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Degradable bioadhesive nanoparticles for prolonged intravaginal delivery and retention of elvitegravir

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ABSTRACT

New methods for long-lasting protection against sexually transmitted disease, such as the human immunodeficiency virus (HIV), are needed to help reduce the severity of STD epidemics, especially in developing countries. Intravaginal delivery of therapeutics has emerged as a promising strategy to provide women with local protection, but residence times of such agents are greatly reduced by the protective mucus layer, fluctuating hormone cycle, and complex anatomical structure of the reproductive tract. Polymeric nanoparticles (NPs) capable of encapsulating the desired cargo, penetrating through the mucosal surfaces, and delivering agents to the site of action have been explored. However, prolonged retention of polymer carriers and their enclosed materials may also be needed to ease adherence and confer longer-lasting protection against STDs. Here, we examined the fate of two poly (lactic acid)-hyperbranched polyglycerols (PLA-HPG) NP formulations – 1) nonadhesive PLA-HPG NPs (NNPs) and 2) surface-modified bioadhesive NPs (BNPs) – loaded with the antiretroviral elvitegravir (EVG) after intravaginal administration. BNP distribution was widespread throughout the reproductive tract, and retention was nearly 5 times higher than NNPs after 24 h. Moreover, BNPs were found to be highly associated with submucosal leukocytes and epithelial cell populations for up to 48 h after topical application, and EVG was retained significantly better in the vaginal lumen when delivered with BNPs as opposed to NNPs over a 24 h period. Our results suggest that bioadhesive PLA-HPG NPs can greatly improve and prolong intravaginal delivery of agents, which may hold potential in providing sustained protection over longer durations.

1. Introduction

Although decades of investigation has led to significant advancements in our understanding of how to treat and prevent human immunodeficiency virus (HIV) infection, 2.1 million cases of newly acquired HIV infection worldwide occurred in 2015 [1]. Moreover, there are nearly 37 million individuals currently infected with HIV, and acquired immunodeficiency syndrome (AIDS) was responsible for 1 million deaths that same year [1]. The HIV epidemic remains at a staggeringly high level in African countries despite the development and optimization of many antiretroviral (ARV) therapies, microbicides, and protective barriers [2]. The persistence of HIV infection in the developing world may suggest the need for new approaches for prevention and treatment in order to dampen the severity of the epidemic, thereby reducing the aforementioned incidence rates of HIV and death rates attributed to AIDS.

The female reproductive tract serves as the site of entry for many bacterial and viral pathogens that can lead to sexually transmitted diseases (STDs). As such, intravaginal administration of therapeutics has long been a strategic approach for preventing and treating STDs such as HIV [3,4]. Though intravaginal administration has certain advantages, such as minimal invasiveness and avoidance of first-pass metabolism [5], delivery of therapeutics in this manner can be challenging. Intravaginal delivery of drugs or other biological molecules must overcome the acidic pH and degradative

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enzymes of the vaginal environment [6,7], the dense, protective layer of mucus that coats the vaginal epithelium [8], poor retention of non-viscous therapeutic formulations in the open-ended anatomical structure [9], and rapid biological changes in tissue and mucus properties correlating to hormonal cycles [10,11].

In order to address barriers to successful microbicide delivery, vehicles must be designed to: 1) increase penetration into the rugae and epithelium to enhance microbicide protection and 2) prolong retention in the reproductive tract to reduce dosing and improve patient adherence, which has plagued many recent clinical trials assessing microbicide effectiveness [12]. Polymer nanoparticles (NPs) can overcome some of these concerns by providing protection for their encapsulated payload. Additionally, such particles can be designed for maximal, effective transport through the mucus barrier [13]. For example, the size and surface properties of particles can be modified to enhance penetration through the mucus barrier layer and increase penetration into the epithelium [14,15]. However, NPs tend to be administered in non-viscous formulations, which can result in leakage and thus, shortened retention times [9]. For maximal effectiveness, drug-containing NPs must remain in the lumen of the reproductive tract long enough to allow diffusion and penetration through the mucosal layer and subsequent association with the underlying cellular layers [8].

Poly (lactide-*co*-glycolide) (PLGA), a degradable polymer that has been used in many FDA approved devices, has long been the standard NP material for intravaginal delivery of therapeutics. PLGA NPs easily encapsulate a wide array of biological agents and can be engineered to provide appropriate release properties [16]. Polyethylene glycol (PEG) has been widely used as a surface addition to PLGA NPs to provide stability and enhanced mucosal penetration and delivery of therapeutic molecules in the treatment of cervicovaginal diseases [9,17–21]. But recently it has been reported that immune responses can develop to PEG [22–24], which could be particularly harmful for repetitive delivery to a mucosal surface. Our group has recently developed a method for dramatically improving the stealth, aggregation-resisting, mucus-penetrating, and local retention properties of NPs, without the use of PEG. This new method involves the conjugation of poly (lactic acid) (PLA) to hyperbranched polyglycerols (HPG) to produce PLA-HPG NPs, with the HPG forming a corona on the NP surface (Fig. 1A) [25]. Because this HPG layer should provide stealth properties to the NPs, HPG was directly compared to particles coated with PEG, which is the most widely used stealth material [26]. The NPs produced from PLA-HPG had a significantly longer half-life after intravenous injection, 10 h, than NPs produced from PLA-PEG, 6 h [25]. Because this stealth property is associated with a lack of adhesion to proteins and protein-rich surfaces, we call these PLA-HPG particles nonadhesive NPs or NNPs.

