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Vaginal Drug Delivery System a Promising Approach for Antiretroviral Drug in the Prevention of HIV Infection: A Review

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Abstract

Vaginal drug administration can improve prophylaxis and treatment of many conditions affecting the female reproductive tract, including sexually transmitted diseases, fungal and bacterial infections, and cancer. However, achieving sustained local drug concentrations in the vagina can be challenging, due to the high permeability of the vaginal epithelium and expulsion of conventional soluble drug dosage forms. Nanoparticle-based drug delivery platforms have received considerable attention for vaginal drug delivery, as nanoparticles can provide sustained release, cellular targeting, and even intrinsic antimicrobial or adjuvant properties that can improve the potency and/or efficacy of prophylactic and therapeutic modalities. Here, we review the use of polymeric nanoparticles, Creams and Gels, tablets and suppositories, Rings for vaginal drug delivery. Although most of the work toward nanoparticle-based drug delivery in the vagina has been focused on HIV prevention, strategies for treatment and prevention of other sexually transmitted infections, treatment for reproductive tract cancer, and treatment of fungal and bacterial infections are also highlighted.

Keywords: Sexually transmitted infections, HIV prevention, Polymeric nanoparticle (NP)

INTRODUCTION

Sexually transmitted infections (STIs) are a significant health problem worldwide. HIV affects over 33 million people worldwide [1]. 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 [2]. 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 [2]. 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 has proven to be insufficient to combat this incurable 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 vaginal 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 [6, 12].

ANATOMY AND PHYSIOLOGY OF THE VAGINA

Based on the pharmaceutical literature, human vagina is often described as a thin-walled slightly S-shaped fibro muscular collapsible tube between 6 and 10 cm long extending from cervix of the uterus [13]. There are three layers which covers the vaginal wall: the epithelial layer, the muscular coat and the tunica adventia. During the menstrual cycle, the thickness of the vaginal epithelial cell layer changes by approximately 200– 300 Am [14]. The surface of the vagina is composed of legion folds, which are often called rugae. The rugae provide dispensability, support and an increased surface area of the vaginal wall. The vagina has an excellent elasticity because of the presence of smooth elastic fibers in the muscular coat. Loose connective tissue of tunica adventia further increases the elasticity of this organ. The network of blood vessels that supply blood to the vagina include a plexus of arteries extending from the internal iliac artery, uterine, middle rectal and internal pudental arteries. In fact, arteries, blood vessels and lymphatic vessels are abundant in the walls of the vagina. Drugs absorbed from the vagina does not undergo first-pass metabolism because blood leaving the vagina enters the peripheral circulation via a rich venous plexus, which empties primarily into the internal iliac veins [15]. There is some drainage to the hemorrhoidal veins as well. The lower part of the vagina receives its nerve supply from the pudental nerve and from the inferior hypogastric and uterovaginal plexuses [16].

Although the vagina does not possess any gland but it secrets a large amount of fluid [17], cervical secretion and transudation from the blood vessels with desquamated vaginal cells and leucocytes mainly constitute the vaginal fluid [18]. Secretions from the endometrium and fallopian tubes also contribute to the vaginal fluid [17]. Like the thickness of the vaginal epithelium, the amount and composition of the vaginal fluid also changes throughout the menstrual cycle. Women of reproductive age produce fluid at a rate of 3–4 g/4 h, while the discharge produced by postmenopausal women is reduced by 50% compared to those produced by women of reproductive age [19]. The human vaginal fluid may contain enzymes, enzyme inhibitors, proteins, carbohydrates, amino acids, alcohols, hydroxyl ketones and aromatic compounds. Sexual arousal may affect the volume and composition of vaginal fluids and that can alter the drug release pattern from vaginal delivery system. Lactic acid produced from glycogen by the Lactobacillus acidophilus present in the vagina acts as a buffer to maintain the vaginal pH between 3.8 and 4.2. During menstruation, the pH of vaginal fluid increases and frequent acts of coitus may also cause an increase in the vaginal pH because both ejaculate and vaginal transudate are alkaline. The presence of cervical mucus and the amount of vaginal transudate may also alter vaginal pH [20]. The vaginal epithelium has a high activity of enzymes that could potentially affect short- and longterm stability of intravaginal delivery systems and devices.

