Non-invasive and selective mesenchymal stem cell transplantation in a 192 IgG-saporin rat model using low-intensity focused ultrasound and the mechanism of transplantation

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Directed by Professor Jin Woo Chang

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Jihyeon Lee

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This certifies that the Doctoral Dissertation of Jihyeon Lee is approved.

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Jihyeon Lee

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Stem cell therapy has been found to be effective against neurodegenerative diseases. However, traditional transplant methods, such as parenchymal or intravenous (IV) injection, have several limitations including the development of secondary injuries and infection and low survival rate of stem cells in the brain.

Recently, the possibility of stem cell therapy using focused ultrasound (FUS) in the brain region has been reported. However, the mechanism underlying stem cell transplantation using FUS and the possibility of cognitive recovery remain unknown. This study was performed to identify the therapeutic effects and the mechanisms underlying stem cell therapy using FUS in a memory-impaired rat model. First, when FUS sonication was performed in the hippocampal region, the efficacy of mesenchymal stem cell (MSC) transplantation increased by 2-fold relative to the control group, and the mean number of intracellular adhesion molecule

(ICAM)-1-positive cells and vascular cell-adhesion molecule (VCAM)-1-positive cells in the control group was significantly lower than that in the FUS group. MMP-2 expression was also significantly higher in the FUS group than that in the control group. These findings suggest that increased expression of ICAM-1, VCAM-1 and MMP-2 induced by FUS might be related to the targeted homing of MSCs.

Second, we found that cholinergic denervation-induced memory impairment was effectively improved by cell transplantation using FUS. In the Morris water maze probe test, the amount of time spent in the platform zone was significantly decreased in the Lesion and FUS only groups compared to that in the normal group. However, it was significantly increased in the FUS + Cell group compared to the Lesion group. Moreover, the number of platform crossings was significantly increased in the FUS + Cell group compared to that in the Lesion group. The number of cholinergic neuron immunopositive cells, brain-derived neurotrophic factor (BDNF), immature neurons and mature neurons were all significantly increased in the FUS + Cell group compared to that in the Lesion group. Taken together, the memory improvement in the FUS + Cell group may be possibly implicated in the enhanced neuroprotective effect of the BDNF and the immunomodulatory effect of the transplanted MSCs, resulting in higher neurogenesis.

The results of this study provide insight into the possibility of stem cell homing and the therapeutic effects of FUS in a rat model of dementia. However, research regarding the function of stem cells transplanted into the brain and more detailed mechanism of stem cell homing by FUS should be further studied.

Key words: Focused ultrasound, mesenchymal stem cell, spatial memory, hippocampus, brain-derived neurotrophic factor, neuroprotection, neurogenesis

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I. INTRODUCTION

Diseases of the central nervous system (CNS), such as Alzheimer's disease (AD) and Parkinson's disease (PD), usually result in degeneration and irreversible damage to the structure and function of the brain, a phenomenon that is often accompanied by serious cognitive or physical impairments. These diseases are major causes of disability and fatality worldwide, accounting for 17% of global deaths and 10% of disability-adjusted life years.[1] The prevalence rate of dementia is constantly increasing, particularly among the aging population. The World Alzheimer Report 2015 stated that among CNS diseases, dementia poses the greatest disease burden globally, with 50 million people living with AD and related disorders worldwide.

Dementia is a term that describes disorders causing cognitive impairment, capable of significantly affecting the functional status and is associated with

symptoms that impair multiple functions, such as memory, thinking, calculation, learning, language, and judgment.[2] It is caused by various brain diseases including AD and vascular dementia. Till date, due to the development of modern medical science, treatments for many CNS diseases are available. A variety of novel therapeutic modalities such as targeted medicine,[3] deep brain stimulation,[4] radiosurgery,[5] and stem cell therapy[6] are being evaluated for treating patients with CNS diseases.[7] However, treatment of some CNS diseases, especially neurodegenerative diseases, remains challenging.

