Helper lipids: impact on immune responses and storage stability of self-amplifying RNA (saRNA) lipid nanoparticle vaccines

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Background: The potential of RNA vaccines as a therapeutic platform has been greatly strengthened by the current pandemic of SARS-CoV-2. The first vaccines to be approved against COVID-19 were based on mRNA-encoding antigens that were encapsulated by lipid nanoparticles (LNPs). LNPs are a type of formulation that contains 4 components: an ionisable lipid, a helper lipid, cholesterol, and a polyethylene glycol (PEG)-lipid. Helper lipids contribute to the stability and delivery efficiency of the formulations. Although many studies focus on the development of novel ionisable lipids to improve and diversify LNP therapeutic applications, less attention has been paid to the role of helper lipids in these formulations. In this study, we have sought to investigate the effect that three different helper lipids have on the elicited immune response, as well as on the LNP's physicochemical characteristics and storage stability.

Methods: LNPs were formulated with two different ionisable lipids (MC3 or C12-200), and each of those was formulated with one of three different helper lipids (DSPC, DOPC, or DOPE) –plus cholesterol and PEG. LNPs' physicochemical characteristics and cell transfection efficiency (via luciferase assay) were analysed on the day of formulation and kept at 4 °C for storage stability analysis for 4 weeks. To investigate the effect on immune responses, mice were immunised with a prime and boost vaccines 21 days apart, at a dose of 1, 0.1, or 0.01 µg of saRNA encoding for SARS-CoV-2 spike protein. Antibody production was analysed by enzyme-linked immunosorbent assay (ELISA) and interferon (IFN)-γ cytokine responses were analysed by enzyme-linked immunosorbent spot (ELISpot).

Results: All LNPs had similar physicochemical characteristics in terms of size and zeta potential. However, the choice of helper lipid had a significant impact on storage stability. LNPs with DSPC had relatively stable levels of transfection over 4 weeks. On the contrary, LNPs formulated with DOPC had an initial high transfection level but dropped down over the course of the weeks. Finally, LNPs with DOPE showed the poorest stability profile, with levels of transfection efficiency dropping 100- to 1000-fold by the end of the study. In terms of *in vivo* immune responses, animals immunised with C12-200 LNPs had almost 10-fold higher antibody titers compared to MC3 LNPs. When comparing different helper lipid formulations, MC3 with DSPC had slightly higher IFN-γ producing cells, but antibody titers were similar between all three formulations. For C12-200 formulations, DSPC had lower antibody titers compared to DOPC and DOPE and, differently from other formulations, C12-200 DOPC had similar levels of IFN-γ ELISpots in all three doses administered to animals (1, 0.1, 0.01 µg).

Conclusions: Overall, this study shows that, even though the immune responses elicited by different saRNA-LNP formulations were not so obviously different, they do tend to have slight differences between groups that might impact the desired outcome for each different vaccine. Also important is the fact that the choice of helper lipid seems to have a significant impact on the storage stability of these formulations. Such parameter may be relevant to product distribution and storage in different global regions. This may improve one of the main hurdles when it comes to accessing and distributing vaccines equally worldwide.