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Pharmacokinetic testing of a first generation cabotegravir prodrug in rhesus macaques

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Summary

Long-acting antiretrovirals can improve therapy and prevention for HIV-1 infection. Current long-acting cabotegravir (CAB LAP) can be administered every other month. Previously, we demonstrated that a myristoylated CAB prodrug encased in poloxamer 407 provided extended plasma drug concentrations. We now demonstrate that this first-generation nanoformulated prodrug can sustain plasma CAB concentrations above the PA-IC_{90} for four months in rhesus macaques. A 2.5-fold extension in CAB half-life and a 1.6-fold increase in area under the concentration-time curve were observed compared to CAB LAP.

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Author contributions

JM: Study design, drug quantitation and analysis, data analyses, graphic design, and preparation and editing manuscript; **AS**: Study design, preparation and characterization of NMCAB using good laboratory practice protocols, preparation of dose solutions, manuscript editing; **TZ**: Prodrug synthesis, NMCAB development, preparation of NMCAB, data analysis and interpretation; **BM,** Study coordination, coordination of sample collection, hematologic and metabolic panel data analysis; **BL, SC**: Study design, administration of NMCAB treatment to animals, blood sample collections, monitoring animal health, hematologic and metabolic panel data analysis; **NG**: Development of validated drug quantitation LC-MS/MS protocols, drug quantitation and pharmacokinetics assessment, data interpretation, manuscript editing; **YA**: LC-MS/MS protocol development, pharmacokinetics assessment, data interpretation, manuscript editing**; BE**: Design of prodrug synthesis scheme, supervision and validation of formulations used in study, data analysis and manuscript editing; **HEG**: Study design, overall project supervision, NMCAB development, data interpretation, writing and editing of the manuscript; **HSF**: Principal investigator of IACUC protocol for NHP studies, study design, supervision of animal work, interpretation of hematologic and metabolic profile data sets, assessment of animal health, data interpretation, manuscript editing.

Conflicts of Interest

There are no conflicts of interest.

Keywords

Long-acting antiretrovirals; LASER ART; cabotegravir; pharmacokinetics; rhesus macaques

Control of human immunodeficiency virus (HIV-1) infection requires lifelong combination antiretroviral therapy [1, 2]. Adherence to any treatment regimen is a common limitation with consequent emergence of viral mutations and comorbid events [3-7]. The development of long acting parenteral antiretroviral drugs presents an important means to improve regimen adherence with secondary benefits in viral prevention. This can be achieved through the deployment of such medicines for pre-exposure prophylaxis[8] as well as the fact that efficacious treatment prevents viral transmission [9]. Cabotegravir (CAB), a novel integrase strand transfer inhibitor, is being developed as a long acting parenteral formulation (CAB LAP) with a half-life of up to 52 days for every month or every other month dosing [10-12]. CAB is effective against a variety of HIV clades and against vaginal transmission of and intravenous challenge with simian and simian-human immunodeficiency virus in non-human primates [13-17]. The combination of CAB and rilpivirine (RPV) present a two-drug regimen for both treatment and prevention against HIV infection [18, 19]. However, limitations for its use include multiple 2 ml intramuscular injection volumes required to deliver an effective dose of 800 mg, injection site reactions and the requirements for health care providers to provide administration education and follow on [11, 18]. To meet needs for reduced volumes and longer therapeutic durations we have developed medicinal chemistry and nanoformulation approaches to improve prodrugs that facilitate what we have coined as long acting slow effective release antiretroviral therapy (LASER ART) that allows the formation of hydrophobic and lipophilic drug nanocrystals stabilized by polymer excipients [20-22]. Myristoyl ester modified cabotegravir (MCAB) and encasement of the prodrug into poloxamer 407 (P407)-stabilized nanocrystals (NMCAB) defines LASER ART and leads to an extended CAB plasma apparent half-life of 278 hours compared to 71 hours for CAB LAP in mice [22]. Intramuscular administration of 45 mg/kg CAB equivalents as NMCAB provided plasma drug levels above 4 times the protein-adjusted 90% inhibitory concentration (4 × PA-IC_{90}, 660 ng/mL) for 56 days in mice and above the 1X PA-IC90 for 89 days in rhesus macaques [22].

