Pharmacodynamic and Antiretroviral Activities of Combination Nanoformulated Antiretrovirals in HIV-1–Infected Human Peripheral Blood Lymphocyte–Reconstituted Mice

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Lack of adherence, inaccessibility to viral reservoirs, long-term drug toxicities, and treatment failures are limitations of current antiretroviral therapy (ART). These limitations lead to increased viral loads, medicine resistance, immunocompromise, and comorbid conditions. To this end, we developed long-acting nanoformulated ART (nanoART) through modifications of existing atazanavir, ritonavir, and efavirenz suspensions in order to establish cell and tissue drug depots to achieve sustained antiretroviral responses. NanoART’s abilities to affect immune and antiviral responses, before or following human immunodeficiency virus type 1 infection were tested in nonobese severe combined immune-deficient mice reconstituted with human peripheral blood lymphocytes. Weekly subcutaneous injections of drug nanoformulations at doses from 80 mg/kg to 250 mg/kg, 1 day before and/or 1 and 7 days after viral exposure, elicited drug levels that paralleled the human median effective concentration, and with limited toxicities. NanoART treatment attenuated viral replication and preserved CD4+ T cell numbers beyond that seen with orally administered native drugs. These investigations bring us one step closer toward using long-acting antiretrovirals in humans.

Long-acting, parenterally administered antiretroviral nanoformulations are of immediate need [1, 2]. Those who would benefit most are patients for whom drug adherence and availability are limited and/or those who cannot ingest drug formulations [2, 3], leading to viral resistance patterns [4]. As monocytes and monocyte-derived macrophages (MDMs) are reservoirs for human immunodeficiency virus type 1 (HIV-1) and can uptake, transport, and release virus into infected tissues, our laboratory developed long-acting nanoformulated antiretroviral therapy (nanoART) in monocyte-macrophage carriers [5–11]. Surfactant composition, size, and charge of the particles were evaluated to optimize cell entry and release of atazanavir (ATV), ritonavir (RTV), and efavirenz (EFV) as these hydrophobic drugs are easily encased and commonly used in the clinic [7, 10–15]. Uptake and release of nanoART into and from MDMs were at drug levels at or beyond the half-maximal effective concentration (EC50) (EFV, 1.7–25 nM; RTV, 3.5–200 nM; ATV, 2–5 nM) [16] with limited or no cytotoxicity. On the basis of these outcomes, the most effective ART nanoformulations were selected for in vivo studies in HIV-1ADA–infected, nonobese diabetic, severe combined immunodeficient common cytokine receptor γ chain–deleted (NOD/scid-γcnull [NSG]) mice reconstituted with human peripheral blood lymphocytes (PBLs) (PBL-NSG mice) [17]. Two schemes were used for testing. First, 1 dose of

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nanoART was injected subcutaneously 1 day before HIV-1\textsubscript{ADA} infection with replicate native drugs given orally. Second, PBL-NSG mice were infected with HIV-1\textsubscript{ADA} before subcutaneous administration of nanoART. ART activity was evaluated by determination of virus suppression and preservation of CD4\textsuperscript{+} T cells. HIV-1\textsubscript{gag} RNA by real-time polymerase chain reaction (RT-PCR) in spleen and immunohistochemical quantitation of infected cells by staining for HIV-1\textsubscript{p24} proteins were assessed with CD4\textsuperscript{+} T-lymphocyte numbers. Systemic toxicities were enhanced as a consequence of graft-versus-host disease (GVHD). These results provide proof-of-concept that stable nanoART formulations provide sustained drug levels in serum and tissues above the EC\textsubscript{50} and afford effective antiretroviral responses independent of ex vivo macrophage loadings.

**MATERIALS AND METHODS**

**Preparation and Characterization of nanoART**

Free base RTV and EFV were obtained from Shengda Pharmaceutical Co and Hetero Labs Ltd, respectively. ATV-sulfate was purchased from Gyma Laboratories of America Inc. The surfactant (excipient) used in generating all formulations was poloxamer-188 (P188; Sigma-Aldrich) with or without 1,2-distearoyl-phosphatidyl-ethanolamine-methyl-polyethylene-neglycol conjugate-2000 (mPEG\textsubscript{2000}-DSPE, Genzyme Pharmaceuticals LLC). Final synthesis of the nanosuspension was achieved by either wet-milling or homogenization [18]. Drug levels were analyzed by reverse-phase high-performance liquid chromatography [7, 10, 12] and by ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) using a Waters ACQUITY UPLC coupled to an Applied Biosystems 4000 Q TRAP quadruple linear ion trap hybrid mass spectrometer [19].