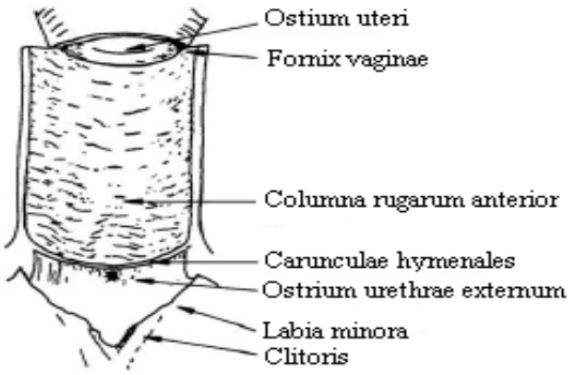

Figure1: Inside the upper vaginal wall.

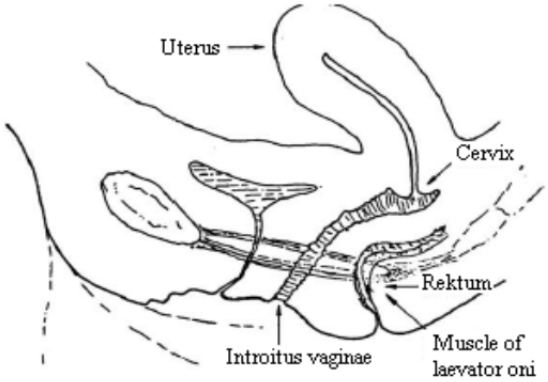

Figure1: Graphical description of the vagina

FACTORS AFFECTING THE VAGINAL ABSORPTION OF DRUGS

Like other mucosal routes of administration, drug is absorbed in the vagina mainly via two routes (i) Transcellularly via concentration dependent diffusion through the cells, (ii) paracellularly mediated by tight junctions and (iii) vesicularly or receptor mediated transport as stated by Richardson and Illum [15]. Drug absorption from vaginal delivery systems occurs in two main steps: drug dissolution in vaginal lumen and membrane penetration. So any factors related to physiology or formulation that affects the above mentioned steps could potentially alter absorption profile from vaginal drug delivery Overall, vast and multifarious factors and processes are involved in drug absorption from the vaginal route [13]

PHYSIOLOGICAL FACTORS

As mentioned above, cyclic changes in thickness of vaginal epithelium, fluid volume and composition, pH and sexual arousal could potentially affect drug release from intravaginal delivery systems and also alter its rate of absorption [20]. For example, the vaginal absorption of steroids is affected by the thickness of the vaginal epithelium [21]. Vaginal absorption of estrogen has been shown to be higher in postmenopausal women compared to premenopausal women [22]. There have been some conflicting reports as to the change in drug absorption with the increase in vaginal epithelium. Studies have shown that the vaginal absorption of steroids is influenced by the

thickness of vaginal epithelium and the epithelial thickness is therefore reduced by long-term estrogen therapy [21].

The volume, viscosity and pH of vaginal fluid may have either negative or positive impact on vaginal drug absorption. The absorption of drug that is poorly water-soluble may be increased when the fluid volume is higher. However, the presence of overly viscous cervical mucus may present a barrier to drug absorption and increased fluid volume may remove the drug from vaginal cavity and subsequently reduce absorption. Since many drugs are weak electrolytes, the pH may change their degree of ionization and affect the absorption of drug. In vitro study has showed that release of PGE2 from vaginal preparations may vary depending on the pH of the media [23]. Any change in the vaginal pH may affect the release profiles of pH sensitive drugs from vaginal drug delivery systems [24].

PHYSICOCHEMICAL PROPERTIES OF DRUGS

Physicochemical properties such as molecular weight, lipophilicity, ionization, surface charge, chemical nature can influence vaginal drug absorption. For example, the vaginal permeability of straight chain aliphatic alcohols increases in a chain length dependent manner [25]. In consideration to permeability literature shows that lipophilic steroids like progesterone and estrone having better permeability than the hydrophilic one like hydrocortisone and testosterone [26]. However, it is generally accepted that low molecular weight lipophilic drugs are likely to be absorbed more than large molecular weight lipophilic or hydrophilic drugs. A study on vaginal absorption of polyvinyl alcohol suggested that the molecular weight cut-off above which compounds are not absorbed may be higher for the vagina than other mucosal surfaces [27]. Since vaginal fluid contains a large amount of water, any drug intended for vaginal delivery require a certain degree of solubility in water. In fact, data on the human vaginal permeability to drugs with different physicochemical properties is very limited; much work needs to be done on the effects of physicochemical parameters of drug on vaginal absorption.

