Targeted gene delivery to the mouse brain by MRI-guided focused ultrasound-induced blood–brain barrier disruption

Qin Huang, Jinmu Deng, Feng Wang, Song Chen, Yingjiang Liu, Zhibiao Wang, Zhigang Wang, Yuan Cheng

Department of Neurosurgery, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China
Department of Biomedical Engineering, Chongqing Medical University, Chongqing, China
Institute of Ultrasound Imaging, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China

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A B S T R A C T
This study aimed to investigate the feasibility of targeted gene transfer into central nervous system (CNS) by MRI-guided focused ultrasound-induced blood–brain barrier (BBB) disruption. Before each sonication, T2-weighted images were obtained to select the target region. Followed by injecting DNA-loaded microbubbles into the tail vein, sonication was performed. The state of local BBB, distribution of plasmid DNA through the opened BBB, the ultrastructural changes of neurons and BDNF expression were detected. The results showed that MRI-guided focused ultrasound (FUS) could accomplish noninvasive, transient, and local BBB disruption, at 1 h after sonication, plasmid DNA across the opened BBB had been internalized into the neurons presenting heterogeneous distribution and numerous transparent vesicles were observed in the cytoplasm of the neurons at the sonicated region, suggesting vesicle-mediated endocytosis. At 48 h after sonication, the expressions of exogenous gene pBDNF-EGFP were observed in the cytoplasm of some neurons, and BDNF expressions were markedly enhanced by the combination of ultrasound and pBDNF-EGFP-loaded microbubbles about 20-fold than that of the control group (P < 0.01). The method by using MRI-guided FUS to induce the local BBB disruption could accomplish effective targeted exogenous gene transfer in CNS. This technique may provide a new option for the treatment of various CNS diseases.

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Introduction

Gene therapy has a powerful potential in the biomedical research and the treatment of various diseases that are due to genomic causes. Accordingly, the development of safe, effective methods of gene transfer is also very important to basic research and clinical science. However, one major challenge of gene therapy in the central nervous system (CNS) is the existence of blood–brain barrier (BBB). The BBB, which is formed by the specialized brain capillary endothelium, plays very important roles in maintaining the homeostasis of the brain through hampering various substances to leak into the brain, but meanwhile excludes more than 98% of small-molecule drugs and approximately 100% large-molecule neurotherapeutics and complicates the treatment of CNS diseases (Kinoshita, 2006; Pardridge, 2005). Only small-molecule drugs with high lipid solubility and lower molecular weights (MW) under 400–500 Da can cross the BBB (Pardridge, 2003), so not to mention the therapeutic genes such as macromolecules with several thousand base pairs and their corresponding MW over 1000 kDa (Liang et al., 2010). In general, the efforts to penetrate the BBB include: 1) trans-cranial brain delivery to bypass the BBB; 2) chemical modification of the macromolecules by increasing the lipid solubility or altering the structure of a lead drug candidate; 3) solvent-mediated BBB disruption by an intraarterial injection of mannitol or other hyposmotic solutions to cause the temporary opening of the tight junction (Pardridge, 2005). However, these methods entail various problems, such as its invasive nature, lack of site specificity and antibody or gene not easily modified to cross the BBB.

Currently great advances in acoustic technology have made ultrasound not only a traditional diagnostic instrument but also a novel therapeutic tool. It has been demonstrated that focused ultrasound (FUS) alone could result in localized BBB disruption without visible damages to the brain parenchyma, in addition the presence of microbubbles (ultrasound contrast agents) injected intravenously in advance could further effectively enhance the cavitation effect and reduce the acoustic energy (Vykhatseva et al., 2008), and especially the combined use of magnetic resonance imaging (MRI) could permit image-guided target selection and real-time temperature monitoring during the sonications (McDannold et al., 2005). There are numerous applications for
incubated in the 37 °C water for 30 min, the vial was vibrated then 450 μl for 30 s while 5:2:1 were prepared by a mechanical vibration method. Briefly, (DPPA) (Avanti Polar Lipids Inc., Alabaster, AL) in a mass ratio of ethanolamine (DSPE) and 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (Sigma-Aldrich, St. Louis, MO, USA) (v/v=3:1) was gently agitated in a 3 ml vial, and then 450 μl of phosphate buffered saline (PBS) was added. After incubated in the 37 °C water for 30 min, the vial was vibrated fiercely for 30 s while filled with perfluorocarbon gases. Then the microbubbles could be separated from unreacted components by centrifugation. The final DNA concentration was adjusted to 1 mg/ml using TE buffer.

