Biomaterials 151 (2018) 53–65

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

# Creation of a nanoformulated cabotegravir prodrug with improved antiretroviral profiles

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ARTICLE INFO

*Article history:*
Received 11 September 2017
Received in revised form
12 October 2017
Accepted 12 October 2017
Available online 15 October 2017

*Keywords:*
Cabotegravir
Long-acting
Prodrug
Nanoformulation
HIV-1

ABSTRACT

Long-acting parenteral (LAP) antiretroviral drugs have generated considerable interest for treatment and prevention of HIV-1 infection. One new LAP is cabotegravir (CAB), a highly potent integrase inhibitor, with a half-life of up to 54 days, allowing for every other month parenteral administrations. Despite this excellent profile, high volume dosing, injection site reactions and low body fluid drug concentrations affect broad use for virus infected and susceptible people. To improve the drug delivery profile, we created a myristoylated CAB prodrug (MCAB). MCAB formed crystals that were formulated into nanoparticles (NMCAB) of stable size and shape facilitating avid monocyte-macrophage entry, retention and reticuloendothelial system depot formulation. Drug release kinetics paralleled sustained protection against HIV-1 challenge. After a single 45 mg/kg intramuscular injection to BALB/cJ mice, the NMCAB pharmacokinetic profiles was 4-times greater than that recorded for CAB LAP. These observations paralleled replicate measurements in rhesus macaques. The results coupled with improved viral restriction in human adult lymphocyte reconstituted NOD/SCID/IL2Rγc$^{-/-}$ mice led us to conclude that NMCAB can improve biodistribution and viral clearance profiles upon current CAB LAP formulations.



## 1. Introduction

Following the 1983 discovery of the human immunodeficiency virus type one (HIV-1), remarkable progress was made in the development of effective diagnostics and treatments [1]. Arguably, the most successful of all is effective antiretroviral therapy (ART). ART has markedly reduced disease-associated morbidities and mortality, enabling a nearly normal quality of life for infected people [2,3]. Nevertheless, life-long treatment is still required to suppress viral replication and contain disease. HIV-1 resistance [4,5], drug toxicities [6,7], and poor patient adherence [8] have also impeded therapeutic effectiveness. Treatment fatigue, lack of financial and social support, co-existing mental symptoms, and substance abuse can result in lack of adherence to ART regimens [9]. Therefore, ways to improve regimen adherence are greatly needed.

An important milestone in recent years to improve ART adherence is long-acting parenteral (LAP) antiretroviral drugs (ARVs) [10]. Changes in treatment patterns from daily oral to monthly or even less-frequent administration may also provide greater patient privacy and satisfaction [11,12]. A survey conducted in 400 HIV-1 seropositive patients indicated that 73% of patients would consider switching from daily pill regimens to LAP regimens [13]. However, not all ARVs can be redeveloped as LAPs based on drug potency and physicochemical properties. In fact, only a few of ARV candidates have been advanced to clinical studies. Cabotegravir (CAB) plus rilpivirine (RPV) is first-ever long-acting combination ART regimen. Monthly or every other month CAB and RPV LAP formulations demonstrated comparable antiretroviral activity to daily oral three-drug combinations for maintenance therapy [14].

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https://doi.org/10.1016/j.biomaterials.2017.10.023

CAB is a novel integrase strand transfer inhibitor (INSTI) with low aqueous solubility, high melting point, high potency, long half-life, and slow metabolic clearance [15,16]. These properties enable CAB to be formulated in a 200-mg/mL suspension (CAB LAP) and administered intramuscularly monthly or even less frequently [14,17]. An additional benefit rests in that CAB is primarily metabolized by uridine diphosphate glucuronosyltransferase (UGT) 1A1, with low potential to interact with other ARVs [16,18]. CAB LAP has also proven to be highly protective against rectal, vaginal, and intravenous SHIV transmission in non-human primates [19–22], and has been advanced into clinical trials for HIV prevention (NCT02720094). Despite such promising drug profiles, dosing pattern has limitations. Specifically, split injections given in 2 mL volumes are required to achieve 800-mg doses, leading to treatment cessations because of intolerable injection site reactions [14,23]. However, even with the high injection volumes, the maximal dosing interval is only 8 weeks. Recently in a phase 2a study investigating safety and tolerability of CAB LAP in HIV-uninfected men (ECLAIR; NCT02076178) [23], 800 mg dose every 12 weeks was selected based on previous clinical studies [17,24], aiming to maintain plasma CAB concentrations above 4 times protein-binding-adjusted 90% inhibitory concentration ($4 \times$ PA-$IC_{90}$, 660 ng/mL), a concentration demonstrated to be protective against new infections in macaques [19–22]. However, two-thirds of participants had faster than anticipated drug absorption from the injection site leading to plasma drug concentrations below targeted concentration of $4 \times$ PA-$IC_{90}$ at 12 weeks. Therefore, follow up study HPTN 083, a study for pre-exposure prophylaxis (PrEP) in HIV-uninfected cis gender men and transgender women, will require shortened dosing intervals (600 mg in 3 mL injection volumes every 8 weeks) to achieve effective protection with relevant antiretroviral plasma drug concentrations. Taken together, a means to extend the dose interval beyond 12 weeks and reduce injection volumes could bring broader usage to such regimens [25].

