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Long-Acting Profile of 4 Drugs in 1 Anti-HIV Nanosuspension in Nonhuman Primates for 5 Weeks After a Single Subcutaneous Injection

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Abstract

Daily oral antiretroviral therapy regimens produce limited drug exposure in tissues where residual HIV persists and suffer from poor patient adherence and disparate drug kinetics, which all negatively impact outcomes. To address this, we developed a tissue- and cell-targeted long-acting 4-in-1 nanosuspension composed of lopinavir (LPV), ritonavir, tenofovir (TFV), and lamivudine (3TC). In 4 macaques dosed subcutaneously, drug levels over 5 weeks in plasma, lymph node mononuclear cells (LNMCs), and peripheral blood mononuclear cells (PBMCs) were analyzed by liquid chromatography–tandem mass spectrometry. Plasma and PBMC levels of the active drugs (LPV, TFV, and 3TC) were sustained for 5 weeks; PBMC exposures to LPV, ritonavir, and 3TC were 12-, 16-, 42-fold higher than those in plasma. Apparent $T_{1/2z}$ of LPV, TFV, and 3TC were 219.1, 63.1, and 136.3 h in plasma; 1045.7, 105.9, and 127.7 h in PBMCs. At day 8, LPV, TFV, and 3TC levels in LNMCs were 4.1-, 5.0-, and 1.9-fold higher than in those in PBMCs and much higher than in plasma. Therefore, 1 dose of a 4-drug nanosuspension exhibited persistent drug levels in LNMCs, PBMCs, and plasma for 5 weeks. With interspecies scaling and dose adjustment, this 4-in-1 HIV drug-combination could be a long-acting treatment with the potential to target residual virus in tissues and improve patient adherence.

Keywords

long-acting; HIV treatment; nanoparticle; tenofovir; lamivudine; targeted delivery

Introduction

Standard HIV treatment relies on orally administered antiretroviral drug combinations, referred to as combination antiretroviral therapy (cART), to suppress plasma viremia.

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Although suppression of plasma viremia is achievable, viral rebound generally occurs upon cART cessation. In 2003, we hypothesized a relationship between limited lymphatic drug exposure and viral rebound, and documented HIV drug levels in the lymph nodes are lower than those in plasma during oral cART.¹ Lymphatic drug insufficiency was confirmed independently by others in a prospective study and was associated with viral persistence.² Although there is ongoing controversy regarding the degree of viral replication during suppressive cART, it appears that residual virus remains in lymphoid tissues.³ Targeting ART to immune sanctuaries and increasing the degree of drug penetration and exposure may be important for viral clearance.

Oral cART has a number of challenges including the daily dosing requirement, which may lead to pill fatigue. Moreover, poor adherence to a therapeutic regimen may lead to ongoing plasma viremia; this may be more common in certain populations, including children, adolescents, and those with untreated depression or active illicit drug use. To overcome these limitations of oral cART, our research focuses on developing long-acting (LA), targeted combination antiretroviral drug formulations.^{1,4-8} Having a combination of drugs, coformulated in a single injection that exhibits LA cellular and plasma pharmacokinetics, would likely improve adherence while maximizing the potential to reduce virus in lymphoid tissues.^{9,10}

Although LA *single* agent formulations including cabotegravir and rilpivirine have been successfully formulated, manufactured, and advanced into clinical development, it is more challenging to coformulate a *combination* of agents that yield synchronous pharmacokinetics. It is even more challenging to coformulate drugs with disparate hydrophobicity and hydrophilicity characteristics. Despite these challenges, we were able to develop and scale-up a novel formulation containing hydrophobic HIV protease inhibitors (lopinavir [LPV] and ritonavir [RTV]) and hydrophilic HIV reverse transcriptase inhibitors (tenofovir [TFV] and lamivudine [3TC]). We have characterized intracellular and plasma pharmacokinetics in nonhuman primates (NHP) for all 4 agents in a drug combination nanoparticle (DcNP) for 5 weeks after a single dose.

Methods

Reagents

TFV, LPV, RTV, and 3TC (Waterstone, Carmel, IN). Good manufacturing practice grade 1,2-distearoyl-*sn*-glycero-3-phosphocholine and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000 (Corden Pharma, Liestal, Switzerland). All other chemicals were of analytical or higher grade.