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is present in high concentration in hippocampus and cerebral cortex and acts on certain neurons of the CNS and the peripheral nervous system, helping to support the survival of existing neurons, and encourage the growth and differentiation of new neurons and synapses (Huang and Reichardt, 2001). It also may play a role in the regulation of stress response and in the biology of mood disorders (Chen et al., 2001). Various studies have shown that BDNF has a great potential for the treatment of many diseases, such as Alzheimer’s disease (Zuccato and Cattaneo, 2009), Parkinson’s disease (Ricci et al., 2010) and depression (Brunoni et al., 2008). However the BDNF is too large to cross the BBB, it could not achieve the therapeutic effect only by conventional delivery methods. Thus, we attempt to transfer exogenous gene BDNF to the targeted region by our technique and provide a new strategy for gene therapy in CNS diseases. In the present study, we first attached the plasmid DNA encoding BDNF on the surface of preformed microbubbles and then investigated the use of MRI-guided FUS, applied through the intact mouse skull, to achieve noninvasive targeted gene delivery across the opened BBB.

Material and methods

Plasmid and microbubble preparation and conjugation

The plasmid pBDNF-EGFP-N1, encoding brain-derived neurotrophic factor and green fluorescent protein, was constructed by insertion of the BDNF gene (760 bp, Gene Bank ID: M61186) cloned from the DNA of human blood leukocytes into the pEGFP-N1 (4.7 kb) at the BamHI and KpnI sites, which was kindly donated by Dr. Qindong Shi (Institute of Neurobiology, Medical School of Xi’an Jiaotong University, Xi’an, China). The plasmid pEGFP-N1, prepared by our own laboratory, was also used in this study as a control. Both plasmids were amplified in DH5α bacteria then purified using EndoToxin-free plasmid extraction kit (Tiangen Biotech, Beijing, China) according to the manufacturer’s protocol. The final DNA concentration was adjusted to 1 mg/ml using TE buffer.

The microbubbles composed of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC), 1,2-distearyoyl-sn-glycero-3-phosphatidyl-ethanolamine (DSPE) and 1,2-dipalmitoyl-sn-glycero-3-phosphate (DPPA) (Avanti Polar Lipids Inc., Alabaster, AL) in a mass ratio of 5:2:1 were prepared by a mechanical vibration method. Briefly, all reagents were dissolved in 50 μl of glycerine in a 3 ml vial, and then 450 μl of phosphate buffered saline (PBS) was added. After incubated in the 37 °C water for 30 min, the vial was vibrated fiercely for 30 s while filled with perfluorocarbon gases. Then the microbubbles could be separated from unreacted components by centrifugation and dilute to a desired concentration by PBS.

Plasmids were conjugated onto the surface of the preformed lipid microbubbles by layer-by-layer (LbL) assembly technique with the help of poly-β-lysine (PLL) as previously demonstrated by Borden et al. (2007). In brief, the solution consisting of microbubbles and PLL (Sigma-Aldrich, St. Louis, MO, USA) (v/v=3:1) was gently agitated in a centrifuge tube for 30 min, and then centrifuged at 800 g for 5 min. Centrifugation forced the bubbles to float at the top of the tube, and the subnatant and remains of bubbles could be removed. After being washed twice, the microbubbles were suspended in the DNA solution, incubating for 30 min at room temperature with gentle shaking. Followed by washing off the residual DNA, DNA-coated bubbles then were suspended in a PLL solution again to start the next cycle. In order to increase the gene loading capacity, this procedure was usually repeated for three times. Finally, the performed multiple layers of DNA-loaded microbubbles were sized and counted using a MasterSizer (Malvern Instruments, Shanghai, China), and DNA surface concentration was measured by a NanoDrop-8000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The final gene package concentration was maintained at 2 mg DNA/5 x 10⁶ microbubbles/ml. Propidium iodide (PI) (Sigma-Aldrich, St. Louis, MO, USA) which can combine with DNA and present the red fluorescent, was utilized to detect the distribution of DNA on the surface of microbubbles using a laser scanning confocal microscope (Leica, Germany). In this study, a total volume of 200 μl DNA-loaded microbubbles containing 400 μg plasmid pBDNF-EGFP/p EGFP was used for each mouse.

