Systemic Delivery of mRNA Therapeutics using Lipid Nanoparticles (LNP): Improved Potency for Novel LNP and Influence of Route of Administration on Protein Expression

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INTRODUCTION

Lipid nanoparticles have been successfully used for systemic delivery of RNAi therapeutics (siRNA) with a clinical candidate for the treatment of transthyretin amyloidosis now in Phase III clinical studies. Such delivery vehicles are also well-suited to the delivery of mRNA therapeutics, which require a carrier to facilitate entry into cells and then allow access to the cytoplasm. The cationic lipid component of the LNP is critical for activity and early studies with RNAi therapeutics demonstrated substantial increases in potency for LNPs with cationic lipids having optimal pKa values [1]. We have built on these earlier studies to evaluate LNP systems for systemic delivery of mRNA encoded reporter and therapeutic proteins.

EXPERIMENTAL METHODS

Synthetic Chemistry and LNP Formulation. Synthesis and characterization of cationic lipids was conducted, as described previously [1]. Formulation of LNP carriers and encapsulation of mRNA constructs was based on the process described previously for siRNA, with minor modifications [1].

mRNA Constructs. Messenger RNA constructs encoding firefly luciferase (FLuc) or human Factor IX (hFIX) were synthesized by Trilink (San Diego) or the Weissman laboratory using modified nucleotides to reduce immune activation and increase translation [2].

In-Vivo Studies. mRNA-LNP formulations were administered to mice via various routes of administration. Expression of the reporter protein FLuc was determined either by ex vivo analysis of luciferase activity (Steady-Glo luciferase assay kit, Promega) or by live imaging (IVIS Imaging System, Perkin-Elmer). Expression of hFIX was determined in mouse plasma samples using an ELISA kit (AbCAM).

RESULTS AND DISCUSSION

Characterization of mRNA-LNP

mRNA constructs could be efficiently loaded into LNP with encapsulation efficiencies ranging from 85-95%. The resulting mRNA-LNP formulations were characterized with respect to several biophysical parameters including particle size distribution and mRNA integrity.

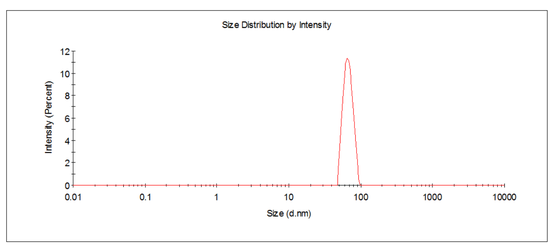

Figure 1. Vesicle size distribution (intensity weighted) for mRNA-LNP containing FLuc determined using Malvern Zetasizer Nano.

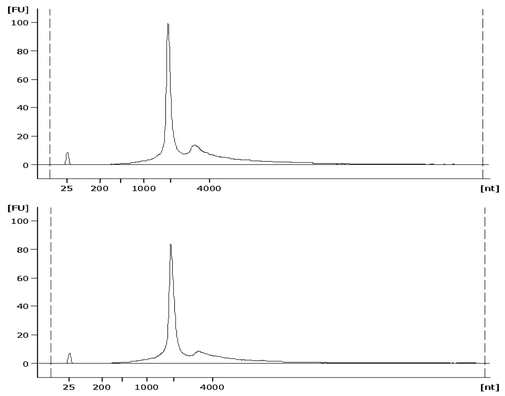

Figure 2. FLuc mRNA Analysis by Agilent 2100 Bioanalyzer. Upper panel shows native FLuc mRNA construct while the lower panel shows FLuc profile after encapsulation in LNPs.

As shown in Figure 1, mRNA-LNPs have a narrow size distribution around approximately 70nm diameter. Further, encapsulation of the mRNA does not result in any degradation or change in the mRNA size profile (Figure 2).

mRNA-LNP Potency In Vivo

Levels of protein expression were compared for mRNA-LNPs containing varying cationic lipids including the benchmark lipid, DLin-MC3-DMA (MC3) [1] and novel Acuitas cationic lipids (ALC-0217 and ALC-0218). These studies were conducted using mRNA encoding either the FLuc reporter protein or the therapeutic protein hFIX.

Following intravenous administration of FLuc mRNA-LNP formulations to mice, luciferase expression in the liver was determined using an ex-vivo analysis at 4 hours post-administration (Figure 3).

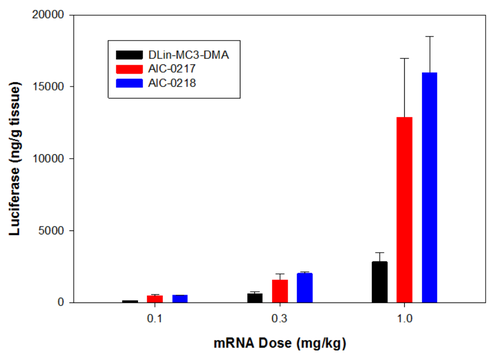

Figure 3. FLuc expression in the liver at 4 hours post-iv administration of FLuc mRNA-LNPs containing DLin-MC3-DMA, ALC-0217 and ALC-0218.