Stem cell therapy is a promising option to combat CNS diseases, and the positive effects and feasibility of stem cell treatment have been verified in recent years.[8] During the early stages of their development, stem cell therapies focused on replacing damaged neural structures with neural stem cells (NSCs). For example, one of the goals of NSC therapy is to replace the dopaminergic neurons in PD, a condition that is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta. Many preclinical and clinical trials have been conducted to achieve this goal. When human NSCs (hNSCs) were transplanted in 6-OHDA-induced parkinsonian mice, the protein profile of the subventricular zone (SVZ) was almost completely restored, and the functional defects were ameliorated by the hNSCs.[9] In a clinical trial, autologous NSCs obtained from patient brain biopsies were injected into patients with PD; these patients showed some motor recovery and increased dopamine uptake in the transplanted putamen, in addition to certain clinical benefits.[10] Although these results show acceptable outcomes, some problems also arose, such as poor long-term efficacy, surgical risks during transplantation, and the high cost of treatment.[11] The application of NSC therapy for

CNS diseases has other limitations. First is the function of the transplanted NSCs. Since these cells rarely connect to the existing neurons, there is little functional improvement after NSC treatment.[12] The second is transplantation yields. If the NSCs are injected into a vein, most of these cells cannot pass through the lungs. Therefore, only a small number of NSCs are able to reach their target area in the brain.[13]

In addition to NSCs, mesenchymal stem cells (MSCs) have emerged as possible candidates for stem cell therapy of CNS diseases.[14] MSCs exert various types of biological effects, including neuroprotective and immunomodulatory effects.[15] Since most neurodegenerative disorders are accompanied by abnormal immunological function, MSCs have been proposed as potential candidates for treating these disorders.[12] For instance, it has been reported that MSCs reduce the number of amyloid-β plaques in a mouse model of AD by secreting soluble intracellular adhesion molecule-1.[16] Further, other studies have confirmed that MSCs enhance neurogenesis in SVZ and differentiation of neural precursor cells into dopaminergic neurons in the substantia nigra in a PD model. The results of some clinical trials support the therapeutic effects of MSCs in PD, stroke, and multiple system atrophy.[17-19] However, similar to NSC therapy, MSC therapy also has limitations, especially with respect to transplantation modalities related to poor efficacy of delivery and survival rate.

Recently, a new treatment for CNS diseases using thermocoagulation by high intensity focused ultrasound (HIFU) has been developed. The therapeutic mechanism underlying focused ultrasound (FUS) treatment for these diseases involves thermal ablation of the area where the FUS energy is concentrated. This technique is clinically used for treating various CNS diseases. MR-guided FUS pallidothalamic tractotomy

(PTT) improved the symptoms of patients with PD.[20] In case of essential tremor, Vim nucleus lesioning with HIFU markedly improved the tremor symptoms in patients,[21] and bilateral thermal capsulotomy with HIFU was used to treat patients with obsessive-compulsive disorder.[22] It is also known that low-intensity FUS has various biological effects such as blood-brain barrier (BBB) modulation, neuromodulation, immune modulation, and alteration in the cerebral microenvironment.[7,23-25] Although the possibility of trans-endothelial migration of NSCs by FUS has been suggested,[26] the mechanism of BBB modulation during cell migration is unknown. Moreover, the approach of combining FUS and stem cells is at an early experimental stage in the CNS disease model.

Therefore, the aims of this study were to determine whether non-invasive FUS can facilitate the migration of MSCs to the target area in the brain and to investigate altered homing molecules induced by FUS. Moreover, the therapeutic effect of MSC transplantation using FUS in a rat model of dementia has not been studied. Therefore, it is necessary to examine whether MSCs transplanted using FUS can treat impaired cognitive function.

II. MATERIALS AND METHODS

1. Rat model of memory impairment

A. Animals

All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and approved by the Institutional Animal Care and Use Committee of Yonsei University, Korea (IACUC number: 2018-0167). The male Sprague-Dawley rats (200-220 g) were housed in groups of three per cage in a temperature/humidity-controlled room with a 12/12 hours light/dark cycle and were provided with *ad libitum* access to food and water. Every effort was made to minimize the number of rats used and overall animal suffering.

