

A LONG-ACTING NANOFORMULATED CABOTEGRAVIR PRODRUG FOR IMPROVED ANTIRETROVIRAL THERAPY

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Background: Long-acting parenteral (LAP) antiretroviral drugs (ARVs) have set a new bar for HIV/AIDS therapeutics. LAP ARVs can improve treatment adherence and positively affect drug resistance patterns and systemic toxicities. A single intramuscular (IM) injection of cabotegravir (CAB), a potent HIV integrase inhibitor, in phase III clinical trials (CAB-LAP), permits up to two month sustained plasma drug levels > 4x protein adjusted IC₉₀ (PA-IC₉₀, 660 ng/mL). We posit that CAB-LAP could be modified to reduce its injection volume and improve its pharmacokinetic (PK) and biodistribution profiles. To this end, a nanoformulated prodrug of CAB (we call NMCAB) was made to extend the drug's half-life and antiretroviral activities.

Methods: CAB was chemically modified with myristoyl chloride to affect its hydrophobic properties. MCAB was encased in poloxamer 407 by high-pressure homogenization. Uptake and retention in human monocyte-derived macrophages (MDM) were tested. Antiretroviral activity was evaluated by reverse transcriptase (RT) activity and HIV-1 p24 antigen levels. NMCAB test results were compared to parent drug formulations in Balb/c mice after a single IM injection of 15 or 45 mg/kg. The plasma drug levels were monitored for two months. Pharmacodynamics of NMCAB was evaluated in HIV-1 infected human PBL reconstituted NOD/scid-IL-2R γ c^{null} (NSG) mice model.

Results: MDM efficiently took up the NMCAB nanoparticles with sustained slow release up to 30 days. Notably, the parent drug formulations were eliminated after the treatment day. Drug crystals were observed by transmission electron microscopy (TEM) in NMCAB treated MDM, but not in cells treated with parent drug formulations. NMCAB elicited sustained antiretroviral activity in MDM determined by RT activity and HIV-1 p24 staining for up to 15 days. In *in vivo* studies, NMCAB showed reduced burst release with reduced rate of elimination, resulting in plasma drug levels at 6-8 weeks being up to 300 times greater than the parent drug formulations with improved abilities to prevent HIV infection in a mouse model of disease.

Conclusion: NMCAB demonstrated improved antiretroviral activities and long-acting slow release properties as compared to CAB-LAP in the testing systems applied. Further refinements in drug delivery platforms will enable LAPs to be more easily administered.

MCAB synthesis and characterization

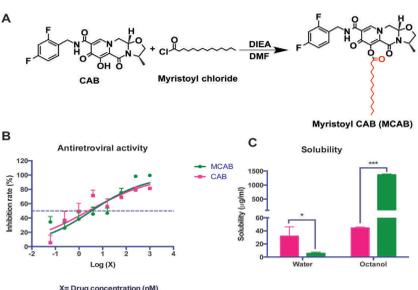


Figure 1. MCAB synthesis and characterization. (A) Synthesis of myristoylated CAB (MCAB). (B) Antiretroviral activity of MCAB versus CAB in HIV-1_{ADA} infected MDM. (C) Solubility of CAB and NMCAB in water and 1-octanol measured at room temperature.

Production of nanoformulated MCAB (NMCAB)

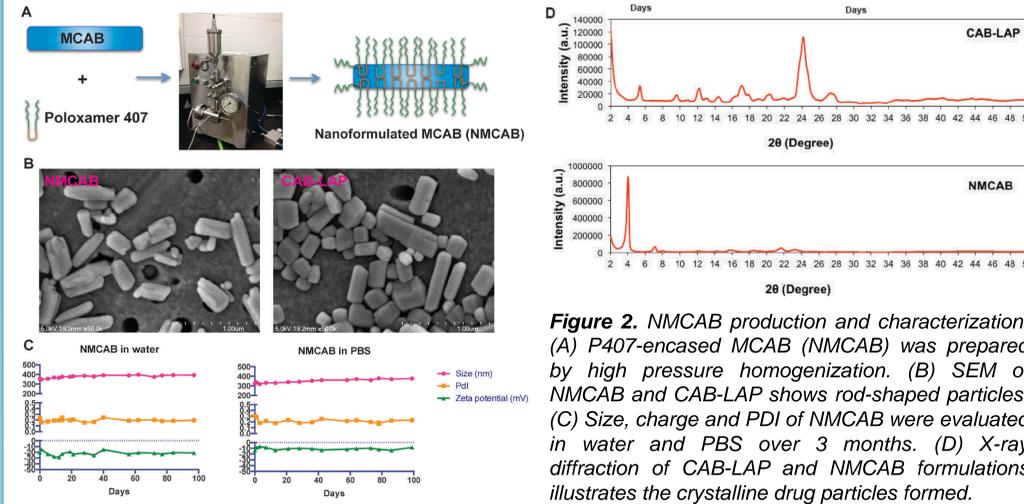


Figure 2. NMCAB production and characterization. (A) P407-encased MCAB (NMCAB) was prepared by high pressure homogenization. (B) SEM of NMCAB and CAB-LAP shows rod-shaped particles. (C) Size, charge and PDI of NMCAB were evaluated in water and PBS over 3 months. (D) X-ray diffraction of CAB-LAP and NMCAB formulations illustrates the crystalline drug particles formed.

