TRANSFORMATION OF DOLUTEGRAVIR INTO A YEAR-LONG PARENTERAL PRODRUG FORMULATION

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BACKGROUND

Antiretroviral therapy (ART) requires lifelong daily dosing to attain durable viral suppression. Limitations remain with long-acting injectables of cabotegravir and rilpivirine (CAB and RPV LA) and include high injection volumes and site reactions, limited drug tissue distribution, and frequent administrations. With the aim of prolonging the apparent half-life of ART our laboratories developed, then characterized, and screened DTG prodrug libraries encased in biocompatible surfactants for sustained plasma concentrations and tissue biodistribution after single intramuscular (IM) injection for a year or longer.

METHODS

A prodrug library of DTG was synthesized by esterification using aliphatic lipid chains. This yielded 14 (MDTG), 18 (M2DTG) and 22 (M3DTG) carbon-modified lipophilic prodrugs. An 18-carbon prodrug carrying two DTG molecules on a single lipid chain and native DTG completed the drug library (M4DTG). Solid prodrug nanoparticles in aqueous suspension were manufactured by high-pressure homogenization. Particle size, homogeneity, and surface charge was assessed prior to tests of human monocyte-derived macrophage (MDM) uptake, retention, cytotoxicity, and antiretroviral activity. A single 45 mg DTG-equivalents/kg IM dose of drug nanoformulation was administered to Balb/cJ mice, SD rats, and rhesus macaques. Release and hydrolysis profiles were investigated.

RESULTS

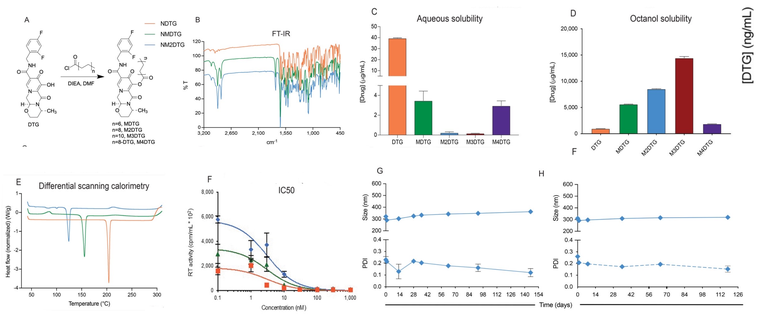


Fig. 1. Synthesis and characterization of DTG prodrugs. (A) DTG was synthesized by esterification of the DTG hydroxyl group yielding lipophilic prodrugs with 14, 18, or 22-carbon chains. They are named MDTG, M2DTG, and M3DTG. A DTG dimer with an 18-carbon chain ester linkage was named M4DTG. **(B)** FT-IR overlays of DTG, MDTG, and M2DTG identified the presence of specific molecular groups in the prodrugs affirmed by spectroscopy. **(C)** Aqueous and **(D)** octanol solubility of DTG and prodrugs are illustrated. Results are expressed as the mean ± SEM for N = 3. **(E)** Differential scanning calorimetry (DSC) thermogram overlays of NDTG, NMDTG, and NM2DTG determined changes in physical state. **(F)** The antiretroviral half maximal inhibitory concentration (IC_{50}) in macrophages were assessed at 0.1-1,000 nM concentrations by measurements of HIV-1 reverse transcriptase (RT) activity in culture supernatants. Results are expressed as the mean ± SEM for N = 3. Nanoformulations that enhance particle properties include **(G-H)** the hydrodynamic diameter (size) of NM2DTG formulations were tested at 22°C over 120 days by dynamic light scattering (DLS). **(G)** NM2DTG (268 mg/mL of M2DTG) formulated with Tween 20 and PEG_{3350} in PBS (pH 7.0). **(H)** NM2DTG (330 mg/mL of M2DTG) formulated with Tween 20 and PEG_{3350} in PBS (pH 7.0).

Hydrophobic prodrug nanocrystals provide plasma DTG levels at or above the PA-IC_{90} for a year following a single IM injection in rodents

