LONG-ACTING PHARMACOKINETICS OF 4 HIV DRUGS COFORMULATED IN ONE NANO-FORMULATION

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UW Targeted Long-Acting Combination Anti-Retroviral Therapy Program (TLC-ART)

Introduction

Daily oral conventional ART, even in fixed dose combination forms, has inherent limitations such as patient adherence and differing pharmacokinetics; both potentially impacting therapeutic outcomes. Oral dosing has also been shown to produce lower and limited tissue exposure where residual virus persists. To address these limitations, we have developed a tissue and cell targeted long-acting anti-HIV nanosuspension containing 4 drugs.

Purpose

The goal of this study is to evaluate the feasibility of formulating 4 drugs, together, in a drug-combinaiton nanoparticle (DcNP) suspension intended for a single subcutaneous injection. A further goal is to characterize the intracellular and plasma pharmacokinetics of this formulation in non-human primates. We therefore developed an anti-HIV DcNP formulation containing a protease inhibitor with a booster[Lopinavir (LPV) and Ritonavir (RTV) and two nucleotide analogue reverse transcriptase inhibitors [Tenofovir (TFV) and Lamivudine (3TC)] for characterization and evaluation.

Methods

- Anti-HIV DcNPs (LPV, RTV, 3TC, and TFV; DSPC, mPEG$_{2K}$-DSPE) were prepared by spray drying (ProCept 4M8-TriX) followed by size reduction utilizing high pressure homogenization (Avestin Emulsiflex C5).
- Drug association efficiency was determined by equilibrium dialysis and drug concentrations by LC-MS/MS
- DcNP diameter was determined by dynamic light scattering.
- A sterile anti-HIV DcNP nanosuspension containing LPV: RTV:TFV:3TC [25 : 6.8 : 11.5 : 10.1 mg/kg] was injected subcutaneously in 4 male *Macaca nemestrina*.
- Blood samples were collected at defined time points up to 5 weeks following drug administration. Axillary lymph node biopsies were performed 24 hours and 8 days after drug administration.
- Time-course plasma and cellular drug concentrations were determined with a validated LC-MS/MS spectrometry[1]

Figure 1. Schematic of the Four Drug Containing anti-HIV DcNP

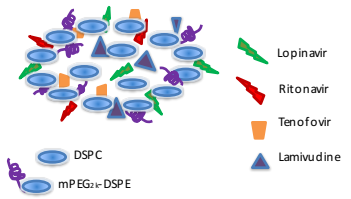
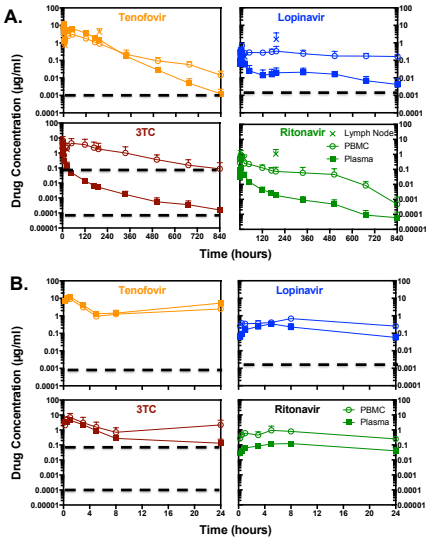


Figure 2. Long-Acting Plasma and Intracellular Profiles of 4 Anti-HIV Drugs 5-weeks Following a Single Administration



(A). The open circles (o) represent the PBMC data, the closed squares (■) are plasma data, and the X represents lymph node data. The data plotted are the mean ±(s.d.), n=4 macaques over 840 hours following a single subcutaneous administration of the 4-in-1 DcNP.

(B). Data are plotted over 24 hours following a single administration.

The dotted lines represent the EC50 concentration for each respective single agent; the two lines on the 3TC graph represent the reported range of EC50 values[3].

Table 1. Long-Acting Plasma and Intracellular Pharmacokinetic Behavior and Enhanced Cellular Exposure as Determined By Noncompartmental Analysis.

