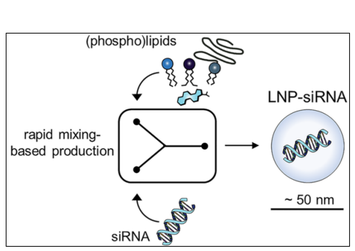
Identifying lipid nanoparticle formulations for nasal RNA delivery targeting the murine respiratory mucosa

A Tam[1,2,3,4], J Kulkarni[1,2], KYT Chan[1,2], L Li[3], DR Dorscheid[4], GK Singhera[4], DD Sin[4], CJ Lim[3], GSD Reid[3], PR Cullis[1,2], D Witzigmann[1,2]

Nanomedicine Innovation Network[1], NanoVation Therapeutics Inc.[2], BC Children's Hospital Research Institute[3], Centre for Heart Lung Innovation[4], University of British Columbia, Vancouver, BC, Canada

Introduction

Lipid nanoparticles (LNP) have been successfully used as platform technology for delivering nucleic acids to the liver. To broaden LNP`s application in targeting non-hepatic tissues, we propose the development of an LNP-based gene silencing RNA (siRNA) therapy for the respiratory tract. Such optimized LNP systems could offer an early treatment strategy for respiratory viral infections such as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).



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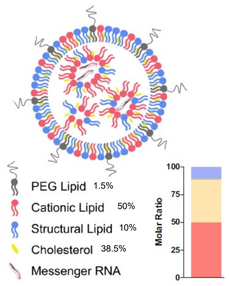

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Hypothesis

Lipid nanoparticles (LNP) can transfect human airway epithelial cells and murine cells that coat the respiratory tracts; thus, offering important clinical utilities for treatment of respiratory infections.

Methods & Materials

- **We first generated a library of LNP formulations with varying helper lipid compositions and characterized their hydrodynamic diameter, size distribution and cargo entrapment properties. (3-4).**
- **Next, we screened these LNP formulations for particle uptake and evaluated their potential for transfecting green fluorescence protein (GFP) or SARS-CoV2 nucleocapsid-GFP fusion reporter gene in a human airway epithelial cell line in vitro.**
- **Following LNP-siGFP delivery GFP protein knockdown efficiency was assessed by flow cytometry to determine %GFP+ cells and median fluorescence intensity (MFI) for GFP.**
- **The potency of lead LNPs candidates was further validated in mice via intravenous (as positive control) and intranasal delivery of a luciferase encoding mRNA.**
- **Gene expression in the nasal and lung cavity was assessed using the bioluminescence in vivo imaging system.**

Results

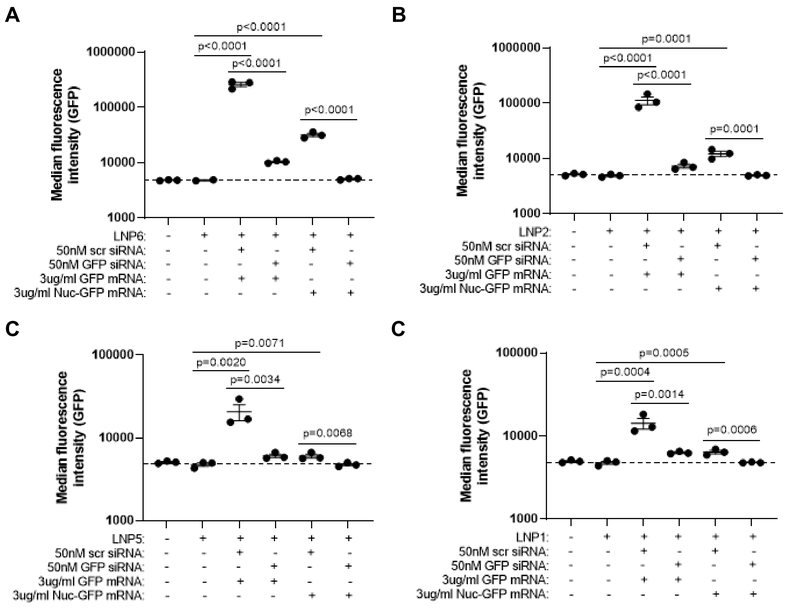
Table 1: Physical properties of lipid nanoparticles (LNP) for *in-vitro* screening

