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Long-acting three-drug combination anti-HIV nanoparticles enhance drug exposure in primate plasma and cells within lymph nodes and blood

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Abstract

Insufficient HIV drug levels in lymph nodes have been linked to viral persistence. To overcome lymphatic drug insufficiency, we developed and evaluated in primates a lipid-drug nanoparticle containing lopinavir, ritonavir, and tenofovir. These nanoparticles produced over 50-fold higher intracellular lopinavir, ritonavir and tenofovir concentrations in lymph nodes compared to free drug. Plasma and intracellular drug levels in blood were enhanced and sustained for 7 days after a single subcutaneous dose, exceeding that achievable with current oral therapy.

Combined antiretroviral therapy (cART) can clear HIV from the blood; however, residual virus remains in lymph nodes [1,2]. Oral cART produces lower drug concentrations in lymphoid tissues than in plasma [3–5], which is linked to persistent virus in lymph nodes [3,6,7] and virus rebound upon therapy cessation [8]. Drug nanoparticles have the potential to overcome lymphatic drug insufficiency [9]. We previously developed and demonstrated in primates a lipid nanoparticle (LNP) containing indinavir (IDV) that enhanced drug levels in all analyzed lymph nodes [4,10]. Also, IDV in LNPs enhanced intracellular drug concentrations in peripheral blood mononuclear cells (PBMCs), prolonged plasma residence time, reversed $CD4^+$ T-cell decline, and suppressed viral RNA in both plasma and lymph nodes [10]. Building on findings with IDV-LNPs, we have developed an anti-HIV LNP containing two protease inhibitors, lopinavir (LPV) and ritonavir (RTV), and a reverse transcriptase inhibitor, tenofovir (TFV), for simultaneous, triple drug delivery to HIV host cells in blood and lymph. LPV and RTV were chosen for their stability and strong hydrophobic interactions with LNPs [11]. Ritonavir enhances the efficacy of LPV through metabolic and drug transporter interactions [12,13]. Inclusion of TFV provides a second target of antiviral action to further suppress drug resistance potential, and intracellular retention of phosphorylated TFV prolongs antiviral activity [14].

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Conflicts of interest

There are no conflicts of interest.

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We evaluated the characteristics of optimized anti-HIV LNPs for primate studies. The aseptically prepared anti-HIV LNPs exhibited 94, 91 and 12% LPV, RTV and TFV incorporation, respectively, with a mean diameter of 52 nm. The well defined unbound drug fractions were included for in-vivo studies. Antiviral potency against HIV-1, evaluated at a fixed LPV: RTV: TFV 1: 1: 0.5 mole ratio, revealed at least a three-fold increase in potency with anti-HIV LNP compared to the drugs in soluble form (LPV, RTV, and TFV EC_{50} 30±0.8, 30±0.8, 15±0.1 nmol/l in LNP form, vs. 98±0.3, 98±0.3, 49 ±0.2 nmol/l in free form).

Primates dosed subcutaneously with LPV, RTV, and TFV (25.0, 14.3, and 17.1 mg/kg) in anti-HIV LNP form exhibited elevated plasma concentrations of LPV and RTV over 7 days (168 h). In contrast, plasma drug levels after administration of free drug in combination subsided to near or below detection limited by 24 h. The increase in total drug exposure [area under the curve (AUC)] provided by the LNP formulation for LPV, RTV, and TFV was 18, 14, and 7-fold, respectively (paired *t* test P =<0.05, 0.07, and 0.173) (Table 1). The AUCs for early (0–8 h) versus late drug exposure (8–168 h) were also analyzed. Primates treated with free drug exhibited 92% of total TFV exposure within the first 8 h, whereas those treated with anti-HIV LNPs exhibited only 9.1% in the first 8 h, with the remaining fraction at 8–168 h (Table 1).

Intracellular drug accumulation is pivotal for antiviral effects. Sustained LPV, RTV and TFV in PBMCs were detected for over 7 days in primates treated with anti-HIV LNPs, whereas those on free drug fell near or below the detection limit by 48 h (Table 1). The ratio of anti-HIV LNP to free drug (LNP/free ratio) was used to compare PBMC drug concentrations between the two test groups. This ratio was greater than 1 at all time points beyond 5 h, and greater than 20 at later time points (Table 1). For TFV, with only 12% LNP association, the LNP/free ratio in blood PBMCs was less than 1 at early time points; by 8 h, however, this value increased to over 50. Anti-HIV LNPs also enhanced intracellular drug concentrations in mononuclear cells of lymph nodes (LNMCs). LNMCs isolated from inguinal lymph nodes at 24 h revealed that no LPV and only low levels of RTV were detectable in animals treated with the free drug combination, whereas those treated with anti-HIV LNPs exhibited over 50-fold higher intracellular concentration (Table 1). For TFV, with limited LNP association, the LNP/free ratio was lower and recorded at 0.7; however, the differences between the two groups of macaques were not statistically significant (P =1.0).