Animals

This study was approved by our Institutional Animal Care and Use Committee. Thirty-four male Kunming mice (4 weeks, 28–32 g) from the Laboratory Animal Center of Chongqing Medical University were anesthetized intraperitoneally with chloral hydrate (400 mg/kg) before the sonication. After removal of the fur on top of the head with an electric razor and a depilatory cream, a catheter (24G; Deltafil, Italy) was inserted in the tail vein for injections. The mice were randomly divided into five groups: control group (n=5) with no treatment, Group 1 (n=5) treated with 200 μl pBDNF-EGFP-loaded microbubbles (containing 400 μg plasmid pBDNF-EGFP), Group 2 (n=5) treated with 200 μl pEGFP-loaded microbubbles and ultrasound exposure, Group 3 (n=5) treated with 400 μg plasmid pBDNF-EGFP, 200 μl microbubbles and ultrasound exposure, and Group 4 (n=14), including three mice for frozen sections, three mice for transmission electron microscopy and three mice for immunofluorescence, treated with 200 μl pBDNF-EGFP-loaded microbubbles and ultrasound exposure. Five mice of every group were used for Western-blot. The mice were prepared as described above and placed on the system. Two-weighted images were obtained to facilitate in the selection of target areas in the brain. Followed by injecting DNA-loaded microbubbles into the tail vein, sonication under MRI guidance was performed. After the sonication procedure, MRI contrast agents were injected to acquire the contrast-enhanced MR images, and Evans blue (EB; Sigma-Aldrich Co., St. Louis, MO; 100 mg/kg) was injected simultaneously to mark and confirm the site of BBB disruption. At 48 h after sonication, the remaining mice were inspected with contrast-enhanced MRI again to learn about the state of BBB.

Experimental equipment

The MRI-guided FUS system was provided by the Department of Biomedical Engineering of Chongqing Medical University similar to that reported previously (Mei et al., 2009). The transducer was mounted in the positioning system and then submerged in a tank of degassed deionized water. The mice were placed supine on a plastic platform above the tank with the head circled by a Flex Loop Small coil (Siemens AG, Erlangen, Germany) and a water bag was used to stuff the area between the tank and the head in order to maximize the transmission of the ultrasound. The whole apparatus was positioned in a 1.5-T clinical MRI scanner (Magnetom Symphony; Siemens AG). The sonication were performed by an in-house-manufactured single-element FUS transducer with a diameter of 150 mm, radius of curvature of 120 mm, and resonant frequency of 1.1 MHz. Sonication was pulsed with a burst length of 10 ms and a repetition frequency of 1 Hz (duty cycle, 1%). The total sonication duration was 40 s and acoustic power was 2.2 W used in this experiment. Ultrasound was propagated through the water to the dorsal surface of the head and focused through the intact skull on the target spot about 4 mm deep from the brain surface, the 6-8 μm beam width and axial focal length of the produced focal spot were 1.5 mm and 4 mm, respectively.
Magnetic resonance imaging

Before each sonication, the target region or BBB disruption was selected on the T2-weighted images (repetition time/echo time: 17/9 ms; FOV: 120 mm × 120 mm; flip angle: 40°; bandwidth: 110 kHz; matrix: 223 × 256; slice thickness: 2 mm) and the transducer repositioned accordingly. Followed by sonication, a gadopentetate dimeglumine MRI contrast agent (Magnevist; Bayer Healthcare Pharmaceuticals, Montville, NJ) was injected intravenously at a dose of 0.2 mol/kg to detect BBB disruption, and T1-weighted contrast-enhanced MR images were acquired in 3 dimensions (repetition time/echo time: 736/24 ms; FOV: 80 mm × 40 mm; flip angle: 150°; bandwidth: 190 kHz; matrix: 102 × 256; slice thickness: 2 mm).