B. Surgical procedure

The rats were anesthetized with a mixture of 75 mg/kg ketamine, 0.75 mg/kg acepromazine, and 4 mg/kg Rompun® and secured in a stereotaxic frame. After scalp incision, rats were injected bilaterally with 8 μL of 192 IgG-saporin (0.63 μg/μL; Chemion; Temecula, CA, USA) into the cerebroventricle as per the Bregma coordinates: AP: −0.8 mm, ML: ±1.2 mm, DV: −3.4 mm. The solutions were injected at the rate of 1 μL/minute, and the syringe was left in place for 5 minutes after injection.

2. Bone marrow-derived (BM)-MSC preparation

A. Isolation and culture of rat BM-MSCs

BM-MSCs were isolated and collected from aspirates of rat femurs and tibias using 10 mL of Dulbecco's Modified Eagle Medium (DMEM)-low glucose supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and 1% penicillin–streptomycin. Mononuclear cells that had been recovered from the interface of Ficoll-Paque PLUS-separated (GE Healthcare; Little Chalfont, UK) BM were washed twice and resuspended in DMEM with 10% FBS and seeded at a density of 5×10^5 cells per 100 mm dish. The cultures were maintained at 37℃ in a humidified atmosphere containing 5% CO_2. After 72 hours, the non-adherent cells were discarded, and the remaining adherent cells were thoroughly washed (twice) with PBS. Fresh MesenPRO RS™ Medium (Gibco, USA) was added and replaced every three days for approximately 10 days to achieve stable status.[29]

B. Pre-staining of rat BM-MSCs with PKH26

The MSC medium was replaced every 2 or 3 days and BM-MSCs were expanded until the fifth passage. Cells at this passage were prestained using the PKH26 red fluorescent cell linker kit (Sigma-Aldrich; St. Louis, MO, USA) to identify PKH26 bound to the cell membrane.

3. FUS

A. MSC transplantation using FUS

For the first experiment, 52 male Sprague-Dawley rats (200-220 g) were randomly assigned to one of the five experimental groups before sonication. Rats in the normal group (n = 9) did not undergo any surgical procedure and those in the IV group (n = 7) underwent stem cell transplantation via the tail vein without any surgical procedure. All rats in the sonication group (n = 36) were bilaterally sonicated with low-intensity FUS energy in the lateral hippocampal area, with 7 rats receiving tail vein injection of BM-MSCs after 3 hours. Rats in the FUS only group (n = 24) underwent sonication only and were euthanized 1, 2, and 3 hours after sonication to determine the expression of cell homing molecules. The remaining rats (n=5) were injected with Evans blue via the tail vein 3 hours after sonication to evaluate the BBB permeability status at the time of MSC infusion.

Animals were deeply anesthetized with a mixture of 75 mg/kg ketamine, 0.75 mg/kg acepromazine, and 4 mg/kg xylazine and secured in a stereotaxic frame. An FUS beam was targeted to the bilateral hippocampal region (AP: −3.5; ML: ±2) using a 3D positioning system. Definity (mean diameter range: 1.1–3.3 μm; Lantheus Medical Imaging; North Billerica, MA, USA) Microbubbles (MBs) were diluted in saline and injected intravenously into the tail vein 10 seconds prior to ultrasound sonication. In case of rats injected with Evans blue, the dye (2%, 100 mg/kg) was injected intravenously 3 hours after sonication, and these rats were sacrificed 30 minutes later to examine the BBB opening.

3 hours after sonication, seven rats in the FUS+ Cell group received 200 μL of

BM-MSCs (3×10^6 cells/200 μL) via tail vein injection. Another seven rats in the IV group received 200 μL of BM-MSCs (3×10^6 cells/200 μL) via tail vein injection only. All rats were immunosuppressed with 12.5 mg/kg cyclosporine via daily intraperitoneal injection starting the day before transplantation and continuing until the day they were sacrificed. These 14 rats were sacrificed 24 hours after stem cell transplantation.

