Development of lipid nanoparticle-enabled gene therapy approaches in the brain

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BACKGROUND

Brain diseases are a significant burden to the Canadian health care system, and can be caused by both heritable and sporadic genetic mutations. Many genetic neurodevelopmental and neurodegenerative diseases are caused by either the toxic gain-of-function of a mutant protein, or a loss-of-function mutation.[1,2]

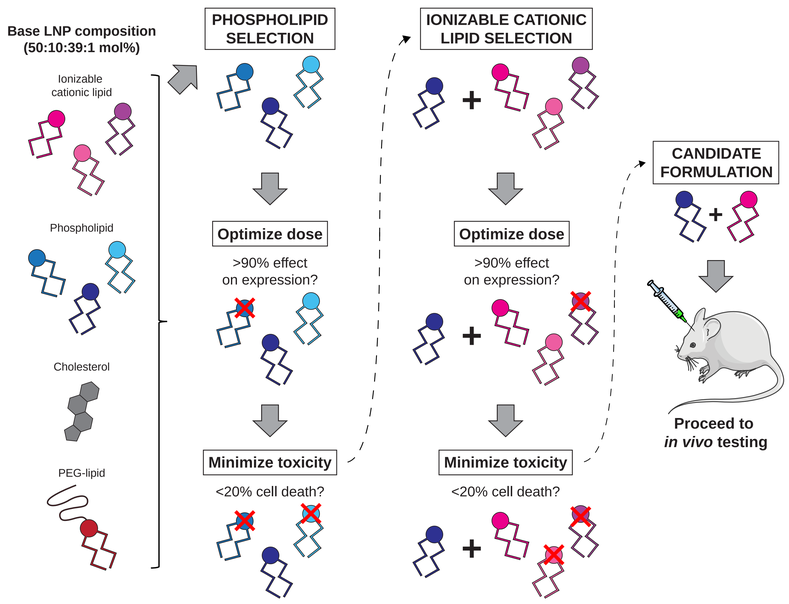
- Brain gene therapy agents must be efficiently delivered to and effective in neurons (the primary cells of interest in the brain)
- Current brain gene therapy approaches are limited by toxicity and immunogenicity
- Neurons are highly amenable to transfection by lipid nanoparticles (LNPs), and LNPs are safe and effective for the treatment of other genetic diseases[3-6]
- In vivo LNP administration will be achieved by direct injection into cerebrospinal fluid or brain tissue, so LNP formulation screening in primary neurons ex vivo will likely translate accurately in vivo

PURPOSE

To identify and optimize lipid nanoparticle formulations and doses for the delivery of gene therapy payloads ex vivo and in vivo to treat genetic brain diseases.

EX VIVO SCREENING STRATEGY

Workflow to optimize LNP formulation and dosage for the efficient delivery of siRNA or mRNA to primary neurons ex vivo.



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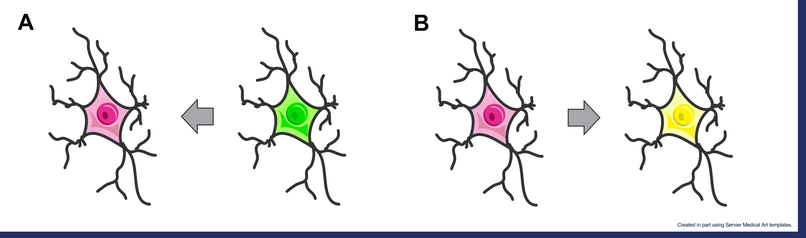
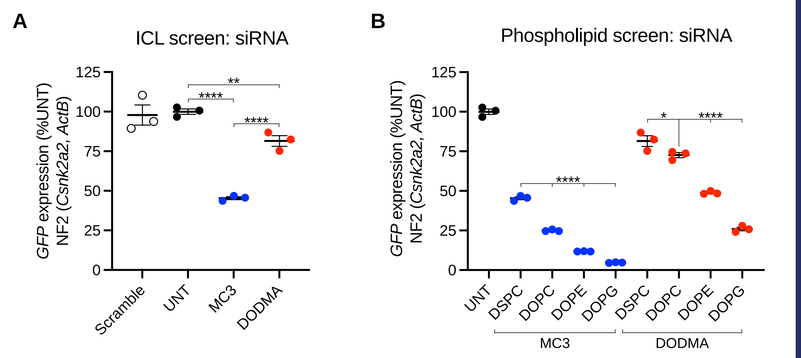
REPORTER SYSTEMS