VAGINAL ENZYMES IN DIFFERENT SPECIES

The external cell layers and the basal cell layers of the vagina retain most of the enzyme activity [1, 22, 23]. Among the enzymes present, proteases are likely to be the prominent barrier for the absorption of intact peptide and protein drugs into the systemic circulation. It has also been reported that the rat vaginal smears have trypsin-like activity, which reaches a maximum level during the proestrus stage [24]. Lee [25] has suggested that most of the exopeptidases and endopeptidases, which digest the peptides and proteins, are present in the vaginal epithelium. The various enkephalins studied in rabbit vaginal epithelium suggest the presence of at least three peptidases viz. aminopeptidase, dipeptidyl peptidase and dipeptidyl carboxypeptidase, which play a vital role in metabolism of enkephalins [26]. Among these enzymes, aminopeptidases were the main enzymes responsible for methionine and leucine enkephalin metabolism, while dipeptidyl carboxypeptidase was the main enzyme for d-ala-met-enkephalin metabolism. Sayani et al. [27] reported the existence of aminopeptidases in rabbit nasal, rectal and vaginal extracts. The highest concentration of these enzymes was in the vaginal extract (0.045 U/ml) of the rabbit. A specific study [28] dealing with the comparison of enzymatic activities of four different aminopeptidases (aminopeptidase N, leucine aminopeptidase, aminopeptidase A and aminopeptidase B) in vaginal homogenates of various species report that the enzyme activity in rat, rabbit and human was significantly lower than that of sheep and guineapig.Overall, the aminopeptidase activity in the species showed the following order of activity: sheep>guinea-pig>rabbit≥human≥rat. The authors conclude that the rat and the rabbit could be used as potential model animals for vaginal enzymatic activity studies and for the determination of degradation of protein and peptide drugs in the vagina.

DRUG DELIVERY SYSTEMS FOR VAGINAL ADMINISTRATION

The vagina has been studied as a favorable site for the local and systemic delivery of drugs, for female associated conditions. 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. More recently, vaginal nanoparticle has been introduced for HIV therapy. Table 1 enlists few novel formulation systems intended for vaginal delivery of different therapeutic agents. Woolfson et al. (2000) also expressed that choice of vaginal drug delivery platforms depends upon multiple variables, spanning from drug properties to clinical requirements to user acceptability [29]

However, by far, it had been difficult to quantitatively measure the distribution of a drug after an intravaginal administration and also it is uncertain if the administered formulation coated the whole organ. In this regard, an interesting study by Chatterton et al. [30], evaluating the distribution of two radio labeled vaginal products, proves helpful. The authors describe the retention and distribution of 99mTc-DTPA vaginal cream (reference product) and gel (experimental) dosage form. Such studies are useful in assessing and comparing different vaginal dosage forms with regard to retention and distribution. Vaginal delivery may be designed for administration of drugs by using an applicator or specifically designed systems for intravaginal administration. Further, vaginal formulations may be designed to produce local effect such as spermicidal or antibacterial effect or to produce a systemic effect by continuous release of drugs such as contraceptives. Readers further interested in vaginal drug delivery are directed to a recent review by Alexander et al. [31]. Few of the commonly used marketed preparations with their intended use are tabulated in Table 2.

Table 1 Formulation systems experimented for vaginal drug delivery

Therapeutic drug	Intended use	Dosage form	Animal model	Comments
Nonoxynol-9	Spermicidal/topical contraceptives	Gel, foam, cream	Rabbit	Detergent type spermicide,irritation and increased risk of infection
Miconazole nitrate	Antifungal	Cream, suppository, swelling controlled release system	In vitro	
Prostaglandin E2	Cervical ripening	Cross linked PEG hydrogel, suppository	IN vitro	Onset of labor not always predictable.
Dapivirine	Anti-HIV	Nanoparticle	Human	Novel safe and effective vaginal microbicides
Tenofovir	Anti-HIV	Nanoparticle	Human	Intravaginal delivery of HIV microbicides for the prevention of HIV transmission.
Liposomes	Dermatological treatment	Gels	Porcine	Good patient compliance.
Metronidazole	Treatment of bacterial vaginosis	Tablet	Rabbits	Side effects are vaginal discharge, vulvovaginal irritation, cervicitis
Oxybutynin	Overactive Bladder	Gel	Rabbit	OXY was well tolerated when administered as a CH_3 gel formulation due to the preventing effect of chitosan against cell damage

Table 2. Few of the commonly used marketed vaginal products

Therapeutic drug (Brand name)	Intended use	Dosage form	Comments	Company
Etonogestrel+ethinyl estradiol(NuvaRing^R)	Contraception	Vaginal ring	Commonly reported adverse events are vaginitis, leukorrhea, weight gain.	Organon
Progesterone (Prochieve^R)	nfertility, secondary Amenorrhea	Bioahesive vaginal gel	Possible side effects are Constipation, pain around Vaginal area, breast pain.	Fleet
Estradiol (EstringR)	Hormone therapy	ring	Frequently reported side effect is increased vaginal secretions	Pharmacia and Upjohn Laboratories
Tioconazole(Vagistat-1R)	Anti-fungal, vaginal candida infection	Ointment	Possible side effects are shortness of breath; swelling of lips, face, or tongue Partners may	Bristol Myers Squibb
Clotrimazole (Trivagizole^R)	Anti-fungal	Cream	experience minor skin irritation	Taro Pharmaceuticals

