HIV Reservoir-Targeted Antiretroviral Nanofabrication Facilitates Viral Clearance

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Abstract

Limitations in adherence, toxicity and reservoir targeting underlie the needs for improvements in antiretroviral therapy (ART). We posit that long-acting nanoformulated antiretroviral therapy (nanoART) can address this need by improving current drug pharmacokinetics (PK) and biodistribution. The development of monocyte-macrophage targeting ligands could affect drug depots and target sanctuaries of viral infections. To this end, we developed a folic acid (FA) crystalline nanofabrication for ritonavir-boosted atazanavir (FA-P407-ATV/r). The PK and pharmacodynamic (PD) properties were, until now, not known. The size, shape, zeta potential, and polydispersity of FA-P407-ATV/r nanoparticles were determined following high-pressure homogenization. NOD/scid-IL-2Rγcnull (NSG) mice were reconstituted with human peripheral blood lymphocytes (hu-PBL) and treated with 10, 50 and 100 mg/kg of native drug, P407-ATV/r, FA- P407-ATV/r or left untreated. The mice were challenged with HIV-1ADA (104 tissue culture infective dose50, TCID50) 24 hours later and sacrificed 14 days after treatment. In further experiments human CD34+ hematopoietic stem cells transplanted NSG (CD34-HSC-NSG) mice at 6-8 weeks age were infected for 10 weeks with HIV-1ADA. The mice were then treated with 100 mg/kg FA-P407-ATV/r or left untreated. A boosting dose with FA-P407-ATV/r (100 mg/kg) was administered 2 and 4 weeks following initial dose. The mice were sacrificed 6 weeks after the first dose. Bioavailability and tissue biodistribution were determined by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). PD was scored by measures of plasma viral RNA copies/ml (Cobas Amplicor V1.5), HIV-1 gag RNA expression by reverse transcriptase polymerase chain reaction and HIV-1p24 expression by immunohistochemistry in spleen, liver, lung and kidney. FA-P407-ATV/r led to an increase in plasma, spleen, liver and lung tissue drug levels up to > 5-fold over uncoated P407-ATV/r. Both treatments showed protection of CD4+ to CD8+ T cell ratios in spleen and blood. Number of infected HIV-1p24 cells and viral RNA levels in liver, spleen and/or lung with FA-P407-ATV/r were reduced up to > 2 logs compared to untreated and > 1 log over P407-ATV/r treated hu-PBL-NSG mice. Three of five CD34-HSC-NSG mice challenged with HIV-1ADA showed no detectable virus with the other two demonstrating viral reductions of up to 2 logs. The spleen tissue sections of FA-P407-ATV/r group also showed significantly reduced HIV-1p24 expression compared to untreated group. FA targeted ART nanofabrications are a significant means to improve PK and PD. The reservoir targeting capacity of these nanoART could lead to platforms for chemical viral eradication measures.

Introduction

Adherence to antiretroviral therapy (ART) remains a limitation towards successful clinical management for human immunodeficiency virus (HIV) infection (1). Maintenance of optimal therapeutic drug levels is essential to preclude viral resistance. As elimination of residual virus in an infected human host remains elusive, drug delivery systems that optimize ART penetrance of viral reservoirs and enhanced suppression of virus are sorely needed (2). Nanomedicine has the potential to achieve such goals. To this end, our laboratories have developed long-acting nanoformulated ART (nanoART) for drug delivery to sites of infection and to maintain drug depots in monocytes and macrophages (3, 4). However, despite such advances, drug dosing issues and toxicities remain (5). We posit that targeting nanoART with folic acid-conjugated poloxamer will enhance macrophage uptake and improve drug pharmacokinetics, biodistribution and antiretroviral activity

Materials and Methods

NanoART manufacture: FA-P407 was synthesized by previously established protocols. Briefly, FA was reacted with DCC/NHS and was linked to amine-P407. For targeted formulations, free-based ATV (1%) and polymer (0.2 %; 3:2 ratio of P407 to FA-P407) were pre-mixed in 10 mM HEPES and homogenized at 20,000 PSI and later purified by centrifugation (5). Particle size, charge and polydispersity were determined by dynamic light scattering.

Pharmacokinetic (PK) and pharmacodynamic (PD) studies: The protocols used were in accordance with University of Nebraska Medical Center Institutional Animal Care and Use Committee (IUCAC). PK and biodistribution was determined in Balb/cJ mice. PD endpoints were measured in Hu-PBL-NSG and CD34-HSC-NSG mouse models (xxxxxxxxxxx). Plasma and tissue drug concentrations at indicated time points were determined by UPLC-MS/MS. PD efficacy of nanoART was assessed from plasma viral loads, flow cytometry analysis of blood and spleen, immunohistochemistry for HIV-1 p24 in tissue sections and PCR analysis for HIV-1gag RNA expression in spleen.