LNP formulations containing either GFP siRNA (A) or luciferase mRNA (B) were tested. Reporter systems were used to avoid disrupting endogenous gene expression.



RESULTS: siRNA

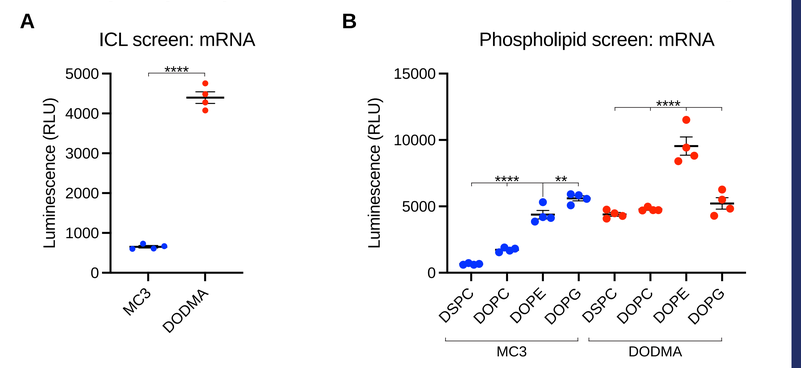
The formulation prepared using the ionizable cationic lipid MC3 and the phospholipid DOPG is most effective for siRNA delivery.



At a dose of 0.01 µg/mL GFP siRNA, the LNP formulation containing the ionizable cationic lipid MC3 is more potent than the formulation containing DODMA (A). LNP formulations containing the phospholipid DOPG are most effective for siRNA delivery, regardless of ionizable cationic lipid type.

RESULTS: mRNA

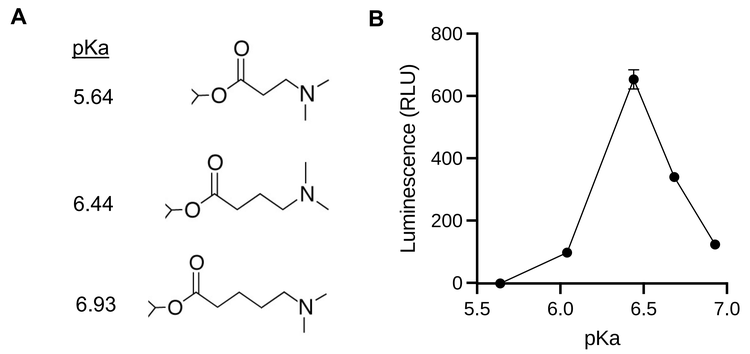
The formulation prepared using the ionizable cationic lipid DODMA and the phospholipid DOPE is most effective for mRNA delivery.



At a dose of 0.3 µg/mL luciferase mRNA, the LNP formulation containing the ionizable cationic lipid DODMA is more potent than the formulation containing MC3 (A). The effect of phospholipid on LNP mRNA formulation potency changes with the ionizable cationic lipid type (B).

RESULTS: EFFECT OF ICL pKa

Ionizable cationic lipid pKa significantly impacts formulation potency.



The ionizable cationic lipid series shown in (A) was used to create a series of five LNP formulations with a range of pKa values. At a dose of 0.3 µg/mL luciferase mRNA, significant variation in formulation potency was observed.

FUTURE DIRECTIONS

- Explore the mechanisms that contribute to observed differential formulation efficacy ex vivo.
- Evaluate the performance of LNP formulations optimized for neuronal delivery of siRNA or mRNA in other primary brain cell types.
- Evaluate the performance of ex vivo-optimized LNP formulations in the brain in vivo.

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