Ganglioside GM3 Functionalized Nanoparticles for Sustained Preexposure Prophylaxis

Björn M. Reinhard[1], Erik B. Schiferle[1], Tiffany K. Chan[2], Chandra N. Roy[3], Wenxu Ge[1], Daniel J. Williams[1], Sonia Zahara[1], Koustav Kundu[1], Andres A. Quinones-Molina[2], Amanda P. Schauer[4], Mackenzie L. Cottrell[4], Zandrea Ambrose[3], Suryaram Gummuluru[2]

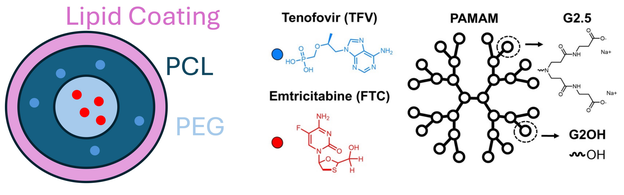
[1]Dept. of Chemistry and The Photonics Center, Boston University, Boston, MA, USA, [2]Dept. of Virology, Boston University, Boston, MA, USA, [3]Dept. of Microbiology and Molecular Genetics, University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA, [3]Department of Pharmacotherapy and Experimental Therapeutics, University of North Carolina Eshelman School of Pharmacy, Chapel Hill, NC, USA

BACKGROUND

Effective PrEP strategies have stringent pharmacological requirements, including high potency, a long half-life that reduces the need for daily dosing, robust tissue penetration both at HIV transmission sites, such as rectal and vaginal mucosa, as well as secondary lymphoid tissue, and low toxicity. Antiretroviral (ARV) loaded nanoparticles (NPs) that selectively target macrophages and install drug depots in these sites can address many of these requirements and provide an innovative strategy for achieving long-acting protection. We have previously demonstrated that GM3 functionalized NPs (GM3-NPs) facilitate targeting of CD169-expressing macrophages and dendritic cells [PMID: 24947940, 34995059]. Here, we evaluate GM3-NPs as vehicles for sustained ARV delivery into rectal and vaginal mucosa, as well as secondary lymphoid tissue in a mouse model.

METHODS

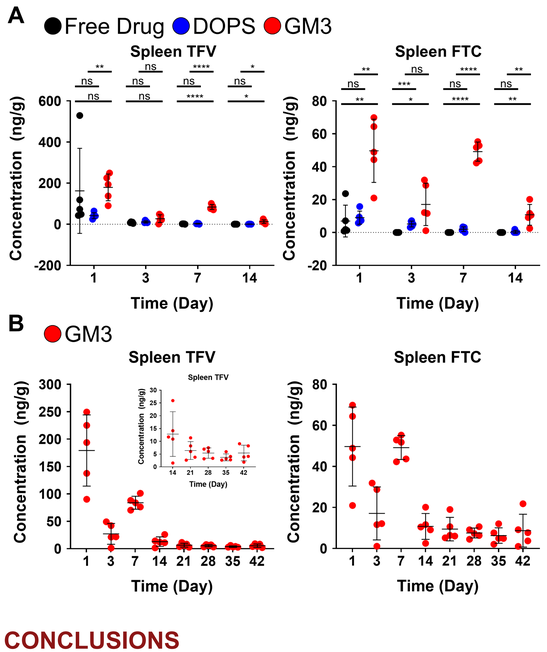
We generated prodrugs of nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) emtricitabine (FTC) and tenofovir (TFV) by covalent attachment to polyamidoamine (PAMAM) dendrimers. Prodrugs were co-loaded into fluorescently labeled lipid (containing either GM3 or DOPS)-coated block co-polymer core NPs and subcutaneously administered to Balb/c mice. Fluorescence and IVIS imaging of lymph nodes and spleen from NP-injected animals were performed on days 1 and 3 post-administration, while NRTI concentrations were measured by mass spectrometry at select time points up to 42 days post inoculation.