For scanning electron microscopic examinations of nanoparticles, 10 µL of nanosuspension was diluted in 1.5 mL of 0.2 µm filtered distilled water and prepared for morphologic examination using a Hitachi S4700 Field-Emission Scanning Electron Microscope (Hitachi High Technologies America, Inc). Human MDM uptake, retention, release, and antiviral activity of nanoART in vitro were determined [10]. Scoring of nanoART, including the polydispersity index (PDI), in vitro activity (particle uptake, drug retention and release, and antiretroviral activity), cytotoxicity, and pharmacokinetics (PK), was made by assessment of decade-weighted ratios (DWRs) [10]. This was determined for each nanof ormulation where an arbitrary maximal (best) score of 10 was assigned for each test performed. DWRs were calculated as areas under the curve (AUC) for each formulation, proportional to 10\textsuperscript{AUC/AUC\textsubscript{best}} ratio. For example, a score of 9.7 reflected an 8-hour nanoART drug “uptake value” of 29.6 µg drug/10\textsuperscript{6} cells of 97% when compared to a “best” test result of 30.5 µg/10\textsuperscript{6} cells seen in all assays. The DWR for in vitro activity of each formulation reflected the averages of differences for nanoART uptake, retention, release, and antiretroviral activity in MDMs for each nanoART as compared to the best performer. DWRs have been described in detail previously[10].

**Cell retention of drug was determined as drug remaining in the cell whereas drug release was the amount of drug released into media. Antiretroviral activity was determined over 15 days. Scoring for in vitro toxicity was calculated as the average of DWR based on highest alamarBlue (AbD Serotec) reduction and lowest macrophage production of tumor necrosis factor α (TNF-α). DWR scoring of PK was determined in vivo as a function of the highest serum drug levels in nanoART-treated mice (250 mg/kg, subcutaneous). Final scores were composite averages of all tests.**

**Animals**

Male NSG mice were purchased from the Jackson Laboratory and housed in filter top cages with free access to food and water under a 12-hour/12-hour light/dark cycle in accordance with ethical guidelines for care of laboratory animals approved by the Institutional Animal Care and Use Committee at the University of Nebraska Medical Center.

**Human Cell Isolation, Transplantation, and Viral Infection**

Human PBLs were purified from leukopaks by countercurrent centrifugal elutriation and used to reconstitute NSG mice [20]. PBLs were injected intraperitoneally into 8-week-old mice at 30 x 10\textsuperscript{6} PBLs per mouse [21]. For viral infections, the HIV-1\textsubscript{ADA} strain was propagated in human MDMs and was found to be negative for endotoxin [22].

**Biodistribution and Antiretroviral Activity of nanoART**

To determine dose-dependent serum drug concentrations and tissue distribution of nanoART, nonreconstituted NSG mice were injected subcutaneously on days 0 and 7 with nanoART (ATV and RTV at 80, 150, or 250 mg/kg). These corresponded to human doses of 6.5–20.3 mg/kg based on an interspecies scaling factor of 12.3 [23]. Blood samples were collected at days 1, 6, and 14 after drug administration (Figure 1A). At study end (14 days after initial injection), tissue samples were collected for drug biodistribution assay. NSG mice were reconstituted with PBLs 7 days prior to subcutaneous nanoparticle administration or oral drug delivery of native drugs (Figure 1B). One day after drug treatment, animals were infected intraperitoneally with HIV-1\textsubscript{ADA} at a dose of 10\textsuperscript{4} median tissue culture infective dose per mouse. Animals were killed on day 9 after drug treatment, and blood and tissues collected for fluorescence-activated cell sorting (FACS) and viral load tests. PBL-reconstituted NSG mice were also infected with HIV-1\textsubscript{ADA} after 7 days. Two doses of nanoART were administered subcutaneously, the first dose at 12 hours (day 0) and the second at 7 days after infection. NanoART was administered as ATV and RTV at 250 mg/kg or ATV, RTV, and
EFV at 100 mg/kg each drug (Figure 1C). Tissues were collected on day 14 for drug level determinations. Serum drug levels on days 1, 6, or 14 were assayed after submandibular or cardiac puncture bleeds. Tissues (liver, spleen, lungs, kidneys, and brain) were collected following phosphate buffered saline (PBS) body perfusion. Drug concentrations in serum and tissues were determined by UPLC-MS/MS [19].