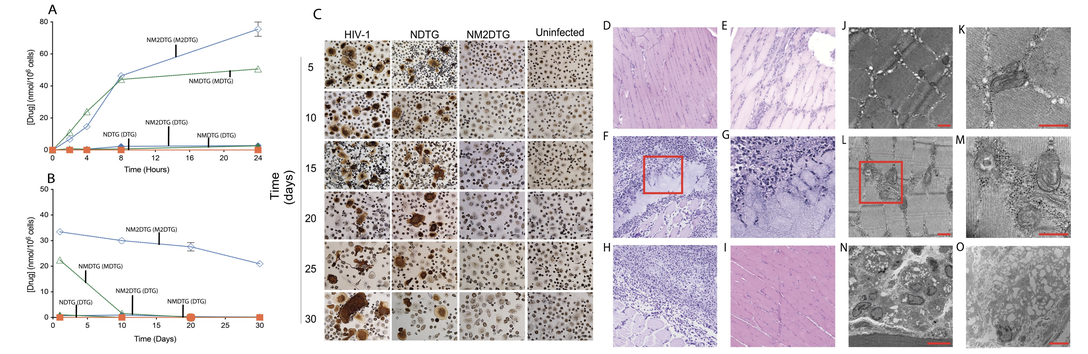


Fig. 2. Biological, histological and electron microscopic characterization of NM2DTG. (A) drug uptake and **(B)** retention in MDM measured over 24 h and 30 days, respectively, after treatment with prodrug nanoformulations at concentration of 25 μM. Results are expressed as the mean ± SEM for N = 3. **(C)** Antiretroviral responses were recorded after HIV-1_{ADA} challenge at a multiplicity of infection (MOI) of 0.1 infectious virions/cell at recorded times following treatment with either NDTG or NM2DTG at 1 μM concentrations for 8 h. HIV-1p24 antigen levels were assessed in fixed MDM by immunohistochemical staining. **(D-H)** H&E staining of caudal thigh muscle in histological sections after dissection from rats 3 days following IM injections. **(D)** Control (uninjected), **(E)** sham (saline-injected) control and **(F-H)** NM2DTG (45 mg DTG-eq./kg) treated muscle sections. **(I)** H&E staining of caudal thigh muscle in histological sections after dissection from rats 57 days following IM injection of NM2DTG at 45 mg DTG-eq./kg. Representative images for panel **(F)** at 10X magnification have been provided at 40X in panel **(G)**. **(J-O)** Replicate muscle samples were examined by transmission electron microscopy (TEM) from rats three days post-treatment. **(J-K)** uninjected and **(L-M)** Sham (saline-injected) controls show normal muscle histology. **(N)** Rats injected with NM2DTG show cell infiltration with ingestion of the nanoformulation into endosomal vesicles at day 3. Representative images for panel **(L)** at 10X magnification have been provided at 40X in panel **(M)**. **(O)** TEM of monkey muscle shows the nanoformulation depot in macrophages at day 364. Scale bars – 500 nm **(J-M)**, 10 μm **(N)**, 2 μm **(O)**.

