PRECLINICAL EVALUATION OF AN ULTRA-LONG-ACTING TENOFOVIR PRODRUG NANOSUSPENSION

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BACKGROUND

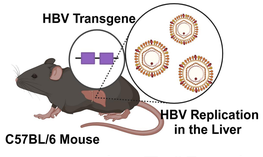
Effective treatment of hepatitis B (CHBV) infection requires adherence to long-term daily antiviral therapies. These include oral tenofovir (TFV) prodrugs [TFV disoproxil-/TFV alafenamide-fumarate - TDF/TAF] or entecavir. Treatment cessation leads to HBV reactivation and disease progression. This underscores the need for long-acting formulations to improve treatment outcomes. Considering these needs, we developed a scalable amino acid-free hydrophobic and lipophilic crystalline phosphonate TFV prodrug (M5TFV). Surfactant-stabilized aqueous nanosuspensions (NM5TFV) were produced at a >300 mg/ml drug concentration. The formulation was stable at room temperature. We now report on possible mechanisms by which NM5TFV sustains suppression of HBV replication in hepatocytes. NM5TFV is well tolerated at the injection site. These promising data support further development and translation of NM5TFV formulation.

METHODS

Scalable M5TFV was formulated into aqueous nanosuspensions using Tween 20 and PEG 3350 as the stabilizing surfactants. In vivo efficacy was evaluated in transgenic (Tg05) mice administered a single intramuscular injection of NM5TFV at either 200 or 400 mg/kg TFV equivalents. NM1TFV, an injectable TFV prodrug shown to be longer acting than TAF (Sci Adv, 2023), was used as a control. HBV viral load in blood was tested biweekly until rebound. At the time of animal sacrifice, liver tissues were collected to evaluate treatment-mediated activation of interferon (IFN)-stimulated genes (ISG) and inflammasomes. For in vitro studies, HepAD38 cells were treated with NM5TFV for eight hours, followed by incubation in drug-free media for 48 hours. HBV DNA and cccDNA were determined by qPCR.

HBV TRANSGENIC MOUSE MODEL

This model is widely used in preclinical antiviral testing due to its ability to produce high levels of hepatitis B virus that closely mimic the viral loads observed in human chronic HBV infections. The C57BL/6 transgenic Tg05 mice, expressing the wild-type HBV genome, were obtained from the University of Southern California. This model demonstrates absence of cytopathology throughout the lifespan of the mice.



A Novel TFV Prodrug Nanosuspension (NM5TFV) Suppressed HBV Replication Over Two Months Following a Single Intramuscular Injection in Transgenic Mice, Without Affecting Innate Immune Responses

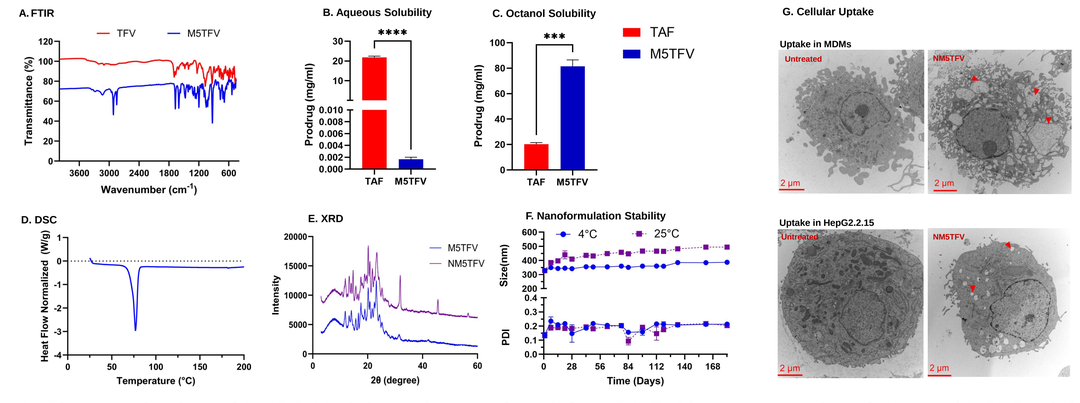


Fig. 1. Characterization of NM5TFV. **(A)** Fourier transform infrared (FT-IR) spectra overlay of TFV and M5TFV. **(B)** Aqueous and **(C)** Octanol solubility of M5TFV and TAF. **(D)** Differential scanning calorimetry (DSC) thermogram of M5TFV. **(E)** The crystallinity of M5TFV and NM5TFV was determined by X-ray diffraction. **(F)** NM5TFV (303 mg/ml) stability was evaluated at 4 °C and 25 °C. **(G)** Transmission Electron Microscopy (TEM) images of Monocyte-derived Macrophages (MDMs) and HepG.2.2.15 cells treated for 8h with 10 µM and 25 µM NM5TFV, respectively.