	$AUC_{(0\text{-}840)}$ (hr•µg/mL)	C_{max} (µg/mL)	$T_{1/2z}$ (hr)
TFV			
PBMC	493.0 (200.0)	9.5 (2.4)	105.9 (13.8)
Plasma	862.5 (228.6)	12.6 (2.3)	63.1 (13.9
PBMC/Plasma	0.6	0.8	1.7
3TC			
PBMC	959.5 (1221.2)	7.8 (4.8)	127.7 (51.1)
Plasma	22.6 (3.1)	4.4 (2.0)	136.3 (42.6)
PBMC/Plasma	42.4	1.8	0.9
LPV			
PBMC	190.8 (92.5)	0.7 (0.2)	1045.7 (699.4)
Plasma	15.6 (5.5)	0.3 (0.1)	219.1 (143.5)
PBMC/Plasma	12.2	2.1	4.8
RTV			
PBMC	59.4 (36.5)	1.1 (0.7)	137.1 (24.2)
Plasma	3.6 (0.8)	0.1 (0.0)	115.2 (44.0)
PBMC/Plasma	16.4	8.6	1.2

Data are presented as mean (s.d.). PBMC and LNMC cell volume was assumed to be 0.2829 pL/cell[2]. AUC, area under the concentration time curve from 0-840 hours. C_{max}, is maximum drug concentration, $T_{1/2z}$ is apparent terminal half-life, PBMC, peripheral blood mononuclear cell.

Table 2. Enhanced intracellular Concentrations of Tenofovir, Lamivudine, Lopinavir, and Ritonavir in LNMCs and These Concentrations Relative to PBMCs and to Plasma, 24 hours and 8 days Following a Single Subcutaneous Administration.

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

Data are presented as mean (s.d.). PBMC and LNMC cell volume was assumed to be 0.2829 pL/cell[2]. PBMC, peripheral blood mononuclear cell; LNMC, lymph node mononuclear cell.

Results

- We have successfully formulated a lipid nanoparticle suspension containing 4 anti-retroviral agents (3 active API and 1 PK booster) with agents of disparate physical chemical characteristics
- The mean particle diameter was 33.3 nm (90% of the total population) and 106.3 nm (10%), the pH was 7.3 and the osmolality was 289 mOsm
- The subcutaneous administration of this 4 drug DcNP in non-human primates was well tolerated and did not result in injection site reactions
- All 4 drugs exhibited long-acting plasma and intracellular pharmacokinetic properties up to 5 weeks following a single administration
- Notable apparent terminal half-lives ($t_{1/2\,z}$) of LPV, TFV, and 3TC were observed in plasma: 219.1 hours (LPV), 63.1 hours (TFV), and 136.3 hours (3TC)
- The intracellular levels of LPV, RTV, and 3TC were higher than plasma at all all time points
- Early (< 360 hours) TFV plasma levels exceed PBMC, but this was reversed after 360 hours whereas TFV exhibited enhanced PBMC drug levels
- Eight days after drug administration, LPV, TFV and 3TC levels in LNMCs were 4.1-, 5.0-, and 1.9-fold higher than those in PBMCs, and much higher than that in plasma.

Conclusions

- This 4-in-1 drug combination, containing antiretroviral agents of varying physical chemical characteristics, was successfully formulated and could be a candidate for future evaluation.
- This formulation demonstrated both cell and tissue targeted properties in addition to sustained pharmacokinetic profiles.
- With appropriate dose scaling, this formulation could be considered for human development.

References:

[1]Koehn J and Ho RJY, *Antimicrob Agents Chemother.* 2014; 58(5): 2675–2680

[2]Simiele M, D'Avolio A, Baietto L, et al. *Antimicrob Agents Chemother.* 2011;55(6):2976-2978

[3]Epivir [package insert]. ViiV Healthcare, Research Triangle Park, NC; 2003, https://www.viivhealthcare.com/media/32160/us_epivir.pdf

Acknowledgements:

The authors wish to express their sincere gratitude to the Washington National Primate Center Research Support Group (Chris English, Michael Gough, and Jason Ogle) for their support of these NHP studies.

Supported by NIH grants UM1AI120176, P51OD010425, and AI 27757