For the second experiment, 48 male Sprague-Dawley rats (200-220 g) were randomly assigned to one of the five experimental groups before sonication. Rats in the normal group (n = 10) did not undergo any surgical procedure and those in the other four groups (n = 38) were injected with 192 IgG-saporin into the cerebroventricle. Those assigned to the Lesion group (n=10) did not undergo any additional treatment. Those in the Cell only group (n=10) received 200 μL of BM-MSCs (3×10^6 cells/200 μL) via tail vein injection only. All rats in the sonication group (n=18) were bilaterally sonicated with low-intensity FUS in the lateral hippocampal area, with 10 rats additionally receiving BM-MSCs via tail vein injection after 3 hours. Rats in the FUS only group (n = 8) underwent sonication only.

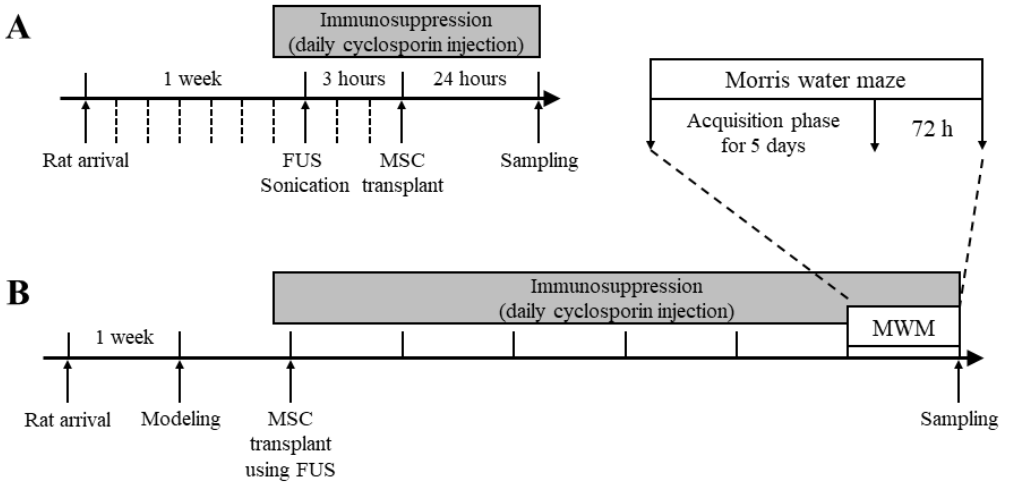

Figure 1. Schematic diagram of the experimental procedure. (A) The first experiment and (B) the second experiment.

B. FUS preparation and sonication parameters

FUS setup and sonication parameters were established based on a previous study.[23] A single-element spherically focused transducer (center frequency: 515 kHz; third harmonic: 1.6 MHz; focal depth: 51.7 mm; and radius of curvature: 63.2 mm; H-107MR; Sonic Concept Inc., Bothell, WA, USA) was driven by a waveform generator (33220A: Agilent Technologies, Palo Alto, CA, USA) and a radio-frequency power amplifier (240L; ENI Inc., Rochester, NY, USA). The transducer electrical impedance was matched to the output impedance of the amplifier (50 ΩS with an external matching network (Sonic Concept Inc., USA). A cone filled with distilled and degassed water was mounted on the transducer assembly, and a needle-type hydrophone (HNA-0400; Onda; Sunnyvale, CA, USA) was used for transducer calibration to measure the acoustic beam profile in the tank filled with degassed water. The sonication parameters involved a 10 ms burst duration at a 1 Hz pulse-repetition frequency for a total duration of 300 seconds to generate 0.25 MPa peak negative pressure on average.