While early screens assessed drug pharmacokinetics to 3 months the prior published report lacked a complete pharmacokinetic analysis of the drug's apparent half-life. Plasma drug decay was not previously defined. As any assessment of long acting antiretroviral drugs needs to include a complete follow on to where plasma drug levels fall below the 50% effective concentration (EC_{50}) values we have extended analyses of both prodrug and native drug levels in NMCAB treated rhesus macaques to 36.6 weeks. To emphasize the importance of our observations, responses were compared to those from data previously published following a single intramuscular dose of 50 mg/kg CAB LAP.[14]. Myristoylated CAB was synthesized and stabilized into P407-coated NMCAB nanocrystals prepared by high pressure homogenization (Avestin EmulsiFlex-C3, Avestin Inc, Ottawa, Ontario, Canada) [22] using good laboratory practice protocols in the Nebraska Nanomedicine Production Plant [20]. Particle size (351.1 nm), polydispersity index (0.18) and zeta potential (−26.8 mV) were determined by dynamic light scattering (Malvern Zetasizer NanoZSP,

Malvern Instruments, Westborough, MA, USA). MCAB concentration in the nanoformulation (131.3 mg/ml) was quantified using ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS; Waters Acquity XevoTQ-S micro system; Waters Corp., Milford, MA, USA) as described [22]. Endotoxin content (Lonza Limulus Amebocyte Lysate Pyrogent-500, Lonza, Walkersville, MD, USA) was < 5 EU/kg. Two rhesus macaques were injected intramuscularly with 45 mg CAB equivalents/kg as NMCAB. Blood was collected over 8 months for cell counts, metabolic profile and drug analyses. Complete blood counts and metabolic panels were performed by the Nebraska Medical Center Pathology and Microbiology Laboratory. Plasma CAB concentrations were determined by UPLC-MS/MS with current drug quantitation sensitivities of 0.5 ng/ml [22].

As illustrated in Fig. 1A plasma CAB concentrations remained above the 1X PA-IC_{90} (166 ng/ml) for 12.7 weeks in both animals given NMCAB. In contrast in the prior report of SIV-infected rhesus macaques treated with 50 mg/kg CAB-LAP [14], the average plasma CAB concentrations dropped below the 1X PA-IC_{90} by 8 weeks and were below the limit of quantification (10 ng/ml) by 16 weeks. In contrast, in our current studies, plasma CAB concentrations in rhesus macaques that received NMCAB remained at or above 10 ng/ml for 33 weeks, and remained above our assay limit of quantitation (0.5 ng/mL) for the study duration. CAB apparent plasma terminal half-life was increased 2.5-fold from 14 days for CAB-LAP to 34-36 days for NMCAB (Fig. 1D). The longer CAB half-life was reflected in the increase in area under the curve, lower clearance (Cl/F) and higher volume of distribution (V/F) for CAB in animals receiving NMCAB compared to those treated with CAB-LAP. In both animals treated with NMCAB plasma MCAB concentrations peaked at 27 ng/ml at 4 days after treatment, dropped to less than 10 ng/ml at 18 days, then remained at the limit of detection (0.5 ng/mL) for the study duration. CBCs and metabolic panels in animals treated with NMCAB remained unchanged from pre-treatment values throughout the study (Fig. 1B and 1C).

These results demonstrate that LASER ART may be used to extend the apparent half-life of CAB with either a decrease in injection volume or extended dosing intervals to 3 months or longer. Further enhancements of drug lipophilicity and hydrophobicity are currently under investigation to further extend dosing intervals beyond six-months. Importantly, lack of observed injection site and systemic reactions indicates that the minimal use of excipients may improve patient acceptance to these regimens. These improvements in chemical composition and administration of CAB could enable improved treatment and preventative measures for HIV-1 infection.