While toxicity of anti-HIV LNPs needs further investigation, complete blood count, serum chemistry panel, C-reactive protein, and complement levels revealed no treatment impact. No anti-HIV LNP-treated animals demonstrated an elevation in C-reactive protein, white blood cell count, blood urea nitrogen (BUN), creatinine, or liver enzymes. Total complement levels were highly variable, but variations were not significant. Also, no significant increase in cholesterol levels was noted with anti-HIV LNP administration (162 ± 16.4 vs. 191 ± 14.8 mg/dl). On physical examination of the injection site, naïve animals receiving free drug demonstrated a local reaction consisting of firm, nonerythematous swellings that resolved over the following weeks. Animals treated with anti-HIV LNP demonstrated no local reaction and their platelet counts remained within normal range.

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In summary, taking advantage of the high LNP incorporation efficiency of two lipophilic protease inhibitors, LPV and RTV, and the ability to encapsulate hydrophilic TFV, we constructed a combination anti-HIV LNP and analyzed intracellular drug concentrations and plasma kinetics in macaques (*Macaca nemestrina*). Primates administered subcutaneously with anti-HIV LNPs exhibited elevated and extended intracellular drug levels in mononuclear cells of both blood and lymph nodes, indicating the potential for this approach to overcome lymph node drug insufficiency and associated viral persistence in patients on oral cART therapy [3–5]. The ability of anti-HIV LNP to extend plasma drug levels for over 1 week with higher total drug exposure supports consideration as a long-acting agent to improve patient compliance. Importantly, three drugs packaged together in anti-HIV LNPs could reduce drug resistance potential by consistently and simultaneously delivering all three drugs above therapeutic levels in the same cell. This reduces the likelihood of varying intracellular concentrations for each drug, as achieved when delivered in free form or in separate particles. Therapeutic efficacy of anti-HIV LNPs could be further enhanced by organelle targeting with pH-responsive drug release [16,17] and expressing $CD4^+$-binding peptide [18] to target HIV host cells; for a review, see Gunaseelan *et al*. [19]. In summary, anti-HIV LNPs show promise for overcoming drug insufficiency in lymphoid tissues and improving patient compliance in search of a cure for AIDS.

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References

1. Lafeuillade A, Chollet L, Hittinger G, Profizi N, Costes O, Poggi C. Residual human immunodeficiency virus type 1 RNA in lymphoid tissue of patients with sustained plasma RNA of <200 copies/mL. J Infect Dis. 1998; 177:235–238. [PubMed: 9419197]
2. Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margo-lick JB, et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4R T cells. Nat Med. 2003; 9:727–728. [PubMed: 12754504]
3. Fletcher CV, Staskus K, Wietgrefe SW, Rothenberger M, Reilly C, Chipman JG, et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. Proc Natl Acad Sci U S A. 2014; 111:2307–2312. [PubMed: 24469825]
4. Kinman L, Brodie SJ, Tsai CC, Bui T, Larsen K, Schmidt A, et al. Lipid-drug association enhanced HIV-1 protease inhibitor indinavir localization in lymphoid tissues and viral load reduction: a proof of concept study in HIV-2287-infected macaques. J Acquir Immune Defic Syndr. 2003; 34:387–397. [PubMed: 14615656]
5. Solas C, Lafeuillade A, Halfon P, Chadapaud S, Hittinger G, Lacarelle B. Discrepancies between protease inhibitor concentrations and viral load in reservoirs and sanctuary sites in human immunodeficiency virus-infected patients. Antimicrob Agents Chemother. 2003; 47:238–243. [PubMed: 12499197]
6. Cavert W, Notermans DW, Staskus K, Wietgrefe SW, Zupancic M, Gebhard K, et al. Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection. Science. 1997; 276:960–964. [PubMed: 9139661]

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7. Pantaleo G, Graziosi C, Demarest JF, Butini L, Montroni M, Fox CH, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature. 1993; 362:355–358. [PubMed: 8455722]