CREAMS AND GELS

Creams and gels are used for topical delivery of contraceptives and anti-bacterial drugs. They can also be used to reduce vaginal irritation, discharge and other sexual problems. This delivery system is messy to apply, uncomfortable and sometimes embarrassing when they leak into the undergarments. Further, creams and gels may not provide an exact dose because of nonuniform distribution and leakage. The desirable properties of vaginally administered cream or gel against microbicides are acceptability and feasibility. They must be easy to use, non-toxic and non irritating to the mucus membrane. metronidazole and clindamycin vaginal cream are used in the treatment bacterial vaginosis and they are found to be nearly as effective as orally administered drugs [32]. Lamont et al.,2004, [32] carried out a randomized, placebo controlled 3-day course study during the second trimester of pregnant women to evaluate the efficacy of an antibacterial vaginal cream in the treatment of bacterial vaginosis[33],. They stated that the clindamycin vaginal

cream was well tolerated and more efficacious than placebo in the treatment. In the absence of an effective prophylactic anti-HIV vaccine or therapy, current efforts are aimed at developing topical intravaginal formulations of anti-HIV agents or microbicides to reduce the mucosal and perinatal virus transmission [34]. Vaginal creams and gels could be based on the principle of emulsion or hydrogel based drug delivery. During the past few years, considerable work has been done on the development of hydrogel controlled release drug delivery systems. These hydrogels, when placed in an aqueous environment, swell and retain large volumes of water in their swollen structure and release drug in a controlled fashion. A swelling controlled release hydrogel delivery system for intravaginal administration of an antifungal drug, miconazole, has been reported [35]. Hydrogels are hydrophilic polymers that have been cross-linked by means of covalent bonds. A 3% alginate gel of nonoxynol-9 has been investigated for intravaginal spermicide delivery [36]. In the study, it was shown that spermicidal activity and diffusion of the agent changes with pH and osmolarity of the formulation. Recently, gel-microemulsions have been proposed as a nontoxic vaginal formulation [37]. A gel microemulsion based formulation of a spermicide with anti- HIV effect, phenyl phosphate derivative of zidovudine, has been developed [38]. Multiple intravaginal application of this drug as microemulsion gel formulation did not cause any damage in the vaginal epithelium in rabbit model. Vaginal gel has also been used for intravaginal vaccine delivery. Intravaginal delivery of cholera vaccine showed a greater mucosal response in female genital tract compared to oral administration of the vaccine [39].

VAGIANL TABLET AND SUPPOSITORIES

There are large number of vaginal medications are 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 vaginal formulations are designed to melt in the vaginal cavity and release the drug for a long period of time. Vaginal suppositories are inserted as solids and dissolve or melt inside the body to deliver the medicine. These are generally rod shaped, conical, or wedge-shaped and may be larger than rectal suppositories (4–8 g) [40]. They are commonly used for topical treatment of vaginal infections such as HIV transmission, human papilloma virus (HPV), herpes simplex virus (HSV), and chlamydia [41]. Various APIs, as miconazole nitrate, are successfully delivered as vaginal suppositories (USP, 200 mg). The main advantages of these dosage forms are self administration, avoidance of first-pass metabolism, localized (topical) action, reduced systemic distribution, and reduced systemic toxicity. However, suppositories have also some limitations, such as mucosal irritation, high cost of manufacture, and patient incompliance [42] Suppository systems are most commonly used to administer drugs like dehydroepiandrosterone sulphate for ripening effect on uterine cervix, miconazole for vaginal candiasis and progesterone for hormonal replacement therapy. Binders, disintegrant and other excipients that are used to prepare conventional oral tablets may contain in the vaginal tablet. It has the advantage of ease of manufacture and insertion. Polymers having mucoadhesive property are sometimes used in vaginal tablet formulation to increase vaginal residence time. Recently Mohd Afftab Alam et al. reported the development of acid-buffering bioadhesive vaginal tablet for the treatment of genitourinary tract infection and was found that acid-buffering bioadhesive vaginal tablet produce better antimicrobial effect than some of the marketed intravaginal delivery system [43].Drugs that are administered as vaginal tablets include itraconazole, clotrimazole and prostaglandins. Literature shows that presence of hydrophobic and release retarding materials may decrease the absorption of a drug from a vaginal formulation. The drugs are too hydrophobic in nature may not be suitable for vaginal tablets. Presence of penetration enhancers such as surfactants, bile salts can significantly enhance absorption.