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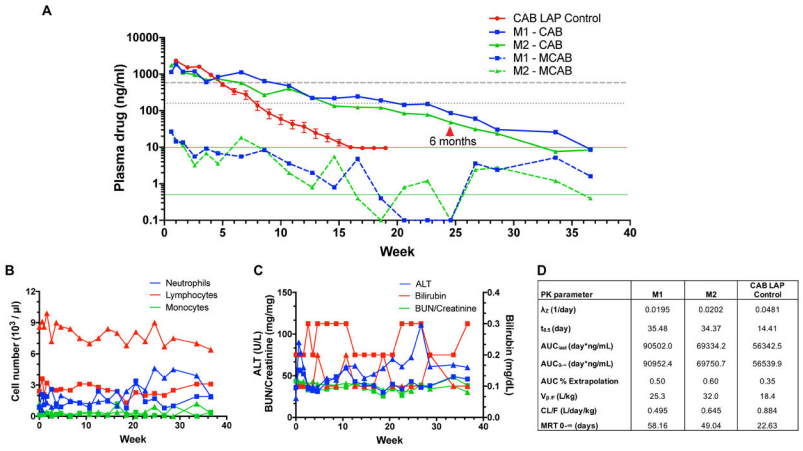

PK parameter	M1	M2	CAB LAP Control
λ_Z (1/day)	0.0195	0.0202	0.0481
$t_{0.5}$ (day)	35.48	34.37	14.41
AUC_{last} (day*ng/mL)	90502.0	69334.2	56342.5
$AUC_{0-\infty}$ (day*ng/mL)	90952.4	69750.7	56539.9
AUC % Extrapolation	0.50	0.60	0.35
V_{β} F (L/kg)	25.3	32.0	18.4
CL/F (L/day/kg)	0.495	0.645	0.884
MRT 0-∞ (days)	58.16	49.04	22.63

Fig. 1. Plasma CAB concentrations, pharmacokinetics, metabolic and hematologic profiles following intramuscular injection of NMCAB or CAB-LAP.

(A) Plasma CAB concentrations were determined over 36.6 weeks after treatment of two rhesus macaques (M1 and M2) intramuscularly with 45 mg CAB equivalents/kg as NMCAB. Data for CAB LAP Control was extracted from [14] with permission, and averages and standard error of the means were determined. CAB LAP was given as a single 50 mg/kg intramuscular injection to SIV-infected rhesus macaques (N=12). The 1X PA-IC_{90} and 4X PA-IC_{90} for CAB in patients are indicated by the small-dashed and large-dashed gray lines, respectively. The solid red line indicates the limit of CAB quantitation for the CAB LAP Control study (10 ng/ml). For our analyses in the current study, CAB and MCAB limits of quantitation (0.5 ng/ml each) are indicated by the solid green line. CAB concentrations = solid lines; MCAB concentrations = dashed lines **(B)** White blood cell counts following NMCAB administration to 2 rhesus macaques. Blood was collected into EDTA tubes and cell counts assessed by manual differentiation. Data from each animal is presented. Square symbols: M1; Triangle symbols: M2. **(C)** Alanine aminotransferase (ALT), total bilirubin, blood urea nitrogen (BUN) and creatinine were determined in plasma following intramuscular NMCAB administration to 2 rhesus macaques. Blood was collected into EDTA tubes and plasma separated by centrifugation at 3,000 g for 10 min. Data from each animal is presented. Square symbols: M1; Triangle symbols: M2. **(D)** Plasma pharmacokinetic (PK) parameters for CAB were determined using noncompartmental analysis in animals treated with NMCAB (M1 and M2) or CAB LAP (control). λ_Z, individual estimate of the terminal elimination rate constant; AUC_{last}, AUC 0 h to last time point; $AUC_{0-\infty}$, AUC from 0 h to infinity; Vβ, volume of distribution at β phase; CL, clearance; MRT, mean residence time.