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Non-invasive, neuron-specific gene therapy by focused ultrasound-induced blood-brain barrier opening in Parkinson's disease mouse model

Chung-Yin Lin [a,b,**], Han-Yi Hsieh [c], Chiung-Mei Chen [d], Shang-Rung Wu [e], Chih-Hung Tsai [c], Chiung-Yin Huang [f], Mu-Yi Hua [g], Kuo-Chen Wei [f], Chih-Kuang Yeh [h], Hao-Li Liu [a,c,*]

[a] Medical Imaging Research Center, Institute for Radiological Research, Chang Gung University/Chang Gung Memorial Hospital, Taoyuan 333, Taiwan
[b] Department of Nephrology, Division of Clinical Toxicology, Chang Gung Memorial Hospital, Lin-Kou Medical Center, Taoyuan 333, Taiwan
[c] Department of Electrical Engineering, Chang Gung University, Taoyuan 333, Taiwan
[d] Department of Neurology, Chang Gung Memorial Hospital, Taoyuan 333, Taiwan
[e] Institute of Oral Medicine, National Cheng Kung University, Tainan 701, Taiwan
[f] Department of Neurosurgery, Chang Gung Memorial Hospital, Linkou Medical Center and College of Medicine, Chang Gung University, Taoyuan 333, Taiwan
[g] Department of Chemical and Material Sciences, Chang Gung University, Taoyuan 333, Taiwan
[h] Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Hsinchu 300, Taiwan

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ABSTRACT

Focused ultrasound (FUS)-induced with microbubbles (MBs) is a promising technique for noninvasive opening of the blood-brain barrier (BBB) to allow targeted delivery of therapeutic substances into the brain and thus the noninvasive delivery of gene vectors for CNS treatment. We have previously demonstrated that a separated gene-carrying liposome and MBs administration plus FUS exposure can deliver genes into the brain, with the successful expression of the reporter gene and glial cell line-derived neurotrophic factor (GDNF) gene. In this study, we further modify the delivery system by conjugating gene-carrying liposomes with MBs to improve the GDNF gene-delivery efficiency, and to verify the possibility of using this system to perform treatment in the 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced animal disease model. FUS-BBB opening was verified by contrast-enhanced MRI, and GFP gene expression was verified via in vivo imaging system (IVIS). Western blots as well as enzyme-linked immunosorbent assay (ELISA) were conducted to measure protein expression, and immunohistochemistry (IHC) was conducted to test the Tyrosine hydroxylase (TH)-neuron distribution. Dopamine (DA) and its metabolites as well as dopamine active transporter (DAT) were quantitatively analyzed to show dopaminergic neuronal dopamine secretion/activity/metabolism. Motor performance was evaluated by rotarod test weekly. Results demonstrated that the LpDNA-MBs (gene-liposome-MBs) complexes successfully serve as gene carrier and BBB-opening catalyst, and outperformed the separated LpDNA/MBs administration both in terms of gene delivery and expression. TH-positive IHC and measurement of DA and its metabolites DOPAC and HVA confirmed improved neuronal function, and the proposed system also provided the best neuroprotective effect to retard the progression of motor-related behavioral abnormalities. Immunoblotting and histological staining further confirmed the expression of reporter genes in neuronal cells. This study suggests that FUS exposures with the administration of LpDNA-MBs complexes synergistically can serve as an effective gene therapy strategy for MPTP-animal treatment, and may have potential for further application to perform gene therapy for neurodegenerative disease.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide, affecting 1% of the elderly population with a higher prevalence [1]. Patients typically develop progressive loss of the nigro-striatal dopaminergic neurons and eventually resulting in wide-spread neuronal cell death [2,3]. Currently no definite treatment approach exists to slow the progression of neurodegenerative disease. Patients suffer a loss of movement controlling and other such

* Correspondence to: H.L. Liu, Department of Electrical Engineering, Chang Gung University, Taoyuan 333, Taiwan.
** Correspondence to: C.Y. Lin, Medical Imaging Research Center, Chang Gung University, Taoyuan 333, Taiwan.
E-mail addresses: winwood5782@gmail.com (C.-Y. Lin), haoliliu@mail.cgu.edu.tw (H.-L. Liu).

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symptoms, mainly due to degeneration of dopaminergic neurons in the substantia nigra (SN), coupled with a depletion of dopamine (DA) and metabolites in the nigrostriatal projections [4,5].

Gene therapy is a therapeutic approach that aims to treat disease by genetically modifying neuronal cells to relieve relevant-symptoms or even reverse PD progression [6,7]. For example, glial cell line-derived neurotrophic factor (GDNF) is a potent agent for PD therapy due to its neuroprotective and neurotrophic effects [8,9]. The overexpression of neuroprotective genes to promote regeneration of DA in activated neurons offers potentially significant symptoms alleviation while slowing disease progression [10]. This strategy allows for treatment using putative neuroprotective-agents prior to significant/irreversible neuronal loss [11]. However, one of the major challenge to this approach is the blood-brain barrier (BBB), in which tight junctions between the endothelial cells block the penetration of molecules >400 Da, thus preventing CNS uptake therapeutic drugs/genes [12]. In addition, therapeutic genes administered intravenously are rapidly degraded through reticuloendothelial system (RES) uptake and clearance, thus typically requiring the invasive intracranial local injection of therapeutic genes [13,14].

