DRUG DELIVERY

Lipid nanoparticles for the inhalation of mRNA

Lipid nanoparticles can be optimized for the efficient delivery of mRNA via nebulization.

Rachel Yoon Kyung Chang and Hak-Kim Chan

It is now clear that messenger RNA (mRNA) encoding the spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) delivered by lipid nanoparticles (LNPs) triggers the efficient protection of lungs from infection by the virus. Generally, mRNA therapeutics could also be used to treat or prevent cystic fibrosis, lung cancer, asthma and other lung diseases[1,2]. LNPs encapsulating mRNAs provide the nucleic acids with protection against degradation by ribonucleases, facilitate their uptake by cells, are largely non-immunogenic and allow for the controlled release of the encapsulated therapeutic[3]. The most direct route (and probably also the safer and most efficient) for administering LNP-encapsulated mRNAs to the lungs is via inhalation, as it maximises the concentration of the therapeutic in these organs and limits its systemic exposure. Writing in *Nature Biomedical Engineering*, James Dahlman, Philip Santangelo and colleagues[4] now describe a screening approach for the optimization of LNPs so that mRNA can be delivered to the lungs in the form of nebulized aerosols.

Dahlman and co-authors employed a cluster-based approach to formulate LNPs by analyzing the effects of different chemical compositions and molar ratios of the nanoparticle constituents — main lipids, neutral or cationic helper lipids, and poly(ethylene glycol) (PEG)) — on the efficiency of nebulized delivery of mRNA to the lungs of mice (Fig. 1). The authors produced LNPs microfluidically and used a previously optimized oligomer–lipid conjugate (termed 7C1) as a main lipid as well as mRNA encoding the enzyme nanoluciferase. They formulated and tested monodisperse LNPs with a diameter smaller than 200 nm in vivo by quantitating the luminescence emitted 48 h after the LNPs were delivered to the lungs of mice via an exposure chamber designed for the noses of the animals (incidentally, nose-only inhalation chambers have a low delivery efficiency, as only about 1.4% of the aerosolized mass is effectively available for inhalation[5]; hence, they require large quantities of aerosolized material). An initial screening round involved the production of six 'extreme groups' of LNPs: LNPs with a high molar fraction of neutral lipid, of 7C1 and neutral lipid, of 7C1 and cholesterol, of cationic helper lipids, of PEG, or of 7C1 and PEG. LNPs with a high fraction of helper neutral lipid or of cholesterol, and hence a low fraction of PEG and (non-helper) lipids, had structural instability, which suggested that the presence of PEG and main lipids is crucial for producing stable 7C1-based LNPs. LNPs with a higher fraction of PEG or cationic helper lipids led to, respectively, the highest and second highest levels of luminescence in the lungs of the mice. To fine-tune the LNP formulation, the authors tested formulations with cationic and neutral helper lipids, cholesterol, 7C1 and PEG at varying molar ratios. For LNPs formulated with cationic helper lipids, LNP size was dependent on PEG–lipid content, with larger LNPs forming when the molar fraction of PEG was low. When administered to the animals through inhalation, LNPs formulated with a higher PEG content led to greater luminescence than those with a low molar fraction of PEG, which confirmed that PEG is a crucial excipient for efficient in vivo mRNA delivery. For LNPs formulated using neutral lipids, the authors tested LNPs formulated with PEG–lipid conjugates of two polymer lengths (chains of 14 or 18 carbon atoms), cholesterol and 7C1. Again, LNP size was dependent on the molar ratios of the PEG–lipid conjugate. Greater luciferase expression was observed for LNPs containing a low molar fraction of PEG and for those with a

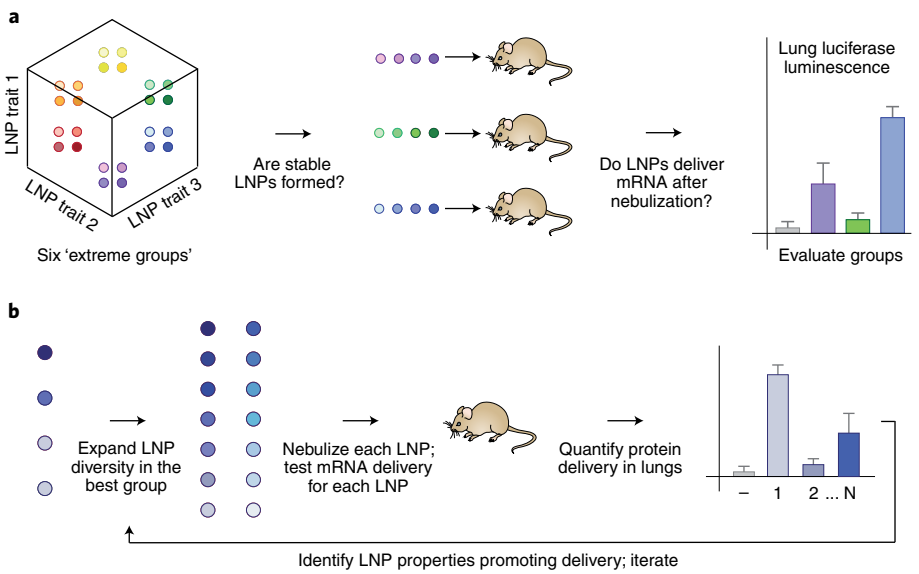

Fig. 1 | A cluster-based approach for the optimization of mRNA-encapsulating LNPs. a, The multidimensional design space of nebulized formulations of LNPs can be defined by 'chemical traits' (three are schematically depicted), such as the molar fraction of cationic helper lipids or the structure of the PEG–lipid conjugates, and can be efficiently explored by first characterizing the stability and delivery efficiency in vivo (via luminescence levels from the lungs of mice that inhaled LNPs encapsulating mRNA encoding nanoluciferase) of combinations of LNP formulations at the 'extremes' of the space (indicated by the coloured circles). **b**, Promising 'extreme' formulations are then 'expanded' around their vicinity in chemical space and characterized, which informs the subsequent optimization steps. Figure reproduced with permission from ref. [4], Springer Nature Limited.