**Serum Biochemistry, Tissue Histopathology, and Immunohistochemical Tests**

Alanine aminotransferase, alkaline phosphatase, creatinine, total serum calcium, and phosphate were determined in blood collected by cardiac puncture at 14 days after initial nanoART treatment using a VetScan comprehensive diagnostic profile disc (Abaxis Inc) and a VetScan VS-2 instrument. Tissue
**Figure 2.** Morphology, in vitro activity, and tissue biodistribution of nanoformulated antiretroviral therapy (nanoART). Serum drug and tissue levels in mice treated with 2 weekly injections of different doses of nanoART (atazanavir [ATV] and ritonavir [RTV]).

### A

Scanning electron micrographs (>15,000) of nanoformulations of ATV formulated by wet-milling (M3001) or homogenization (H3001), RTV formulated by wet-milling (M2001) or homogenization (H2001), and homogenized efavirenz (EFV) (H4001) on 0.2-µm polycarbonate filter membranes. Bar = 1 µm.

### B

Selection criteria for formulations used.

<table>
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<th>Drug</th>
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<th>PDI</th>
<th>Activity</th>
<th>Toxicity</th>
<th>PK</th>
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<td>10.0</td>
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</table>

### C

Serum levels of ATV and RTV over 15 days with different doses (80, 150, 250 mg/kg).

### D

Tissue levels of ATV and RTV in different organs (Liver, Spinal, Kidney, Lung, Brain) with different doses (80, 150, 250 mg/kg).
samples were collected on day 14, placed in 10% neutral buffered formalin, and embedded in paraffin. Five-micrometer-thick sections were cut and mounted on glass slides. For histopathological analysis, tissue sections were stained with hematoxylin and eosin. Histopathological evaluations were performed in accordance with the guidelines of the Society of Toxicologic Pathology. Immunohistochemical staining used mouse monoclonal antibodies (Dako) for HLA-DR (clone CR3/43, 1:100) and HIV-1p24 (clone Kal-1, 1:10) and the polymer-based horseradish peroxidase–conjugated antimouse Dako EnVision systems were used for secondary detection, then developed with 3,3-diaminobenzidine and counterstained with hematoxylin. Images were obtained with a Nikon DS-Fi1 camera fixed to a Nikon Eclipse E800 microscope using NIS-Elements F 3.0 software (Nikon Instruments). The number of HIV-1p24+ cells per section was determined and expressed as the percentage of total HLA-DR+ cells. For HIV-1 gag RNA measurements, RNA from spleen sections was extracted with TRIzol (Invitrogen) and reverse transcribed to complementary DNA with random hexamers and Moloney murine leukemia virus reverse transcriptase (Invitrogen), and quantitative RT-PCR was performed [24].

**FACS Analyses of T-Cell Subsets**

At study termination, blood samples were collected into ethylenediaminetetraacetic acid–coated tubes (BD Diagnostics), and spleen cells were resuspended in PBS. Blood leukocyte and spleen cell suspensions were tested for expression of human...
CD45, CD3, CD4, and CD8 markers as 4-color combinations [25].

**Statistical Analyses**

Because of limitations in sample size, descriptive statistics used medians. Outcomes were compared between 2 groups using a Mann-Whitney test and data assessed among 3 or more groups using 1-way analysis of variance after performing a rank transformation on the variables. Post hoc comparisons were conducted using the Tukey method to adjust for multiple comparisons. A \( P \) value \( \leq .05 \) was considered statistically significant.

