Lipid Nanoparticles Targeting Neutrophil and NETs to Mitigate Lung Injury in SARS-CoV-2 Mice

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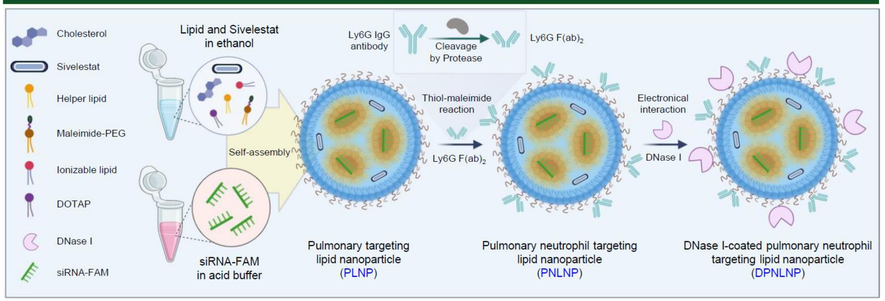
Background

The need to understand key players driving pulmonary inflammation and fibrosis in COVID-19 patients leading to effective preventive strategies is imminent. Excessive neutrophil activation, including extracellular trap (NET) formation, is associated with severe COVID-19 and long-term pulmonary sequelae. However, the clinical applications of neutrophil-targeting therapies are challenging due to short bioavailability and lack of cell-type specificity

Method

The lipid nanoparticle (LNP) platform designed to deliver two NET inhibitors, DNase I and Sivelestat (Siv), referred to as DPNLNPs, specifically to lung neutrophils. The LNP design was optimized by incorporating the cationic lipid into a conventional LNP formulation to enhance lung-specific delivery. To enable neutrophil targeting, Ly6G antibodies were cleaved into F(ab')2 fragments and conjugated to the LNPs. We evaluated the therapeutic efficacy DPNLNP in the K18-hACE2 mouse model.

Schematic illustration of the synthesis of different LNPs



Results

Characterization of DPNLNPs

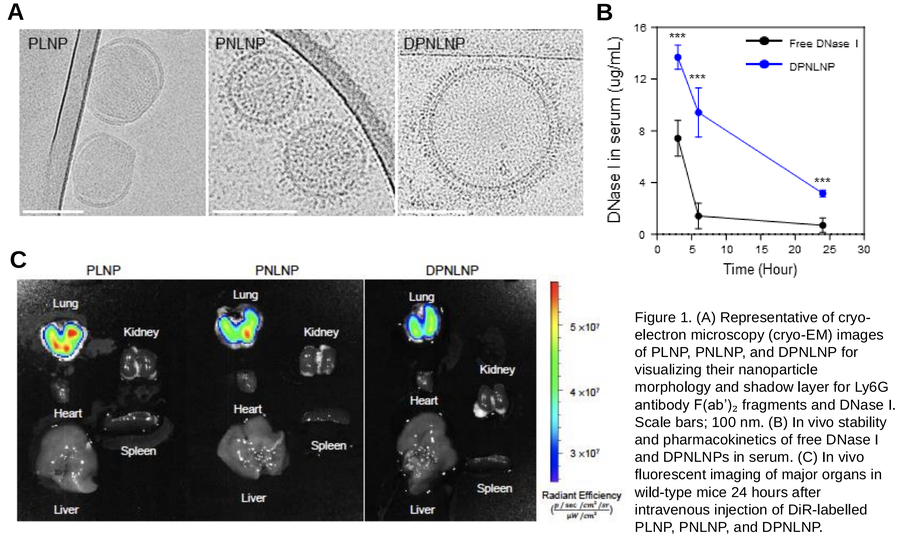


Figure 1. (A) Representative of cryo-electron microscopy (cryo-EM) images of PLNP, PNLNP, and DPNLNP for visualizing their nanoparticle morphology and shadow layer for Ly6G antibody F(ab')$_2$ fragments and DNase I. Scale bars; 100 nm. (B) In vivo stability and pharmacokinetics of free DNase I and DPNLNPs in serum. (C) In vivo fluorescent imaging of major organs in wild-type mice 24 hours after intravenous injection of DiR-labelled PLNP, PNLNP, and DPNLNP.

This study serve as a proof-of concept for adapting the LNP platform to deliver more than the one immunomodulatory drug in a cell-specific manner to manage NET-associated complication in COVID-19 and other respiratory diseases.

