delivery system, which avoids the "messy" discharge associated with product leakage. The films rapidly dissolve once in contact with the vaginal fluids with no introduction of additional fluids, thus reducing leakage. Additionally, their rapid dissolving nature ensures quick release once inserted. Polymeric vaginal films offer an advance in vaginal formulation technology. Vaginal films have the potential advantages of easier application, lower product cost, increased patient acceptability, increased retention time, and increased drug stability. An acceptability study conducted by investigators at the University of Alabama (111) showed that film formulations are preferred by women over other vaginal formulations, such as gels, foams, and suppositories. The small size of the film and the lack of the need for applicators results in a less expensive product that is easier to store. Reduced cost is an important consideration in developing nations as well as in the USA.

Advances in the field of polymer sciences have increased interest in the development of drug delivery systems which utilize newly available polymeric materials. Polymeric films are increasingly being used as a means of drug delivery (112–115). A well-known example is the Listerine® pocket pack breath strip which provides similar oral antibacterial agents as found in the liquid formulation of Listerine®. Films for the oral delivery of vitamins, minerals, herbal remedies, supplements, cold remedies, pain medications, and gastric disturbance medications have been developed (114). Polymeric films provide rapid drug release and bioadhesive properties that may increase retention time at the target tissue. Film-formulated products are very convenient for the user as well as easy to apply. Vaginal films have been investigated for use as contraceptives and more recently as microbicide formulations (116–118).

More recently, research has been conducted for the use of polymeric vaginal films as contraceptives and as microbicide formulations (116–118). There are several attributes which make vaginal films attractive as a microbicide product dosage form. However, few reports have been published using vaginal films as microbicides. A polystyrene sulfonate (PSS) microbicide film has been recently developed. PSS is a novel noncytotoxic antimicrobial contraceptive agent, which has been shown to be safe for vaginal administration in phase I clinical trials in a gel dosage form. The films were colorless, transparent, thin, soft, and tough and rapidly dissolve in physiologic fluid to form a smooth, viscous, and bioadhesive solution. Formulation of these films comprised a polymeric base with polyvinyl alcohol (PVA) combined with either hydroxyl ethylcellulose or hydroxyl propyl methylcellulose and the addition of a plasticizer that varied among glycerin, poly ethylene glycol, and sorbitol (116).

Another study investigated the effects of cellulose acetate 1,2-benzenedicarboxylate (CAP), a film-coating excipient, to inhibit infection by cell-free and cell-associated

HIV-1 isolates (119–121). CAP was formulated with hydroxyl propyl cellulose into a polymeric film by organic solvent evaporation method (122), and safety and efficacy studies are planned.

Most recently, an RC-101 vaginal film has been developed, and results were presented at the Microbicides 2008 annual meeting held in New Delhi, India. RC-101 is a synthetic analog of retrocyclin that has shown activity against X4 and R5 strains of HIV-1 *in vitro*, indicating potential as a microbicide. A PVA polymeric vaginal film formulation containing RC-101 (100 μg/film) was developed by aqueous solvent precasting. RC-101 film product was clear, flexible, had smooth surfaces, and dissolved in less than 5 min in water. *In vitro* permeability studies in a Franz cell model using excised ectocervical tissue were also conducted. These studies compared permeability for tissue exposed to an RC-101 solution and tissue exposed to RC-101 formulated in a film dosage form. Both permeability evaluations resulted in no detectable amounts of RC-101 in the receptor chamber after 6 h as quantified by high-performance liquid chromatography analysis, suggesting no potential for systemic absorption.

Vaginal Tablets

Only one vaginal tablet has been investigated as a microbicide dosage form. Praneem polyherbal was originally formulated with purified ingredients from Neem (*Azadirachta indica*) leaves, *Sapindus mukerossi* (pericarp of fruit), and *Mentha citrata* oil into a pessary delivery device for spermicidal and contraceptive purposes (123). Later on, the formulation was investigated and proven to be effective against some sexually transmitted infections (124). The polyherbal was further developed into a vaginal tablet. The formulation consisted of purified extracts of dried leaves of *A. indica* (Neem tree; 80 mg), along with purified saponins from *S. mukerossi* (40 mg), *M. citrata* oil (20 mg), and quinine hydrochloride (30 mg) with other inactive ingredients such as sodium lauryl sulfate, sodium bicarbonate, carbopol 934P, ethyl cellulose 20 M, starlac, microcrystalline cellulose, croscarmellose sodium, colloidal silicon dioxide, magnesium stearate, and purified talc (125,126). In clinical trials, this product was shown to be safe for vaginal use for up to 6 months with minor adverse effects (125).