Transcranial focused ultrasound exposure with microbubbles (MBs) can temporally and locally open the BBB to allow large therapeutic substances to penetrate the targeted CNS regions [15–18] in various species ranging from small to larger animal [19–21]. Regarding gene-vector delivery, we have previously shown that the technique can be synergistically combined with a liposome-containing plasmid DNA (LpDNA) system to significantly promote GDNF transfection (5–10 fold increase in GDNF measures as compared to controls) at target CNS sites in normal animals [22]. It is reasonable to surmise that GDNF gene delivery and expression in SN via LpDNA system combined with FUS-BBB opening should be beneficial to PD progression control, since previous studies have confirmed that supplemental GDNF supplement can help prevent neuronal death and can retard PD progression [23,24]. In addition, FUS-induced BBB opening relies on the administration of MBs to provide cavitation-induced sheer stress/radiation force in capillaries to trigger tight-junction opening. A more efficient design involves forming LpDNA-MBs complex [25,26] to maximize gene-vector delivery into the brain since the MB-generated force directly radiates LpDNA toward tight-junctional crafts. Therefore, we hypothesize that the LpDNA-MBs complex with an FUS-induced BBB opening can effectively deliver genes to the brain to provide effective PD treatment.

The study develops and evaluates the efficacy of a CNS gene delivery system via the synergistic use of FUS-induced BBB opening with the GDNF-gene-vector/MBs complex to perform noninvasive GDNF gene delivery and evaluate its treatment efficacy in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. We propose a novel GDNF-gene-vector/MBs complex design by synthesizing biotinylated liposome-containing pDNA (LpDNA) to bind avidin-MBs via biotin-avidin linkages. Once in the cell, the LpDNA eventually enters the nucleus and enhances the protein expression. The designed LpDNA contains green fluorescence protein (GFP) genes and GDNF genes to allow for in vivo detection of gene expression. Fig. 1 shows the proposed FUS gene delivery system. Biophysical/biochemical analysis was conducted to characterize the LpDNA-MBs complex system. We measured the expression levels of proteins and metabolites, conducted pathological examinations, assessed motor performance of MPTP-treated mice, and compared the proposed LpDNA-MBs complex system with separated LpDNA/MBs administration. We also present immunohistochemistry (IHC) staining evidence that neuronal cells are the major target to be transduced via the proposed system.

2. Material and methods

2.1. Plasmid DNA (pDNA) preparation

A single bacterial colony containing a plasmid encoding both the GFP gene (marker gene) and the glia-derived neurotrophic factor (GDNF) gene (therapeutic gene) was cultured and inoculated in 500 mL LB medium. The mixture was then incubated for about 24 h at 37 °C with shaking at 300 rpm. The bacteria cells were harvested by centrifugation at 3000 ×*g* for 30 min at 4 °C. Following to the manufacturer's instructions, the samples were centrifuged at 15,000 ×*g* for 10 min and the supernatant was decanted. Then, 200 μL of double-distilled autoclaved water (DDAC) was added to the pellet, followed by 20 μL of sodium acetate along with 550 μL of cold ethanol. The mixture was centrifuged at 4 °C for about 15 min. Finally the supernatant was gently removed and

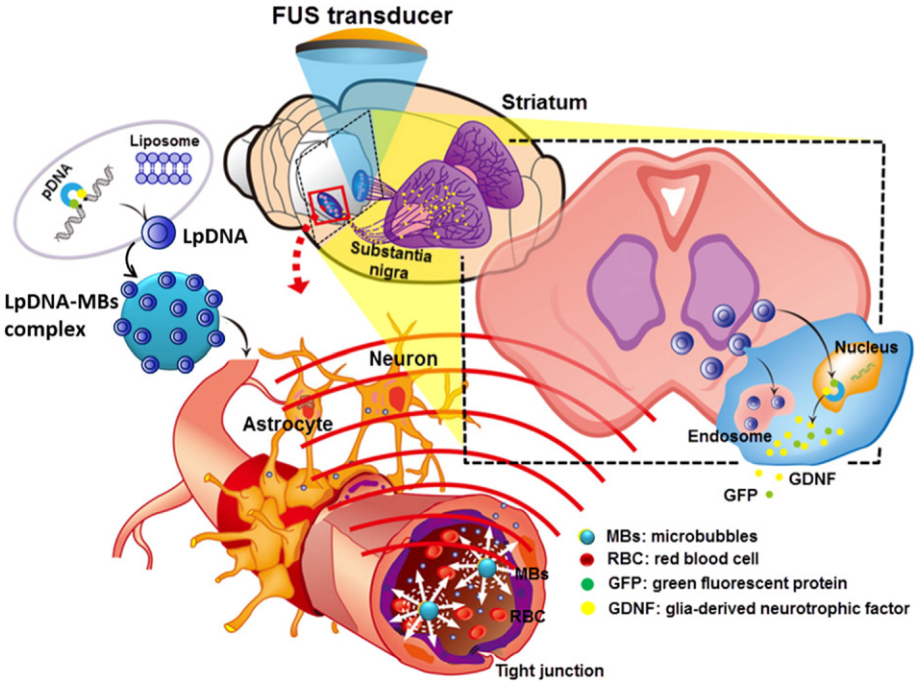

Fig. 1. Schematic representation of the synergistic use of the LpDNA-MBs complex to assist FUS-induced BBB opening to perform noninvasive and targeted GDNF gene delivery for MPTP-treated animals. FUS = focused ultrasound; BBB = blood-brain barrier.