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Biophysical characterization of small molecule antiviral-loaded nanolipogels for HIV-1 chemoprophylaxis and topical mucosal application



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ARTICLE INFO

Article history:

Received 3 November 2015

Received in revised form 20 January 2016

Accepted 22 February 2016

Available online 3 March 2016

Keywords:

Nanoparticles

Liposomes

Chemoprophylaxis

Maraviroc

TDF

ABSTRACT

Nanocarriers are versatile vehicles for drug delivery, and emerging as platforms to formulate and deliver multiple classes of antiretroviral (ARV) drugs in a single system. Here we describe the fabrication of hydrogel-core and lipid-shell nanoparticles (nanolipogels) for the controlled loading and topical, vaginal delivery of maraviroc (MVC) and tenofovir disoproxil fumarate (TDF), two ARV drugs with different mechanisms of action that are used in the treatment of HIV. The nanolipogel platform was used to successfully formulate MVC and TDF, which produced ARV drug-loaded nanolipogels that were characterized for their physical properties and antiviral activity against HIV-1 BaL in cell culture. We also show that administration of these drug carriers topically to the vaginal mucosa in a murine model leads to antiviral activity against HIV-1 BaL in cervicovaginal lavages. Our results suggest that nanolipogel carriers are promising for the encapsulation and delivery of hydrophilic small molecule ARV drugs, and may expand the nanocarrier systems being investigated for HIV prevention or treatment.

Statement of Significance

Topical, mucosal intervention of HIV is a leading strategy in the efforts to curb the spread of viral infection. A significant research thrust in the field has been to characterize different dosage forms for formulation of physicochemically diverse antiretroviral drugs. Nanocarriers have been used to formulate and deliver small molecule and protein drugs for a range of applications, including ARV drugs for HIV treatment. The broad significance of our work includes evaluation of lipid-shell, hydrogel-core nanoparticles for formulation and topical, vaginal delivery of two water-soluble antiretroviral drugs. We have characterized these nanocarriers for their physical properties and their biological activity against HIV-1 infection *in vitro*, and demonstrated the ability to deliver drug-loaded nanocarriers *in vivo*.

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1. Introduction

Nanoparticles have been widely investigated as carrier systems to deliver small molecule drugs and biologics to improve cell or tissue targeting, reduce side effects, control release, and promote intracellular uptake of agents that have sites of action within cells [1,2]. Hydrophobic small molecule encapsulation within nanocarriers has been employed with notable success in numerous clinical applications, most commonly for cancer therapy [2]. Recently, sim-

ilar nanocarriers that encapsulate and deliver antiretroviral (ARV) drugs have been investigated for application in HIV prevention and therapy [3–7]. Strategies used for topical prevention of sexually transmitted infections (STIs) have demonstrated that local delivery of ARV drugs to the vaginal mucosa may sustain higher drug concentrations in the genital tract and reduce systemic exposure [8]. Local delivery may also limit off-target effects that result from systemic delivery. However, there are few vaginal dosage forms that have been tested for co-delivery of water-soluble small molecule ARV drugs. Previous groups have shown that formulating drugs within nanoparticles is one strategy for drug delivery to the vaginal mucosa that prolongs drug retention in the reproductive organs and reduces systemic delivery of ARV drugs [9]. To date, only a few nanocarriers have been investigated as microbicides,

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and these have been limited to the delivery of single ARV drugs in most cases. Expanding the number of nanocarriers that are amenable to vaginal mucosal delivery may expand the number of single and combination agents that can be used for topical HIV prevention.

Generally, the most widely investigated nanoparticle systems that have been evaluated for ARV drug delivery include liposomes and polymer nanoparticles. A key advantage to liposomes is the ability to formulate hydrophobic and hydrophilic drugs within the lipid shell and aqueous core [10]. Liposomes have been used to formulate hydrophilic and hydrophobic ARV agents such as indinavir and zidovudine within the aqueous core or lipid bilayer at ~3–30% drug encapsulation efficiency [4,11,12]. As an alternative to liposomes, polymeric nanocarriers have also been investigated for formulating ARV drugs. Formulation of hydrophobic ARV drugs such as efavirenz, saquinavir, and dapivirine in polymer-based nanocarriers have shown promising results with high encapsulation efficiencies nearing 100% [5,9]. In contrast, studies evaluating hydrophilic ARV drug delivery (zidovudine and tenofovir) in poly (lactic-co-glycolic acid) (PLGA), poly(isobutylcyanoacrylate), and chitosan nanoparticles showed that these systems exhibited encapsulation efficiencies of 5–10% [13–15]. These studies highlight the capacity of different nanomaterials to formulate a range of physicochemically diverse ARV drugs, and motivate the need for further characterization of nanoparticle systems for ARV drug formulation and delivery.

