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Injectable long-acting human immunodeficiency virus antiretroviral prodrugs with improved pharmacokinetic profiles

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ABSTRACT

While highly active antiretroviral therapy (HAART) has significantly reduced mortality rates in patients with human immunodeficiency virus type 1 (HIV-1), its efficacy may be impeded by emergence of drug resistance caused by lack of patient adherence. A therapeutic strategy that requires infrequent drug administration as a result of sustained release of antiretroviral drugs would put less burden on the patient. Long-acting antiretroviral prodrugs for HIV therapy were synthesized through modification of the active drugs, emtricitabine (FTC) and elvitegravir (EVG), with docosahexaenoic acid (DHA) in one-step, one-pot, high-yielding reactions. The *in vitro* drug release profiles of these synthetic conjugates demonstrated sustained and controlled release of the active drug over a period of 3–4 weeks attributable to the hydrolysis of the chemical linker in conjunction with the hydrophilicity of the parent drug. Both conjugates exhibited superior antiviral activities in tissue culture models of HIV replication as compared to those of the free drugs, strengthening their role as potent prodrugs for HIV therapy. Pharmacokinetic analysis in CD1 mice further confirmed the long-acting aspect of these conjugates with released drug concentrations in plasma detected at their respective IC_{90}/IC_{95} values over a period of 2 weeks and discernable amounts of active drug even at 6 weeks. Our findings suggest that the injectable small molecule conjugates could be used as long-acting controlled release of FTC and EVG in attempts to mitigate adherence-related HIV resistance.

1. Introduction

Approximately 36.7 million people worldwide are affected by human immunodeficiency virus type 1 (HIV-1), of which 20.9 million receive highly active antiretroviral therapy (HAART; World Health Organization) (HIV/AIDS). Since the first antiretroviral, zidovudine, hit the market 32 years ago, there has been a steady growth of available treatment options (~35 drugs) across seven distinct drug-classes, based on their molecular mechanism and resistance profiles (HIV/AIDS). Due to the increased number of currently available therapies, it has become even more imperative to tailor a treatment plan that addresses tolerability, safety, and convenience. Conventional antiretroviral therapy regimen requires daily oral intake of a combination of at least three anti-HIV drugs that have the potency to suppress the viral replication and prevent development of drug resistance (Clinical Guidelines). While HAART is not a cure for HIV, it prolongs and improves the quality of life, and reduces the risk of HIV transmission (Cohen et al., 2011).

With more anti-HIV FDA-approved drugs currently on the market, there has been tremendous progress in improving access of these drugs to high-risk individuals in low- and middle-income countries. However, one of the primary challenges of HAART is poor adherence (50–75%) to medications, which is extremely common in patients taking oral treatment for chronic diseases (Rajoli et al., 2015; Coleman et al., 2012; Kim et al., 2018). Such noncompliance renders the drug combination

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ineffective against suppressing HIV replication and can lead to the emergence of drug resistance (Paydary et al., 2013; Gross et al., 2001; Genberg et al., 2012). In resource-constrained environments, especially with limited drug options, it becomes increasingly difficult to effectively address the development of acquired resistance and switch regimens in a timely manner (World Health Organization, 2010). All these challenges underscore the need for long-acting therapeutics that can replace the daily pill burden by being administered on a more infrequent basis (Boffito et al., 2014). Simplification of HIV therapeutic regimens can lead to improvement in patient adherence and make lifelong HIV management easier for the patients (Kerrigan et al., 2018).

As an alternative to the more conventional oral HAART regimen, long-acting injectables (LAIs) can be administered on a more infrequent basis, reduce patient non-compliance, and potentially minimize the development of drug resistance (Owen and Rannard, 2016). Although HIV-targeted injectable crystalline nanoparticle formulations have progressed to clinical trials, some concerns include prolonged systemic exposure and challenges in removing drug in the event of toxicity (Spreen et al., 2013). Other drug nanoparticle formulations required multiple doses to achieve an enhanced pharmacokinetic (PK) effect over the corresponding soluble drugs (Gautam et al., 2013). Attempts at encapsulating the active drugs into carriers have been limited by poor drug loading (Mandal et al., 2017) and "leaky" particles resulting from passive diffusion (Mandal et al., 2017; Tshweu et al., 2014). Our lab has previously developed an injectable biopolymeric microconfetti delivery system for one week controlled release of saquinavir from a single injection (Collier et al., 2016). However, a more sustained drug release profile is required to achieve fewer administrations to address the previously mentioned practical challenges faced in HIV therapy.

Direct chemical modification of an antiretroviral drug that transforms them into a LAI could circumvent the barriers that exist with the aforementioned injectable systems. To this end, we identified two current drugs with reactive functional moieties that can be used as chemical handles for further modification: emtricitabine (FTC) and elvitegravir (EVG). FTC, a nucleoside reverse transcriptase inhibitor (NRTI), was approved by the FDA in 2003 for the prevention and treatment of HIV infection in adults and children (Saag, 2006). EVG, belonging to a different HIV drug class, integrase inhibitors, was FDA-approved in 2014 as a single pill formulation. EVG prevents HIV transmission by blocking an essential HIV enzyme required by the virus for integration of its genetic code into the host DNA (Unger et al., 2016). Due to their high potencies, both drugs are part of several combination therapies that are currently being used worldwide by patients. However, their long-term efficacy is limited by daily administration (Truong et al., 2015), poor bioavailability (Bastiaans et al., 2014), and metabolism by cytochrome P450 3A4 (CYP3A4) (Klibanov, 2009). LAI versions of these drugs could improve their systemic circulation and reduce the daily intake requirement, ultimately reducing the overall drug cost (Barnhart, 2017). To realize this goal, we sought to develop two standalone LAI-based derivatives of antiretrovirals (FTC and EVG), with the potential for fewer drug administrations compared to traditional HAART therapy.