Therapeutic effect of DPNLNP treatment on SARS-CoV-2-infected mice

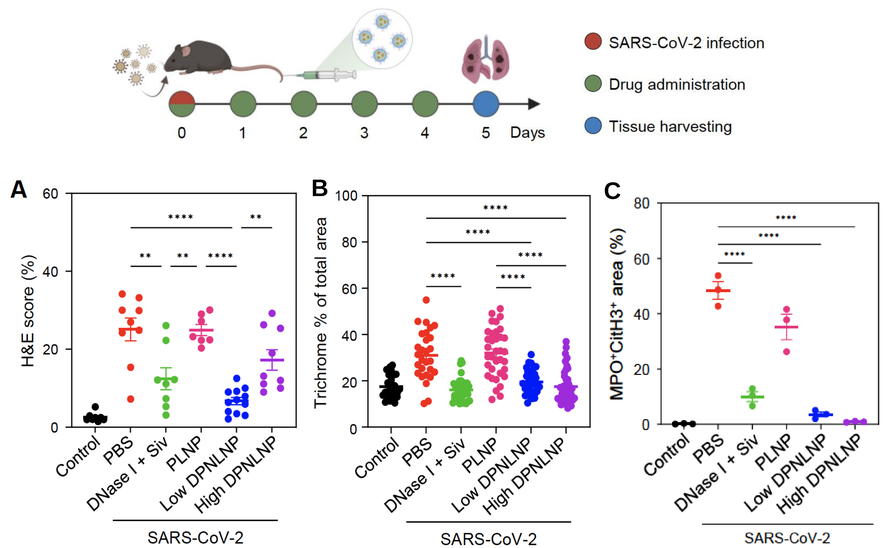


Figure 2. (A-B) Histological analysis of the lungs stained with H&E(A) and Gomori's Trichrome (B) to assess inflammation, tissue damage, and ECM deposition (n = 4 mice per group) (C) Quantification of NET markers by staining for MPO (red) and CitH3 (green) in the lungs from infected mice treated with different LNPs or PBS (n = 3 mice per group, and each data point is the average of counts from 2 different fields from each mouse). (D) The survival of infected mice were monitored daily for 14 days. Kaplan-Meier survival curve in infected mice treated with different LNPs was analyzed by the log-rank (Mantel-Cox) test (n = 10 mice per group).

NET inhibition in the lungs by DPNLNPs alleviates systemic inflammation in SARS-CoV-2-infected mice

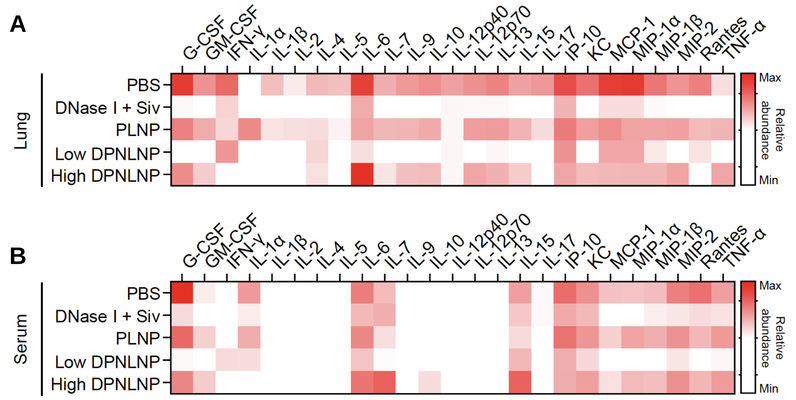


Figure 3. (A-B) The levels of cytokines and chemokines in the lungs (A) and serum (B) from infected mice with different LNP treatments were measured using Luminex assay (n ≥ 3 mice per group). Unsupervised clustering was applied to generate the cytokine/chemokine profile heatmap to illustrate relative levels compared to relevant controls. Data were log10 transformed and normalized.

Identify the therapeutic window of the DPNLNP treatment

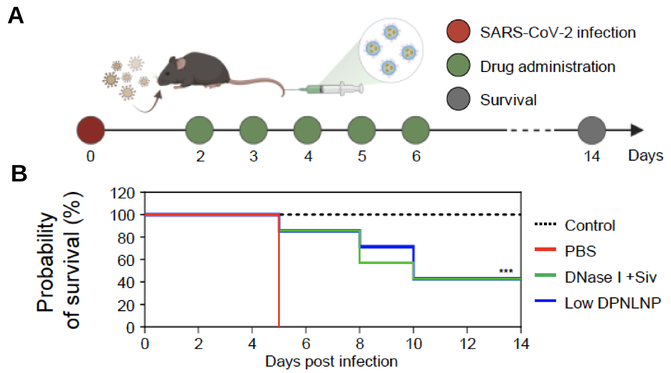


Figure 4. (A) Schematic illustration of the DPLNP treatment schedule in infected K18-hACE2 mice. (B) Kaplan-Meier survival curve analysis of the mice treated with PBS, free DNase I (100 μg) and Siv (5 μg), and Low-dose DPNLNPs (10 μg DNase I and 0.5 μg Siv) daily from D2-D6 (n = 7 mice per group, ***p < 0.001)

Conclusion

Our study show that combinatory treatment by DPNLNPs requires much lower drug dosages to attenuate overall inflammation and improve disease outcomes thus advancing the NET inhibitor treatment strategies. These findings highlight the role of neutrophil-derived NETs in promoting lung injury and systemic inflammation and also provide a novel therapeutic LNP platform for combinatory treatment in a cell-specific manner within the lung.

Acknowledgements

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