Transmission electron microscopy (TEM)

At 1 h after the sonications, three mice in Group 4 were re-anesthetized and transcardially perfused with 100 ml of 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/l phosphate buffered saline (PBS, pH = 7.4) and the brains removed. A mouse brain matrix (ASI Instruments, Warren, MI, USA) was used to bisect the brain at 4 mm from the brain surface to expose the blue spots at the targeted locations. Five tissue blocks of approximately 1 mm² from each blue spot and from corresponding areas in the nonsonicated hemisphere were sampled and then fixed by immersion in 2.5% glutaraldehyde for more than 2 h. After washing in PBS, the blocks were postfixed in 2% osmium tetraoxide for 2 h, dehydrated in alcohol, passed through propylene oxide and embedded in Epon-Araldite. Ultrathin sections stained with uranyl acetate and lead citrate were observed with a JEM-1200EX electron microscope at 80 kV.

Immunofluorescence

The immunofluorescence was used to detect the distribution of BDNF-EGFP protein in the brain. Three mice in Group 4 were sacrificed 48 h after the sonications. Then the brain was quickly removed, embedded in paraffin and sectioned serially into 10-μm-thick slices. After deparaffinization in xylene and rehydrated, the sections were blocked with goat serum for 20 min at room temperature, and then incubated with mouse anti-EGFP monoclonal antibody (diluted 1:500, Millipore, Billerica, MA) at 4 °C overnight. After rinsing with PBS, the sections were incubated with anti-mouse IgG conjugated with FITC (diluted 1:100, Sigma-Aldrich) for 1 h at 37 °C in dark, then counterstained with PI for 2 min. At last, the sections were coverslipped with anti-fade fluorescent mounting medium and images were captured using a laser scanning confocal microscope (TCS SP2, Leica, Wetzlar, Germany). In addition, every fifthtieth section was stained with HE for histological examination.

Western-blot

Western-blot analysis was performed to detect the levels of BDNF protein in different groups. At 48 h after the sonications, five mice in each group were sacrificed and the brains (100 mg) were rapidly homogenized in 1 ml of RIPA lysis buffer (Beyotime, Jiangsu, China), and the supernatants were collected after centrifugation. Equal amounts of proteins (20 μg) were separated by SDS-PAGE and proteins were then transferred onto a PVDF membrane (Millipore, Billerica, MA). Blots were blocked with 5% nonfat milk for 1 h at room temperature and incubated overnight at 4 °C with primary anti-BDNF polyclonal antibody (diluted 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) and a polyclonal antibody β-actin (diluted 1:3000; Santa Cruz) respectively. After incubation with HRP-conjugated secondary antibody (diluted 1:3000, Zhongshan Goldenbridge Biotechnology, Beijing, China) for 1 h at room temperature, immune complexes were visualized by enhanced chemiluminescence (ECL kit; Pierce Biotechnology, Rockford, USA). Using Quantity One software (Bio-Rad), the levels of BDNF and β-actin were calculated by measuring the band density at 14 and 43 kDa, respectively and the results were normalized to β-actin.

Distribution of PI-dyed plasmid DNA in vivo after sonications

Three mice in Group 4 were used to detect the distribution of PI-dyed plasmid DNA in vivo. At 1 h after ultrasonic irradiation, the mice were re-anesthetized and perfused with 50 ml of saline and 50 ml of 4% paraformaldehyde via the left ventricle. The brains were quickly removed and frozen, then sectioned serially into 10-μm-thick slices by a Cryostat Microtome (CM 1900, Leica, Heidelberger, Germany). After coverslipped, the sections were observed directly by a laser scanning confocal microscope (TCS SP2, Leica, Wetzlar, Germany). In addition, every thirtieth section was stained with hematoxylin and eosin (HE) for histological examination.

Statistical analysis

Data are presented as the mean ± SD for each group. One-way ANOVA was used to test the statistical significance of differences among groups. Multiple comparisons between all groups were performed by the Tukey–Kramer test. In all comparisons, a P value of less than 0.05 was considered statistically significant.