VAGINAL RINGS

Vaginal rings are circular ring type drug delivery devices designed to release drug in a controlled fashion after insertion in the vagina. The advantages of vaginal ring are that it is under user controlled, does not interfere with coition, does not require daily intake of Pills and allows continuous delivery of low dose Steroids. They are approximately 5.5 cm diameter with a circular cross section diameter of 4–9 mm and the ring is inserted in the vagina. In simple vaginal rings, drug is homogeneously dispersed within a polymeric ring. Ring design, solubility of drug in the elastomer and the molecular weight of the drug are important factors that regulate the release pattern of the drug. Very high release rates can be attained by using a high drug load at the ring. Sometimes, drugs in the outermost layer provide an initial burst release. To obtain a constant release of a drug from vaginal ring, sandwich or reservoir type rings have been developed. Sandwich type devices consist of a narrow drug-containing layer located below the surface of the ring and positioned between a non-medicated central core and a nonmedicated outer band. In reservoir type rings, drugs are generally dispersed in a centralized core, which is then encapsulated by a polymer containing drug free layer. In a single ring, it is possible to have several cores of different drugs and thereby allowing administration of several drugs from the same device. The rate of drug release can be modified by changing the core diameter or thickness of the non medicated coating. The material used for making vaginal ring is usually polymeric in nature. literature shows that polymer used for much of the vaginal ring formulation are poly(dimethylsiloxane) or silicone devices, although other elastomeric polymers such as ethylene vinyl acetate and styrene butandiene block copolymer have been tested in recent years [44,45]. Ethylene vinyl acetate polymers are classified by the content of vinyl acetate. The addition of vinyl acetate units in the polyethylene provides the following advantages: increased flexibility, improved optical properties, greater adhesion, and increased impact and puncture resistance. Further, the clinical acceptability of rings made of ethylene vinyl acetate is very high [44, 46]. In a study by Roumen

and Dieben [44], evaluating the tolerability of ethylene vinyl acetate non medicated vaginal ring of diameter 54 mm, the acceptability percent among the subjects involved in the study was 91%. The ring was to remain inserted for 21 consecutive days after insertion, permitting temporary removal during coition. Most of the women judged the ring easy to insert and remove. No adverse effects were experienced among the test group during the study period.

Vaginal rings are used for contraceptive and hormone replacement therapy [47–49]. For most contraceptive applications, the rings are placed in vagina for 21 days followed by a week of ring free period. NuvaRing ^{R} is the only combined contraceptive vaginal ring available in the US market. NuvaRing^{R} is a flexible, transparent, contraceptive vaginal ring containing two active components, etonogestrel and ethinyl estradiol. The ring releases 120 Ag/day of etonogestrel and 15 Ag/day of ethinyl estradiol over a 3-week period of use. Clinical trials show that NuvaRing^{R} is an effective contraceptive ring with good cycle control and user acceptability [46]. Femring^{R} and Estring^{R} are estrogen releasing rings used for estrogen therapy. Femring^{R}, which is made up of silicone elastomer, contains acetate derivate of estradiol, which is placed in the vagina once every trimester. Estradiol acetate is hydrolyzed to estradiol after being released from the delivery device. Estring^{R} is made of silicone polymers and when inserted in the vagina releases 7.5 Ag of estradiol per day.

POLYMERIC NPS (P-NPS)