4-1 Drug Nanosuspension

DcNPs were prepared as previously reported.^{1,6,8} The 4-in-1 DcNP formulation contains LPV (11.83 mg/mL), RTV (1.69 mg/mL), TFV (5.63 mg/mL), and 3TC (5.06 mg/mL). The percent of particle-associated drug were 90.1 (LPV), 85.7 (RTV), 16.9 (TFV), and 13.2 (3TC); 90% of drug particles were less than 500 nm in diameter, and the osmolality of the formulation was 244 mOsm/kg.

All studies were approved by the University of Washington Institutional Animal Care and Use Committee. Four male *Macaca nemestrina* each received a single subcutaneous injection of the DcNP in the upper midscapular region (25.0, 7.0, 10.6, and 10.6 mg/kg of LPV, RTV, TFV, and 3TC, respectively). Blood was obtained at 0, 0.25, 0.5, 1, 3, 5, 8, 24, 48, 120, 168, 192, 336, 504, 672, and 840 h, and an axillary lymph node was collected at 24 and 192 h. Sample processing, drug level analyses, and pharmacokinetic modeling were conducted as previously described using a validated combination liquid chromatography–tandem mass spectrometry assay.^{6,8,11}

Results

Administration of a single subcutaneous dose of the 4-drug DcNP was well tolerated in all animals, and no injection site re-actions were noted. To determine the plasma and intracellular pharmacokinetics, we evaluated the time-course of all the 4 drugs (LPV, RTV, TFV, and 3TC) for 5 weeks. As shown in Figure 1a, all the 4 drugs exhibited notable concentrations in both plasma and peripheral blood mononuclear cells (PBMCs) over the 5-week (840 h) study. Although both TFV and 3TC doses were equivalent (10.6 mg/kg), plasma TFV levels are higher than 3TC levels over the 5-week study. As RTV is used as a booster for LPV, we administered LPV (25.0 mg/kg) at a much higher dose than RTV (7.0 mg/kg). Thus, LPV plasma levels persisted longer and at a higher concentration than RTV. Compared with plasma drug levels, the PBMC LPV, RTV, and 3TC levels were much higher throughout (Fig. 1a). Although PBMC levels of TFV were slightly lower than those in plasma early in the time course, this is reversed by week 2, and TFV intracellular levels were higher for the remaining 3 weeks (Fig. 1a), similar to the other drugs.

These time-course data were analyzed for total exposures (area under the concentration time curve from 0–840 h [AUC_{0-840h}]) and terminal half-lives (Fig. 1b). Interestingly, with equivalent doses of TFV and 3TC, the plasma AUC of 3TC is much lower than TFV (22.6 vs. 862.5 $\mu\text{g h/mL}$). However, the 3TC intracellular AUC is approximately double the TFV value (959.5 and 493.0 $\mu\text{g h/mL}$). The cell-to-plasma ratios of LPV, RTV, and 3TC exposure as measured by $AUC_{\text{PBMC}}/AUC_{\text{plasma}}$ were 12.2, 16.4, and 42.4, respectively, (Fig. 1). Because of higher plasma concentrations of TFV in early time points, the $AUC_{\text{PBMC}}/AUC_{\text{plasma}}$ was lower (0.6). By 336 h and for the remaining 3 weeks, TFV PBMC concentrations exceed plasma levels. The sustained levels of all the drugs are further reflected in the apparent terminal half-life values (Fig. 1b) that range from 63.1 h (TFV in plasma) to 1045.7 h (LPV in PBMCs).

We also evaluated the drug levels in lymph nodes at 24 h and day 8 (192 h). LPV concentrations in the lymph node mononuclear cells (LNMCs) were similar to those observed in plasma and PBMCs at 24 h but exhibited a much higher degree of LNMC accumulation 8 days following drug administration (LNMC/PBMC and LNMC/plasma ratios at 192 h of 4.1 and 102.8, respectively; Fig. 1a and Table 1). TFV exhibited somewhat delayed LNMC accumulation characteristics relative to other drugs in which the LNMC/PBMC and LNMC/plasma ratios were less than 1.0 at 24 h (0.4 and 0.2, respectively) and higher at 192 h (5.0 and 2.9). Of note, 3TC exhibited striking intracellular accumulation relative to plasma, with LNMC/plasma ratios of 23.8 and 352.2 (24 and 192 h, respectively).

