ULTRA-LONG-ACTING BICTEGRAVIR PRODRUG NANOFORMULATIONS

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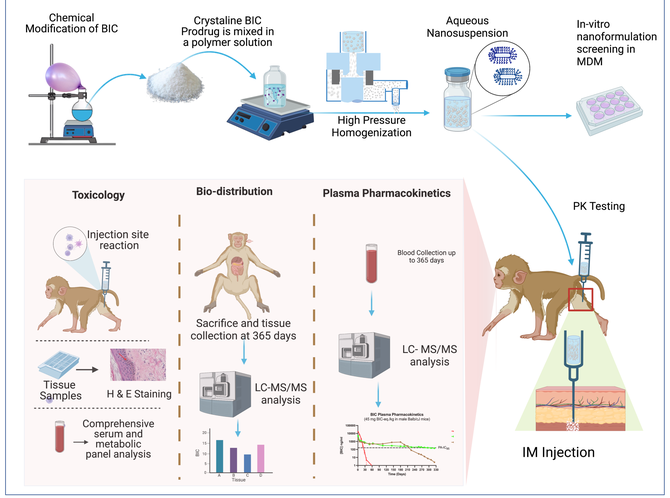
INTRODUCTION

Antiretroviral therapy (ART) suppresses HIV-1 replication which leads to reduced disease mortality and associated co-morbidities. Viral transmission from infected to susceptible persons is lowered. However, therapeutic outcomes remain affected by suboptimal adherence, adverse ART events and tolerability, and viral resistance. To these ends, the development of ultra-long-acting (ULA) antiretroviral drug (ARV) formulations with improved half-lives and pharmacokinetic (PK) profiles is timely. Herein, we report the transformation of bictegravir (BIC) into prodrug nanoformulations with extension of the drug's apparent half-life. The BIC prodrugs encased into nanocrystals were tested for changed physicochemical and PK properties. The overarching goal led to the creation of ULA BIC formulations with reduced injection volumes and a shorter PK tail.

METHODS

Dimeric (MXBIC) and monomeric (MBIC, M2BIC, M3BIC) BIC prodrugs were synthesized by one-step dimerization and mono-esterification. Prodrugs were nanoformulated by high-pressure homogenization then evaluated for stability, particle size, homogeneity, and surface charge after formulation of aqueous solid drug nanocrystals. Prodrug cellular uptake and retention, antiretroviral activity, and cytotoxicity were tested in primary human monocyte-derived macrophages (MDM). Following a single intramuscular (IM) injection, PK and biodistribution (BD) profiles were evaluated in Balb/cJ mice, SD rats, and rhesus macaques.



Conversion of BIC into Ultra-Long -Acting Prodrug Nanosuspensions with Potential for Every Six-Months Parenteral Dosing Regimen

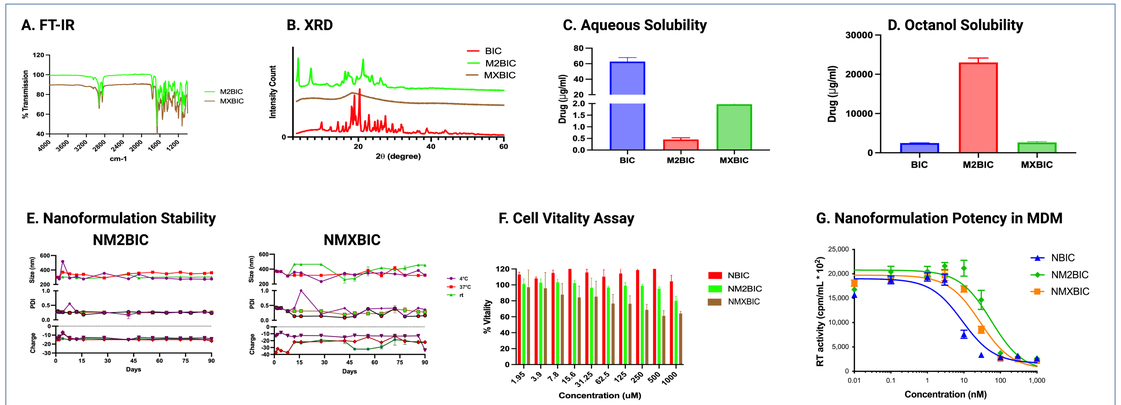


Figure 1. Characterization of BIC prodrugs. (A) Fourier-transform infrared spectra (FT-IR) overlay of M2BIC and MXBIC identified distinct functional groups in M2BIC and MXBIC **(B)** Differences in the solid states of BIC, M2BIC and MXBIC as determined by Powder X-ray diffraction (XRD) analyses at 2Ø = 2- 60 at 1/min **(C)** Aqueous and **(D)** octanol solubility of native BIC and prodrugs. **(E)** The stability of BIC and prodrug nanoformulations was evaluated over 90 days following manufacture at room temperature, 4ºC, and 37ºC as determined by size (nm), polydispersity index (PdI), and zeta potential (mV). **(F)** Cell vitality was assessed in MDM by MTT assay at 24 h after NBIC, NM2BIC, or NMXBIC treatment over a range of concentrations (1.95 - 1000 µM). Results were normalized to untreated control cells. **(G)** The antiretroviral half-maximal inhibitory concentration (IC_{50}) in MDM was assessed at 0.1-1,000 nM concentrations by measuring HIV-1 reverse transcriptase (RT) activity in culture supernatants. Results are expressed as the mean ± SEM for N = 3 The experiments were repeated twice.

