MONTHSLONG HBV SUPPRESSION BY TFV DOUBLE ESTER PRODRUGS

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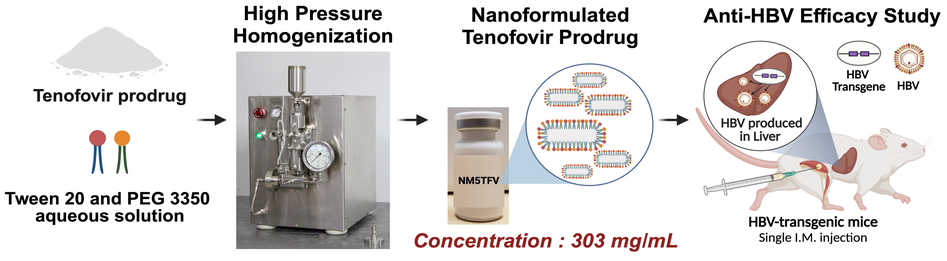
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BACKGROUND

Tenofovir (TFV) prodrugs [TFV disoproxil-/TFV alafenamide- fumarates (TDF/TAF)] are recommended daily as oral treatments for chronic hepatitis B (HBV) infection. Given the growing popularity of long-acting (LA) therapies for treatment of chronic viral infections, an ever-increasing number of potent medicines will come available. One, developed in our own laboratories, is a lipophilic ProTide of TFV called M1TFV that exhibited sustained suppression of HBV DNA in mouse models of human disease [Sci. Adv.8,eade9582(2022)]. However, like other ProTide prodrugs, the reported chemical synthesis of M1TFV necessitates costly chiral catalysts and time-consuming separation of the Sp and Rp diastereomers generated at the phosphorous atom. To overcome these limitations, we have developed alternative amino acid free hydrophobic and lipophilic double ester crystalline TFV prodrugs. Two nanoformulated diester candidates elicited enhanced and sustained HBV DNA suppression in transgenic C57BL/6 Tg05 (called Tg05) mice compared to limited efficacy for TAF ProTide. Each nanoformulated compound was administered as a single intramuscular (IM) injection.

METHODS

Three amino acid free double ester prodrugs of TFV were synthesized through a non-catalytic esterification of TFV monophenyl ester with either an aryl substituted lipid (M4TFV), octadecanol (M5TFV) or docosanol (M6TFV). The synthesized prodrugs were characterized and nano-formulated using biocompatible surfactants to produce stable aqueous solid drug nanosuspenisons (NM4TFV, NM5TFV and NM6TFV). Control treatment consisted of TFV alafenamide solid drug nanosuspensions (NTAF). Additional controls were infected and untreated animals. Antiviral efficacy was tested in HBV Tg05 mice containing a stably integrated HBV transgene. Drug formulations were administered as single intramuscular injections at 168 mg/kg TFV equivalents. HBV DNA viral load in blood was measured every other week.



Animal model of HBV infection. C57BL/6 transgenic Tg05 mice expressing wild-type HBV genome (HBV genomic information plus a terminal redundancy, 1.3 times the size of HBV genome) were obtained from J.-H. James Ou, University of Southern California. These mice replicate and express the virus in hepatocytes and secrete HBV DNA into blood at levels comparable to that of infected liver and blood of human patients with chronic hepatitis viral infection. HBsAg and HBeAg are detectable in the serum and urine of these mice, while HBcAg is found in the liver and kidney where high levels of HBV gene are expressed, and at lower levels in other tissues. This model shows no evidence of pathological changes in any organ, especially the liver and kidney during the lifetime of these mice. This provides the opportunity to screen the antiviral potential of pharmacological agents and immune response to control HBV replication for prolonged durations of time in a genetically defined small-animal system which would naturally be uninfected by HBV infection.

A single dose of a novel crystalline nanosuspension of NM5TFV phosphonate prodrug produces monthslong suppression of HBV DNA in Tg05 mice without recorded adverse events.

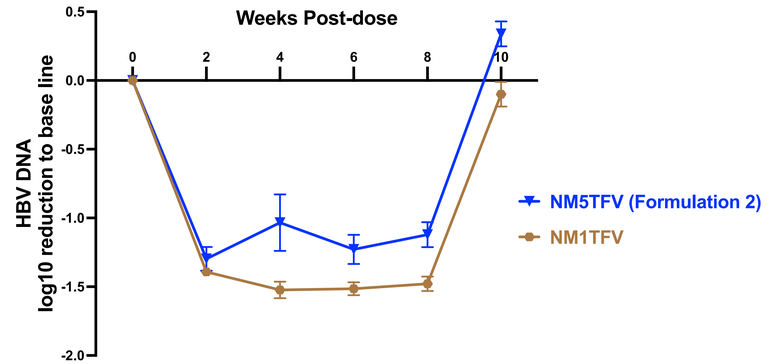


Figure 1. Comparative analysis of HBV suppression by NM5TFV phosphonate prodrug and NM1TFV ProTide in HBV transgenic Tg05 mice. A single intramuscular injection of NM5TFV or NM1TFV (**n=8** NM5TFV and **n=6,** NM1TFV, equal male and female) shows comparable HBV DNA suppression in peripheral blood. Notably, Formulation 2 of NM5TFV phosphonate prodrug (produced at a drug concentration of 303 mg/ml) was non-inferior to NM1TFV ProTide nanosuspension in sustaining HBV DNA suppression. Results are expressed as Mean ± SEM.