Discussion

A stable and scalable 4-in-1 drug formulation based on a novel nanosuspension platform, 4–6,8,12 with 4 well-established antiretroviral drugs, and sustained plasma and intracellular drug levels for up to 5 weeks. Despite 4 drugs having disparate physiochemical properties, we observed LPV, RTV, TFV, and 3TC levels over this extended period. This is a distinct improvement over drugs delivered in the absence of lipid excipients where they are eliminated within 48 h (hydrophobic agents) and 8 h (hydrophilic agents).^{5,6}

It should be noted that we chose the dose levels in the present study, with species differences in metabolism in mind, to achieve meaningful drug levels in NHPs. Translation of these doses for human applications will require interspecies dose scaling and an understanding of how our formulation behaves in a number of species to allow reasonable human dose selection. We wish to emphasize, nevertheless, that the dosing interval of our LA formulation would be less frequent than currently available *daily* oral regimens. Although the mg/kg doses may seem high in the present study, they are a fraction of what would be administered for any of our agents over 7 days of daily oral administration, for example. More specifically, Kaletra[®] is typically prescribed at 800 mg LPV daily (or approximately 13 mg/kg for a 60 kg person). In contrast, our formulation was administered at 25 mg/kg LPV, which is equivalent to approximately 3.6 mg/kg/d LPV over a 7-day period. This is a similar situation for both TFV and 3TC (300 mg of TFV or 3TC in a 60 kg human is ~5 mg/kg; over 7 days this is 35 mg/kg and our studies used 10.6 mg/kg).

The higher intracellular (PBMC and LNMC) drug levels we observed relative to plasma for all 4 drugs, particularly the high LNMC/plasma ratios, suggest that DcNPs deliver these anti-retrovirals into cells with high efficiency. These findings indicate that this multi-drug lipid-stabilized DcNP formulation administered subcutaneously may overcome the lymphatic insufficiency reported for oral cART.¹ Although it is true that LPV/RTV is no longer a first-line choice for HIV treatment in the United States, it is still used globally, especially in resource-limited settings, and we chose this protease inhibitor based on its continued global relevance. TFV and 3TC remain important drugs and are widely prescribed, both in the US and globally. With these 3 active antiretroviral agents and 1 pharmacokinetic booster, we target multiple HIV proteins and reduce the risk of developing viral drug resistance.

The observed persistence of LPV, RTV, TFV, and 3TC over 5 weeks in our study may be shorter than the sustained plasma duration achieved with LA cabotegravir or LA rilpivirine in NHP and humans.¹³ This could be an advantage for our DcNP formulation because the long duration of subtherapeutic levels of LA cabotegravir has raised concerns about the risk for the development of drug resistance if treatment is stopped abruptly without initiation of a suppressive oral cART regimen. Moreover, with our 4-in-1 DcNP, the initial peaks of the active agents are reached within 8 h (Fig. 1). This is in contrast to the delay in achieving therapeutic plasma levels with more traditional LA formulations that deposit drugs at the injection site. Because the time-to-peak or time to reach therapeutic levels could be less predictable, in conjunction with concerns regarding potential intolerance to cabotegravir and/or rilpivirine, these formulations are currently administered with an oral lead-in phase.

The early time to peak of the 4 drugs in our formulation coupled with their long history of use may obviate the need for an oral lead-in. Furthermore, having a combination of antiretroviral agents in a single DcNP delivered to the cells of lymph nodes may increase antiviral potency and diminish the potential for developing drug resistance.

We have previously reported half maximal effective concentration (EC_{50}) values for LPV and TFV (formulated in a 3 drug DcNP called TLC-ART101) to be 3.0 ± 0.8 and 1.5 ± 0.1 nM, respectively.⁶ Five weeks following a single injection of the DcNP in the present study, the LPV and TFV concentrations in PBMCs were approximately 14.4 and 154 ng/mL (or 22.9 nM and 537 nM), respectively; both well above the EC_{50} values. Although the EC_{50} for 3TC in the current formulation remains to be directly determined, it is likely to be similar or lower than the reported value of approximately 1–120 nM.¹⁴ The intracellular 3TC concentration of 88.5 ng/mL or 380 nM observed at 5 weeks is well above the 120 nM 3TC EC_{50} value.