Furthermore, PLA-HPG NPs can be made bioadhesive. In these bioadhesive NPs (BNPs), the surface coating of the NNPs were oxidized by simple exposure to sodium periodate for brief periods; this procedure converts the vicinal diols on HPG to aldehydes [27]. Aldehydes on the BNPs are capable of forming stable Schiff base interactions with amine groups, such as N-terminal or lysine side chain of proteins [27,28]. Our group has demonstrated that these BNPs, through amine interactions, can serve as an effective topical sunscreen through enhanced skin bioadhesion, which resists vigorous washing with water, thereby serving as a long-lasting potent UV protectant for the skin [27]. Additionally, we have recently reported the effectiveness of these BNPs in reducing systemic toxicity of the potent chemotherapeutic agent epothilone B (EB), enhancing retention of EB after intraperitoneal administration, and increasing therapeutic efficacy against the development of high-grade ovarian and endometrial carcinomas [28].

This current report describes the development and characterization of PLA-HPG NNP and BNP formulations (Fig. 1A) encapsulating elvitegravir (EVG) (Fig. 1B), a strand transfer inhibitor of HIV [29]. We explore whether these NP formulations can increase particle penetration and retention in the reproductive tissue, thereby prolonging the local dose and therapeutic efficacy of ARV prophylactic treatments against HIV. To test this hypothesis, we examine the distribution and retention of fluorescent NPs in the reproductive tracts after vaginal administration in mice. Specific nanoparticle-cell association was also explored to give insight into mucus penetration and tissue association as well as intravaginal EVG retention to assess long-term efficacy. In summary, we demonstrate that BNPs have significantly longer retention times after vaginal delivery than NNPs. The superior performance of BNPs makes sense: these nanoparticles can similarly penetrate mucus but once in contact with epithelial cells or leukocytes, they become immobilized and are retained for long periods. In contrast, the NNPs – lacking bioadhesive properties – are readily cleared by natural turnover of mucus or lymphatic drainage if they reach the tissue space (Fig. 1C).

2. Materials and methods

2.1. Materials

Elvitegravir was provided by Gilead Sciences (Foster City, CA) via CONRAD (Arlington, VA). PLA-HPG was synthesized as previously described [25]. IR-780 iodide, glycerol, $NaIO_4$, Na_2SO_3, bovine serum albumin (BSA), lactic acid, acetic acid, mucin, urea, glucose, Tween 80, Solutol (Kolliphor) HS 15, and HPLC-grade formic acid were obtained from Sigma-Aldrich. The 4-(4-(dihexadecylamino) styryl)-N-methylpyridinium iodide salt (DiA) and 4,6-diamidino-2 phenylindole (DAPI) stain were ordered from Invitrogen. Antibody stains anti-CD45 (ab10558) and anti-EpCAM (ab71916) and corresponding isotype controls for flow cytometry were purchased from Abcam. HPLC grade acetonitrile and water were purchased from VWR (J.T. Baker).

2.2. Nanoparticle preparation

To prepare NNPs, 100 mg of PLA-HPG was dissolved in 2.4 ml of ethyl acetate. For dye loaded particles, either IR-780 iodide dye or DIA (0.5 wt %) was dissolved in 0.6 ml of dimethyl sulfoxide (DMSO). For drug loaded particles, EVG (20% w/w) was dissolved in 0.6 ml of DMSO. The drug or dye solution was then combined with the polymer solution resulting in a polymer/dye or polymer/drug solvent mixture (ethyl acetate: DMSO = 4:1). The resulting solution was added to 4 ml deionized (DI) water under vortex and sonicated with a probe sonicator (4x, 10s each). Then, the emulsion was diluted in 20 ml of DI water and subsequently placed on a rotavapor for 30 min. The particle solution was washed by filtration using Amicon ultra-15 centrifugal filter units (100 K cut-off) 3 times before being suspended in DI water. NNPs were flash frozen with liquid nitrogen and stored at −20 °C until use [25,27].

To convert NNPs into BNPs, NNPs were incubated with 0.1 M $NaIO_4$ and 10× phosphate buffered saline (PBS) (1:1:1 vol ratio) for 20 min. The reaction was quenched with 0.2 M Na_2SO_3 (1:3 vol ratio). BNPs were washed by filtration three times with DI water using Amicon ultra-0.5 ml filters (100 K cutoff) and resuspended in DI water [27].

2.3. In vitro characterization of nanoparticles

The surface charge, diameter, and polydispersity index (PDI) of NP formulations (n = 5) were determined by laser doppler electrophoresis and dynamic light scattering using a Zetasizer Nano ZS