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Research paper

pH-responsive nanoparticles releasing tenofovir intended for the prevention of HIV transmission

Tao Zhang [a], Timothy F. Sturgis [b], Bi-Botti C. Youan [a,*]

[a] Laboratory of Future Nanomedicines and Theoretical Chronopharmaceutics, University of Missouri-Kansas City, Kansas City, USA
[b] Department of Environmental Health and Safety, University of Missouri-Kansas City, Kansas City, USA

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ABSTRACT

This study is designed to test the hypothesis that tenofovir (TNF) or tenofovir disoproxil fumarate (TDF) loaded nanoparticles (NPs) prepared with a blend of poly(lactic-*co*-glycolic acid) (PLGA) and methacrylic acid copolymer (Eudragit® S-100, or S-100) are noncytotoxic and exhibit significant pH-responsive release of anti-HIV microbicides in the presence of human semen fluid simulant (SFS). After NPs preparation by emulsification diffusion, their size, encapsulation efficiency (EE%), drug release profile, morphology, and cytotoxicity are characterized by dynamic light scattering, spectrophotometry, transmission electron microscopy, and cellular viability assay/transepithelial electrical resistance measurement, respectively. Cellular uptake was elucidated by fluorescence spectroscopy and confocal microscopy. The NPs have an average size of 250 nm, maximal EE% of 16.1% and 37.2% for TNF and TDF, respectively. There is a 4-fold increase in the drug release rate from the 75% S-100 blend in the presence of SFS over 72 h. At a concentration up to 10 mg/ml, the PLGA/S-100 NPs are noncytotoxic for 48 h to vaginal endocervical/epithelial cells and *Lactobacillus crispatus*. The particle uptake (~50% in 24 h) by these vaginal cell lines mostly occurred through caveolin-mediated pathway. These data suggest the promise of using PLGA/S-100 NPs as an alternative controlled drug delivery system in intravaginal delivery of an anti-HIV/AIDS microbicide.

1. Introduction

According to the recent report of the global AIDS epidemic, it was estimated that there were 2.7 million new infections and 2.0 million HIV/AIDS related deaths, in 2008 [1]. Unprotected, heterosexual, vaginal intercourse has become one of the major routes of infection. Although the global percentage of women among people living with HIV has remained stable (50%), women are considered more susceptible to sexually acquired HIV infection due to physiological, social, and economical factors [1]. Several HIV transmission prevention methods, such as condoms and circumcision, have been implemented, especially in developing countries. However, the results have been unsatisfactory, since it was reported in many regions that men were reluctant to use either method [2,3]. Besides these facts, a successful HIV vaccine has yet to be developed. It is critical to design a topical delivery strategy of microbicides that women can use as a pre-exposure prophylaxis (PrEP) method. Some examples of new drug delivery system (DDS) designed for the delivery of anti-HIV drugs have been reported [4–6], but none of them are currently used clinically for the purpose of the prevention of HIV transmission.

Extensive research activities have been dedicated to the field of HIV microbicides development, such as detergents/pH buffers [7], entry/fusion inhibitors [8], and HIV reverse transcriptase inhibitors [9,10]. Tenofovir (TNF, {[(2*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl]oxy}methylphosphonic acid) is a nucleotide analog HIV reverse transcriptase inhibitor whose prodrug (tenofovir disoproxil fumarate, TDF) is now marketed in an oral dosage form (Viread®, Gilead Science), and its 1% vaginal gel formulation has recently been proven effective in clinical trial [11]. Other vaginal delivery strategies of tenofovir along with dapivirine and emtricitabine have also been studied [12,13]. However, factors affecting the acceptability of the gel formulation include the ease of incorporation into typical sexual practices and type of sexual partnership [14].

