Facilitating DNAzyme Transport Across the Blood-Brain Barrier with Nanoliposome Technology

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Introduction

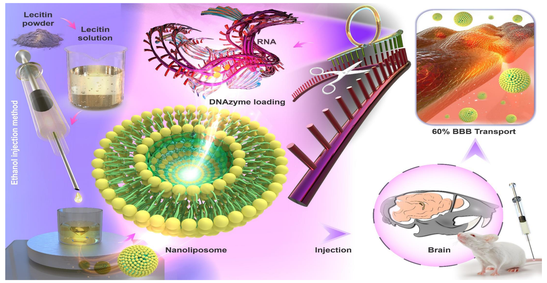
The presence of the blood-brain barrier (BBB), a tightly interconnected network of blood vessels and endothelial cells that restricts the entry of foreign particles larger than 100 nm into the brain, poses considerable challenges and complexities in advancing therapeutic strategies for neurodegenerative diseases (NDs) 1. 2. Gene therapies, specifically DNAzymes, post-transcriptional gene silencing technique, have shown promising results in treating neurodegenerative diseases 5. DNAzymes are single-stranded DNA-based catalytic molecules functioning as efficient RNA-silencing agents; they target, bind to, and degrade disease-associated mRNAs, reducing the levels of proteins implicated in specific pathological conditions 6. Thus, the study aimed to synthesize nanoliposomes from soy lecithin using the ethanol injection method, serving as carriers to deliver DNAzymes for targeted brain modification and enhancing their ability to cross the BBB by more than 6%. To this end, after synthesizing and investigating the physicochemical properties of DNAzyme-loaded nanoliposomes (DNZ-NLPs), both in vitro and in vivo studies were performed using SH-SY5Y brain cells and BALB/c mice, respectively.

Methods

Preparation of DNZ-NLPs was performed by optimizing an ethanol injection method 25. Basic Physicochemical Properties of DNZ-NLPs such as the mean particle size (Z-Average), polydispersity index (PDI), zeta potential, (ATR-FTIR), in vitro release profile, scanning electron microscope (SEM) , Entrapment efficiency (EE) and Drug loading (DL) of DNZ-NLPs were investigated and cellular assay of this system such as Cytocompatibility assay, Intracellular uptake; to assess the ability of DNZ-NLP's cellular uptake in SH-SY5Y cells, DAPI staining, the release kinetics of Lip-DNAzymes and the BBB permeability and the distribution of DNZ-NLPs within brain tissues were examined by intravenously injecting female BALB/c mice with fluorescently labeled free-DNAzymes and DNZ-NLP. The animals were sacrificed at 4 and 24 hours post-DNZ-NLP injection, and their brains were harvested, sectioned, and viewed under a fluorescent microscope.

Results

The investigation of physicochemical characteristics of fabricated nanoliposomes, particularly size, morphology, and surface charge, revealed that the size of DNZ-NLPs was ~68 nm, an optimum size for brain delivery. Cellular uptake and cytocompatibility studies using SH-SY5Y human neuroblastoma cells demonstrated that both blank nanoliposomes (B-NLPs) and DNZ-NLPs were cytocompatible, and DNZ-NLPs had a stable biphasic release profile in 48 h. Most importantly, about 60% of intravenously administered DNZ-NLPs to the healthy mouse were found in the brains of the animals. These findings confirmed that DNZ-NLPs passed the BBB. The controlled release of DNAzymes, the maximal cytocompatibility, and significantly improved BBB permeability suggest that our DNZ-NLPs offer a promising formulation for delivering all types of oligonucleotides to the brain for neurodegenerative disease treatments.



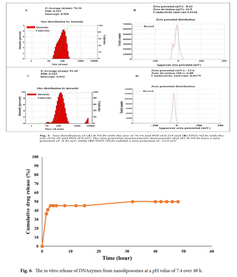


Fig. 6. The in vitro release of DNAzymes from nanoliposomes at a pH value of 7.4 over 48 h.