8. Wong JK, Gunthard HF, Havlir DV, Zhang ZQ, Haase AT, Ignacio CC, et al. Reduction of HIV-1 in blood and lymph nodes following potent antiretroviral therapy and the virologic correlates of treatment failure. Proc Natl Acad Sci U S A. 1997; 94:12574–12579. [PubMed: 9356491]

9. Freeling JP, Ho RJ. Anti-HIV drug particles may overcome lymphatic drug insufficiency and associated HIV persistence. Proc Natl Acad Sci U S A. 2014; 111:E2512–E2513. [PubMed: 24889644]

10. Kinman L, Bui T, Larsen K, Tsai CC, Anderson D, Morton WR, et al. Optimization of lipid-indinavir complexes for localization in lymphoid tissues of HIV-infected macaques. J Acquir Immune Defic Syndr. 2006; 42:155–161. [PubMed: 16760797]

11. Koehn J, Ho RJ. Novel liquid chromatography-tandem mass spectrometry method for simultaneous detection of anti-HIV drugs lopinavir, ritonavir, and tenofovir in plasma. Antimicrob Agents Chemother. 2014; 58:2675–2680. [PubMed: 24566184]

12. Hsu A, Granneman GR, Ritonavir Bertz RJ. Clinical pharmacokinetics and interactions with other anti-HIV agents. Clin Pharmacokinet. 1998; 35:275–291. [PubMed: 9812178]

13. Storch CH, Theile D, Lindenmaier H, Haefeli WE, Weiss J. Comparison of the inhibitory activity of anti-HIV drugs on P-glycoprotein. Biochem Pharmacol. 2007; 73:1573–1581. [PubMed: 17328866]

14. Piliero PJ. Pharmacokinetic properties of nucleoside/nucleotide reverse transcriptase inhibitors. J Acquir Immune Defic Syndr. 2004; 37 (Suppl 1):S2–S12. [PubMed: 15319664]

15. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of monuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand J Clin Lab Invest Suppl. 1968; 97:77–89. [PubMed: 4179068]

16. Choi SU, Bui T, Ho RJ. pH-dependent interactions of indinavir and lipids in nanoparticles and their ability to entrap a solute. J Pharm Sci. 2008; 97:931–943. [PubMed: 17546665]

17. Straubinger RM, Duzgunes N, Papahadjopoulos D. pH-sensitive liposomes mediate cytoplasmic delivery of encapsulated macromolecules. FEBS Lett. 1985; 179:148–154. [PubMed: 2578112]

18. Endsley AN, Ho RJ. Enhanced anti-HIV efficacy of indinavir after inclusion in CD4-targeted lipid nanoparticles. J Acquir Immune Defic Syndr. 2012; 61:417–424. [PubMed: 22743598]

19. Gunaseelan S, Gunaseelan K, Deshmukh M, Zhang X, Sinko PJ. Surface modifications of nanocarriers for effective intracellular delivery of anti-HIV drugs. Adv Drug Deliv Rev. 2010; 62:518–531.10.1097/QAD.0000000000000421 [PubMed: 19941919]

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Table 1

The effect of anti-HIV lipid nanoparticle[a] on intracellular levels of lopinavir, ritonavir, and tenofovir in peripheral blood mononuclear cells and inguinal lymph nodes, as well as overall drug exposure in plasma[b].

