Cell Penetrating Peptide based gene delivery system targeting Amyloid Precursor Protein for the treatment of Alzheimer's disease.

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Background: Alzheimer's disease (AD) is characterized by amyloid-β (Aβ) plaque accumulation, derived from the amyloid precursor protein (APP). Monoclonal antibodies such as lecanemab, which target aggregated Aβ, have received FDA approval but show limited efficacy and serious side effects, including amyloid-related imaging abnormalities (ARIA). Unlike these downstream approaches, gene therapies such as small interfering RNA (siRNA) and splice-switching oligonucleotides (SSOs) is able to directly reduce APP expression. Our study evaluates the use of cell-penetrating peptides (CPPs)—FLR and its PEGylated version (PEG-FLR)—to deliver APP-targeted siRNA and SSOs, aiming to develop a safe and effective non-viral gene therapy for AD. PEG-FLR is FLR modified with 5 kDa polyethylene glycol (PEG) and is intended to enhance in vivo delivery by improving mucus penetration, biodistribution, and nanoparticle colloidal stability.

Methods: We evaluated the delivery of SSO and siRNA targeting the APP gene in SH-SY5Y cells using FLR and PEG-FLR as non-viral vectors. Lipofectamine 2000 served as a positive control. Transfection efficiency was assessed through human APP protein ELISA, PCR, and quantitative PCR (qPCR), measuring APP exon 17 skipping at the transcript level and reductions in APP mRNA and protein expression.

Results: Among the two nucleic acid drugs, siRNA-FLR exhibited stronger transfection efficiency compared to SSO-FLR. Optimization of siRNA-FLR showed that a 0.5 μg dose achieved the highest APP gene silencing at 24 hours post-transfection, with charge ratios of 3, 5, and 10 all show significant gene knockdown with no detectable cytotoxicity. For SSO-FLR, transfection efficiency was detected as early as 4 hours, peaking at 48 hours. The optimal formulation—250 ng SSO with a charge ratio of 5—showed effective APP exon skipping with low cytotoxicity. A chronic delivery schedule of four consecutive daily doses further enhanced SSO-FLR transfection without increasing toxicity.

However, co-delivery of siRNA and SSO using FLR did not improve therapeutic outcomes compared to siRNA delivery alone, possibly due to competitive uptake, intracellular saturation, or divergent processing mechanisms between the two oligonucleotide types.

In vitro studies demonstrated that FLR significantly outperformed PEG-FLR in delivering both siRNA and SSOs. However, PEG-FLR may hold greater potential for in vivo applications, as our previous research has shown its enhanced diffusivity in lung tissue.

Conclusions: FLR-based delivery systems provide efficient, low-toxicity transfection of both siRNA and SSOs targeting the APP gene in vitro, with siRNA-FLR showing better silencing efficacy. While PEG-FLR demonstrated limited performance in vitro, its known in vivo delivery advantages highlight its potential for future therapeutic application. Co-delivery of siRNA and SSOs did not yield additive effects, suggesting distinct intracellular processing pathways may limit synergy. These findings support further in vivo evaluation of FLR-mediated gene silencing, particularly siRNA-based approaches, as a promising strategy for Alzheimer's disease therapy.