Dose-responsive expression of FLuc was for all LNP formulations tested. In the case of LNP formulations containing the cationic lipids ALC-0217 and ALC-0218, much higher FLuc expression was seen at all doses.

The timecourse of expression of Factor IX following iv administration of hFIX mRNA-LNPs is shown in Figure 4. Consistent with FLuc data, significantly higher levels of protein expression were seen for LNPs comprising ALC-0217 and ALC-0218 compared to systems containing MC3.

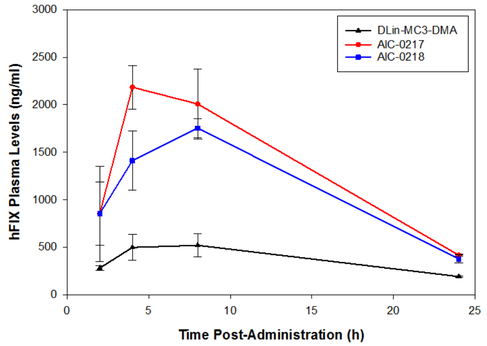

Figure 4. hFIX expression time-course in plasma post-iv administration of LNPs containing DLin-MC3-DMA, ALC-0217 and ALC-0218.

mRNA-LNP Route of Administration

The influence of route of administration (ROA) on the expression profile of FLuc was evaluated. These studies compared the subcutaneous (SC), intradermal (ID), intramuscular (IM), intraperitoneal (IP) and intravenous (IV) routes. Using live-imaging we examined the influence of ROA on the site, level and duration of FLuc expression (Figures 5 and 6).

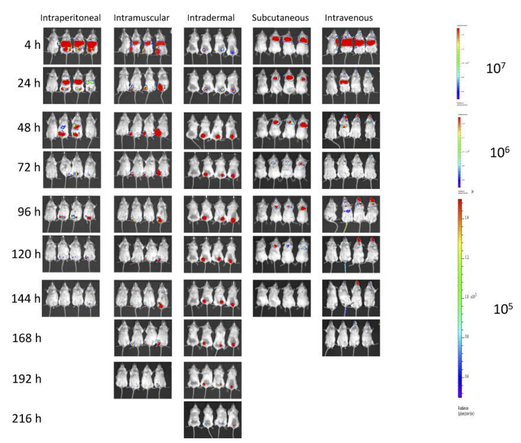

Figure 5. Time-course and site of FLuc expression in mice as a function of the route of administration.

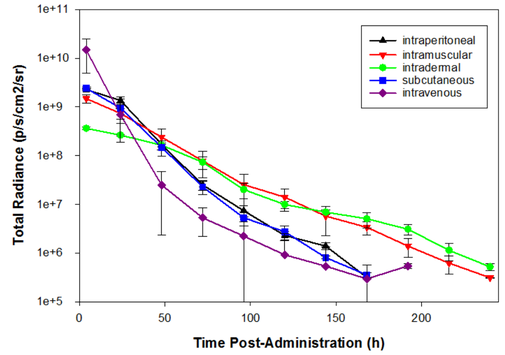

Figure 6. Quantitative FLuc expression time-course up to 10 days for the routes of administration tested.

The highest levels of FLuc expression were seen after iv administration with expression predominantly in the liver. After IP administration, expression is seen at both the site of administration and in the liver. This is consistent with local access to peritoneal tissue with concomitant drainage via the lymphatic system to the blood compartment and subsequent uptake in the liver. Similarly after IM administration, some liver expression is seen however this appears to be at a lower level than for the IP route of administration. SC and ID administration of mRNA-LNP predominantly results in local expression. An extended duration of translation with the SC and ID routes is observed.

CONCLUSIONS

Lipid nanoparticles can be prepared exhibiting a narrow size distribution and stable encapsulation of mRNA constructs. We show that novel cationic lipids (ALC-0217 and ALC-0218) can provide substantially higher levels of reporter protein expression compared to LNP compositions containing benchmark lipids such as MC3. Reporter protein expression is seen following dosing of mRNA-LNP via different routes of administration. Total expression levels are highest following IV administration with luciferase expression predominantly shown in the liver. Substantial levels of luciferase expression are also seen following IP, IM, SC and ID administration with the longest durations of expression seen after IM and ID administration (up to 10 days).

REFERENCES

1. Jayaraman et al. (2012) "*Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo"* Angew Chem Int Ed Engl **51**(34|): 8529-33.

2. Karikó et al. (2012) *Increased erythropoiesis in mice injected with submicrogram quantities of pseudouridine-containing mRNA encoding erythropoietin*. Mol Ther. May;**20**(5):948-53.