Results

PrEP treatment paradigm

- FA-P407-ATV/r improved plasma ATV levels and improved biodistribution
- Protection of CD4+ T cells was seen for FA-P407-ATV/r and P407-ATV/r
- FA-P407-ATV/r elicited reduced HIV-1 gag RNA levels compared to P407-ATV/r
- Reductions in HIV-1p24 antigen by FA-P407-ATV/r were observed.

Chronic infection

- FA-P407-ATV/r treated mice had plasma ATV levels above the minimal drug inhibitory concentration (150 ng/ml) for 6 weeks
- Viral loads were significantly reduced in the FA-P407-ATV/r mice over untreated mice
- CD4+ T cells with FA-P407-ATV/r treatment were similar to uninfected controls
- FA-P407-ATV/r treated mice expressed fewer HIV-1 p24 antigens
- FA-P407-ATV/r showed PD efficacy at 2.5 times less dose and ½ the dose frequency in comparison to non-targeted formulation

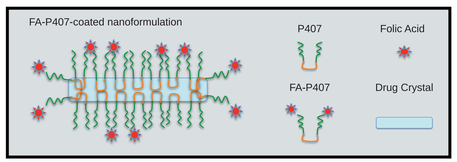

Figure 1: A cartoon structure of FA-P407 coated nanoformulation and its components. Nanoformulations of ATV and RTV were prepared by high-pressure homogenization.

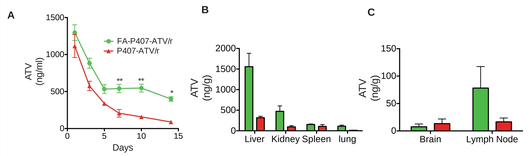

Figure 2: Pharmacokinetics of FA-P407-ATV/r and P407-ATV/r. Balb/cJ mice were injected with 50 mg/kg of either FA-P407-ATV/r or P407-ATV/r and sacrificed 14 days later. (A) Plasma ATV levels were determined throughout the study and (B, C) tissue ATV levels were determined at day 14 by UPLC-MS/MS.

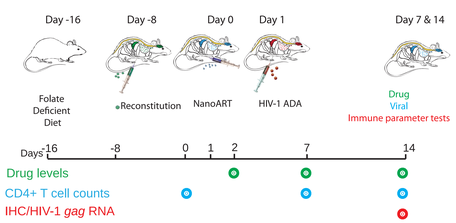

Figure 3: PrEP treatment was studied in NSG mice reconstituted with human peripheral blood lymphocytes. The scheme of infection, treatment and PD end points measured are shown.

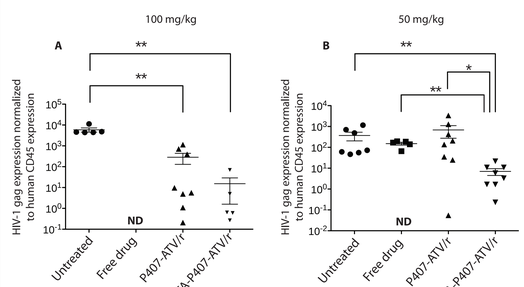

Figure 4: Comparison of antiretroviral activities of FA-P407-ATV/r and P407-ATV/r in hu-PBL reconstituted NSG mice infected with HIV-1ADA. Mice were treated on day 0 with PBS (untreated), free drug ATV/r, P407-ATV/r or FA-P407-ATV/r at doses of (A) 100 mg/kg or (B) 50 mg/kg or untreated. Mice were then infected with HIV-1ADA 24 hours later and sacrificed on day 14. HIV-1gag RNA expression in spleen was determined and normalized to human CD45 expression as a marker for human cell reconstitution. Data are expressed as mean ± SEM and considered *, ** statistically different, at P < 0.05 and P < 0.01, by the Mann-Whitney U-test.

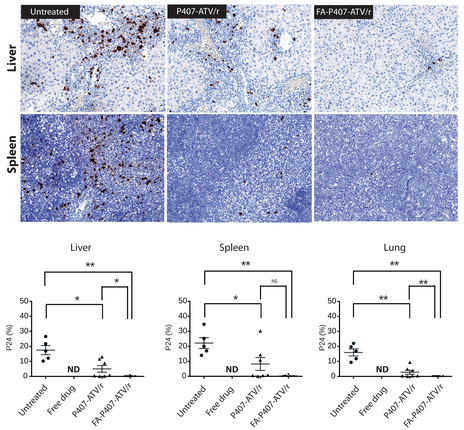

Figure 5: Immunohistochemistry for HIV-1p24 antigen in tissue sections of Hu-PBL-NSG mice. FA-P407-ATV/r and P407-ATV/r (100 mg/kg each drug) were administered intramuscularly on day 0 to huPBL-NSG mice. Mice were infected with HIV-1ADA on day 1 and sacrificed on day 14. Liver and spleen sections were immunostained with antibodies specific for HLA-DR or HIV-1p24, and visualized by DAB. Antiretroviral efficacy was determined by counting the number of HIV-1p24+ cells per section and expressed as percent of HLA-DR+ cells. Data are expressed as mean ± SEM and *, ** statistical differences were determined at P < 0.05 and P < 0.01 by the Mann-Whitney U-test.

