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Nanoparticles-in-film for the combined vaginal delivery of anti-HIV microbicide drugs

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ABSTRACT

Combining two or more antiretroviral drugs in one medical product is an interesting but challenging strategy for developing topical anti-HIV microbicides. We developed a new vaginal delivery system comprising the incorporation of nanoparticles (NPs) into a polymeric film base – NPs-in-film – and tested its ability to deliver tenofovir (TFV) and efavirenz (EFV). EFV-loaded poly(lactic-*co*-glycolic acid) NPs were incorporated alongside free TFV into fast dissolving films during film manufacturing. The delivery system was characterized for physicochemical properties, as well as genital distribution, local and systemic 24 h pharmacokinetics (PK), and safety upon intravaginal administration to mice. NPs-in-film presented suitable technological, mechanical and cytotoxicity features for vaginal use. Retention of NPs *in vivo* was enhanced both in vaginal lavages and tissue when associated to film. PK data evidenced that vaginal drug levels rapidly decreased after administration but NPs-in-film were still able to enhance drug concentrations of EFV. Obtained values for area-under-the-curve for EFV were around one log10 higher than those for the free drugs in aqueous vehicle (phosphate buffered saline). Film alone also contributed to higher and more prolonged local drug levels as compared to the administration of TFV and EFV in aqueous vehicle. Systemic exposure to both drugs was low. NPs-in-film was found to be safe upon once daily vaginal administration to mice, with no significant genital histological changes or major alterations in cytokine/chemokine profiles being observed. Overall, the proposed NPs-in-film system seems to be an interesting delivery platform for developing combination vaginal anti-HIV microbicides.

1. Introduction

There is an urgent need to reinforce the battle against HIV/AIDS, namely by investing more in preventing new infections [1]. Scientific and medical evidence produced over recent years supports that both oral and topical pre-exposure prophylaxis (PrEP) are promising approaches that can reduce sexual transmission of the virus [2]. Data from different clinical trials motivated the approval of oral tenofovir disoproxil fumarate/emtricitabine regimens for individuals at high risk of infection in various countries, while the World Health Organization has just recently endorsed oral PrEP [3]. Additionally, the body of knowledge on topical PrEP is increasing and providing momentum towards the development of vaginal and/or rectal microbicides. The use of such products may be preferential over oral PrEP due to the ability to allow the rapid onset of potentially protective drug levels at mucosal sites following administration, as well as to diminish systemic drug exposure and, consequently, reduce adverse effects. Results from the CAPRISA 004 [4] and the parallel MTN-020/IPM 027 [5,6] clinical trials provided evidence that the vaginal use of a tenofovir (TFV) gel or a dapivirine-releasing ring, respectively, could afford protection from HIV-1. Despite encouraging, overall risk reduction rates were only moderate (27–39%) and most likely associated with poor user adherence. Inconsistent utilization of microbicides probably leads to important gaps in protective windows provided by microbicides (*i.e.*, drug levels below those required for viral inhibition at mucosal sites), thus allowing for transmission to more easily occur [7]. Consequently, microbicide products should comply as much as possible with women's preferences as this may radically influence acceptability and, ultimately, adherence [8]. Another relevant problem of already tested microbicides may be the inability of one single antiretroviral compound to completely inhibit transmission. Following the path of current antiretroviral therapy, the development of microbicides comprising two or more active

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compounds is becoming increasingly accepted as the way forward in the field [9]. Still, combining antiretroviral drugs with different physicochemical properties into one single product may be challenging and require significant effort at the pharmaceutical development stage [10–12].

Antiretroviral drug formulation and delivery may significantly affect the performance of microbicides, particularly genital drug distribution and pharmacokinetics (PK) [13]. Various approaches have been pursued in order to engineer sophisticated systems that could overcome problems associated with more traditional vaginal dosage forms. In particular, different groups have been engaged in the development of nanotechnology-based microbicides, namely comprising the use of polymeric nanoparticles (NPs) as antiretroviral drug carriers [14,15]. This last approach has been proven advantageous regarding the enhancement of drug protection from degradation, vaginal retention and distribution, mucosal tissue penetration, intracellular delivery and genital safety, which can ultimately contribute to protection from viral transmission. However, microbicide drug nanocarriers require their incorporation into adequate platforms that allow for convenient intravaginal self-administration. Gels have been typically used [16,17] but semisolid dosage forms present several disadvantages including the requirement for an applicator, risk of microbial contamination, and leakage from the vagina after administration. Moreover, drug release from NPs incorporated into gels may occur during storage due to the presence of water. We have recently proposed the incorporation of poly(lactic-*co*-glycolic acid) (PLGA)-based NPs in a thin, quick dissolving polymeric film (NPs-in-film) as an innovative and safe system for vaginal antiretroviral drug delivery [18]. Films are widely recognized as valuable vaginal dosage forms and are being currently tested in microbicide clinical trials [19]. NPs-in-film systems share the same attributes as common vaginal films, being expected to allow easy and convenient administration, while providing a potential on-demand microbicide product that could be used precoitally.