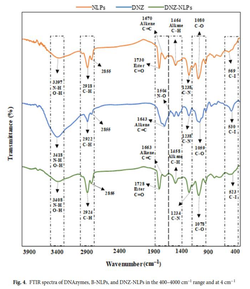


Fig. 4. FTIR spectra of DNAzymes, B-NLPs, and DNZ-NLPs in the 400–4000 cm⁻¹ range and at 4-cm⁻¹

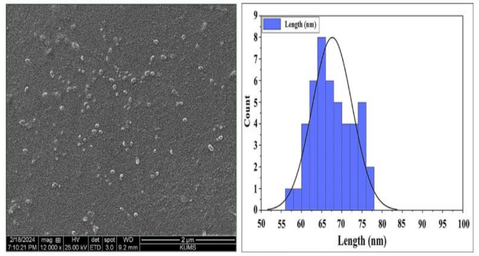


Fig. 3. SEM analysis of DNZ-NLPs with morphologically uniform particles averaging 68 nm in diameter at a scale of 2 µm.

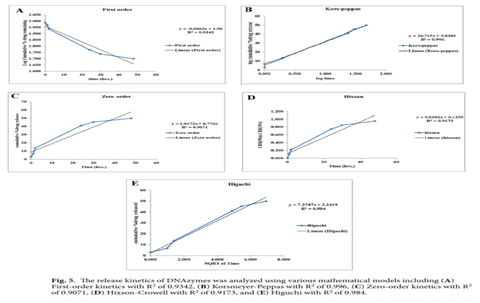


Fig. 5. The release kinetics of DNAzymes was analyzed using various mathematical models including (A) First-order kinetics with R^2 of 0.9342, (B) Korsmeyer-Peppas with R^2 of 0.996, (C) Zero-order kinetics with R^2 of 0.9071, (D) Hixson-Crowell with R^2 of 0.9173, and (E) Higuchi with R^2 of 0.984.

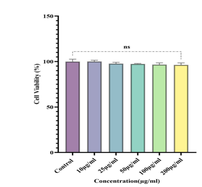


Fig. 7. Cell viability of the SH-SY5Y cells after treatment with 10, 25, 50, 100 and 200 µg/ml of DNZ-NLPs. Data are presented as mean ± SD. Results were reported as mean ± SD (*p ≤ 0.1, **p ≤ 0.01, ns three independent replicates.

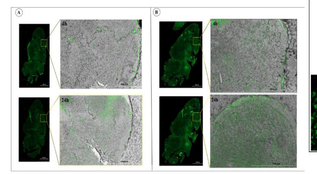


Fig. 9. UV microscopy results of mouse brains at (A) mice-brain tissue after being treated by free DNAzymes labeled FITC and (B) mice-brain tissue after being treated by DNZ-NLPs labeled FITC (X 1000 µm scales) at two times of 4 and 24 h after treatment.

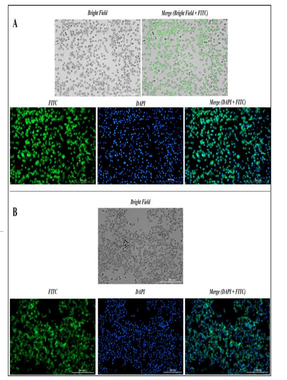


Fig. 8. Bright field, Bright field and FITC merged, FITC, DAPI and DAPI and FITC merged images of SH-SY5Y cells treated with (A) DNZ-NLPs and (B) DNAzyme, with a final concentration of 200 µg/mL at pH 7.4 with a 200 nm bar scale.

Conclusions

In this study, to explore the potential of nanoliposomes for targeted and controlled DNAzymes delivery in brain disorders therapy, DNZ-NLPs were successfully prepared, using the ethanol injection method. Our analysis of nanoliposomes and DNZ-NLPs confirmed their concern for suitable size, surface charge, and morphology, supporting their potential for effective brain drug delivery. Chemical and conformational analyses via FTIR further validated the successful DNAzyme encapsulation in nanoliposomes. The substantial drug release from nanoliposomes over 48 hours highlights their effectiveness in controlled drug delivery. In vitro experiments revealed no cytotoxic effects of DNAzymes and DNZ-NLPs on SH-SY5Y cells even in high concentrations of 100 and 200 µg/mL, emphasizing the system's potential. Also, in vivo investigations confirmed the capability of DNZ-NLPs to effectively pass through the BBB in mouse models. This study represents the combination of DNZ-NLPs as a significant advancement in targeted brain drug delivery, highlighting natural lipid-based nanotechnology's role in the treatment of neurodegenerative diseases such as Huntington's disease (HD).