NMCAB cellular reservoir formation

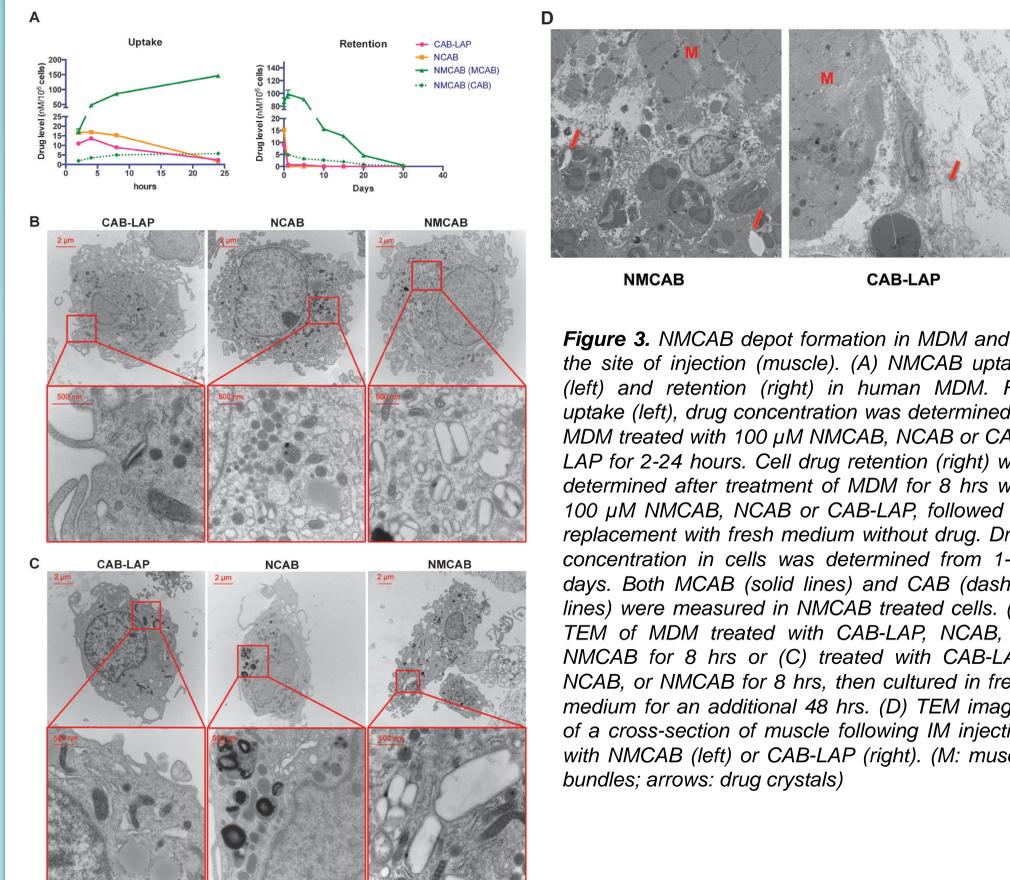


Figure 3. NMCAB depot formation in MDM and at the site of injection (muscle). (A) NMCAB uptake (left) and retention (right) in human MDM. For uptake (left), drug concentration was determined in MDM treated with 100 μ M NMCAB, NCAB or CAB-LAP for 2-24 hours. Cell drug retention (right) was determined after treatment of MDM for 8 hrs with 100 μ M NMCAB, NCAB or CAB-LAP, followed by replacement with fresh medium without drug. Drug concentration in cells was determined from 1-30 days. Both MCAB (solid lines) and CAB (dashed lines) were measured in NMCAB treated cells. (B) TEM of MDM treated with CAB-LAP, NCAB, or NMCAB for 8 hrs or (C) treated with CAB-LAP, NCAB, or NMCAB for 8 hrs, then cultured in fresh medium for an additional 48 hrs. (D) TEM images of a cross-section of muscle following IM injection with NMCAB (left) or CAB-LAP (right). (M: muscle bundles; arrows: drug crystals)