The nucleoside inhibitors TFV and 3TC are phosphorylated intracellularly to TFV diphosphate (TFV-DP) and 3TC triphosphate (3TC-TP), and these metabolites inhibit HIV replication. Following administration of several different DcNP formulations, intracellular TFV-DP levels and 3TC-TP in NHPs were found to be 60% of total TFV and 40% of total 3TC, similar to literature reported values.^{15–19} Our observed PBMC levels of TFV-DP are greater than those reported for the 2 oral TFV prodrugs, tenofovir alafenamide, and tenofovir disoproxil fumarate, at equivalent doses indicating the utilization of these prodrugs present no advantages in our platform.⁸ Although previously reported data indicate ~90% of intracellular 3TC exists as 3TC-TP at steady-state after oral 3TC administration, there was also high intrasubject variability in these measures,^{15,16,18,19} and our observed 40% is within range. Taken together, our data indicate sufficiently high intracellular metabolite levels of both TFV and 3TC.

These higher intracellular drug levels, relative to plasma, suggest roles of the formulation properties and route of administration in enhancing tissue and cell exposure. Specifically, higher LNMC to PBMC drug levels occurring weeks after injection indicate that DcNPs are likely to first distribute from the subcutaneous injection site into lymphatic system.^{20,21} We postulate that these DcNPs are trapped in the sinuses of lymph nodes, taken up by the resident lymphocytes, and excess is distributed widely into the entire lymphatic system. This hypothesis is consistent with the similar LNMC drug levels we have observed in axillary and inguinal nodes.¹ These and other mechanisms are currently under investigation. Although it is of interest to determine the efficacy and long-term safety of this and other combination ART LA nanosuspensions, such studies are beyond the scope of this brief report. We also recognize there are inherent limitations of using an NHP model in preclinical pharmacokinetic studies for possible translation to human use. Chief among these are species differences in drug metabolism and lymphatic system architecture. Nonetheless, we feel that this study is a necessary first step in the development of targeted LA therapeutics.

In summary, a single subcutaneous dose of this combination provided persistent LPV, RTV, TFV, and 3TC levels for 5 weeks in LNMCS, PBMCs, and plasma. In addition to the hydrophobic drugs LPV and RTV, our platform enabled 2 hydrophilic drugs, TFV and 3TC,

to be combined into a single DcNP with a similar degree of LA and enhanced intracellular drug levels. This higher intracellular drug exposure, coupled with multi-week persistence, may help improve therapeutic outcomes in those unsuccessful with oral ART and address potential lymphatic drug insufficiency. The DcNP platform should be considered for developing targeted, LA antiretroviral drug combinations with diverse hydrophobic and hydrophilic characteristics.

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Figure 1.

Plasma, PBMC, and LNM drug concentration versus time plots. (a) The open circles (○) represent the PBMC data, the closed squares (■) are plasma data, and the crosses (X) represent lymph node data. The data plotted are the mean $\pm$ SD, $n = 4$ macaques over 840 h (5 wks) following a single subcutaneous administration of the 4-in-1 DcNP. Time scales are interrupted to show more detail from the first 8 h. (b) PBMC and LNM cell volumes were assumed to be 0.2829 pL/cell.²² The dotted lines represent the EC_{50} concentration for each respective single agent, and the shaded section in the 3TC graph is the reported range of EC_{50} values.¹⁴ C_{max} , maximum drug concentration; $T_{1/2}$, apparent terminal half-life.

Table 1

Concentrations of TFV, 3TC, LPV, and RTV in LNMCs and These Concentrations Relative to PBMCs and to Plasma, 24 h and 8 d Following a Single Subcutaneous Administration of the 4-in-1 DcNP in 4 NHPs

Drug Concentration (ng/mL)				
	TFV	3TC	LPV	RTV
LNMC				
C _(24h)	724.3 (617.7)	1748.4 (731.6)	159.0 (69.4)	530.9 (384.1)
C _(192h)	4216.5 (2283.4)	2143.7 (2947.1)	1597.0(2092.3)	1063.7 (1041.3)
Drug Concentration Ratios				
	TFV	3TC	LPV	RTV
[LNMC]/[PBMC]				
24 h	0.4 (0.3)	2.0 (1.8)	0.6 (0.2)	2.3 (1.4)
192 h	5.0 (2.3)	1.9 (1.7)	4.1 (3.1)	23.1 (18.7)
[LNMC]/[Plasma]				
24 h	0.2 (0.2)	23.8 (25.2)	3.2 (2.4)	19.6(18.7)
192 h	2.9 (2.1)	352.1 (380.9)	102.8 (83.8)	572.0 (585.6)

Data presented are the mean (SD) of LNMC concentration of respective drugs expressed in ng/mL. LNMC volume assumed to be 0.2829 pL/cell.

C_(24h) and C_(192h), drug concentrations at 24 and 192 h.