Progress in medicinal chemistry and nanotechnology has provided new opportunities to improve ARV delivery. Diverse strategies, such as chemical modification, cell-based drug delivery, HIV reservoir targeting, alternative delivery methods, are being tested to improve ARV pharmacokinetic (PK) profiles and biodistribution (BD) [26–28]. Modifications in ARV structures and delivery platforms can facilitate drug depot formation and lymphoid targeting [29–31], and have the potential to better penetrate HIV reservoirs, lower drug dosing, and reduce systemic toxicities [31–33]. To these ends, we designed a CAB nanoformulated prodrug, aiming to improve the drug's lipophilicity, hydrophobicity, and cellular entry and retention to improve the drug's ability to access anatomical reservoirs while maintaining high plasma concentrations. The result was a nanoformulated myristoylated CAB (NMCAB) with tailored formulation modifications that enhance tissue access and improve its antiretroviral activities.

## 2. Materials and methods

### 2.1. Materials

Native CAB and CAB LAP (200 mg/mL) were graciously obtained from ViiV Healthcare (Research Triangle Park, NC, USA). Myristoyl chloride, poloxamer 407 (P407), *N,N*-diisopropylethylamine (DIEA), dimethylformamide (DMF), 1-octanal, ciprofloxacin, paraformaldehyde (PFA), and 3,3′-diaminobenzidine (DAB) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Corning Life Sciences (Tewksbury, MA, USA). Heat-inactivated pooled human serum was purchased from Innovative Biologics (Herndon, VA, USA). Cell counting kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies, Inc. (Rockville, MD, USA). Gentamicin, HPLC grade acetonitrile (ACN), HPLC grade methanol, and Optima grade LC/MS water was purchased from ThermoFisher Scientific (Waltham, MA, USA). FITC mouse anti-human CD45, Alexa Fluor 700 mouse anti-human CD3, APC mouse anti-human CD4, and BV421 mouse anti-human CD8 were purchased from BD Biosciences (San Jose, CA, USA). Monoclonal mouse anti-human HIV-1p24 (clone Kal-1), monoclonal mouse anti-human leukocyte antigen (HLA-DR; clone CR3/43), and the polymer-based HRP-conjugated anti-mouse EnVision + secondary were purchased from Dako (Carpinteria, CA, USA).

### 2.2. Myristoyl CAB (MCAB) synthesis

Synthesis of MCAB was conducted according to the scheme shown in Fig. 1A. Briefly, a precooled (0 °C) solution of CAB (4.9 mM, 1 equivalent) in anhydrous DMF (35 mL) was deprotonated using DIEA (9.8 mM, 2 equivalents) and then reacted with myristoyl chloride (9.8 mM, 2 equivalents) for 16 h at room temperature. After completion of the reaction, the mixture was concentrated and the product isolated by flash silica gel chromatography using a mixture of ethyl acetate and hexane (4:1). The fractions containing UV active MCAB were dried and washed with diethyl ether, followed by drying under vacuum to get the final product.

### 2.3. MCAB physicochemical characterizations

Proton nuclear magnetic resonance ($^1$H NMR) spectra of CAB and MCAB were recorded on a Varian Unity/Inova-500 NB (500 MHz; Varian Medical Systems Inc., Palo Alto, CA, USA). $^1$H NMR data is reported in parts per million (ppm) downfield from tetramethylsilane as an internal standard. Fourier transform infrared spectroscopy (FT-IR) analysis was performed using a Spectrum Two FT-IR spectrometer (PerkinElmer, Waltham, MA, USA). Comparative crystallographic analyses of CAB and MCAB by powder X-ray diffraction (XRD) were carried out in the 2θ range of 2–50° using PANalytical Empyrean diffractometer (PANalytical Inc., Westborough, MA, USA) with Cu-Kα radiation (1.5418 Å) at 40 kV, 45 mA setting. A mask of 20 mm and a divergence slit of 1/8° were used on the incident beam path. A nickel foil filter was used to eliminate the diffraction peaks due to possible $K_b$ wavelength.

### 2.4. Solubility test

The solubilities of CAB and MCAB in water and 1-octanol were determined by adding excess drug to each solution at room temperature then mixing for 24 h. Samples were centrifuged at $20{,}000 \times g$ for 10 min to pellet insoluble drug. The supernatants containing solubilized drug were vacuum-dried then re-dispersed in methanol for drug concentration measurement using a Waters ACQUITY ultra performance liquid chromatography (UPLC) H-Class System with TUV detector and Empower 3 software (Milford, MA, USA). CAB and MCAB samples were separated on a Phenomenex Kinetex 5 μm C18 column (150 × 4.6 mm) (Torrance, CA) using either 65% 5.0 mM $KH_2PO_4$, pH 3.2/35% ACN or 90% ACN/10% water with a flow rate of 1.0 mL/min and detected at 254 and 230 nm, respectively. Drug content was quantitated by comparison of peak area to those of known standards (0.05–50 μg/mL in methanol).

### 2.5. Nanoformulated MCAB (NMCAB) manufacture and characterization

NMCAB and nanoformulated CAB (NCAB) were manufactured by high-pressure homogenization (Avestin EmulsiFlex-C3; Avestin Inc., Ottawa, ON, Canada). Briefly, MCAB or CAB (5% w/v) was premixed in a P407 solution (0.5% w/v in endotoxin free water) for 16 h