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Synthesis and Characterization of a Long-Acting Tenofovir ProTide Nanoformulation

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Abstract

Antiretroviral therapy (ART) has significantly improved the quality of life of Human Immunodeficiency Virus (HIV) patients; but adverse side effects and poor patient compliance to lifelong daily pills remain major challenges. To this end, the need for long acting (LA) therapies that can improve treatment adherence, positively affect drug resistance patterns in addition to limiting drug toxicities cannot be overstated. Tenofovir alafenamide (TAF), a nucleotide reverse transcriptase inhibitor of HIV infection and prodrug of tenofovir (TFV), is characterized by potent antiretroviral activities and high genetic barrier to viral resistance making it a suitable candidate for long-acting antiretroviral therapy. However, the inherent physicochemical features of TAF that includes high water solubility and susceptibility to degradation in aqueous buffers has limited its transformation into long-acting sustained release formulations. With these limitations in mind, this work sought to produce a stable TFV prodrug that would facilitate development of a long-acting formulation without compromising on TAF's antiretroviral activity and safety profile. A lipophilic and hydrophobic prodrug of TFV (M1TFV) was therefore developed through chemical synthesis making it possible to formulate the drug as a stable aqueous nanosuspension to improve upon drug dissolution. The aqueous poloxamer stabilized TFV prodrug nanosuspension (NM1TFV) was characterized for physicochemical properties, chemical stability, cellular drug uptake and retention. The average particle size of the nanoparticles was 220-270 nm with a polydispersity index of <0.5, suggesting uniform particle size distribution within the formulation. Compared to TAF, the synthesized M1TFV prodrug demonstrated improved prodrug stability in water and enhanced intracellular drug uptake in monocyte derived macrophages and was also efficiently converted into the active metabolite (TFV-DP) that competitively inhibits the activity of HIV reverse transcriptase enzyme to stop the virus from replicating. These results are a major step towards producing a novel long acting tenofovir formulation that could potentially facilitate treatment and prevention of HIV infection.

Methods

M1TFV Synthesis and Characterization: The monophosphorylated prodrug of TFV was synthesized through a modified ProTide approach(1). Successful prodrug synthesis was confirmed using nuclear magnetic resonance (NMR) and mass spectroscopy.

Nanocrystal Development: The hydrophobic and lipophilic M1TFV prodrug was nanoformulated in an aqueous buffer by high pressure homogenization using poloxamer 407 (P407) as the stabilizing surfactant.

Drug uptake and Retention in MDM : Human monocyte derived macrophages (MDM) were obtained by differentiating primary monocytes with macrophage colony stimulating factor. For drug uptake studies, MDM were treated with 10 µM of M1TFV and collected at various time intervals over 24 hours for intracellular drug quantitation by UPLC-UV/Vis. For drug retention studies, MDM were treated with 10 µM of drug for 8 hours, then washed twice with phosphate buffered saline and cultured in media without drug until days of collection and analyses by UPLC-UV/Vis. Since the active form of the nucleotide analog is a diphosphate, both uptake and retention samples were analyzed for TFV-DP levels.