Nanolipogels are an emerging platform being investigated as an alternative to the more widely used liposomes or polymer nanoparticles. These dual structure nanoparticles have a distinct lipid bilayer encompassing a polymer core and have been synthesized by UV-induced gelation of a hydrogel network within liposomes using various processes [16,17]. In general, the approaches promote gelation within a liposome reactor to generate nanogel particulates, which are subsequently isolated [16–18]. Lipid-shell, polymer-core nanoparticles have been utilized previously for the encapsulation of several types of agents, including small molecule cancer drugs, and small and large proteins in various biomedical applications [19–24]. Nanolipogels have been used to incorporate physicochemically diverse agents within the lipid bilayer and the aqueous core, including a small hydrophobic TGF- β inhibitor and IL-2 protein, for a tumor immunotherapy application [19]. In addition, active loading can be used to drive drugs that are weak bases into the acidic hydrogel core of nanolipogels, where they become entrapped and are less likely to partition across a neutrally charged lipid membrane [24]. This has been shown to result in high encapsulation efficiency and provide controlled release of the encapsulated drug. These reasons make nanolipogels an interesting platform to investigate for physical and biological attributes that facilitate ARV drug delivery as topical microbicides for HIV chemoprophylaxis.

Here, we investigate the use of nanolipogels for formulation and mucosal delivery of the hydrophilic ARV drugs maraviroc (MVC) and tenofovir disoproxil fumarate (TDF). MVC and TDF are two HIV antiviral drugs with distinct mechanisms of action against HIV viral replication. MVC is a small molecule agonist, which specifically inhibits CCR5-dependent viral entry of HIV-1 [25]. TDF is a nucleotide reverse transcriptase inhibitor and a prodrug of tenofovir. Once internalized, TDF is converted to tenofovir diphosphate, which is the active form of the drug capable of inhibiting reverse transcription [26]. MVC and tenofovir are hydrophilic drugs that have been tested in a range of clinical trials as topical microbicides [8,27–30]. Although tenofovir has been primarily used in clinical trials of microbicides, the prodrug TDF has been investigated in non-human primate models and has shown to protect against infection when released from an intravaginal ring [31]. Specific advantages of TDF include higher cell and tissue

permeability and increased potency of up to 100-fold over tenofovir [32]. Additionally, TDF has been shown to protect against vaginal herpes infection, making it an interesting agent for dual protection against two sexually transmitted pathogens [32,33]. Both drugs have been formulated into semi-solid dosage forms for vaginal drug delivery (e.g., rings and gels) for the purposes of HIV pre-exposure prophylaxis. However, recent research has shown that encapsulation of ARV drugs, such as dapivirine in nanocarriers, improves local retention of drug in the reproductive organs resulting in decreased systemic biodistribution of drug [9]. To this end, we evaluated the utility of nanolipogels for encapsulation and delivery of MVC and TDF.

We observed that nanolipogels exhibited high levels of ARV drug encapsulation and this study demonstrates the biological utility of these vehicles for use in HIV chemoprophylaxis. Specifically, we highlight the potential of ARV drug-loaded nanolipogels to inhibit cell-free and cell-cell HIV-1 infection *in vitro*. Finally, we demonstrate the *in vivo* application of MVC- and TDF-nanolipogels as a topical vaginal microbicide. The implications of our results support nanolipogels as a system for delivery of hydrophilic ARV drugs for HIV-1 prevention and treatment that is worthy of further investigation in future studies.

2. Materials and methods

2.1. Materials

Egg phosphatidylcholine (EPC) was obtained from Avanti Polar Lipids (Alabaster, AL, USA). Acrylamide (AAm), N,N'-methylenebis (acrylamide) (MBA), 2,2-diethoxyacetophenone (DEAP), and cholesterol were obtained through Sigma Aldrich (St. Louis, MO, USA). MVC was synthesized, purified, and generously donated by the Suydam Lab at Seattle University. TDF was obtained through the NIH AIDS Research and Reference Reagent Program (<http://www.aidsreagent.org/>). Water used in buffer solution was purified using a Milli-Q purification system (Millipore Corporation, Billerica, MA, USA). TZM-bl cells, PM-1 cells, CEMx174 lymphocytes, and HIV-1 BaL isolate were also obtained through the NIH AIDS Research and Reference Reagent Program. Medroxyprogesterone acetate was purchased through the University of Washington pharmacy (Greenstone LLC, Peapack, NJ, USA). Media used in cell culture assays was complete Dulbecco's Modified Eagle Medium (cDMEM) (Invitrogen), made by supplementing DMEM (Invitrogen) with 10% fetal bovine serum (FBS) (Hyclone), 5% of 100 \times Penicillin/Streptomycin (Invitrogen), and 5% of 200 mM L-glutamine (Invitrogen).

2.2. Synthesis and preparation of nanolipogels

Nanolipogels were synthesized by modifying previously established methods for photopolymerization of a hydrogel core within a lipid vesicle reactor [16,17]. EPC and cholesterol were dissolved in chloroform at 20 mg/ml and 10 mg/ml. EPC and cholesterol at a mass ratio of 3:1 were transferred to a round-bottom flask, which was attached to a rotary evaporator (Buchi, Flawil, Switzerland) rotating at 120 rpm over a 35 °C water bath until all solvent was removed. AAm, MBA, and DEAP were dissolved in 50 mM Tris-HCL buffer and used to rehydrate the lipid film to 5 mg lipids/film, forming multilamellar vesicles (MLVs). ARV drugs were dissolved in DI water and were incorporated into the rehydration buffer at 9.1% or 4.77% (w/w) of the total material mass. MVC and TDF were loaded separately. MLVs were extruded with a hand-held needle extruder (Avanti Polar Lipids, Alabaster, AL) involving 21 passes with syringes through a 200 nm polycarbonate membrane. This produced unilamellar vesicles (ULVs) ~170–220 nm in diameter,

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