Nebulization of siRNA-containing lipid-based delivery vectors produced by microfluidic mixing

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Background: Small interfering RNA (siRNA) has enormous therapeutic potential owing to its ability to inhibit gene expression via the phenomenon of RNA interference. Pulmonary delivery is an effective, non-invasive administration route due to the lungs' large surface area and the potential to deliver siRNA directly to the site of action for lung-specific conditions. Nebulization, in particular, is an attractive method as it can deliver aerosolized drug during tidal breathing and in larger dose quantities compared to other inhalation devices. The aim of this study was to investigate the impact of nebulization on the physical properties (size and encapsulation efficiency) of several siRNA-containing liposomal and LNP formulations produced via microfluidic mixing.

Methods: siRNA formulations were manufactured using a microfluidic staggered herringbone mixer by injecting an aqueous buffer phase containing non-targeting, negative control siRNA and an organic phase containing lipid components dissolved in ethanol. A Flow Rate Ratio (FRR) of 5:1 (aqueous: organic phase) was used with a Total Flow Rate (TFR) of 3.6ml /min. Three formulations were produced: 1) non-PEGylated liposomes comprised of DOTAP:Cholesterol, 2) PEGylated liposomes (DOTAP:Cholesterol: DMPE-PEG2000) and 3) LNPs (C12-200:Cholesterol: DSPC: DMPE-PEG2000). siRNA was encapsulated at a nitrogen: phosphate (N:P) ratio of 5:1. Samples were nebulized via direct pipetting into the reservoir of an Aerogen Pro Vibrating Mesh Nebulizer and collected in a 2ml Eppendorf tube. Samples were tested prior to and post nebulization for size using a DLS Zetasizer Nano and encapsulation efficiency using the RiboGreen RNA quantification assay.

Results: On average, the size diameter of the non-PEGylated liposomes was found to be considerably larger than that of the PEGylated liposomes and LNPs. Nebulization also had a significant impact on the physical properties of all three formulations resulting in a notable increase in both size and PDI. The LNPs, displayed desirable characteristics with an approximate size of 82 nm or less, a maximum PDI of 0.24 and satisfactory batch to batch reproducibility. The LNPs also remained of a sufficiently small size post nebulization for siRNA delivery (<200 nm). The siRNA encapsulation efficiency of all three formulations was high at ≥98%. Post nebulization encapsulation efficiency was ≥78% demonstrating that the lipid carriers remained sufficiently stable after nebulizing to retain a large proportion of their siRNA cargo.

Conclusions: This study has demonstrated the potential of lipid-based carriers as stable vectors for siRNA delivery via nebulization. The important role of a PEG-lipid in helping to achieve an appropriately small particle size is also apparent. The LNP formulation chosen (C12-200:Cholesterol: DSPC: DMPE-PEG2000) is particularly promising given the results of its physical characterization pre and post nebulization. Further experiments are warranted, to explore the feasibility of these formulations for nebulized siRNA including aerodynamic droplet size distribution analysis and A549 lung cell transfection studies.