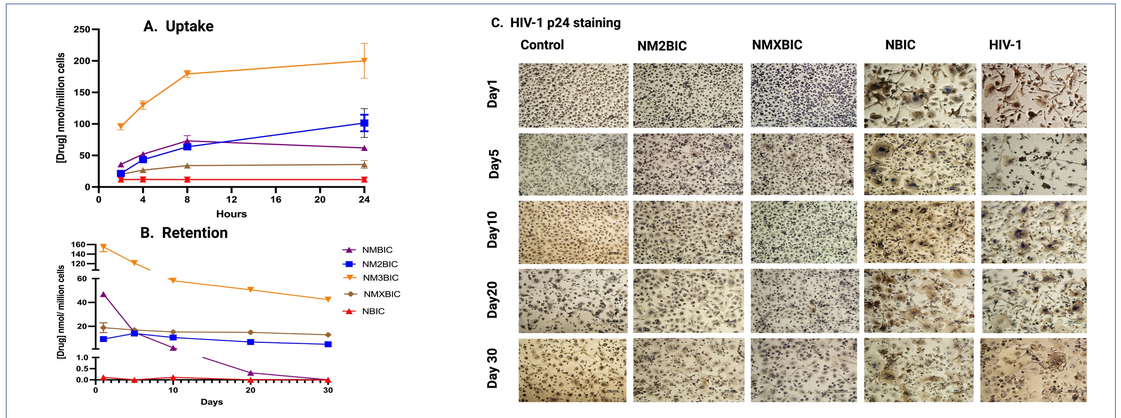


Figure 2: Cellular-Nanoformulated drug interactions at equimolar concentrations. A) Drug uptake was measured over an 8h period after treatment with 25 µM of either formulation **B)** Intracellular drug retention after a single 8 h drug treatment was measured over 30 days. **C)** Antiretroviral responses 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 NM2BIC or NMXBIC or NBIC at 10 µM concentrations for 8 h. HIV-1p24 antigen levels were assessed in fixed MDM by immunohistochemical staining.

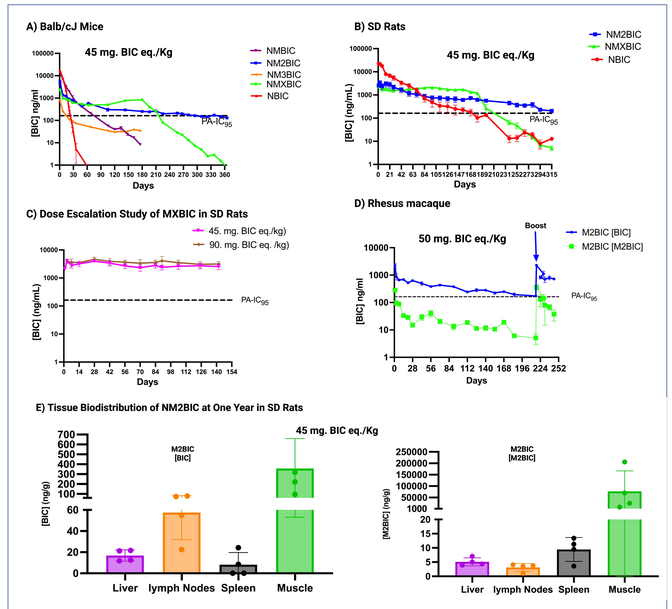


Figure 3: Pharmacokinetics in Balb/cJ mice, Sprague Dawley rats, and Rhesus Macaques. (A) Balb/cJ mice and **(B)** Sprague Dawley rats were administered a single parenteral 45 mg BIC- eq./kg dose of nanoformulated native NBIC, NMBIC, NM2BIC, NM3BIC or NMXBIC. **(C)** Plasma BIC levels in SD rats after a single IM injection of a more concentrated NMXBIC formulation at a dose of either 45 or 90 mg. BIC eq./kg. **D)** Plasma BIC (blue line) and M2BIC (green line) levels in rhesus macaques given a 50 mg BIC-eq./kg IM dose of NM2BIC in the quadriceps muscles, followed by an equivalent booster dose on day 217 (arrow). Plasma samples were collected, and drug levels were determined. **(E)** Tissue biodistribution of NM2BIC in SD rats was assessed at one year. N prefix refers to the encasement of drug into nanoparticles. From day 1 plasma drug levels were monitored weekly. The dotted line is 1X PA-IC_{95} of 162 ng/ml. Drug levels were determined by LC-MS/MS. The animal numbers in each group were N= 5 for rodents and 3 for RM. Standard errors of the mean (SEM) are shown for each recorded value.

CONCLUSION

- Chemical modification of BIC enhanced the drug's hydrophobicity and lipophilicity. The synthesized prodrugs were safe and retained BIC's potency.
- Nanoformulated BIC prodrugs exhibited sustained drug retention in MDM and protected against viral challenge to 30 days.
- The PK profile for NMXBIC demonstrated a shorter terminal phase PK tail.
- The data supports the development of NMXBIC and NM2BIC as a once every six-months parenteral antiretroviral therapy

ACKNOWLEDGEMENT

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