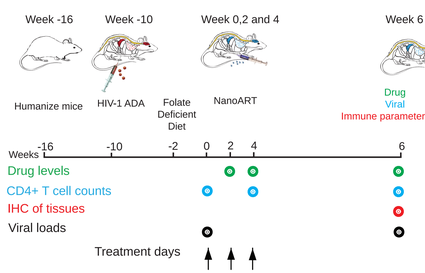

Figure 6: Antiretroviral efficacy of FA-P407-ATV/r was studied in CD34+ hematopoietic stem cell transplanted NSG mice. The treatment, scheme of infection and PD end points measured are shown

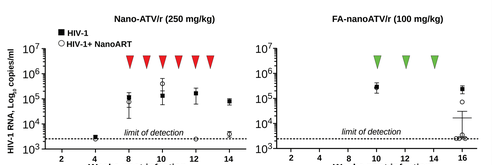

Figure 7: Plasma viral load in CD34+ hematopoietic stem cell transplanted and HIV-1 infected humanized mice during and following FA-nanoATV/r treatment. Humanized NSG mice were either treated with (A) 6 doses of 250 mg/kg poloxamer 188-ATV/r every week from weeks 8 to 13 following HIV-1 infection or (B) 3 doses of 100 mg/kg FA-nanoATV/r at 10, 12 and 14 weeks following HIV-1 infection. Plasma viral loads from nanoATV/r or FA-nanoATV/r (open circle) and untreated (closed box) groups were determined before and after treatment as illustrated. Data are expressed as mean ± SEM.

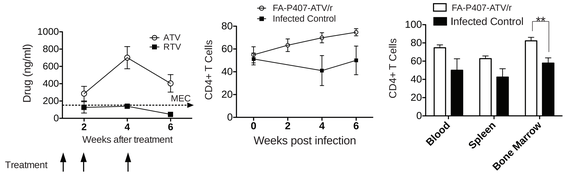

Figure 8: Plasma drug levels and flow cytometric analyses following FA-nanoATV/r treatment in CD34-HSC-NSG mice. Plasma ATV/RTV levels were determined during the study by UPLC-MS/MS. The percent of CD4+ T cells among CD3+ T cells and the CD4+ to CD8+ T cell ratios were determined using fluorescence-activated cell sorting between treated (green) and untreated infected control (black) groups. (A) CD4+ T cells and (B) CD4+ to CD8+ ratios in blood over time are shown along with (C) CD4+ T cell ratios and (D) CD4+ to CD8+ ratios in blood, spleen and bone marrow at the end of the study. Data are expressed as mean ± SEM. ** Statistically different at P<0.01 using unpaired Student's t-test.

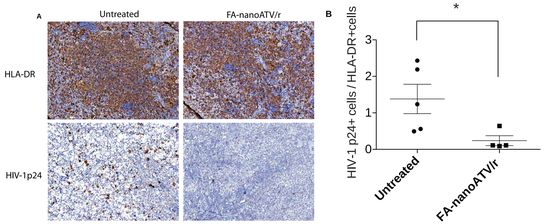

Figure 9: Spleen immunohistochemistry for HIV-1p24 following FA-nanoATV/r treatment. Humanized NSG mice were infected with HIV-1ADA for 10 weeks and then treated with 100 mg/kg FA-nanoATV/r at 0, 2 and 4 weeks. (A) Spleens were collected from treated and untreated groups and immunostained with antibodies for either HLA-DR or HIV-1p24, then visualized with DAB staining. (B) Antiretroviral efficacy was determined by counting the number of HIV-1p24+ cells in each 20X field of spleen sections and divided with rank scores of HLA-DR+ cells in the same microscopic field. * Statistically different at P<0.05 using unpaired Student's t-test.

Conclusions

- Folate receptors on macrophages can be targeted by nanoART
- FA-P407 coating improved PK, PD and biodistribution.
- A dose of 50 mg/kg achieved optimal therapeutic drug concentrations
- FA-P407-ATV/r showed significant improvements in PD in HIV-1 PrEP and chronic infection mouse models
- FA-P407 coating can be used to formulate nanoformulations of drugs with extended half-life and to develop long-acting parenteral therapy
- These laboratory findings have translational potential for moving the concept of targeted drug delivery from the bench to the patient bedside.

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