Abbreviations: C-6, coumarin-6; MTS, [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] solution; DDS, drug delivery system; DLS, dynamic light scattering; EE%, encapsulation efficiency; LDH, lactate dehydrogenase; S-100, methacrylic acid copolymer; NPs, nanoparticles; F127, Pluronic F127; PBMCs, peripheral blood mononuclear cells; PLGA, poly(lactic-*co*-glycolic acid); PI, propidium iodide; PrEP, pre-exposure prophylaxis; SFS, semen fluid simulant; TNF, tenofovir; TDF, tenofovir disoproxil fumarate; TEM, transmission electron microscopy; TEER, transepithelial electrical resistance; VFS, vaginal fluid simulant.

* Corresponding author. Laboratory of Future Nanomedicines and Theoretical Chronopharmaceutics, Division of Pharmaceutical Science, University of Missouri-Kansas City, Kansas City, 2464 Charlotte, MO 64108, USA. Tel.: +1 816 235 2410; fax: +1 816 235 5779.
E-mail address: youanb@umkc.edu (Bi-Botti C. Youan).

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The vaginal route has been a site of local delivery as well as systemic delivery. Several dosage forms have been investigated as vaginal delivery systems, such as vaginal rings [15,16], films [17], and gels [13]. Nanoparticles (NPs) also potentially provide one possibility of such drug delivery system due to their unique characteristics, such as small size, protection of native drug, ability to reduce irritation at delivery site, and the ability of targeted delivery and controlled release of drugs. The concept of so-called nanomicrobicides has embraced the advanced potential of nanomedicine, and efforts have been made to address the major health problem of HIV prevention [18,19]. Several insightful studies of NPs vaginal formulation have indicated that it is a promising strategy towards the delivery of peptides and even SiRNA [20,21].

Since the HIV virus can be present in human semen during the intercourse, it is promising to design a semen-triggered topical delivery system. The ambient human vagina pH varies from 4 to 5, whereas human semen has a higher pH (typically 7.5) as well as higher buffer capacity [22]. Therefore, the local acidic pH will be altered during intercourse, which has been utilized in semen triggered delivery and pH-sensitive hydrogel [23]. However, vaginal retention of such a delivery system is also important. Otherwise, the short duration of the drug action requires the user to apply microbicide formulation hours before sex (coital dependence), which leads to significant patient compliance issues as recently observed in the clinical trial of tenofovir gel [11,14].

Based on the above consideration, the focus has been given to the preparation of a semen-triggered delivery system having a sustained release characteristic for vaginal delivery. It is hypothesized that a semen-triggered polymeric nanoparticulated delivery system can be engineered using poly(lactic-*co*-glycolic acid) (PLGA) and the methacrylic acid copolymer. PLGA, is a FDA-approved and widely accepted biodegradable copolymer used in NPs formulation, which can also provide the sustained release of an encapsulated drug. Various blends of Eudragit® and PLGA have been described in the preparation of heparin [24], antibiotics [25,26], gene encoding mouse interleukin-10 [27], salmon calcitonin [28], and diclofenac sodium [29] loaded nanoparticles. Eudragit® S-100 (hereafter referred to as S-100) would be effective and safe polymeric matrix in the prevention of HIV transmission by vaginal route. Basically, S-100 is a methacrylic acid–methyl methacrylate copolymer (1:2) synthesized from methacrylic acid and methacrylic acid methyl ester, which is soluble in an alkaline environment [30]. Therefore, it has been widely used in intestine or colon delivery systems where pH is above 7 [30].

The present investigation is aimed at testing the hypothesis that tenofovir or tenofovir disoproxil fumarate loaded nanoparticles prepared with a blend of poly(lactic-*co*-glycolic acid) (PLGA) and methacrylic acid copolymer (S-100) are noncytotoxic and exhibit significant pH-responsive release of anti-HIV microbicides in the presence of human semen fluid simulant. Basically, the TNF and TDF loaded, pH-sensitive NPs were prepared, and physicochemical characteristics, as well as biological responses were investigated. The results of this study demonstrate that a PLGA/S-100 NPs formulation may provide semen-triggered delivery and sustained release of an encapsulated microbicide for the prevention of HIV/AIDS transmission.