Table 1. Head to head efficacy comparison of NM1TFV ProTide vs NM5TFV phosphonate in HBV transgenic mice

Treatment / weeks	log10 reduction in serum HBV DNA levels from baseline				
	2	4	6	8	10
NM5TFV	-1.30	-1.03	-1.23	-1.12	0.34
NM1TFV	-1.39	-1.52	-1.51	-1.48	-0.10
P value	0.35	0.23	0.29	0.49	0.52

Drug levels were sustained in NM5TF treated HBV-transgenic mice for over 77 days

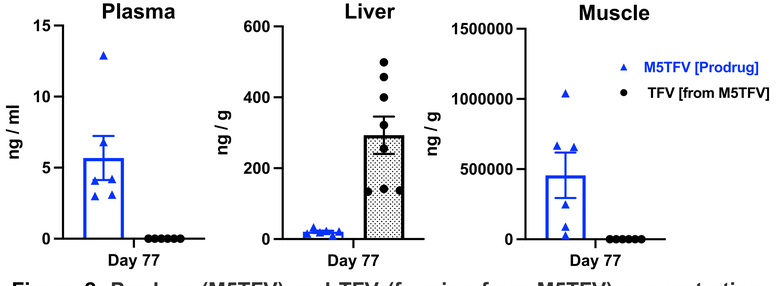


Figure 2. Prodrug (M5TFV) and TFV (forming from M5TFV) concentration in plasma and tissues. Notably, lower TFV levels were detectable in plasma and at the muscle injection site. A significant amount of TFV was detected in the liver. Data are expressed as means ± SEM, and dots represent individual mice.

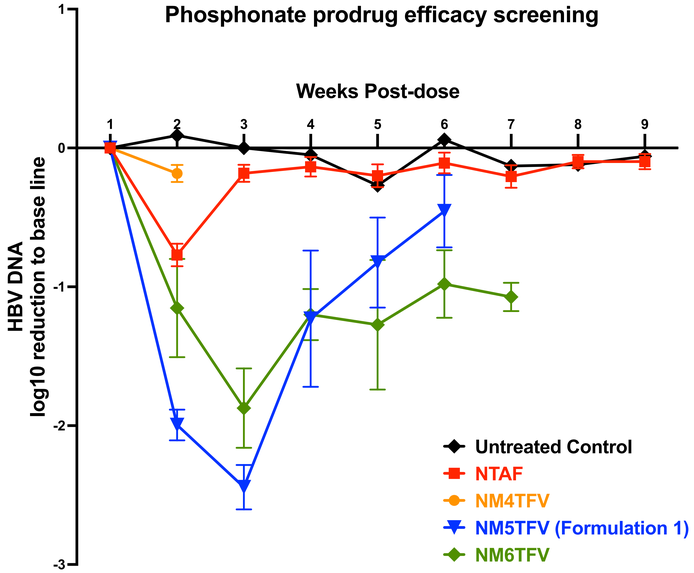


Figure 3. Suppression of HBV replication in HBV transgenic Tg05 mice (C57BL/6). A single intramuscular injection of NM5TFV (Formulation 1, produced at a drug concentration of 164.56 mg/ml) or NM6TFV (**n=4 per group**) suppressed HBV DNA in peripheral blood by two orders of magnitude compared to untreated, nanoformulated TAF ProTide and NM4TFV phosphonate prodrug. Results are expressed as Mean ± SEM.

RESULTS SUMMARY

Three diester prodrugs of TFV were synthesized in high chemical yields and with one of them forming a single isomer at the phosphorous chiral center. The synthesized prodrugs were crystalline and compatible with scalable formulation approaches for producing surfactant stabilized aqueous nanosuspensions. Notably, a single IM injection of lipophilic NM5TFV or NM6TFV diester prodrug nanosuspensions to Tg05 mice demonstrated enhanced and sustained efficacy compared to limited anti-HBV activity for TAF ProTide or arylated NM4TFV diester prodrug.

CONCLUSIONS

NM5TFV phosphonate prodrug nanosuspension produced sustained monthslong suppression of HBV DNA in Tg05 mice without recorded adverse events.

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