**RESULTS**

**Characterization of nanoART Formulations**

The morphologic (shape), physicochemical (PDI and charge) and biologic characteristics (uptake, retention, release, and antiretroviral activity in MDMs) of the nanoARTs were assessed [7, 10, 12] (Figure 2). ATV nanoARTs were thin and rod-
Figure 5. Antiretroviral activities of nanoformulated antiretroviral therapy (nanoART) administered prior to human immunodeficiency virus type 1 (HIV-1) exposure. NanoART was delivered on day 0 as a combination of atazanavir (ATV) and ritonavir (RTV) (250 mg/kg each H3001 and H2001, subcutaneously) to peripheral blood lymphocyte (PBL)–reconstituted NSG mice. Native drugs (250 mg/kg each ATV and RTV in PBS) were delivered by oral gavage on day 0. Mice were infected with HIV-1ADA on day 1. Spleens were harvested on day 9 after drug injection. A, Fluorescence-activated cell sorting analysis shows the percentage of human CD4+ among total CD3+ T cells (left panel) and CD4+/CD8+ T-cell ratios (right panel) for spleens from individual mice. B, Percentages of HIV-1p24–expressing cells among human HLA-DR+ cells were determined from cell counts of immunohistochemistry from splenic tissues (left panel). HIV-1gag RNA expression normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) messenger RNA expression in NanoART-Treated HIV-1–Infected Mice • JID 2012:206 (15 November) • 1583
Figure 6. Antiretroviral activities of nanoformulated antiretroviral therapy (nanoART). NanoART was delivered on days 0 and 7 as a combination of atazanavir (ATV) and ritonavir (RTV) (250 mg/kg each H3001 and H2001, subcutaneously) or a combination of ATV, RTV, and efavirenz (EFV) (100 mg/kg each H3001, H2001, and H4001, subcutaneously) to peripheral blood lymphocyte (PBL)–NSG mice infected intraperitoneally with human...
shaped whereas RTV nanoARTs were plumper particles. EFV particles were short and rectangular-shaped. Particle sizes ranged from 281 nm (M3001) to 470 nm (H4001) (Supplementary Table 1), with PDI being lower for homogenized than for wet-milled particles (0.200 [H2001] to 0.288 [M3001]). All particles were negatively charged (~31.6 mV [H3001] to ~13.5 mV [M2001]). MDM uptake of the nanoparticles and intracellular retention and release were similar for wet-milled or homogenized formulations (Figure 2B). Antiretroviral efficacies of the nanoformulations in MDM infected with HIV-1ADA were drug-dependent [11].

Based on in vitro screening, the nanoART formulations M2001, H2001, M3001, H3001, and H4001 were selected for animal studies [6, 10, 18]. The “M” prefix designation in the formulation nomenclature refers to those produced by wet-milling, while the “H” refers to those prepared by homogenization (Figure 2B). Milled formulations performed within the top quartiles in uptake, retention, release, and antiretroviral efficacy tests in MDMs [10]; however, the formulations showed more limitations in size and PDI and greater toxicities as determined by decreased alamarBlue reduction and increased MDM TNF-α production (Figure 2B) [6, 11]. Formulations were administered to BALB/cJ mice in a pilot survey. A partial PK evaluation demonstrated that formulations containing the P188 surfactant produced sustained serum and tissue drug concentrations (Figure 2B; Supplementary Figure 1) through 7 days after injection. Taken together, these data serve as selection criteria for the P188-containing nanoformulations (M2001, H2001, M3001, H3001, and H4001) for use in the current animal investigations.

**Nanotoxicology Profiling**

Serum chemistry metabolic profiling and tissue histopathological analyses were conducted in control NSG mice injected subcutaneously with M3001 and M2001 (combination ATV and RTV) at ≤250 mg/kg for each drug. Treatment was administered in 2 doses, on days 0 and 7. Normal serum chemistry profiles (Supplementary Table 2) were seen. Of interest, histopathology of both untreated and nanoART-treated mice showed minor extramedullary hematopoiesis [26] and uneven liver glycogenation. Supplemental studies, performed in normal BALB/cJ mice, showed a 2-fold increase and decrease in platelet counts and blood lymphocyte counts, respectively, at 14 days following treatment with 100 mg/kg nanoART (data not shown). The decline in lymphocyte counts was also observed following treatment with native drugs.

We next assessed whether PBL reconstitution and/or HIV-1 infection might exacerbate any nanoART toxicity. Thus, HIV-1–infected PBL-NSG mice were administered PBS (no drug); nanoART (ATV and RTV, 250 mg/kg, or ATV, RTV, and EFV, 100 mg/kg, subcutaneously) (Figure 1C). In PBS-treated, HIV-1–infected PBL-NSG mice, serum transaminase levels were approximately 2-fold (149.4 U/L) higher than normal (Supplementary Table 2). Histopathological assessment of liver sections confirmed substantive numbers of lymphocytes in the pericentral regions of PBS- and nanoART-treated mice (Figure 3B). A few necrotic hepatocytes were also observed, consistent with GVHD [27, 28].

**Pharmacodynamic Analyses**

To determine the nanoART dose(s) required to achieve therapeutic serum drug concentrations, pharmacodynamic analyses were performed in normal NSG mice following subcutaneous administration of 80, 150, or 250 mg/kg ATV and RTV. Median drug concentrations declined 2 orders of magnitude (log_{10}) from day 1 to day 6 (Figure 2C) and at day 6 were 94.8 ng/mL and 132 ng/mL, respectively, in the 250mg/kg-dose group. This drop was due to drug metabolism. Notably, ATV concentrations at day 14 were higher than day 6 levels. In the high-dose group, ATV concentrations at day 14 were 417 ng/mL above the median plasma concentrations seen in ATV-treated patients [29]. In contrast, there were no differences in serum concentrations of RTV at day 14 compared with day 6.

