Rational Design of Anti-Inflammatory Lipid Nanoparticles for mRNA Delivery

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Statement of Purpose: Lipid nanoparticles (LNPs) play a crucial role in delivering messenger RNA (mRNA) therapeutics for clinical applications, including COVID-19 mRNA vaccines[1]. While mRNA can be chemically modified to become immune-silent and increase protein expression, LNPs can still trigger innate immune responses and cause inflammation-related adverse effects. Inflammation can in turn suppress mRNA translation and reduce the therapeutic effect. Dexamethasone (Dex) is a widely used anti-inflammatory corticosteroid medication that is structurally related to cholesterol, a key component of LNPs. Here, we developed new LNP formulations with anti-inflammatory properties (**Figure 1A**) by partially substituting cholesterol with Dex as a means to reduce inflammation in target cells.

Methods: LNPs were formulated by mixing an aqueous phase containing mRNA and an organic phase containing an ionizable lipid, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPC), PEG conjugated lipid (C14PEG-2000), cholesterol, and dexamethasone in a microfluidic device. To avoid immune activation by the mRNA, purified 1-methylpseudouridine-containing mRNA was used throughout this study. MC3 LNPs in the absence of Dex (C10D0) and Dex-incorporated LNPs (C9D1) were prepared and evaluated in *in vitro* and *in vivo* studies (**Figure 1B, 1C**). LNPs encapsulating mRNA encoding for luciferase – in the presence or absence of Dex – were used to treat HepG2 cells to assess transfection efficiency and cytotoxicity. To verify whether the incorporation of Dex can suppress the immune response triggered by the LNPs themselves, the anti-inflammatory effect of C9D1 LNP on murine macrophages was evaluated. C57BL/6 mice were used to investigate the inflammatory response and mRNA delivery of LNPs.

Results: Replacement of 10% cholesterol with Dex resulted in robust LNP formation with minimal effect on LNP size, polydispersity, and mRNA encapsulation efficiency. LNPs without Dex (C10D0 LNPs) significantly stimulated the production of TNF-α by approximately 2.6 folds in macrophages, while those with Dex (C9D1 LNPs) only marginally increased TNF-α levels. *In vivo*, C10D0 LNP-treated mice showed significantly higher TNF-α levels than untreated mice. Interestingly, the serum TNF-α concentration of C9D1 LNP-treated mice was significantly reduced compared to C10D0 LNP-treated mice (**Figure 1B**). These results suggest that C9D1 LNPs can successfully reduce the inflammatory response triggered by LNPs *in vivo*. In terms of mRNA delivery, strong luciferase expression in the liver was observed for both C9D1 LNP- and C10D0 LNP-treated mice (**Figure 1C**), however quantification of the luminescence signal showed a 1.5-fold increase in mice treated with C9D1 compared to C10D0 LNPs, suggesting that these anti-inflammatory LNPs also act to enhance mRNA delivery.

Conclusions: Dex-incorporated LNPs (C9D1) were successfully prepared and demonstrated potent anti-inflammatory effects. C9D1 LNPs were found to suppress the pro-inflammatory cytokine TNF-α to a near-basal level *in vitro*, and significantly down-regulated TNF-α levels *in vivo* compared to the native C10D0 LNP. Due to the reduced inflammatory responses, the overall mRNA transfection was improved by 1.5-fold in C9D1 LNP-treated mice. Therefore, Dex substitution within LNPs could be a potentially promising strategy to reduce inflammation-related adverse effects of LNPs while enhancing protein expression of mRNA therapeutics.

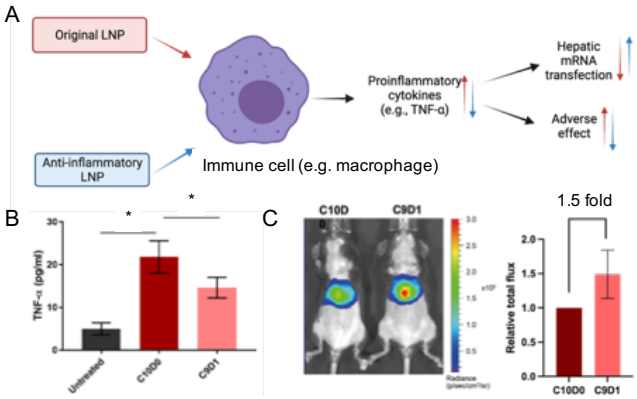

Fig. 1: (A) Schematic illustration of anti-inflammatory LNP to reduce adverse effects and improve mRNA transfection. Anti-inflammatory LNPs containing dexamethasone (Dex) suppress the local inflammation caused by LNPs in immune cells leading to reduced adverse effects and enhanced hepatic mRNA transfection. LNPs are proposed to stimulate immune cells such as macrophages. Dex can reduce the release of pro-inflammatory cytokines (e.g., TNF-α), and thus improve hepatic transfection and minimize the adverse effects of LNPs. (B,C) *In vivo* TNF-α levels and mRNA delivery following treatment with LNPs without Dex (C10D0) and those with Dex (C9D1). (B) Serum TNF-α levels following treatment with C10D0 (middle bar) or C9D1 LNPs (right bar) in mice. Serum was collected 20 h after treatment. Data is presented as mean ± SD (n = 3). *P<0.05. (C) *In vivo* mRNA delivery and luciferase expression following treatment with C10D0 (left mouse and bar) or C9D1 LNPs (right mouse and bar) in mice. For each mouse, 4 μg of LNP-formulated luciferase mRNA was injected intravenously.

Ref: 1. Mitchell et al. Nature Rev Drug Discov. 2021;20:175-196.