Creation of Long - Acting Nanoformulated Tenofovir Prodrugs

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Abstract

Background: Despite the success of existing antiretroviral therapy (ART) in controlling human immunodeficiency virus (HIV) replication, treatment requires life-long adherence to medicines. Compliance to ART can be compromised by frequency of dosing and adverse drug reactions. To this end, tenofovir (TFV), a nucleoside analog inhibitor of HIV, was modified into two different lipophilic prodrugs (M1TFV and M2TFV) to extend the apparent drug half-life, improve potency and facilitate access to viral replication sites. Polymer- coated M1TFV and M2TFV nanocrystals were prepared to further improve drug biodistribution and longevity.
Methods: TFV was modified and formulated into long acting lipid nanocrystals by high-pressure homogenization. The created TFV prodrugs were purified by silica column chromatography and characterized by ^{1}H and ^{13}C-NMR, ^{31}P-NMR, and FTIR spectroscopy. Nanoparticles were produced by high-pressure homogenization (NM1TFV, NM2TFV). Human monocyte derived macrophages (MDM) and CEM-ss CD4+ T cells were used as a biological platform to measure drug uptake and retention. Drug levels were quantitated in cell lysates by UPLC-TUV. After treatment with 1- 1^{-9} µM M1TFV, M2TFV, NM1TFV, NM2TFV, TAF, or TFV cells were challenged with HIV-1_{ADA} (in MDM) or NL4-3 (in CEM-ss CD4+ T cells) at a multiplicity of infection (MOI) of 0.1 for 4 hours. Culture fluids were collected at 10 days and assayed for reverse transcriptase (RT) activity. To assess the pharmacokinetic (PK) profile of these tenofovir prodrug formulations, Male Sprague Dawley (SD) rats were injected with 75-mg/kg TFV equivalents of NM1TFV and NM2TFV while TAF required a dose of 168 mg/kg TFV equivalents to achieve a similar sustained drug release profile. Plasma, blood, and peripheral blood mononuclear cells (PBMCs) were collected weekly after injection of drug formulations. At the end of the four-weeks, organs and tissues were collected for detection of prodrug, native drug, and tenofovir diphosphate (TFV-DP) by liquid chromatography tandem mass spectrometry (LC-MS/MS).
Results: TFV prodrugs showed increased uptake in MDM compared to TFV alefenimde fumarate (TAF). NM1TFV and NM2TFV facilitated retention of prodrug in MDM for 30 and 15 days respectively; whereas TAF was hydrolyzed within a day. NM1TFV and NM2TFV also demonstrated sustained TFV-DP levels in MDM for one month. Both M1TFV and M2TFV prodrugs and their nanoformulations showed enhanced potency over TFV in MDM and CEM-ss CD4+ T cells. Importantly, TFV prodrug formulations demonstrated sustained drug concentrations in plasma, and TFV-DP in PBMCs over 4 weeks in SD rats after a single intramuscular dose. At day 28, TFV-DP levels observed following a single intramuscular injection of 168 mg/kg TAF, were less than or equal to TFV-DP levels in animals receiving a single intramuscular injection of 75 mg/ kg of NM1TFV or NM2TFV.
Conclusion: TFV was modified into two different lipophilic prodrugs (M1TFV and M2TFV). The chemical modifications extended the drug's apparent drug half-life, improved its potency and facilitated its access to viral replication sites. Surfactant stabilized M1TFV and M2TFV aqueous nanocrystals were prepared to further improve drug biodistribution and longevity. In SD rats, these novel prodrug nanoformulations demonstrated sustained prodrug and TFV-DP concentrations. Moreover, TAF required double the dose given for NM1TFV and NM2TFV treatments to achieve comparable TFV-DP levels. In all, these data sets demonstrate that NM1TFV and NM2TFV can be developed as long-acting ARV agents for treatment and prevention of HIV infections.