LNP formulation	charge	membrane	Hydrodynamic size (nm)	Size distribution (PDI)
LNP 1	neutral	rigid	64.09	0.061
LNP 2	neutral	fluid	68.95	0.114
LNP 3	neutral	rigid	66.23	0.064
LNP 4	negative	fluid	77.36	0.086
LNP 5	neutral	fluid	72.31	0.047
LNP 6	negative	rigid	73.90	0.110

Table 2: Ranking of LNP by GFP transfection efficiency in 1HAEo cells

Conditions	% GFP	MFI GFP	% DiD	MFI DiD	rank
Empty lipid carrier	1.63	5,242	NA	NA	NA
Lipo-GFP mRNA	43.2	23,673	NA	NA	7
LNP1-GFP mRNA	72.7	14,228	99.98	50,008	6
LNP2-GFP mRNA	92.5	112,337	99.93	241,905	2
LNP3-GFP mRNA	85.8	47,556	100	234,195	4
LNP4-GFP mRNA	91.5	39,066	99.98	86,880	3
LNP5-GFP mRNA	84.9	20,580	99.97	114,048	5
LNP6-GFP mRNA	96.5	260,930	99.98	235,359	1

Figure 1. Transfection and knockdown efficiency of GFP or Nuc-GFP mRNA using GFP siRNA encapsulated in the top 2 (A-B) and bottom 2 (C-D) LNPs identified in Table 2 in 1HAEo cells when assessed by flow cytometry.



Results

Figure 2. Intravenous (IV) delivery of LNP-luciferase mRNA as positive control for liver transfection *in-vivo*.

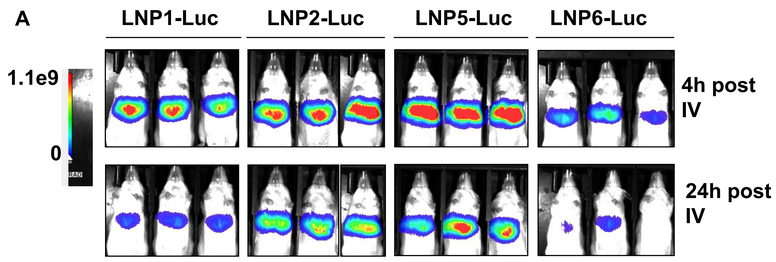
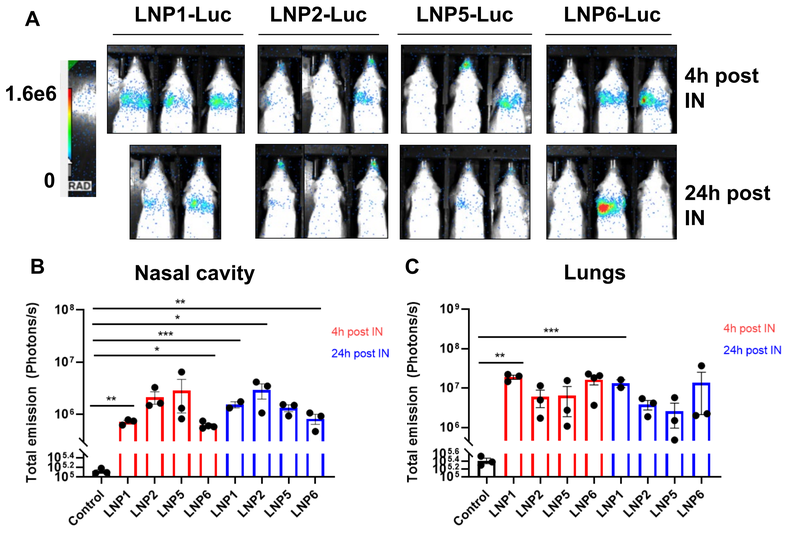


Figure 3. Intranasal delivery of LNP-luciferase mRNA target the nasal cavity and lungs in *in-vivo*.



Conclusion

LNP formulations enable the delivery of RNA payloads into human airway epithelial cells, and in the murine respiratory system via intranasal delivery. We found that changing the helper lipid type can enhance transfection efficiencies, suggesting that for optimal RNA delivery to a specific target tissue the LNP composition needs to be optimized.

References

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3. Kulkarni et al. ACS Nano. 2018;12(5):4787-95.
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