Intranasal delivery of siRNA targeting ApoE4 as potential treatment of Alzheimer's disease

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Background:

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by memory loss, cognitive decline, and impaired daily functioning. Despite decades of research, effective treatments for AD remain elusive, and current therapies only offer symptomatic relief without addressing the underlying disease process. Apolipoprotein E (ApoE) is the strongest genetic risk for late-onset AD, with ApoE4/4 genotype increasing the risk by 14-fold, while ApoE2/2 decreases it by 87%. A promising approach to targeting the AD pathology involves the investigation of gene therapies utilizing siRNA to selectively silence ApoE4 without affecting ApoE2/3. However, the efficacy of siRNA therapies for AD is hindered by challenges like transport instability, inadequate delivery to target regions within the brain due to the blood-brain barrier (BBB), and the risk of off-target effects. To overcome these challenges, intranasal delivery of siRNA selectively targeting ApoE4 has been developed as a gene therapy strategy for AD treatment.

Methods:

An *in vitro* nasal mucosa model was established with human RPMI-2650 cells and validated. Intranasal formulations were prepared via thin film hydration and microfluidics, then characterized by particle size, ζ-potential and TEM imaging, to encapsulate FAM-labelled siRNA for *in vitro* permeability assay, which was then observed with a confocal microscope. Designed siRNAs targeting ApoE4 were evaluated for specificity and knockdown efficiency utilizing RT-qPCR in normal H4 cells and human ApoE4 knock-in H4 cells.

Results:

The RPMI-2650 in vitro nasal mucosa model was successfully validated using TEER measurements and permeability assays (fluorescein sodium, isothiocyanate-dextran). FAM-labelled siRNA formulations were prepared and characterized, such as siRNA liposome, including particle size, zeta-potential and transmission electronic microscopy (TEM) imaging, following the permeability assessments of siRNA-loaded formulations on an *in vitro* nasal mucosa cell model and an *ex vivo* porcine nasal mucosa tissue model.

Optimized siRNA intranasal formulations were also assessed via confocal imaging and magnetic resonance imaging (MRI) technologies. Effective siRNA that downregulates expression of ApoE4 mRNA specifically was identified, and related function pathways and interplays of ApoE4 siRNA have been analyzed through proteomics assay, which has shown the interplay and potential pathways related to ApoE4 function in AD pathology, including the role in the metabolism of post-translational protein phosphorylation.

Conclusions: The optimized ApoE4 siRNA3 demonstrated high efficiency and selectivity for ApoE4 mRNA. Optimized siRNA formulations exhibited enhanced permeability in the *in vitro* nasal mucosa model, supporting their potential for intranasal delivery.