TFV prodrug characterization & formulation

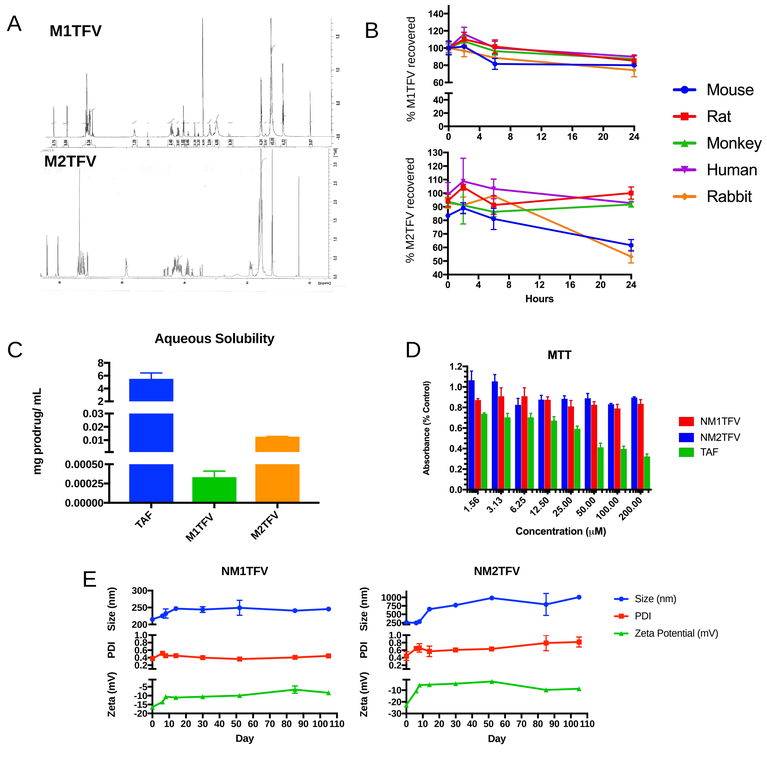
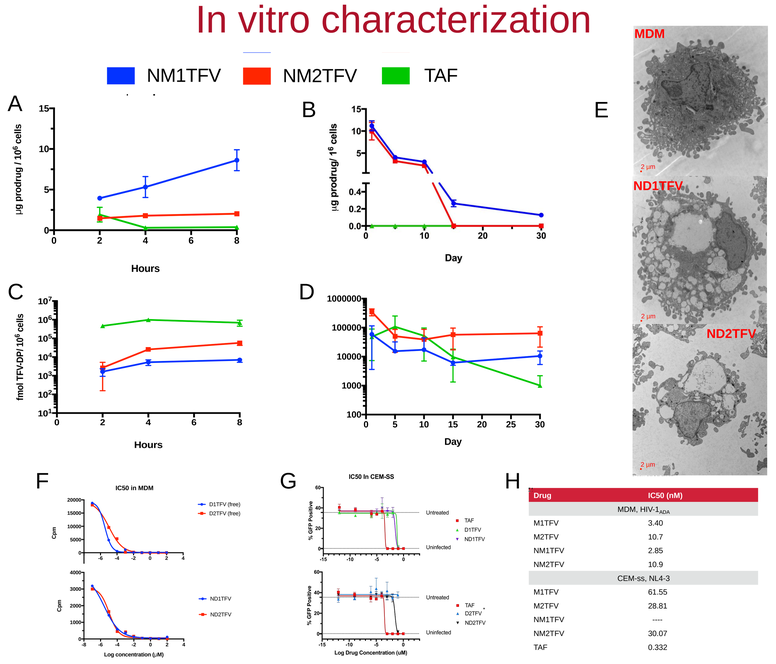


Figure 1: (A) ^{1}H-NMR spectra of M1TFV and M2TFV, (B) Stability of M1TFV and M2TFV in mouse, rat, monkey, human, and rabbit plasma over 24hrs at 37°C. (C) Aqueous solubility of M1TFV, M2TFV and TAF at room temperature. (D) Evaluations of mitochondrial function in MDM by MTT assay demonstrated that the created TFV prodrugs exerted no adverse effects to cell viability at 200 µM of drug or less. (E) Surfactant stabilized NM1TFV and NM2TFV aqueous nanosuspensions were produced at a drug loading of 75%. Formulation stability was determined by measurement of particle size, PDI and zeta potential.

In vitro characterization



Drug	IC50 (nM)
MDM, HIV-1_{ADA}	
M1TFV	3.40
M2TFV	10.7
NM1TFV	2.85
NM2TFV	10.9
CEM-ss, NL4-3	
M1TFV	61.55
M2TFV	28.81
NM1TFV	----
NM2TFV	30.07
TAF	0.332

Figure 2: Uptake and retention of TAF, NM1TFV and NM2TFV in MDM were measured with equal 10 µM drug concentrations. (A) For uptake, MDM were treated with TAF or TFV prodrug formulations from 1-8 hrs. (B) Drug retention in MDM after an 8 h drug loading and a 30-day observation. Intracellular drug and TFV-DP levels (C and D) by LC-MS/MS. Data are expressed as mean ± SEM for n = 3 samples/group. (E) Intracellular drug nanoparticles determined by transmission electron microscopy (TEM) after an 8 h treatment. (F) IC_{50} was determined in MDM following treatment with 1- 1^{-9} µM M1TFV, M2TFV, NM1TFV, or NM2TFV. Cells were infected with HIV-1_{ADA} or NL4-3 in MDM or CEM-ss cells at a MOI of 0.1 for 4 hours. Culture fluids were collected at 10 days and assayed for RT activity. Data are expressed as mean ± SEM for n = 4 samples per group (G) IC_{50} in CEM-ss cells was determined. On day 10, cells were fixed in 2% PFA and analyzed by flow cytometry for GFP. (H) IC_{50} values were derived from figures F & G.