Corresponding tissue drug levels in nanoART-treated animals were determined at day 14. Livers of animals treated with 250 mg/kg nanoART contained 1656 ng/g ATV and 758 ng/g RTV (Figure 2D). ATV levels were 120–183 ng/g tissue in spleen, kidney, and lung. RTV levels were 276 and 264 ng/g tissue in spleen and kidney, respectively, and 141 ng/g in lung. Drug levels in brain were at the limit of detection. Of interest, ATV and RTV levels in liver, spleen, kidney, and lung differed by dose. On the basis of these results, a dose of 250 mg/kg ATV...
and RTV (H3001 and H2001) was chosen for assessment of antiviral efficacy. Here, NSG mice were reconstituted with human PBLs 7 days prior to a single dose of nanoART, and 24 hours later were infected with HIV-1<sub>ADA</sub>. In the second experimental paradigm, nanoART was administered subcutaneously, 12 hours after HIV-1<sub>ADA</sub> infection (day 0) and again on day 7. Biodistribution studies were used to confirm that nanoART treatment achieves therapeutic serum concentrations of ATV in PBL-reconstituted, HIV-1–infected mice (Figure 4A). Serum ATV concentrations decreased from day 1 to day 6, but increased to 256 ng/mL and 88 ng/mL on day 14 in animals treated with ATV and RTV or ATV, RTV, and EFV, respectively. Of note, median ATV concentrations on day 14 in the ATV and RTV group were above the effective ATV serum concentration for humans of 150 ng/mL [30]. Serum EFV concentration was 4–6-fold lower than that of RTV or ATV (Figure 4A) and was significantly lower than the minimal plasma therapeutic concentration of 1000 ng/mL [31].

Next, we determined tissue drug levels in HIV-1–infected PBL-NSG mice on day 14 after nanoART treatment on days 0 and 7 (Figure 4B). In contrast to nanoART treatment in normal NSG mice (Figure 2D), median spleen ATV levels were 3-fold higher than in liver (Figure 4B). Spleen ATV levels were commonly variable and in several mice were 16,000–17,000 ng/g [27, 28]. Median liver ATV levels were 761 ng/g and 287 ng/g in animals treated with ATV and RTV and ATV, RTV, and EFV, respectively. In mice treated with ATV and RTV or ATV, RTV, and EFV, ATV levels were 3–4-fold higher in liver (900 ng/g and 268 ng/g, respectively) than in spleen (211 ng/g and 90/g, respectively). Levels of drug (ATV and RTV with EFV) in the skin at the site of injection (s) were 1.1–1.6 mg drug per gram tissue serving as a potential depot for drug.

**Antiviral Activities of nanoART Administered Before HIV-1 Infection**

Antiviral activities of nanoART together with PK and biodistribution studies were performed. PBL-reconstituted NSG mice were treated with nanoART or native drugs 1 day prior to HIV-1 infection, and viral activity was determined 9 days after drug administration. HIV-1 infection results in loss of human CD4<sup>+</sup> T cells in PBL-reconstituted immunodecient mice [21, 32–35]. Thus, as an indicator of viral infection, levels of human CD4<sup>+</sup> and CD8<sup>+</sup> T cells as percentages of total human CD3<sup>+</sup> T cells and CD4<sup>+</sup>/CD8<sup>+</sup> T-cell ratios were determined from FACS analyses of spleen cells taken at study termination (9 days after drug treatment). Levels of human CD4<sup>+</sup> T cells were similar in all treatment groups (1.7% ± 0.4%, 2.2% ± 0.4%, and 1.4% ± 0.3% of total cells for PBS-, native drug-, and nanoART-treated mice, respectively). Low spleen CD4<sup>+</sup> T cells and CD4<sup>+</sup>/CD8<sup>+</sup> T-cell ratios were observed in PBS-treated HIV-1–infected animals (Figure 5A). To determine the level of HIV-1 infection, HIV-1p24 expression among HLA-DR<sup>+</sup> cells in spleen was determined. NanoART treatment significantly reduced HIV-1p24<sup>+</sup> cells in the spleen and reduced spleen HIV-1 gag gene expression (Figure 5B). In contrast, treatment with native drug did not decrease the number or expression levels of HIV-1p24<sup>+</sup> cells in the spleen. At day 9, drug levels in serum and peripheral tissues were higher in nanoART-treated mice than in those given native drug (Figure 5C).