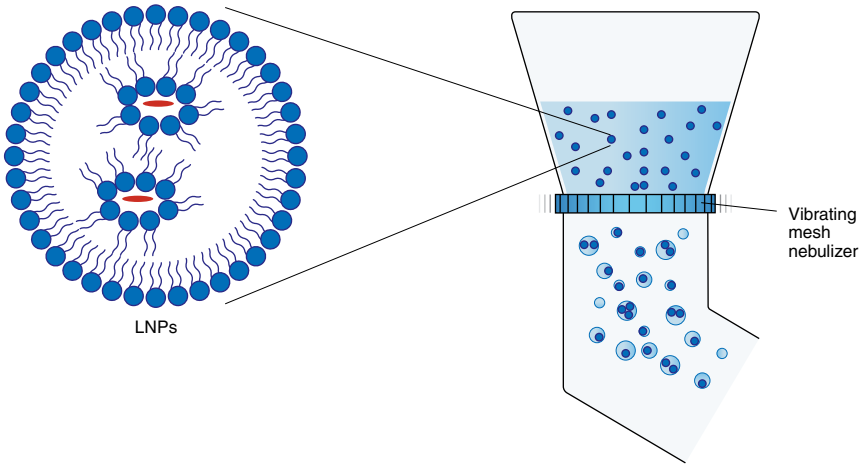

Fig. 2 | Vibrating mesh nebulizers influence the properties of LNP formulations. The design of the device can affect the structural and physicochemical stability of the nanoparticles and hence of the encapsulated payload (red), the average size of the nanoparticles and their size distribution, and the efficiency of encapsulation of the payload.

longer polymer. On the basis of all the data gathered, the authors arrived at three design rules: the presence of a PEG–lipid conjugate is crucial to the formation of stable LNPs, nebulized mRNA delivery can be improved with the use of cationic helper lipids and a higher molar fraction of PEG, and more PEG is needed for formulations with cationic helper lipids than for those with neutral lipids.

Dahlman and colleagues' optimized LNPs consisted of 7C1, cholesterol, PEG with 14 carbon atoms, and the helper lipid DOTAP (1,2-dioleoyl-3-trimethylammonium-propane) at molar fractions of 35%, 5%, 55% and 5%, respectively. Compared with LNPs designed for systemic delivery[6,7], the optimized LNPs exhibited greater structural stability after nebulization and substantially higher luminescence in mouse lungs. Also, the optimized LNPs were mostly uniformly distributed throughout the lung tissue of the treated mice, with preferential accumulation at alveolar spaces. The researchers also show that mRNA could be detected in lung epithelial cells (ciliated and club bronchial) and in alveolar type-I and type-II cells, that the treated mice did not experience any adverse events and that mRNA expression could not be detected in organs other than the lung. Luminescence was detected for up to seven days after exposure, with the highest luminescence levels in the lungs measured two days after nebulized delivery. Moreover, they show that LNPs encapsulating mRNA encoding a broadly neutralizing antibody and delivered to the mice by inhalation a few days before the animals were inoculated with a lethal dose of influenza A virus protected all of the animals from death.

Nanoparticles dispersed into inhalable aerosol droplets experience shearing stress, which can cause the particles to fragment[8], affecting the stability of the payload, particularly for hydrophilic molecules such as mRNA[9]. Although nebulized LNPs can maintain their size and charge via appropriate formulation design, significant drops can occur in mRNA encapsulation efficiency (from higher than 80% to around 40%; ref. [8]). In general, vibrating mesh nebulizers (Fig. 2), which generate aerosols through a perforated plate with micrometric apertures that vibrates at frequencies of ~100 kHz (ref. [10]) and which Dahlman and colleagues used, are gentler on LNPs than air-jet and ultrasonic systems. However, vibrating mesh nebulizers typically lead to increases in the measured size of the LNPs, which probably reflects structural LNP damage. Nebulizers with larger mesh apertures minimize LNP damage[11] and improve the retention of their payload; however, larger mesh apertures imply larger droplet sizes and hence a lower density of inhalable fine droplets. Newer nebulizers relying on surface acoustic waves are not subject to these issues[12,13].

Dahlman and co-authors' LNP-design approach is in principle adaptable for the delivery of other types of RNA and nucleic acids and for LNPs formulated as inhalable dry powders[14,15]. As gene therapies advance to the clinic, strategies for the local administration of therapeutics to the lungs via inhalation of nebulized or powdered formulations will become increasingly important for the treatment of respiratory diseases. Naturally, formulations developed for systemic delivery cannot simply be adopted for inhalation, and formulations developed for inhalation are also subject to their own stability, manufacturing, scale-up and long-term-storage challenges. Plenty of optimization lies ahead. ❐

Rachel Yoon Kyung Chang and Hak-Kim Chan ✉

Advanced Drug Delivery Group, Sydney Pharmacy School, Faculty of Medicine and Health, The University of Sydney, Sydney, New South Wales, Australia.

✉*e-mail: kim.chan@sydney.edu.au*

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Competing interests

The authors declare no competing interests.