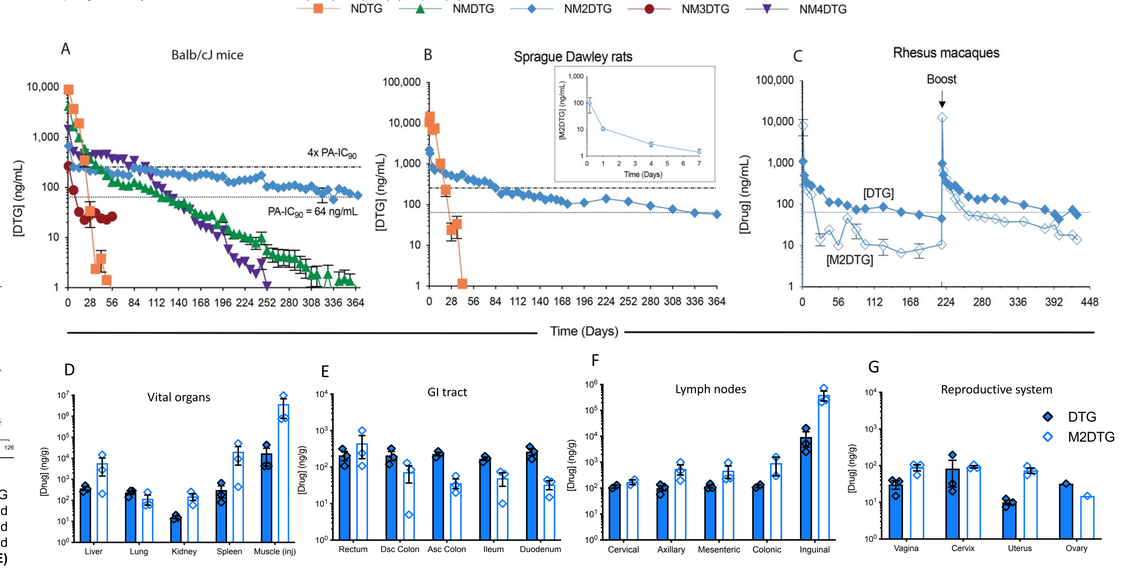


Figure 3. DTG PK studies in Balb/cJ mice, Sprague Dawley rats, and rhesus macaques. (A) Plasma DTG levels in male Balb/cJ mice administered a single IM dose of NDTG, NMDTG, NM2DTG, NM3DTG, or NM4DTG. Drug formulations were injected at concentrations of 45 mg DTG-eq./kg IM in the caudal thigh and native drug levels monitored to day 367. The dotted line indicates the DTG protein-adjusted IC_{90} (PA-IC_{90} = 64 ng/mL) and dashed line indicates four-times the PA-IC_{90} (4x PA-IC_{90} = 256 ng/mL). **(B)** Plasma DTG levels in male SD rats administered a single 45 mg DTG-eq./kg IM dose of NDTG or NM2DTG in the caudal thigh were recorded. Plasma prodrug (M2DTG) levels are shown in the insert to day 7. **(C)** Plasma DTG (solid line) and M2DTG (dashed line) levels in female rhesus macaques given a 45 mg DTG-eq./kg IM dose of NM2DTG in the quadriceps muscles, followed by an equivalent booster dose on day 217 (arrow). Plasma samples were collected, and drug levels determined up to day 428. **(D-G)** Tissue biodistribution of NM2DTG in rhesus macaques was assessed at one year. Parent drug (DTG) and prodrug (M2DTG) levels were determined in (**D**) Vital organs **(E)** GI tract, **(F)** lymph nodes, and **(G)** reproductive system.

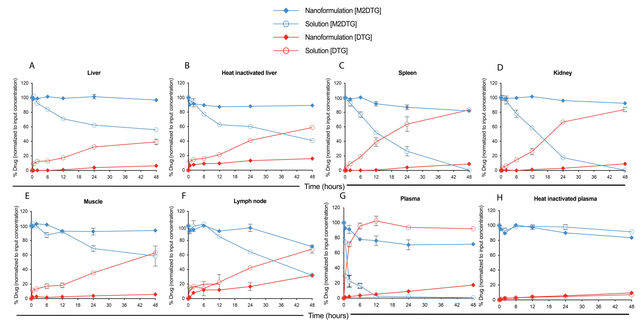


Figure 4. NM2DTG prodrug cleavage studies in tissue drug depots. Cleavage of M2DTG solution (dissolved in 1% (v/v) methanol) or NM2DTG nanoformulation in SD rat tissue homogenates **(A-F)** and plasma **(G-H)**. Prodrug (M2DTG) and parent drug (DTG) were quantified following M2DTG or NM2DTG incubation over 48 h in **(A)** liver, **(B)** heat-inactivated liver, **(C)** spleen, **(D)** kidney, **(E)** muscle, **(F)** lymph node, **(G)** plasma, and **(H)** heat inactivated plasma. All drug levels were quantified by UPLC-MS/MS. Results are expressed as mean ± SEM for N = 3.

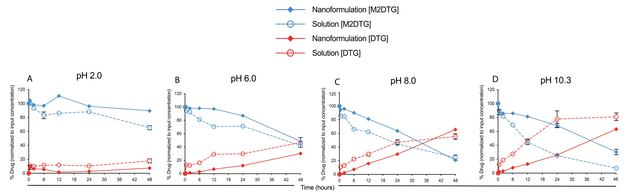


Figure 5. pH-dependent prodrug hydrolysis. Hydrolysis of M2DTG solution (dissolved in 1% (v/v) methanol) or NM2DTG nanoformulation in buffers of various pH. Prodrug (M2DTG) and parent drug (DTG) were quantified following M2DTG or NM2DTG incubation over 48 h in buffers at **(A)** pH 2.0, **(B)** pH 6.0, **(C)** pH 7.0, and **(D)** pH 10.3. All drug levels were quantified by UPLC-MS/MS. Results are expressed as mean ± SEM for N = 3.

CONCLUSIONS

- A single intramuscular injection of the NM2DTG prodrug nanocrystal generated DTG levels at or above the PA-IC_{90} for up to one year. These PK data sets were recorded in rodents and rhesus macaques.
- DTG prodrug depots in spleen, inguinal lymph node, liver and muscle tissues were 20, 400, 6,and 3800 μg/g at a year in rhesus.
- The extended NM2DTG PK profile is associated with prodrug nanocrystal tissue dissolution and pH.
- NM2DTG is readily was retained and provides protection beyond 30 days in macrophages challenged with HIV-1_{ADA}.

FUNDING SOURCES

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