Results

1 Synthesis and Characterization of M1TFV

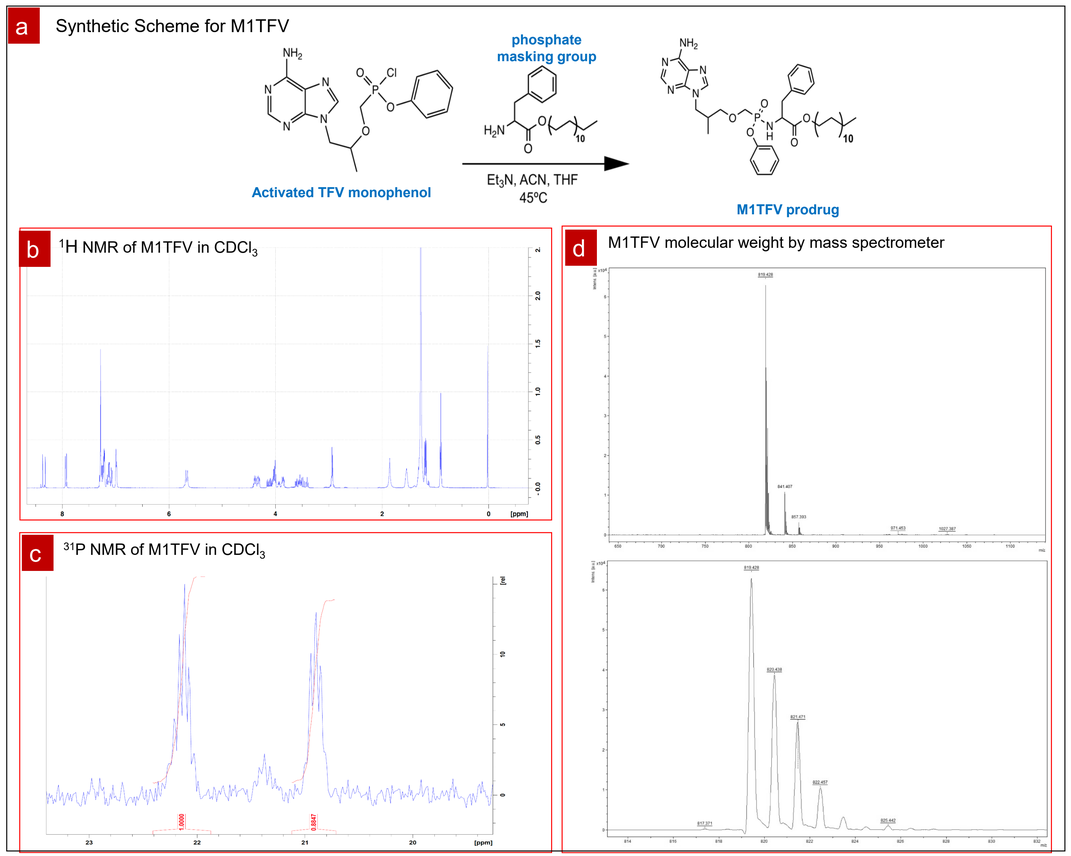

Figure 1. (a) Synthesis scheme for M1TFV. (b) ^{1}H NMR spectra of M1TFV was acquired in deuterated chloroform (c) ^{31}P NMR spectrum of M1TFV representing the two diastereomers (R_P and S_P) of the prodrug. (d) Mass spectrometric analyses (Bruker Autoflex Max MALDI-TOF) of M1TFV showed the desired molecular ion peaks at 819.4 $[M+H]^+$

2 M1TFV Nanoformulation

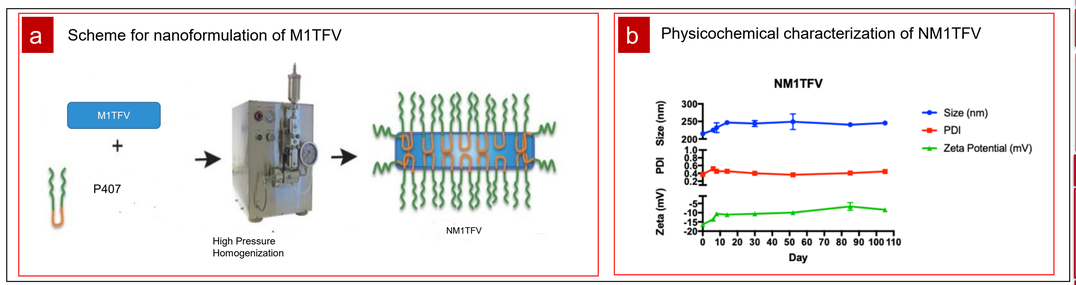

Figure 2. (a) Schematic representation of nanoformulation of M1TFV. The formulation was produced by high pressure homogenization using poloxamer as the surfactant. (b) M1TFV nanoparticles were characterized for particle size, homogeneity (PDI) and surface charge (zeta potential) by dynamic light scattering on a Malvern Zetasizer Nano-ZS.

3 In vitro characterization of M1TFV

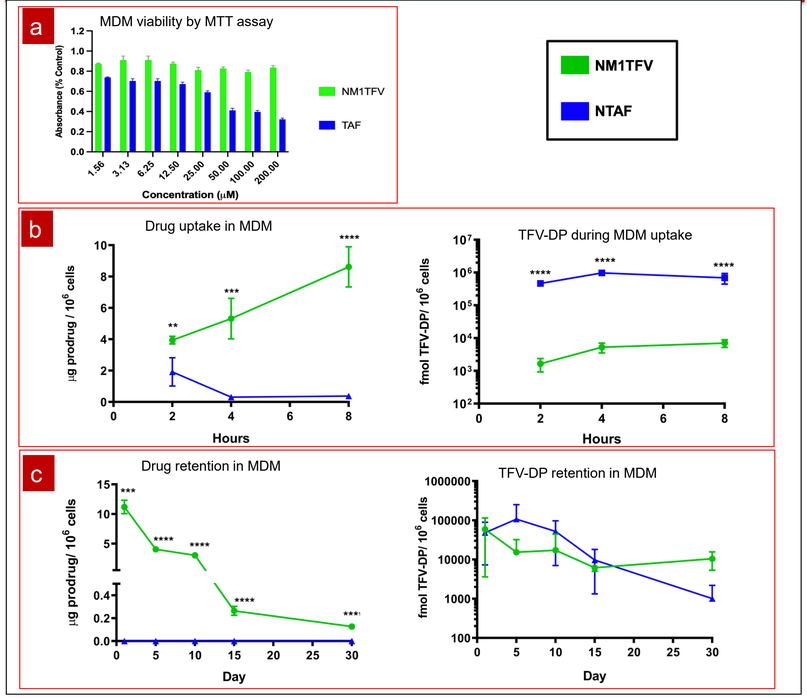

Figure 3. (a) Evaluation of mitochondrial activity demonstrated that NM1TFV exerted no adverse effects to cell viability at 200uM of drug or less.(b) Following 10 µM treatments of NM1TFV or NTAF, intracellular prodrug (left panel in b) and TFV-DP (right panel) concentrations in MDM were measured over an 8hr period. Prodrug levels for NM1TFV were significantly greater than for NTAF. However, TAF treatment results in faster prodrug conversion to TFV-DP (right panel), compared to NM1TFV. (c) Concentrations of prodrug (left panel) and TFV-DP (right panel) following 10 µM treatments of NM1TFV or TAF were determined in MDM over 30-day period.

Summary

(1) M1TFV was synthesized and its physicochemical properties were characterized.
(2) Surfactant stabilized M1TFV nanoparticles exhibited stable particle sizes and narrow polydispersity indices
(3) NM1TFV demonstrated enhanced MDM drug uptake and retention compared to TAF.

Future Studies

(1) Anti-retroviral efficacy studies in MDM and CD4+ T cells to determine whether the prodrug formulations would lead to sustained efficacy.
(2) In vivo pharmacokinetics, biodistribution and drug release studies.
(3) In vivo pharmacodynamic studies.

References

(1) McGuigan, C., Pathirana, R.N., Balzarini, J., and DeClercq, E. 1993. Intracellular delivery of bioactive AZT nucleotides by aryl phosphate derivatives of AZT. J. Med. Chem. 36:1048-1052.

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