Polymeric NPs are nano-sized spheres (usually range between 10–200 nm or more, or they can also be larger [in the um range], in which case they are characterized as microspheres or microparticles), which are composed of various polymers mixed with stabilizers and can be loaded with drug molecules. P-NPs are known to be able to enhance local drug delivery while limiting systemic exposure. Various types of P-NPs have been tested in order to evaluate their possible use as vaginal drug delivery systems, from which the main types with some examples will be presented here. Between the various types of polymers for P-NPs, poly-dl-lactide-co-glycolic acid (PLGA) has been extensively used. After being endocytosed PLGA P-NPs can escape liposomal disruption and deliver their cargo into the cytoplasm. PSC-RANTES, a CCR5 chemokine receptor inhibitor, was loaded in PLGA P-NPs and ex vivo permeation studies demonstrated that the NPs significantly increase drug uptake (4.8 times) compared to the free drug [50], a fact that was attributed to high cellular uptake of P-NPs and protection of the encapsulated drug fromacidic/enzymatic degradation. Intravaginal administration of various types of fluorescent NPs revealed that PLGA NPs without surface modification and avidin bearing PLGA NPs demonstrated lower retention in the vagina than PEGylated PLGA NPs, which were found in the cervicovaginal lavage 24 h after administration, but were not evaluated for longer time periods [51]. Acyclovir-loaded PLGA-NPs administered intravaginally, were found to protect more mice (53%) than the equivalent acyclovir solution (16%). Additionally, a solution with a 10 times higher concentration of acyclovir demonstrated only 30% protection [52]. The lack of success of the VOICE clinical trial triggered the need for development of an NP delivery systemfor tenofovir. PLGA and a pH-sensitive polymer, Eudragit S100, formed NPs, which were compatible with the vaginal epithelium. Eudragit (insoluble at pH 4.5, but soluble at pH 7.6) presence minimized the release of TFV from the NPs in simulated vaginal fluids, but increased the release in simulated seminal fluid (since Eudragit S100 is soluble at pH 7.6). The Eudragit- PLGA NPs uptake by vaginal epithelial cells was 50% after 24 h of incubation [53], and they were noncytotoxic (up to 1 mg/mL) [54]. Tenofovir was also loaded in chitosan. NPs which were demonstrated to penetrate mucus [55]. Furthermore, TFV-loaded NPs (consisted of chitosan and PLGA) have been prepared [56], but not yet investigated as intravaginal delivery systems. Of course all of the proposed P-NPs for vaginal delivery (or targeted delivery) ofmicrobicides, require a suitable vehicle to deliver the NPs, in the vagina. Unfortunately, there are only a few successful efforts in this direction. It is well known that gelling agents may drastically affect NP stability and size, and thereby sufficient gelling agents thatwould prevent such effects should be identified. Some relevant studies considered a Thermosensitive gel (liquid at room temperature and highly viscous at 37 °C) mixed with Raltegravir and Efavirenz-loaded PLGA-NPs. In this case, the NP incorporation in gels did not modulate any of their physicochemical characteristics, while the NPs were rapidly endocytosed by HeLa cells (within 30 min) [57]. Furthermore, after vaginal administration of fluorescently labelled NPs to mice, significant fluorescence levels were detected in the vaginal epithelium even 24 h post-administration. After treatingHIV-1-infectedH9monocyte cellswith Efavirenz (EFV) and lopinavir combination-loaded PLGA-NPs, considerably higher drug levels were detected in the cytoskeleton and nucleus of the cells, compared with cells treated with the free drugs. Furthermore the NP-dosage forms were non-cytotoxic and effective to block HIV-1, demonstrating their efficacy for delivery of combinations of ARV drugs to inhibit HIV-1 replication [58]. PLGA NPs of efavirenz (EFV) or saquinavir (SQV) were demonstrated to have a substantial HIV inhibitory effect (IC50 values of NPs were 50 times lower than free drug), and were also non-cytotoxic and nontoxic towards ectocervical explants. Furthermore a strong synergistic effect was demonstrated when NP-EFV or NP-SQV were combined with TFV [59]. Various types of Poly-epsilon-caprolactone (PCL) consisted and Dapivirine-loaded NPs have also been preclinically evaluated for their effects on the drug permeability (compared to that of free drug). When the NP surface was coated with cetyltrimethyl ammonium bromide (CTAB), the drug transport was enhanced, it was unchanged when the surface was coated with sodium lauryl sulfate (SLS), and reduced by a poly(ethylene oxide) (PEO) coating, while all three NP types increased the retention of the drug on the monolayer/tissue compared to the free drug, a fact attributed to increased cell uptake and mucosal penetration of Dapivarine from the NP formulations. Additionally, the

cytotoxicity of the dosage form was different for the different NP-types; the SLS and CTAB coatings demonstrated increased toxicity, but the PEO coating resulted in lower toxicity (in vitro, but not ex vivo). Thereby, it was concluded that PCL NPs can enhance the mucosal uptake of Dapivirine and more specifically that PEO-modified Dapivirine-loaded PCL NPs may increase the topical bioavailability of the drug and subsequently its efficacy as a microbicide [60]. These NPs were also evaluated for their ability to modify the pharmacokinetics and genital distribution of Dapivirine and were found to provide favorable genital drug levels, while also being safe after daily administration for 14 days. Thereby, such NPs should provide advantages when used for vaginal drug delivery of HIV prophylaxis [61].