2. Materials and methods

2.1. Materials

Tenofovir (TNF) was purchased from Zhongshuo Pharmaceutical Co. Ltd. (Beijing, China). Tenofovir disoproxil fumarate (TDF) was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, Rockville, Maryland. Poly(D,L-lactide-co-glycolide) Resomer® RG756 with L/G ratio of 75:25 (MW 76–116 kDa) was purchased from Boehringer Ingelheim Inc. (Ingelheim, Germany). Eudragit® S-100 or S-100 (Methacrylic acid–methyl methacrylate copolymer 1:2) was purchased from Evonik Industries (Darmstadt, Germany). Poloxamer 407 (Pluronic® F127) was a gift from the BASF Corporation (Rhom, Germany). Coumarin-6, propidium iodide (PI) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). CytoTox-ONE™ and CellTiter 96™ Aqueous kits were purchased from Promega (Madison, WI, USA). All other chemicals used in this study were of analytical grade and used without further purification.

2.2. Nanoparticle preparation

TNF and TDF loaded PLGA/S-100 nanoparticles were prepared at room temperature using a previously described emulsification solvent diffusion method [31]. Briefly, TNF (5 mg) and the polymer (100 mg, PLGA/S-100 ratio 25:75, 50:50, and 75:25) were co-dissolved in 4 ml DMSO. This mixture served as the organic phase for the NPs preparation. The organic phase was added drop wise to 25 ml of aqueous phase, containing 0.6% (w/v) Pluronic F127, under homogenization at 13,500 rpm for 10 min (IKA ULTRA-TURRAX T-25, Staufen, Germany). The suspension was further ultracentrifuged at 15,000 rpm for 1 h (Beckman L8-70M Ultracentrifuge, Brea, CA, USA) to collect NPs and then washed three times with distilled water to remove the surfactant. The supernatant was used for the determination of drug encapsulation efficiency (EE%). Finally, the NPs were first frozen in liquid nitrogen then lyophilized for 12 h using a lab-scale freeze dryer (Labconco Corporation, Kansas City, MO, USA) under −46 °C and stored at 4 °C until use. Blank NPs and coumarin-6 (C-6) loaded PLGA/S-100 NPs were prepared using the same method.

2.3. Nanoparticle characterization

2.3.1. Particle size and zeta potential

The particle size and size distribution of the various NPs solutions were measured at 25 °C by dynamic light scattering method (Zetasizer Nano ZS, Malvern Instruments Ltd., Worcestershire, UK). The particle size of different samples (nanosuspension before lyophilization and suspension of lyophilized NPs powder) was evaluated and represented as *Z*-average diameter. The zeta potential of the PLGA/S-100 NPs suspension was measured using the zeta potential analysis mode of the instrument. Nanosphere™ size standard (59 ± 2.5 nm) and zeta potential standard (−68 ± 6.8 mV) were used to calibrate the instrument prior to the analysis.

2.3.2. Morphology

The images of the NPs formulation were taken by transmission electron microscopy (TEM). Particles were diluted in 2.5% uranyl acetate (UA), sonicated, and then 8 μl of the solution was put on a carbon coated grid and allowed to equilibrate for 5 min; excess solution was wicked off. Then, 5% UA was put on the grid to increase contrast. The grids were viewed under a JEOL JEM 1400 transmission electron microscope (JEOL Inc., Peabody, USA) and photographed digitally with a Gatan axis-mount $2k \times 2k$ digital camera.

2.3.3. Encapsulation efficiency

The encapsulation efficiency (EE%) was measured at a wavelength of 260 nm by UV spectrometer (Spectronic Genesys 10 Bio, Thermo Electron Corporation, WI, USA). The standard curves of TNF and TDF were prepared using drug concentration ranging from 2 to 100 μg/ml. The amount of encapsulated drug was calculated using mass balance by subtracting the amount of the free