Pharmacokinetics (PK)

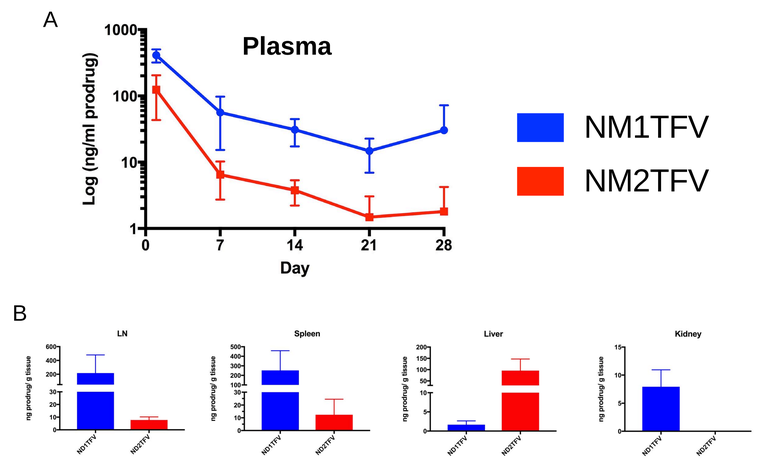


Figure 3: Drug PK was evaluated in Sprague Dawley male rats that received a single 75-mg/kg intramuscular injection of NM1TFV or NM2TFV. Blood and plasma was collected weekly for 4 weeks. At 4 weeks, animals were sacrificed and tissues analyzed for drug content by LC-MS/MS. (A) Drug concentrations (ng/ mL) of M1TFV and M2TFV in rat plasma and (B) in rat lymph node (LN), spleen, liver, and kidney were measured. Data are expressed as mean ± SEM for n = 5 animals/group.

PK comparisons based on TAF dosing

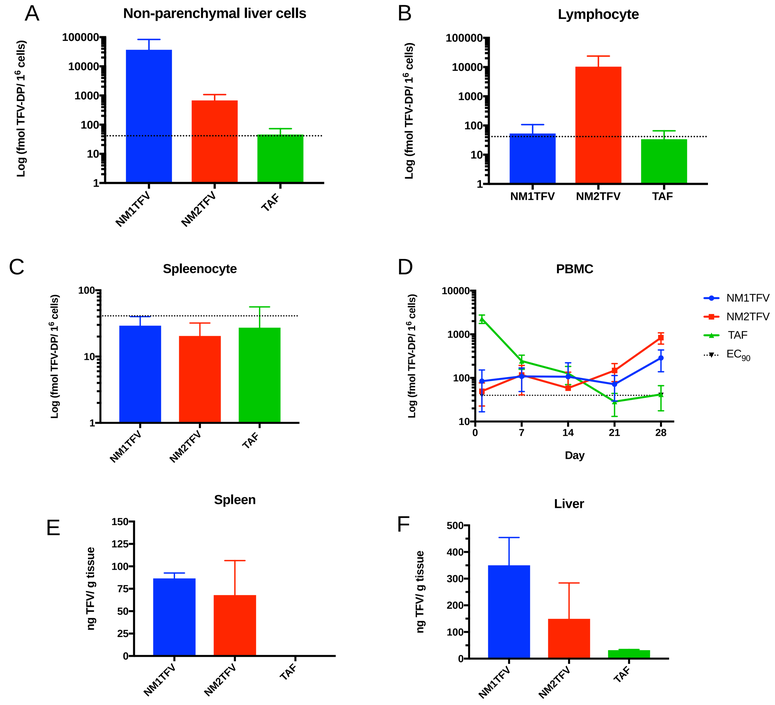


Figure 4: TAF dose required to elicit equivalent TFV-DP levels as NM1TFV and NM2TFV was determined. Male SD rats received a single 75-mg/kg intramuscular injection of NM1TFV, NM2TFV, or a single 168-mg/kg intramuscular injection of TAF. Blood was collected weekly for 4 weeks. At week 4, animals were sacrificed. Tissues, PBMCs, splenocytes, non-parenchymal liver cells, and lymphocytes were collected for drug content.

Conclusions

M1TFV and M2TFV were formulated into aqueous nanosuspensions by high pressure- homogenization. NM1TFV and NM2TFV prodrug and TFV-DP levels were observed for 30 days in MDM. The long-acting TFV prodrugs demonstrated sustained plasma concentrations over 4 weeks in SD rats after a single dose. At day 28, TFV-DP levels that followed a single intramuscular injection of 168-mg/ kg TAF, were less than or equal to TFV-DP levels in rats receiving a single intramuscular injection of 75-mg/ kg of NM1TFV or NM2TFV. Thus, long-acting TFV prodrugs may facilitate dose reductions and extend half-lives. In all, these data sets support further investigation of NM1TFV and NM2TFV as long-acting ARVs for treatment and prevention of HIV-1 infection.

Acknowledgement

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