Recently, chitosanNPswere proposed as anti-HIV therapeutic systems [62], since chitosan is a natural polymer with anti-microbial properties and low immunogenicity. Furthermore, chitosan NPs have a cationic surface which may lead to improved targeting efficacy towards negatively

charged cells (compared to other NPs). Other advantages of chitosan NPs may be the efficient delivery of drugs to macrophages (which serve as HIV-1 reservoirs) via chitosan, as well as their instability in the acidic environment of endosomes (which should result in fast drug release, after NP uptake by cells)[63]. Saquinavir-loaded chitosan NPs with high drug encapsulation efficiency (75%) and enhanced cell targeting efficiency (N92%), demonstrated superior control of virus proliferation in Jurkat and CEM-CCR5 cells, compared to the free drug. A more advanced Tenofovir NP-type, hyaluronidase-sensitive NPs composed of hyaluronic acid (HA-NPs) were very recently proposed for triggered release of Tenofovir under the influence of hyaluronidase (HAase) enzyme. Unlike the case of HA-based gel, HAase significantly triggered TFV release and HA degradation, ~90% w/w and 65% w/w, respectively, after 24 h, when the same values in HAase absence, were only ~39% w/w and 26% w/w, respectively. The NPs were noncytotoxic to cell-types and Lactobacillus crispatus. The data demonstrated the potential of HAase-sensitive HA-NPs for controlled vaginal delivery of anti-HIV microbicides [64].

BIOADHESIVE DELIVERY SYSTEMS

Bio-adhesive vaginal formulations those are capable of delivering the active agent for an extended period at a predictable rate. Bio-adhesive formulations have been found to reduce the conventional treatment time of fungal infections by at least 25%. A bio-adhesive formulation might not necessarily contain a therapeutic agent and can be used as a moisturizer for the treatment of dry vagina. Tablets that are placed directly between the vaginal mucosal surfaces have been demonstrated to be excellent bio-adhesive formulations .Most of the conventional vaginal formulations are associated with disadvantages of low retention to the vaginal epithelium, leakage and messiness thereby causing inconvenience to the user. To circumvent these challenges, bioadhesive drug delivery systems are being propagated. Bioadhesive polymers that have been used for vaginal formulation include chitosan, sodium-alginate, polycarbophil, polycaprolactone, polyethylene glycol hydroxypropylcellulose and polyacrylic acid. A bioadhesive polycarbohil gel, ReplensR, is available in the market, which is used to retain moisture and lubricate vagina.The formulation remains in the vagina for 2–3 days and maintains the vagina at healthy, acidic pH. Various peptide and protein drugs have also been attempted to administer via bioadhesive microparticulate vaginal delivery system. Hyaluronic acid based intravaginal deliveries of calcitonin, a polypeptide used in the treatment of postmenopausal osteoporosis, have been shown promise for intravaginal administration of drugs for the systemic effect [65].

A mucoadhesive controlled release drug delivery system for nonoxynol-9, a spermicidal agent, has been reported [66]. This gel type system consisting of varying levels of nonoxynol-9 and EDTA, a chelating agent, were formulated using carbopol 934P polymer [67, 68]. The carbopol 934P polymer system provided a high burst release of nonoxynol-9 in the first 2 min and controlled release for 6 h. Gel type dosage form has the advantage over the tablet type dosage form, in that the former has greater surface contact and less irritation [69]. A modified simulated vaginal fluid was used to simulate vaginal conditions for bioadhesion. Isolated lamb vaginal epithelium and cellophane saturated with simulated vaginal fluid were used a model membranes. The principle of bioadhesion is based on the measurement of tensile strength or shear stress required to break the adhesive bond between a model membrane and test formulation. The delivery system is placed between two model membranes fixed on flexible supports in the assemblies for a certain period of time. After the adhesive bond is formed, the force required to separate the bond is measured and calculated as bioadhesive strength. Such assemblies are useful for comparative evaluation of various polymers for bioadhesion and retention properties in vitro.

VAGINAL IMMUNIZATION

In recent years, there have been several reports of successful immunization with DNA vaccines administered via various mucosal routes [70]. Mucosal sites, including the vaginal route, represent the primary site of entry of pathogens into the human body. Most of the conventional vaccines are administered via the oral or parenteral route resulting in systemic rather than mucosal immunity. On the other hand, mucosal immunization causes mucosal as well as systemic immunity [71]. In this regard, several vaginal vaccine formulations are being continuously researched against a variety of pathogens, including the human immunodeficiency virus (HIV). A recent study reports the development of a novel HIV-CCR5 receptor vaccine for the control of mucosal simian (SIV) and human forms of the virus. The vaccine, which targets both the virus and its CCR5 receptor, was administered in female rhesus monkey either by the vaginal route or by targeting the proximity of the draining iliac lymph nodes [72]. This immunization strategy through the vagina was found to significantly inhibit SIV/HIV infection in the animal model and shows