Results

Local BBB disruption induced by MRI-guided FUS

Successful BBB disruption was confirmed by localized regions of enhanced signal intensity on contrast-enhanced T1-weighted MRI after sonication, due to the penetration of the MR contrast agent through the BBB (Fig. 1A). In addition, the leakage of EB into the brain parenchyma marked the site of BBB disruption in the corresponding brain samples (Fig. 1B). At 48 h after sonication, contrast-enhanced MRI showed no localized signal enhancement at the previously sonicated region, indicating that the initial transient BBB disruption had recovered (Fig. 1C). And no apparent microscopic tissue damages related to BBB disruption were seen in the corresponding HE staining (figure not shown).

Character of DNA-loaded microbubbles, localized distribution of plasmid DNA in vivo and TEM analysis

Plasmid DNA was conjugated onto the surface of the preformed lipid microbubbles through electrostatic interactions to increase the DNA loading capacity. By measurement, the final diameter of DNA-loaded microbubbles was 3.60 ± 0.33 μm, the density was 5.03 ± 0.28 × 10⁹/m and the DNA surface concentration was 0.067 ± 0.005 pg/μm². After dyed by PI, the plasmid DNA presented red fluorescence throughout the surface of microbubbles (Fig. 2A).

After BBB opening was confirmed, we further investigated the distribution of plasmid DNA in vivo in the short term. At 1 h after sonication, there was no obvious red fluorescence observed in the cytoplasm of the neurons at the contralateral side of the brain except for a very few scattered red dots (Fig. 2B). However, a large amount of heterogeneous distribution of red fluorescence plasmid DNA was observed in the cytoplasm of the neurons throughout the sonicated region (Fig. 2C). And corresponding TEM showed numerous transparent vesicles in the cytoplasm of the neurons at the sonicated region (Fig. 2E). There were no significant ultrastructural changes observed in neurons of the contralateral brain (Fig. 2D).
Fig. 1. MR monitoring of the BBB opening and photographs of the corresponding brain samples. (A) Contrast-enhanced T1-weighted MRI after sonication. BBB disruption was monitored by localized signal enhancement (arrows) due to the penetration of MR contrast agent into the target regions (AX: axial, COR: coronal, SAG: sagittal). (B) The brain was harvested 1 h after sonication. The location of the BBB opening was confirmed by leakage of Evans blue from the vasculature into the target brain (the volume of BBB disruption noted with red). (C) At 48 h after sonication, contrast-enhanced MRI showed no localized signal enhancement at the previously sonicated region.

Fig. 2. Confocal images of the PI-dyed DNA-loaded microbubbles and localized distribution of plasmid DNA after sonications and corresponding TEM examination. (A) Owing to combined with PI, the plasmid DNA absorbed on the surface of microbubbles presented red fluorescence (inset magnification of two typical DNA-loaded microbubbles). (B, C) At 1 h after sonication, the distribution of PI-dyed plasmid DNA in the sonicated and the contralateral side of the brain were presented (cont. means contralateral). In (B), except for a very few scattered red dots, there was no obvious red fluorescence diffused into the cytoplasm of the neurons. In (C), uptake of PI-dyed plasmid DNA in neurons presented numerous punctate regions of higher concentration in the cytoplasm of the neurons throughout the sonicated region (inset magnification showed more heterogeneous distribution in the cytoplasm of the neurons). (D, E) TEM examination corresponding to B, C. (D) There were no significant ultrastructural changes observed in neurons of the contralateral brain. (E) Numerous transparent vesicles were observed in the cytoplasm of the neurons at the sonicated region (arrows). N: nucleus of the neuron.
MRI-guided focused ultrasound enhanced exogenous gene expression in the target brain region

Immunofluorescence and Western blot were used to detect the exogenous gene expression 48 h after transfection. The results of the immunofluorescence revealed that the use of MRI-guided FUS could make the exogenous gene BDNF-EGFP be expressed in the cytoplasm of some neurons at the targeted region (Fig. 3). According to the analysis of Western blot, expressions of BDNF were markedly enhanced by the combination of ultrasound and pBDNF-EGFP-loaded microbubbles about 20-fold than that of the control group (P<0.01). As compared with the control group, there were no statistical significances of BDNF expression among the other groups, despite a slight increase in the group treated with FUS and separate injection of pBDNF-EGFP and microbubbles (Fig. 4).