**Suppression of Acute HIV-1 Infection by nanoART**

To determine whether nanoART could suppress preexisting HIV infection, HIV-1–infected PBL-NSG mice were treated with nanoART 12 hours and 7 days after infection, then sacrificed on day 14. Levels of human CD4<sub>5</sub><sup>+</sup> T cells (as percentage of total cells in peripheral blood and spleen [blood, 59.2% ± 6.5% to 73.9% ± 2.9%; spleen, 22.3% ± 7.2% to 29.8% ± 4.9%]) did not differ significantly among treatment groups. In HIV-1–infected animals not treated with nanoART, percentages of CD4<sup>+</sup> T cells and CD4<sup>+</sup>/CD8<sup>+</sup> T-cell ratios were significantly decreased in both peripheral blood (Figure 6A) and spleen (Figure 6B) compared with uninfected animals. Importantly, in nanoART-treated (ATV/RTV or ATV/RTV/EFV) HIV-1–infected animals, there was no difference in levels of CD4<sup>+</sup> T cells and CD4<sup>+</sup>/CD8<sup>+</sup> T-cell ratios from those observed in uninfected mice. The level of HIV infection was determined by immunohistochemical quantitation of HIV-1p24–expressing cells among HLA-DR<sup>+</sup> cells in spleen. Both ATV/RTV and ATV/RTV/EFV treatments significantly reduced the number of HIV-1p24<sup>+</sup> stained cells in the spleen, although a few were detected in some sections from the ATV and RTV treatment group (Figure 6C and 6F). Suppression of HIV-1 replication was also determined in spleen by PCR measurements for HIV-1 gag gene expression. Treatment with ATV/RTV and ATV/RTV/EFV reduced viral gene expression by 2 and 3 orders of magnitude (log<sub>10</sub>), respectively (Figure 6D), although the difference between the 2 treatments was not statistically significant. Taken together, these results demonstrate that weekly dosing with nanoART can reduce HIV-1 viral infection to nearly undetectable levels.

**DISCUSSION**

We manufactured injectable long-acting antiretroviral combinations and tested them in a disease-relevant animal system. Our data demonstrate that nanoformulations of commonly used protease inhibitors that show efficacy in cell-based laboratory tests [7, 10] can be used successfully in rodent models of HIV-1 disease to halt loss of CD4<sup>+</sup> T cells and attenuate viral replication. The results are in line with drug concentrations detected in sera and tissue. Toxicology profiles reveal that the drugs are well-tolerated.
The need for alternative antiretroviral formulations is clear based on complexities of many existing regimens, difficulties with adherence, lack of targeting viral reservoirs, emergence of drug resistance, and toxicities [2, 13]. Moreover, in patients who abuse drugs and show poor adherence to ART regimens, accelerated virologic resistance and transmission can be seen [36]. Adding to such concerns is the availability of ART in resource-limited settings [37–39]. Thus, the need for alternative therapies is timely [30, 40].

Cell-mediated drug delivery is a novel concept that employs intracellular recycling and late endosomes as reservoirs for drug [8]. Monocyte-macrophages offer a particularly attractive cell delivery system. Although the role played by macrophages in the current study is not certain, the established reservoirs and drug stability suggest that these cells serve as nanoparticle depots. Moreover, the observation that drugs delivered by nanoformulations can be stored in recycling endosomal compartments in macrophages may explain the observed long-acting nature of the drugs [9]. Although the promise of cell-mediated delivery of drugs is real, its perils demand equal consideration. This includes immune reactions elicited against the particle, untoward reactions against the excipients, and effects on the cell and tissues, as well as effects on its function consequent to harboring the crystal for periods of weeks [41, 42].

Also limitations are acknowledged in the experimental approach. For example, NSG mice as recipients of human PBLs commonly develop GVHD [27]. Thus, it is difficult to draw a firm conclusion on any nanoART hepatic toxicities in these animals. Nonetheless, such systems, as developed in this report, represent a new strategy for treating HIV-1 disease. The further design of nanoparticle carriers for such drug delivery may include decorating the particle with specific cell ligands that target virus-infected cell surface antigens. Moreover, the particle compositions used in these studies make nanoART translation to humans viable. Taken together, targeted drug delivery and ease of access to ART as nanoformulated medicines are real and convincing and demand further investigation for human use.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://www.oxfordjournals.org/our_journals/jid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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