Antiretroviral Responses

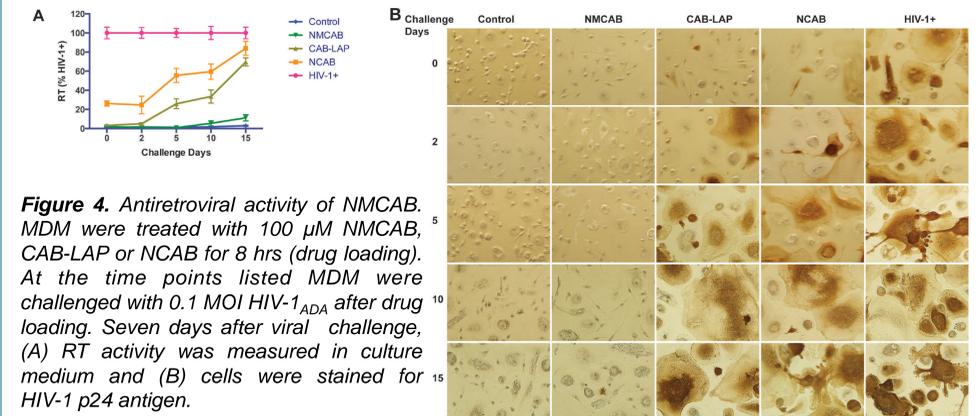


Figure 4. Antiretroviral activity of NMCAB. MDM were treated with 100 μ M NMCAB, CAB-LAP or NCAB for 8 hrs (drug loading). At the time points listed MDM were challenged with 0.1 MOI HIV-1_{ADA} after drug loading. Seven days after viral challenge, (A) RT activity was measured in culture medium and (B) cells were stained for HIV-1 p24 antigen.

Pharmacokinetics

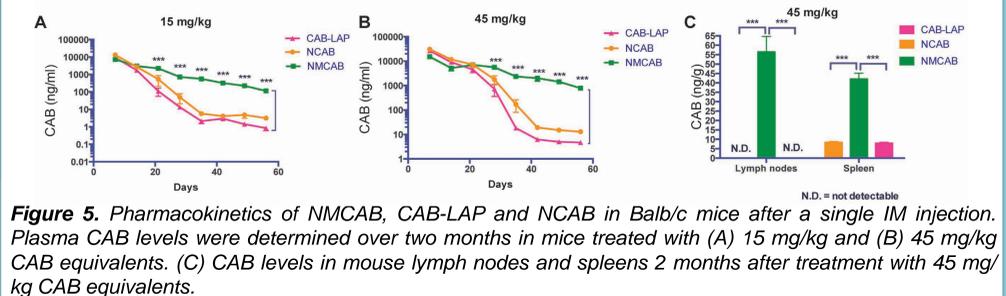


Figure 5. Pharmacokinetics of NMCAB, CAB-LAP and NCAB in Balb/c mice after a single IM injection. Plasma CAB levels were determined over two months in mice treated with (A) 15 mg/kg and (B) 45 mg/kg CAB equivalents. (C) CAB levels in mouse lymph nodes and spleens 2 months after treatment with 45 mg/kg CAB equivalents.

Pre-Exposure Prophylaxis

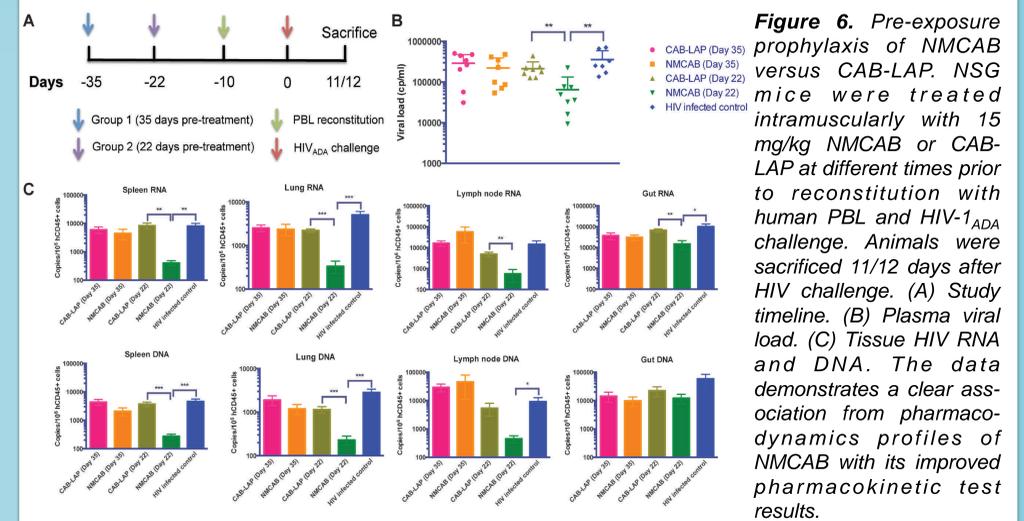


Figure 6. Pre-exposure prophylaxis of NMCAB versus CAB-LAP. NSG mice were treated intramuscularly with 15 mg/kg NMCAB or CAB-LAP at different times prior to reconstitution with human PBL and HIV-1_{ADA} challenge. Animals were sacrificed 11/12 days after HIV challenge. (A) Study timeline. (B) Plasma viral load. (C) Tissue HIV RNA and DNA. The data demonstrates a clear association from pharmacodynamics profiles of NMCAB with its improved pharmacokinetic test results.

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