promise for a novel approach in the prevention of HIV transmission. In another study [73], intravaginal infection of mice with influenza A virus resulted in mucosal and systemic immunity against HIV type 1 epitope. DNA vaccines represent a newer approach to the control of infectious diseases. A recent study [74] demonstrates the formulation and application of plasmid DNA vaccine to mucosal inductive tissues, including the vagina. The female genital tract has the capacity to produce humoral and cellular immune responses against locally encountered antigens [74]. Vaginal immunization of rodents, human and non-human primates has been shown to elicit serum and secretory IgA and IgG responses in cervico-vaginal washes . Further, this route of immunization was ineffective at eliciting immune responses in other mucosal compartments. Rather than simple application of vaccine formulation at the target site, an example of vaccine administration via vagina specific dosage form could best be illustrated by a seminal paper on mucosal immunity by Loehr et al. [75]. In an attempt to induce immunity against bovine herpes virus-1 in cattle, the researcher's immunized cows intravaginally with suppositories containing plasmid coding for the glycoprotein D. The level of immunity obtained, as assessed by the level of IgG in serum and IgA in both serum and nasal fluids, was of sufficient magnitude to minimize weight loss and significantly reduce the duration of virus shedding. Such successful noninvasive DNA immunization in large animals could open avenues for a new area of mucosal immunization in humans.

RECENT RESEARCH WORK ON VAGINAL NANOPARTICLE DRUG DELIVERY SYSTEM

Zhang T, et al,[76] 2011, have been developed pH-responsive nanoparticles releasing tenofovir intended for the prevention of HIV transmission and stated that by using PLGA/S-100 NPs as an alternative controlled drug delivery system in intravaginal delivery of an anti-HIV/AIDS microbicide

Santos SS, et al,[77] 2014, have been developed a formulation and *in-vitro* evaluation of coconut oil-core cationic nanocapsules intended for vaginal delivery of clotrimazole and stated that the nanocapsules were more active than free clotrimazole against Candida albicans and Candida glabrata strains susceptible and resistant to fluconazole. Hence, clotrimazole-loaded coconut oil-core nanocapsules represent promising alternatives to the treatment of vulvo vaginal candidiasis.

Yang M,[78] 2014, have been developed Nanoparticle penetration of human cervicovaginal mucus: The effect of polyvinyl alcohol and stated that conventionally formulated PVA-coated particles are strongly immobilized in human CVM provides implications for identifying or developing new muco-inert surfactants or new processes for formulating mucus penetrating particle.

Jian J,[79],2014,have been developed Mussel-inspired protein-mediated surface functionalization of electrospun nanofibers for pH-responsive drug delivery and stated that results of fluorescein diacetate staining and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays show the viability of cancer cells after treatment with doxorubicin-released media at different pH values qualitatively and quantitatively, indicating that the media containing doxorubicin that were released in solutions at low pH values could kill a significantly higher number of cells than those released in solutions at high pH values.

Das Neves J, Sarmento B, [80], 2015, have been developed dapivirine-loaded nanoparticles for the development of anti-HIV vaginal microbicides Dapivirine transport across cell monolayers was significantly decreased when mucin was present at the donor side with either NPs or the free drug, thus evidencing the influence of this natural glycoprotein in membrane permeability

CONCLUSIONS

The vagina still remains to be an underutilized route of drug delivery. Over the years, the vaginal route has been used for the local application of drugs, but is now becoming a potential route for noninvasive, controlled transmucosal delivery of both local and systemic therapeutically active compounds. Various therapeutically important drugs such as insulin, calcitonin and sex hormones, antiretroviral drugs have been attempted to deliver via vaginal route but there is not much success in the development of safe and viable vaginal formulations for these macromolecular drugs. Among the drug delivery systems available for this route, intravaginal gel form labor induction has been found to be potential vaginal drug delivery systems mainly because of their bearing on childbirths. Bioadhesive vaginal formulations are likely to introduce as new vaginal formulations for both local and systemic delivery. With the increasing number of novel polymers each year, challenge remains to design appropriate bioadhesive vaginal formulations. Vaginal rings have shown significant role and are well accepted formulation within female population. Several combination vaginal contraceptive rings have been found to provide excellent contraceptive efficacy with minimum risk of adverse effects. More sophisticated and programmable vaginal rings could be developed in the near future for systemic delivery of therapeutically important macromolecules. Another area that needs to be investigated in detail is the application of immunization via the vagina. With the pandemic increase in the number of HIV infected individuals every year worldwide, the development of an effective vaginal vaccine rendering local immunity becomes imperative. One of the real challenges for future vaginal drug delivery will be to recognize ways to subjugate the complex biological barriers that limit the delivery of small and macromolecular drugs.

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