However, important questions remain, particularly regarding the usefulness of NPs-in-films to deliver multiple drugs presenting different solubility profiles and to understand the impact of such systems on local and systemic PK. The co-delivery of hydrophobic and hydrophilic compounds may be a significant challenge when formulating drug products, often requiring unconventional approaches or complex systems that require multiple manufacturing steps and/or excipients [20–22]. Poor design of delivery platforms may lead to problems such as drug crystallization/precipitation, drug-drug incompatibility, inadequate drug release or poor stability. In the specific case of vaginal microbicides, most strategies for co-delivery have been based on the increase of the water dispersibility or solubility of hydrophobic compounds namely by using their micronized forms [23,24], including solubilizers/co-solvents [25,26] or developing multiphase systems (*e.g.*, emulsions) [27]. Nevertheless, the use of these last often requires the addition of potentially harmful pharmaceutical ingredients that, even in small amounts, may lead to safety issues [28]. Segmented and pod/tablet insert vaginal rings that are able to accommodate compounds presenting different solubility have also been recently proposed [29]. Such systems, however, are only possible for developing products intended to be used for prolonged time periods in order to provide coitus-independent protection. In this work, we tested the ability of NPs-in-film to successfully incorporate two model antiretroviral drugs, namely TFV (hydrophilic; included directly into the film matrix) and efavirenz (EFV, hydrophobic; associated to NPs before incorporation into the film matrix), and studied its safety and PK upon vaginal delivery to mice.

2. Materials and methods

2.1. Materials

PLGA (Purasorb PDLG 5002, 50:50 D,L-lactide:glycolide, Mw ≈ 17 kDa) was kindly provided by Corbion (Gorinchem, The Netherlands), TFV monohydrate by Gilead Sciences (Foster City, CA, USA) and poloxamer 407 (Kolliphor® P 407) by BASF (Ludwigshafen, Germany). EFV was from BDR Lifesciences (Vadodara, India). Hydroxypropyl methylcellulose (HPMC; Methocel E4M) was acquired from Colorcon (Dartford, UK), glycerin from Aliand (Mem Martins, Portugal), PLGA-fluorescein (50:50 D,L-lactide:glycolide, Mw ≈ 30–40 kDa, Cat No. AV04) from PolySciTech (West Lafayette, IN, USA), and poly(vinyl alcohol) (PVA; 87–90% hydrolyzed, 30–70 kDa), phosphate buffered saline (PBS) tablets, purified type II mucin and ethyl acetate from Sigma-Aldrich (St. Louis, MO, USA). All other materials and reagents were of analytical grade or equivalent.

2.2. Production and characterization of efavirenz-loaded nanoparticles

EFV-loaded PLGA NPs (EFV-NPs) were obtained by adapting a previously reported emulsion-solvent evaporation method [30]. Briefly, EFV (5 mg) and PLGA (40 mg) were dissolved in ethyl acetate (2 mL) and mixed with 0.1% (w/v) poloxamer 407 (5 mL) using a Vibra-Cell™ VC 50 ultrasonic processor (Sonics & Materials, Danbury, CT, USA) for 60 s at 70% intensity. The resulting oil-in-water emulsion was promptly diluted with 0.1% (*w/v*) poloxamer 407 (15 mL) and kept under magnetic stirring for 4 h at 200 rpm. NPs were concentrated by centrifugation (4000 × *g*, 30 min) and washed twice with 15 mL of water by using Amicon Ultra-15 filter units (MWCO 100 kDa; Merck Millipore, Tullagreen, Ireland). The percentage of drug association efficiency (AE%) and loading degree (LD%) was indirectly estimated by assaying the amount of EFV in fluids recovered during purification by high-performance liquid chromatography (HPLC) with UV detection (*Supplementary Materials*; *S1. HPLC-UV analysis)*. Empty NPs (*i.e.*, without EFV) were also prepared using the same procedure but skipping the addition of EFV, while fluorescent NPs were obtained by simply replacing 5 mg of PLGA by PLGA-fluorescein during the preparation process (no EFV was added). All particles were characterized for hydrodynamic diameter, polydispersion index (PdI), and zeta potential at 25 °C and dispersed in water using a ZetaSizer Nano ZS (Malvern, Worcestershire, UK). Transmission electron microscopy of NPs stained with uranyl acetate was performed at 80 kV using a JEOL JEM-1400 (JEOL Ltd., Tokyo, Japan). Also, cytotoxicity of NPs was assessed as detailed in *Supplementary Materials* (*S2. Cytotoxicity potential of nanoparticles and films*).

2.3. Manufacturing and characterization of films

Films were prepared according to an optimized solvent-casting method [18]. In brief, 1.5 g of matrix-forming excipients (72% HPMC, 18% PVA, 10% glycerin) were dissolved in ultrapure water (q.s. 50 g) and the mixture cast onto 12 × 12 cm polystyrene molds. When required, an appropriate amount of excipients was substituted by drug(s) and/or NPs, which were dissolved/dispersed just before casting. Drying was performed for 72 h at 35 °C in a BM-500 drying oven (Memmert, Schwabach, Germany), and the obtained film sheet cut into samples of appropriate dimensions using a precision knife. Films without any drug or NPs (Film), or incorporating TFV and EFV (TFV/EFV-film), empty NPs (NPs-in-film), TFV and EFV-NPs (TFV/EFV-NPs-in-film), or fluorescent NPs (FL-NPs-in-film) were produced. When applicable, TFV and EFV were incorporated at 1.6 mg/cm^2 and 0.16 mg/cm^2, respectively. In order to do so, TFV was dissolved and EFV was dispersed with the aid of a minimum amount of dimethyl sulfoxide (<0.02%) in the water/polymers/glycerin mixture before casting. Films were characterized by assessing the following features: (i) organoleptic features; (ii) mass per area; (iii) thickness using a digital micrometer (I.C.T, Lardero, Spain); (iv) moisture content with a MX-50 moisture analyzer (A&D, Tokyo, Japan); (v) mechanical properties by using a TA.XT*plus* texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) and performing puncture tests (HDP/FSR support rig, 5 mm spherical P5/S probe, speed of 1 mm/s in compression mode): puncture strength was calculated as the ratio between the breaking force and