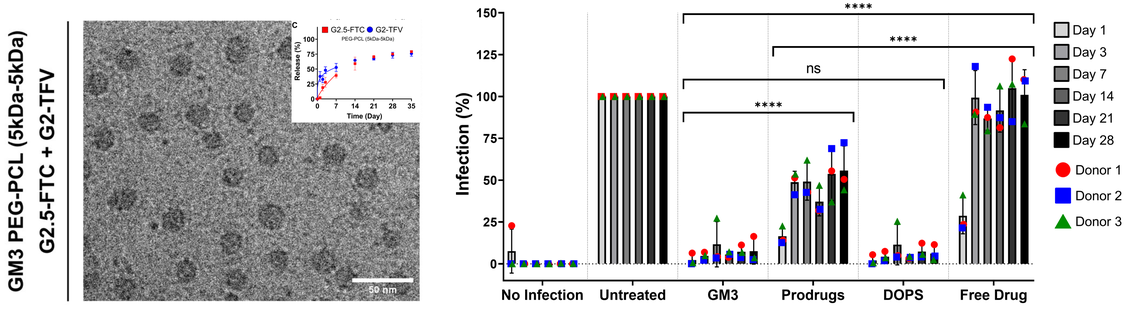


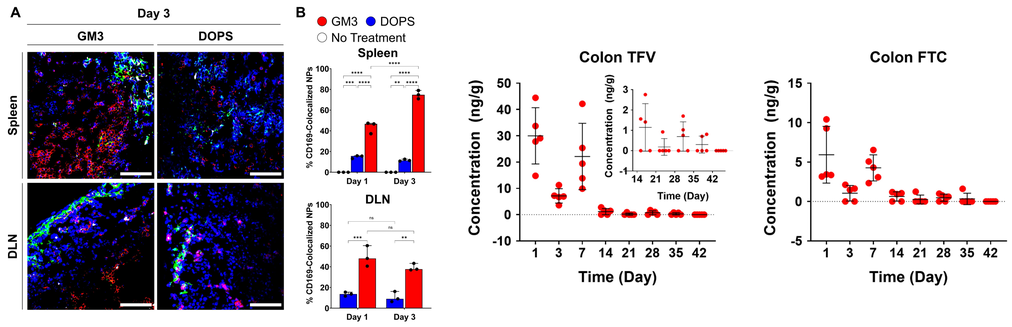
GM3-NPs with block-copolymer core provide sustained release of co-loaded ARV-prodrugs (TFV or FTC loaded dendrimers) and inhibited HIV-1 infection in primary human monocyte derived macrophages for 28 days. GM3-NPs achieved targeting of CD169-expressing myeloid cells in a mouse model, and yielded increased TRF, FTC concentrations in spleen and colon.

RESULTS

GM3-NPs with block co-polymer core achieved a gradual but steady release of FTC and TFV, with retention of 20% of ARV payload up to 35 days. NPs loaded with FTC or TFV prodrugs successfully inhibited HIV infection ($p < 0.0001$) over 28 days in CD169+ monocyte derived macrophages *in vitro*. A direct comparison of the TFV and FTC concentration measured in the spleen after a single administration of GM3-NPs or no GM3 control NPs confirmed that the GM3-NPs achieved significantly higher levels of drugs over a duration of 14 days ($p < 0.05$ (TFV); $p < 0.01$ (FTC) on day 14). Optical colocalization experiments confirmed colocalization of GM3-NPs with CD169 and CD45 expressing cells in the spleen. Importantly, quantification of drug concentrations in spleen and lymph nodes demonstrated detectable TFV and FTC levels for mice injected with GM3-NPs up to 42 days.







CONCLUSIONS

Lipid coated block-copolymer NPs provide a platform for independently tuning i) targeting and ii) loading and release properties. GM3-NPs as carriers of dendrimer-based prodrugs achieve sustained FTC and TFV release and delivery of FTC and TFV to HIV transmission sites. Improving the delivery and release profile of established and well characterized ARVs provides new opportunities for overcoming limitations of well-established ARVs in PrEP.

ADDITIONAL KEY INFORMATION

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PLAIN LANGUAGE SUMMARY

New formulations of established PrEP medications can provide opportunities for overcoming challenges associated with dosing frequency. This work investigated prodrugs of TFV and FTC formulated in polymer nanoparticles as strategy to achieve sustained drug concentrations in spleen and colon.