Exploring novel lipid nanoparticle compositions for effective delivery of mRNA

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INTRODUCTION

In the past two decades, lipid nanoparticles (LNP) have gained particular interest as carriers for the effective delivery of nucleic acids *in vivo*. Their structure is based on four different lipids: a cationic (or ionizable) lipid, a sterol, a phospholipid, and a PEGylated lipid. The role of an ionizable lipid is to encapsulate the RNA, which is accommodated by the electrostatic interaction of a positively charged cationic lipid's head and negatively charged phosphate group on the RNA chain. Sterols (usually cholesterol) and phospholipids contribute to the cellular uptake of RNA, the LNP structuring, and encapsulation efficiencies. The PEG-lipids protect the LNPs from aggregation in the aqueous environment to increase the storage shelf-life (1,2).

LNPs can be applied to encapsulate various RNA modalities used in preventive or therapeutic medicine, e.g., vaccine development, oncology, etc. There are a few commercially available products that contain either messenger RNA: Spikevax (Moderna), BNT162b2 (Pfizer-BioNTech), or small interfering RNA: Onpattro (Alnylam Netherlands B.V.).

In this work, we divulge an *in vitro-in vivo* screening method to select the best ionizable lipid for intravenous LNP composition, effectively encapsulating a model mRNA and producing superior *in vivo* performance. The LNPs containing various ionizable lipid compositions were screened for three distinct characteristics: (a) physicochemical properties (particle size, polydispersity index, encapsulation efficiency), (b) luminescence intensity after *in vitro* transfection and (c) *in vivo* study on mice. The tested ionizable lipids were designed and synthesized in Celon Pharma S.A.

MATERIALS AND METHODS

Physicochemical properties

Particle size and polydispersity index (PDI) were measured using Zetasizer Ultra (Malvern). Encapsulation efficiency and mRNA concentration were assessed by Ribogreen assay.

In vitro transfection method

Transfection with the LNP containing luciferase as model mRNA was performed using HEK293 cells. The read-out was performed 24 hours after transfection. Naked mRNA and lipofectamine were used as controls.

In vivo study

For each lipid, three mice were taken for the experiment. They were given an LNP-mRNA formulation containing 1µg of luciferase-coding mRNA/mouse. Before the imaging, they received luciferin at 150 mg/kg b.w. intraperitoneally. The mice were then anesthetized with isoflurane and transferred to the imaging chamber of the IVIS Spectrum CT device. The luminescence intensity was measured at the 4th, 24th, 48th, 72nd, and 144th hours of the experiment.

RESULTS

Celon Pharma S.A. synthesized and screened over 150 different ionizable lipids. All the lipids were stable in ethanol solution for at least 24 hours at room temperature required for the downstream formulation process. Next, LNP formulations were prepared by microfluidics. Each formulation contained the same sterol, phospholipid, and PEG-lipid but differed in ionizable lipid. The lipid ratio was kept fixed for all formulations. LNPs were then analyzed in terms of particle size, PDI, EE (encapsulation efficiency), and luminescence intensity after *in vitro* transfection. The best formulations (PDI<0,2; *in vitro* luminescence>10^7) were selected for *in vivo* study. Here, we reveal the results for 7 different lipid formulations. Their physicochemical properties and the total luminescence intensity measured from the in vitro transfection assay are shown in Table 1.

Formulation number	LNP513	LNP514	LNP515	LNP516	LNP517	LNP518	LNP519
Size (nm)	66	64	66	86	67	62	65
PDI	0,081	0,056	0,038	0,091	0,032	0,077	0,074
EE (%)	77,9	86.6	93,8	87,4	93,0	85,4	83,4
Luminescence intensity *in vitro*	4,6E+07	2,1E+08	1,5E+08	1,7E+08	5,0E+07	2,6E+08	5,5E+07

Table 1. Particle size, polydispersity index (PDI), encapsulation efficiency (EE), and luminescence intensity *in vitro* for LNPs number 513-519, used in *in vivo* study.

