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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 produg 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 produg 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 demonstrated improved produg 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 produg of TFV was synthesized through a modified ProTide approach1. Successful produg synthesis was confirmed using nuclear magnetic resonance (NMR) and mass spectroscopy. Nanocrystal Development: The hydrophobic and lipophilic M1TFV produg was nanoformulated in an aqueous buffer by high pressure homogenization using poloxamer 407 (P407) as the stabilizing surfactant.

Results

Figure 1. (a) Synthesis scheme for M1TFV. (b) 1H NMR of M1TFV in CDCl3. (c) 31P NMR of M1TFV in CDCl3. (d) Mass spectrometric analyses (Bruker Autolx Max MALDI-TOF) of M1TFV showed the desired molecular ion peaks at 819.4 [M+H]+.

Figure 2. (a) Scheme for nanoformulation of M1TFV. (b) Physicochemical characterization of NM1TFV.

Figure 3. (a) Evaluation of mitochondrial activity demonstrated that NM1TFV exerted no adverse effects to cell viability at 200µM of drug or less. (b) Following 10 µM treatments of NTAF and TAF, 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 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.

Synthesis and Characterization of M1TFV

1. High pressure homogenization using poloxamer 407 (P407) as the stabilizing surfactant.

2. M1TFV nanoformulation

3. In vitro characterization of M1TFV

Future Studies

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

References


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