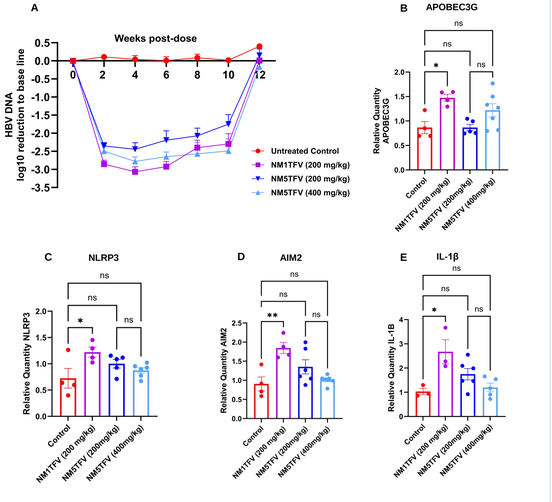


Fig. 2. The effect of NM5TFV on HBV replication and innate immune response stimulation in transgenic mice. **(A)** NM5TFV and NM1TFV suppressed viral replication over 10 weeks, with comparable efficacy. **(B-E)** In contrast to NM5TFV, NM1TFV increased the expression of ISGs in the livers of Tg05 mice. Results are expressed as Mean ± SEM. *P<0.05, **P<0.01 by one-way ANOVA.

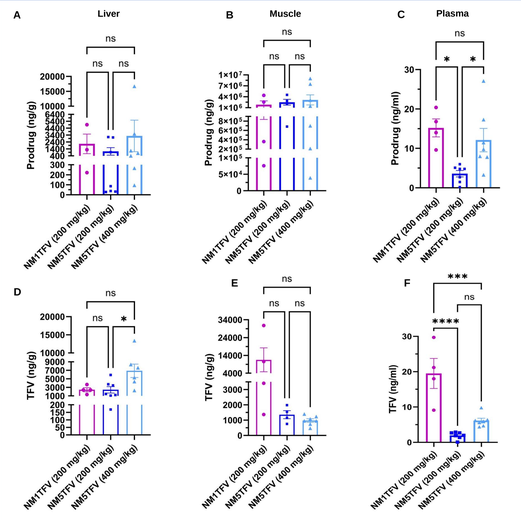


Fig. 3. TFV and prodrug concentrations in tissues and plasma of transgenic mice treated with either NM5TFV or NM1TFV. Biodistribution of prodrug and TFV was assessed in the liver **(A and D),** injection site **(B and E)** and plasma **(C and F).** Data are expressed as Mean ± SEM and dots represent individual mice. *P < 0.05 by one-way ANOVA

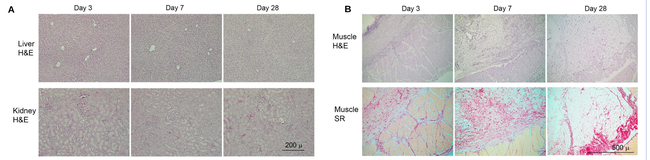


Fig. 4. Histological evaluation of NM5TFV in the tissues and the injection site in Sprague Dawley rats. (A) H&E staining of the liver and kidney. **(B)** Injection site characterization (upper panel: H&E staining, lower panel: Sirius Red staining).

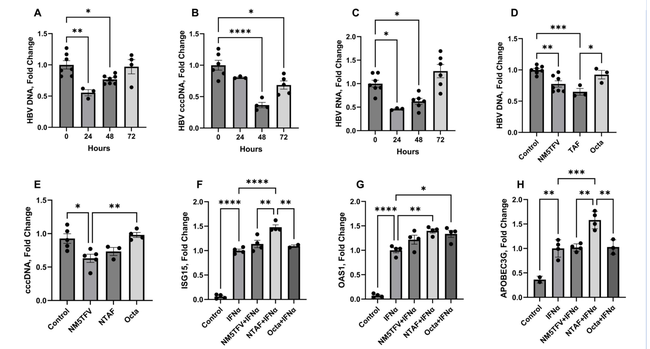


Fig. 5. Effect of NM5TFV on HBV replication and induction of interferon-stimulated genes. Treatment with 25 µM NM5TFV for 8 hours reduced the levels of **(A)** HBV DNA, **(B)** cccDNA, and **(C)** HBV RNA in HepAD38 cells. NM5TFV, but not octadecanol, suppressed **(D)** HBV DNA and **(E)** cccDNA. The addition of IFNα (200 IU) 6 hours before cell harvesting increased the expression of **(F)** ISG15, **(G)** OAS1, and **(H)** APOBEC3G. NM5TFV and octadecanol did not alter the expression of these genes. Results are expressed as mean ± SEM for four replicates. *P<0.05, **P<0.01, ***P<0.001. ***P<0.0001, ****P<0.00001 by one-way ANOVA

CONCLUSIONS

- A single dose of NM5TFV sustained suppression of HBV DNA in mice for over two months.
- NM5TFV achieved prolonged viral suppression without activating ISGs and inflammasome markers.
- NM5TFV was well tolerated at the injection site.
- These promising findings support further development of NM5TFV as an ultra-long-acting formulation candidate.

ACKNOWLEDGEMENTS

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