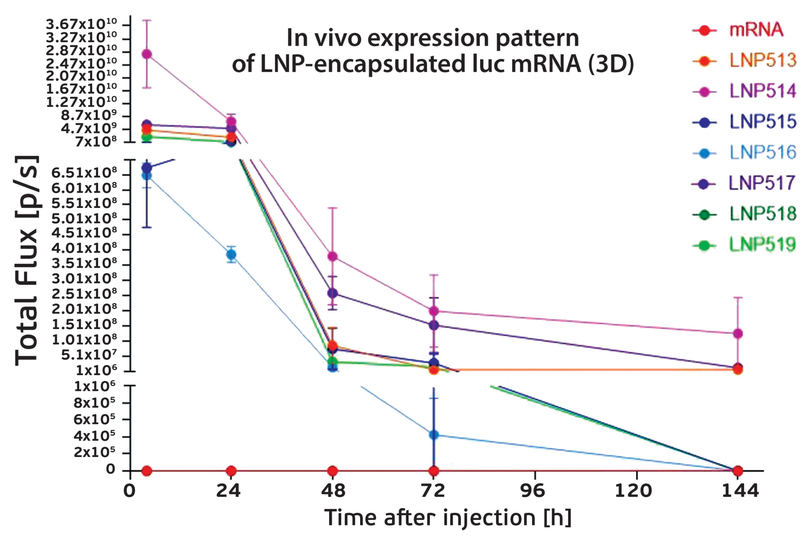

Figure 1. Luminescence intensities of LNP compositions containing different ionizable lipids after intravenous administration. One formulation contained a naked mRNA (red line).

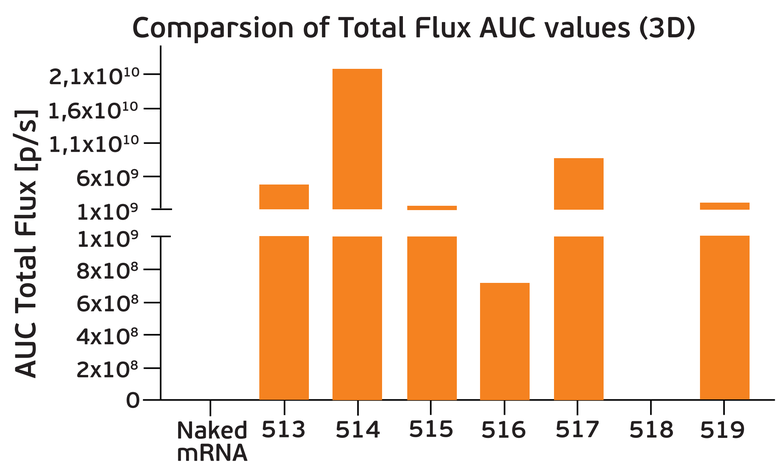

Figure 2. AUC of luminescence intensity of LNP compositions containing different ionizable lipids after intravenous administration.

All of the tested compositions were homogeneous (PDI<0,1) and had similar particle sizes, ca. 65 nm (apart from LNP516 with the result of 86 nm). The encapsulation efficiency was over 70%, clearing an acceptable criterion for starting the animal study. The distinct formulation compositions exhibited various in vitro luminescence intensities ranging from 4,6E+07 to 2,6E+08.

The formulations were further injected into mice according to the protocol described in the METHODS section. The luminescence intensity upon time for the tested compositions is shown in Fig. 1. We further calculated the areas under the curve (AUC) as a surrogate for the total in vivo response (Fig. 2). Strikingly, the AUC was higher for the compositions LNP514 and LNP517 compared to the reference sample (LNP513), which was the LNP composition formulated with the D-Lin-MC3-DMA lipid. The rest of the compositions exhibited similar (LNP515, LNP519) or lower (LNP516, LNP518) AUC values in comparison to the reference (LNP513).

Additionally, we were unable to draw a consistent correlation among the *in vitro* and *in vivo* data. The LNP517 formulation exhibited comparable *in vitro* and *in vivo* responses to the control LNP513. By contrast, the LNP518 and LN516 exhibited high in vitro response yet significantly inferior or moderate performance *in vivo*. In summary, the superior *in vivo* performance of the LNP514 and LNP517 compared to the reference (LNP513 containing D-LIN-MC3-DMA lipid) is a promising outcome that requires further investigation to validate the mechanism behind the improved efficiency.

CONCLUSION

In the presented case study we reported a thorough in vitro-in vivo screening method to discover novel ionizable lipids that can be utilized to formulate unique LNP-mRNA compositions for intravenous injection. The analysis of physicochemical properties and luminescence intensity after in vitro transfection served as a primary screening toolbox. Nevertheless, due to the lack of precise correlation between the in vitro and in vivo response, intravenous administration remains a more powerful screening approach to measure the LNP-mRNA composition superiority performance.

REFERENCES

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MEDICAL RESEARCH AGENCY

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