Discussion

Over the past decade, ultrasound-mediated gene transfection has been broadly utilized as a gene delivery system both in vitro and in vivo researches, such as the heart, blood vessel, muscle, skin, liver, lung, kidney, pancreas, salivary, spine, neurons and tumor, however there is few research about this technique applied in CNS diseases. In this study, we initially demonstrated that MRI-guided FUS made it possible to deliver exogenous gene BDNF across the opened BBB, then expressed its corresponding protein in the cytoplasm of some neurons at the targeted regions. Moreover, using this technique, the expressions of BDNF at the targeted region were markedly enhanced than that of the control group, in contrast, the expressions of BDNF at the contralateral non-sonicated region remained low at a normal level. It was noteworthy that after the plasmids not encoding BDNF gene were transfected by the same technique, the BDNF level at the sonicated region didn’t change significantly. These results showed that MRI-guided focused ultrasound could achieve gene transfer at the targeted regions but not cause the enhancement of the endogenous BDNF of this region.

In this study, the successful plasmid transfer into CNS must undergo two key steps: at first, after sonication, the plasmids released from the surface of microbubbles must pass through the opened BBB in some way into the brain interstitial, immediately, the plasmids diffused in the brain interstitial should be internalized into the target cells to express its own protein through a series of autonomous replication, transcription and translation. For the first step, similar to the other studies on drug delivery by use of MRI-guide focal BBB opening (Kinoshita et al., 2006a, 2006b; Liu et al., 2010; Mei et al., 2009; Treat et al., 2007), we have identified the BDNF disruption by the increase of MRI signal intensity at the sonicated region and the leakage of EB in the corresponding brain samples. So far, there are intensive researches on the mechanisms of macromolecular trans-BBB after sonication, but the widely accepted theory was put forward by Sheikov et al. (2004), who suggested that the mechanisms included transcytosis, opening of tight junctions, endothelial cell fenestration and channel formation and free passage through injured endothelium. For the second step, no similar studies were reported previously. In order to investigate the mechanisms about the process of therapeutic gene into neurons in vivo, we observed the distribution of plasmid DNA having passed through the opened BBB into the brain interstitial in the short term. At 1 h after sonication, PI-dyed plasmid DNA had been internalized into neurons, presenting numerous punctate regions of higher concentration within the cytoplasm and TEM results showed that there were lots of vesicles formed in the cytoplasm of the neurons at the sonicated region, but no changes were observed in the contralateral side. Together, these results preliminarily indicated the intracellular uptake of plasmid DNA via vesicle-mediated endocytosis after sonication, which was consistent with the conclusion provided by Meijering et al. (2009). They used the fluorescent-tagged dextrans with different molecular weight to

Fig. 3. Use of the immunofluorescence to detect the expression of plasmid BDNF-EGFP in the targeted region 48 h after the sonication. (A) Detection of enhanced green fluorescent protein (EGFP) transgene in paraffin-embedded sonicated brain sections. (B) PI staining was used to reflect the location of the nucleus. (C) Merged image showed EGFP was only expressed in the cytoplasm of some neurons at the sonicated region.

Fig. 4. Use of Western-blot to investigate the levels of BDNF protein 48 h after treatment. (A) Expression bandings of BDNF in the different experimental groups. (B) Statistical analysis of BDNF band density according to (A). Con: control group with no treatment, Group 1: only with pBDNF-EGFP-loaded microbubbles, Group 2: with pEGFP-loaded microbubbles and sonication, Group 3: with pBDNF-EGFP, microbubbles and sonication, and Group 4: with pBDNF-EGFP-loaded microbubbles and sonication. 4a: samples from the contralateral non-sonicated region, 4b: samples from the sonicated region, **P<0.01 compared to Con, 1, 2, 3, and 4a groups. Each bar represents the mean±SD (n=5).
study the exact mechanisms behind intracellular delivery of therapeutic compounds and pointed endocytosis is a key mechanism in US and microbubbles targeted delivery besides transient pore formation, with the contribution of endocytosis being dependent on molecular size. Thus, we provided a new evidence for the FUS and microbubble-induced endocytosis to improve the uptake of macro- molecular in vivo, however, there are many aspects that need to be further studied, such as which kind of protein mediated endocytosis and the spatio-temporal scope of endocytosis.