Nanoparticles for Targeted Microbicide Delivery

A recently investigated alternative for the targeted delivery of microbicides into the vaginal tissue utilizes nanoparticles. Nanoparticles formulated from poly(D,L-lactide-*co*-glycolide (PLGA)) are currently being investigated for many therapeutic applications to overcome typical drug issues, such as biological half-life, conformational stability, physicochemical stability, solubility, and immunogenic response, all of which may result in reduced activity. In the case of vaginal microbicides, nanoparticles can serve not only to protect the active agent but also facilitate penetration into the vaginal and ectocervical mucosa, allowing drug to reach HIV target cells. A biodegradable nanoparticle drug delivery system for PSC-RANTES, a CCR5 chemokine receptor inhibitor, has been recently developed (127). In these studies, PSC-RANTES was encapsulated via a double-emulsion solvent-evaporation method, and *ex vivo* targeting studies were performed in a Franz cell system with human ectocervical tissue. It was shown PSC-RANTES had a 4.8 times greater uptake into the tissue over nonencapsulated PSC-RANTES during a 4-h exposure time. Once they entered the tissue, the PSC-RANTES loaded PLGA nanoparticles targeted the basal layer of the epithelium.

CONCLUSIONS AND FUTURE PERSPECTIVES

Despite extraordinary advances in treatment, HIV/AIDS continues to increase and spread worldwide. Anti-HIV microbicides represent an important and accessible means to reduce the sexual transmission of HIV. However, facile microbicide development requires design of appropriate dosage forms and physiologically relevant *in vitro* models for the evaluation of product functionality and the effect of formulated microbicidal agents on HIV transmission and normal physiological conditions. Acceptable formulations of microbicidal candidates are needed if this approach is to succeed in limiting HIV spread.

Several factors, defined in this review, are essential for consideration during development of a successful microbicide drug delivery system. Of primary importance is safety and efficacy. *The physical and chemical stability of the drug delivery system is crucial.* In addition, patient acceptability and compliance should contribute significantly to decision making in the design of microbicide drug delivery systems. Patient acceptability, ability, and willingness to use a product directly impacts efficacy.

To ensure successful use of female-controlled products, vaginal microbicides need to be designed for women's convenience. In recent years, women's preferences regarding vaginal formulations have been researched (61,70). The consumer's perspectives and their choice of formulation vary depending on the individual, partners, cultural norms, age, and economical, social, and climate conditions of the specific geographical region. Women's preferences regarding the ideal characteristics of a microbicide product have been extensively studied (5,128–130). Several important criteria for vaginal microbicides with regard to efficacy have been established. Principally, the product must have potent activity against most HIV strains. It may or may not provide broad activity against other STIs. It is essential that the compound retain its activity in the presence of vaginal fluids and semen as well as over the broad pH range which can occur in the vagina. Ideally, the drug product would be effective for several hours minimally. With regard to safety, several criteria exist. The product must not disrupt the normal vaginal flora nor compromise the vaginal mucosa in any way. The importance of conducting microbiologial evaluations to ensure that vaginal products are not toxic to the normal vaginal flora, most important of which is *Lactobacillus*, has been demonstrated in several studies (131–134). Microbicide vaginal products should also be compatible with the existing prevention strategy, the male condom, and have no adverse effects on reproductive health. From an acceptability standpoint, ultimately the product must have adequate shelf life, with tropical conditions and the potential for lack of proper storage being considered. Guidance for microbicide product stability should follow International Conference on Harmonisation guidelines, which include revisions that address standardization

of storage conditions for various climate zones (135). The product must be economically feasible as well as easy to use without interfering with sexual pleasure.

As previously mentioned, the majority of clinical trials to date have used gel-based drug delivery systems. However, these dosage forms may have several drawbacks. Among these is poor retention in the vaginal lumen which results in product leakage and the complaint of "messiness" by the user (116). Economic and social conditions as well as consumer/patient preference may interfere with acceptability of these traditional dosage forms. This would result in products not acceptable to consumers, resulting in overall reduced effectiveness of such microbicide products. This demonstrates the critical need for a thorough scientific evaluation of the chemical and physical properties of a vaginal microbicide product. This data may ultimately impact not only patient acceptability but also safety, functionality, and efficacy of the product. Given that women's preference, social environment, and economic status vary, it will be necessary to develop multiple drug delivery platforms to ensure use, compliance, and ultimately product efficacy.

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