Herein, we report the synthesis of polyunsaturated fatty-acid (PUFA) modified-FTC and EVG as long-acting derivatives of the parent drugs. Success of this approach is dependent upon the cleavage rate of the chemical bond between the parent drug and the derivatizing moiety. We evaluated their antiviral activities against HIV-infected cells and compared to those of the free drugs. *In vitro* drug release profiles were generated in attempts to demonstrate sustained release of the active antiretrovirals. Pharmacokinetic analyses were also conducted in mice to discern the long-acting aspect of these drug conjugates.

2. Materials and methods

2.1. General considerations

Deuterated solvents were purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and used without further purification. Compounds FTC-DHA and EVG-DHA were prepared according to literature procedures, with slight modifications. (Agarwal et al., 2013) All reagents were purchased from Sigma Aldrich (St. Louis, MO, USA) and used without further modification, unless otherwise indicated. Emtricitabine (FTC) was purchased from 1 ClickChemistry, Inc. (Kendall Park, NJ, USA). Elvitegravir (EVG) was purchased from PharmaBlock, Inc. (Sunnyvale, CA, USA). Reactions were performed under a dry nitrogen atmosphere, unless otherwise noted. All flash chromatography was carried out using a 75-mm inner diameter column containing 100-mm length of silica gel under a positive pressure of laboratory air. The following reagents were obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: HIV-1_{NL4-3} Infectious Molecular Clone (pNL4-3) from Dr. Malcolm Martin, TZM-bl from Dr. John C. Kappes, Dr. Xiaoyun Wu, and Tranzyme Inc.

2.2. Instrumentation

^{1}H and ^{13}C NMR spectra were recorded on an Inova 400 FT-NMR spectrometer (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR). ^{1}H NMR data are reported as follows: chemical shift (multiplicity (b = broad, s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, and m = multiplet) and peak assignments). ^{1}H and ^{13}C chemical shifts are reported in ppm downfield from tetramethylsilane (TMS). Electrospray-ionization mass spectrometric (ESIMS) data was obtained on a Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (ThermoFisher Scientific, Waltham, MA, USA). UV–vis absorption spectra for all samples were obtained on a SpectraMax M2 Multi-Mode Microplate Reader (Molecular Devices, San Jose, CA, USA).

2.3. Synthesis of ((2R,5S)-5-(4-amino-5-fluoro-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolan-2-yl)methyl (4E,7E,10E,13E,16E,19E)-docosa-4,7,10,13,16,19-hexaenoate (FTC-DHA)

FTC (800 mg, 3.24 mmol), docosahexanoic acid (DHA) (2.13 gm, 6.47 mmol), and *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate, *O*-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU) (2.45 gm, 6.47 mmol) were dissolved in dry dimethylformamide (DMF, 20 mL) in a 100 mL round-bottom flask. Diisopropylethylamine (DIPEA) (18.6 mL, 0.11 mol) was added to the reaction mixture *via* a gas tight syringe, and the reaction was allowed to proceed for 18 h at room temperature. The reaction mixture was then concentrated under reduced pressure and purified by flash chromatography (50:50 v/v ethyl acetate/hexanes) to afford compound FTC-DHA as a colorless oil (1.18 gm, 65% yield). ^{1}H NMR (Fig. S1 in ESI†, $CDCl_3$, 400 MHz, δ ppm): 8.17 (d, 1H, CFC*H*), 6.22 (s, 2H, NH_2), 5.18–5.68 (m, 1H, NC*H*CH$_2$, 1H, OC*H*CH$_2$, 12H, C*H* vinylic protons), 4.38 (dd, 2H, CH_2OCO), 3.19 (dd, 2H, NCHCH_2S), 2.69–2.87 (m, 10H, CH_2 vinylic protons), 2.31–2.47 (m, 4H, OCOCH_2CH_2), 1.95–2.07 (m, 2H, CH_2CH$_3$), 0.92 (t, 3H, CH_3). ^{13}C NMR (Fig. S2 in ESI†, $CDCl_3$, 100 MHz, δ ppm): 172.33 (C═OO), 158.76 (*C*NH$_2$), 152.15 (C═O), 131.98 (*C*F), 126.97 (CF*C*H), 127.29–129.72 (11C, CH, vinylic carbons), 87.54 (N*C*HO), 85.66 (O*C*HS), 62.88 (*C*H$_2$OC═O), 39.23 (NCH*C*H$_2$S), 33.75 (CH$_2$*C*═OO), 25.53–25.71 (5C, *C*H$_2$ vinylic carbons), 22.07 (*C*H$_2$CH$_2$C═OO), 20.56 (CH$_2$*C*H$_3$), 14.23 (*C*H3). ESIMS (Fig. S3 in ESI†): *m/z* calculated for $C_{30}H_{40}FN_3O_4S$ $(M+H)^+$: 558.73. Found: 558.28.