	Drug concentration								
	Lopinavir			Ritonavir			Tenofovir (TFV)		
Time (h)	**Free drug**	**LNP**	**LNP/free ratio[c]**	**Free drug**	**LNP**	**LNP/free ratio[c]**	**Free drug**	**LNP**	**LNP/free ratio[c]**
Intracellular drug concentration of mononuclear cells in blood (ng/10^6 mononuclear cells)[d]									
0	0.00 ± 0.00	0.00 ± 0.00	**1.0**	0.00 ± 0.00	0.00 ± 0.00	**1.0**	0.00 ± 0.00	0.00 ± 0.00	**1.0**
0.5	0.67 ± 0.31	0.28 ± 0.24	**0.4**	0.96 ± 1.67	0.41 ± 0.58	**0.4**	5.01 ± 5.12	1.29 ± 1.83	**0.3**
1	1.93 ± 1.18	1.85 ± 1.11	**1.0**	2.92 ± 2.62	2.57 ± 1.04	**0.9**	3.55 ± 4.21	1.29 ± 0.93	**0.4**
3	0.91 ± 0.53	2.39 ± 1.15	**2.6**	2.85 ± 1.50	3.05 ± 1.06	**1.1**	2.57 ± 2.62	1.19 ± 0.92	**0.5**
5	0.32 ± 0.28	2.59 ± 1.44	**8.1**	0.90 ± 0.60	2.20 ± 1.43	**2.4**	0.27 ± 0.21	1.24 ± 1.16	**4.6**
8	0.70 ± 0.86	4.23 ± 2.34	**6.0**	0.74 ± 0.99	4.57 ± 3.04	**6.2**	0.01 ± 0.01	0.59 ± 0.27	**59.0**
24	0.16 ± 0.28	3.26 ± 2.25	**20.4**	0.00 ± 0.00	4.09 ± 2.90	**>409.0**	0.01 ± 0.01	0.70 ± 0.14	**70.0**
48[c]	0.01 ± 0.01	0.03 ± 0.00	**3.0**	0.00 ± 0.00	0.00 ± 0.00	**1.0**	0.00 ± 0.00	2.27 ± 0	**>227.0**
120[c]	NA	0.02 ± 0.00	**NA**	NA	1.37 ± 0.00	**NA**	NA	0.64 ± 0	**NA**
168	0.00 ± 0.00	0.08 ± 0.10	**>8.0**	0.00 ± 0.00	1.32 ± 1.14	**>132.0**	0.00 ± 0.00	0.25 ± 0.25	**>25.0**
Intracellular drug concentrations in mononuclear cells of inguinal nodes (ng/10^6 mononuclear cells)[e]									
24	0.00 ± 0.00	4.85 ± 0.04	**>485.0**	0.13 ± 0.13	6.57 ± 0.08	**50.5**	1.02 ± 0.13	0.76 ± 0.09	**0.7**
Plasma drug exposure analysis (AUC in μgh/ml)[f]									
0–168	3.83 ± 4.04	69.6 ± 10.7	**18.2**	1.39 ± 1.18	19.4 ± 12.2	**14.0**	56.6 ± 17.04	395.0 ± 344.5	**7.0**
0–8 (%)	19.6	9.9		30.2	11.3		91.8	9.1	
8–168 (%)	80.4	90.1		69.8	88.7		8.2	90.9	

LNP, lipid nanoparticle. NA, not available.

[a] Anti-HIV LNPs composed of 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), *N*-(carbonyl-methoxypolyethyleneglycol-2000)-1,2-distear-oyl-*sn*-glycero-3-phosphoethanolamine, sodium salt (MPEG-2000-DSPE), lopinavir, ritonavir, and tenofovir (TFV) were prepared by thin film hydration method, as previously published [4]. All drugs were dried with the lipid film, rehydrated in bicarbonate-buffered saline, and size-reduced by high-pressure homogenization under aseptic conditions to produce drug-lipid nanoparticles with a mean 52 nm diameter. Free drug suspension dosages were prepared in bicarbonate-buffered saline using biocompatible solvent and surfactant to suspend the highly hydrophobic drugs.

[b] Four primates (*Macaca nemestrina*) were administered anti-HIV LNPs and free drug in a cross-over study at a normalized dose of 25 mg/kg LPV, 14.3 mg/kg RTV, and 17.1 mg/kg tenofovir (TFV) subcutaneously. All experiments were done under an approved Institutional Animal Care and Use Committee (IACUC) protocol. Blood and lymph nodes were collected over 7 days at indicated time points

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and drug concentrations were determined with a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) published assay [11]. Data are expressed as mean ± SD. Ratios for comparative analysis are shown in bold.

[c] LNP/free ratio is the mean anti-HIV LNP drug concentration divided by mean free drug concentration. In cases where the drug level was below detectable limits, the number was taken as '0.01' to calculate ratio.

[d] PBMCs were isolated from whole blood by density gradient method [15], and cell pellets of 2 million PBMCs each were analyzed. (*n* = 4/group; at 48 h and 120 h, *n* = 2/group).

[e] An inguinal lymph node was collected at 24 h (*n* = 2/group) and mononuclear cells were isolated by pressing tissue through a 200 μm cell strainer. Pellets of 2 million cells each were analyzed.

[f] Area under the curve (AUC) was calculated from plasma drug concentrations using the trapezoidal rule. The fractional percentage of total AUC in the early phase (0–8 h) and late phase (8–168 h) was calculated from the mean of the total AUC and the mean AUC in the indicated time range. Values are expressed as a percentage of the total AUC (*n* = 4/group).

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