The choice for gene vectors plays a vital role in gene therapy. Although viral vector systems have occupied more than half of clinical trials, largely because of the high transfection efficiency, the potential of immunogenicity and the deleterious consequences caused by the integration of administered DNA into the host chromosome limit their clinical applications (Yoon and Park, 2010). In contrast, naked plasmid DNA is much safer, cheaper and easier to manipulate, but its transfection efficiency is extremely low. Moreover, the plasmid DNA via systemic administration can’t pass through the BBB and is easily degraded and eliminated by nucleases and the mononuclear phagocyte system (Meier-Humbert and Guy, 2005); even though direct injections of large amount of plasmid DNA into targeted brain sites have been reported to demonstrate successful transgene expression (Sekiguchi et al., 2003), this method usually needs craniotomy and increases risks of neurologic damage, bleeding, and infection. The above problems accelerate the pursuit of a safer and more effective system of gene delivery. In recent years, US and microbubbles in the application of CNS have attracted wide attention. The main advantages of MRI-guided FUS-mediated gene delivery in this study are shown as follows: easy to manipulate, no radiation and toxicity, the precise anatomical targeting under the MRI guidance, noninvasive and local BBB disruption without damages to brain tissues, effective gene expression at the targeted regions and no effect on the non-target tissue. In addition, microbubbles also had an outstanding contribution to gene transfer, which not only maintained its own acoustic activity, but also acted as gene carrier through electostatic interactions to increase the DNA loading capacity and lower the possibility of premature elimination or enzymatic degradation. And the result of Western blot also confirmed both receiving FUS exposure, compared with injection of pBDNF-EGFP-loaded microbubbles, the group treated with separate injection of plasmids and microbubbles didn’t cause significant BDNF increase.

Although gene transfer using MRI-guided FUS-induced BBB disruption was effective, there are some limitations of the present study. First, we only confirmed that this technique could achieve exogenous gene expression, but it’s still unknown about the trend of protein content changing over time and the protein level at which could meet the therapeutic needs. Ongoing work at our institutions is aimed at demonstrating the therapeutic effects of exogenous gene in some animal models with CNS disorders. Second, numerous previous studies revealed that FUS-induced BBB disruption could be transient and reversible (McDannold et al., 2005), so it is reasonable to assume that repeated use of this technique is feasible in the experimental setting. Yang et al. (2011) pointed that EB extravasation could be enhanced by repeated sonication, however compared to a single sonication, the pattern of MRI contrast enhancement was changed and histological examination showed that vacuolation occurred after repeated sonication. Therefore, whether this technique could be repeatedly applied in gene therapy needs to be further studied. Finally, it is well known that the skull severely hampers the acoustic propagation because of its high acoustic attenuation coefficient (Hynynen and Jolesz, 1998), therefore the conventional craniootomy was needed to perform an acoustic window in the earlier studies. In this study, our acoustic equipment could make the ultrasound wave pass through the skull then successfully focus in the deep brain. But it was just applied to the four-week old mice with the thickness of skull less than 1 mm, at present how to translate this finding to the larger animals or humans is also under our consideration, mainly including the following two aspects. One is the optimization of acoustic parameters, such as much lower frequency (few hundred KHz) especially needed for human skull penetration compared with 1.1 MHz used in this study (Yin and Hynynen, 2005). The other is the determination of gene dosage. The issue that how much dose of DNA is required to produce measurable clinical result needs to be resolved through numerous trials later on.

In conclusion, our present study suggests that the MRI-guided FUS-induced BBB disruption method is a promising technique for gene delivery to the CNS. Although many aspects still need a lot of in-depth studies, the ability to deliver the therapeutic gene non-invasively and effectively to the target region provides a new option for the treatment of various CNS diseases.

Conflict of interest

There are no potential or actual, personal, political, or financial interests by any of the authors in the material, information, or techniques described in the paper.

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