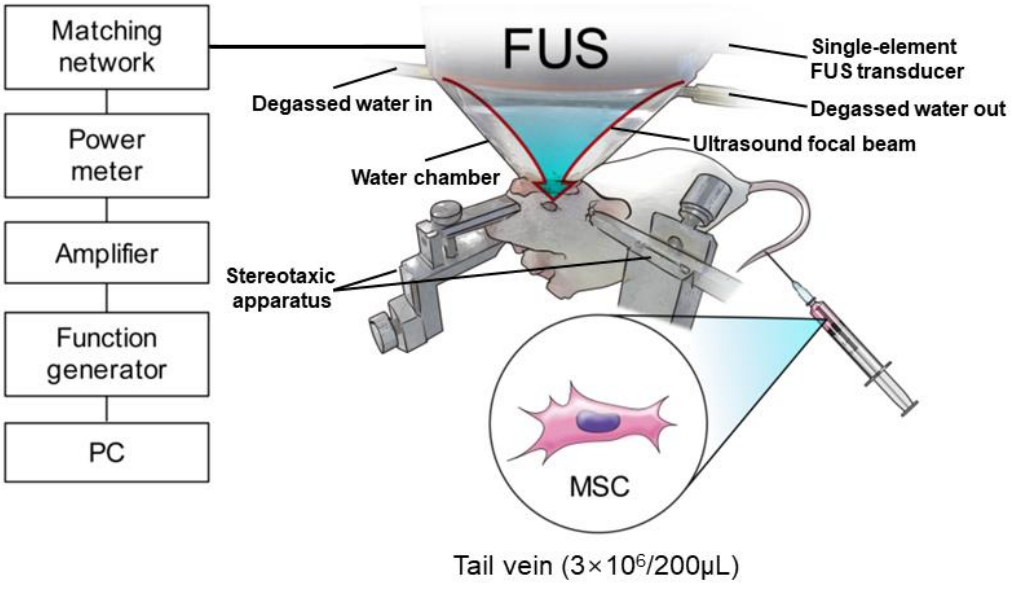

Figure 2. The FUS experimental setup.

4. Behavioral test: Morris water maze

5 weeks after MSC transplantation, the rats were trained on the Morris water maze. The water maze consisted of a circular pool, 2 m in diameter and 0.5 m in depth, filled with opaque tap water (23℃); a circular (0.15 m in diameter) black escape platform was submerged 2 cm below the water surface. The rats were trained in 4 trials per day for 5 consecutive days with a fixed platform. For each training trial, the rats were semi-randomly placed into the pool at one of the four start points and given 60 seconds to reach the platform. Any rat that did not reach the platform within 60 seconds was led to the platform by the experimenter and allowed to remain on the platform for 10 seconds. 72 hours after the last training trial, the rats were given probe trials, lasting 60 seconds. The platform was removed from the pool. Swimming speed, distance, path, time in each zone, and path were recorded using a video tracking system (Harvard Apparatus; Holliston, MA, USA).

5. Histology

Half of the rats in each group were anesthetized with a mixture of ketamine, acepromazine, and Rompun® and perfused with normal saline and cold 4% paraformaldehyde (Duksan; Seoul, South Korea). For immunohistochemistry, the brains were stored in 4% paraformaldehyde for three days at 4℃ and transferred to 30% sucrose (Duksan, South Korea). The brains were sectioned into 30 μm sections using a freezing microtome (Leica Biosystems; Wetzlar, Germany) and stored in a cryoprotectant solution (0.1 M phosphate buffer (pH 7.2), 30% sucrose, 1% polyvinylpyrrolidone (Sigma-Aldrich, USA), and 30% ethylene glycol (Thermo Fisher Scientific; Rockford, IL, USA)) at -20℃. Anatomical landmarks from a

stereotaxic atlas[30] were used to localize the medial septum (MS) and hippocampus.

Fluorescence immunohistochemistry was performed to detect intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, microglial cells, astrocytes and endothelial cells. The sections were blocked with 5% normal goat serum (Vector Labs; Burlingame, CA, USA) for 1 hour at room temperature and incubated with primary antibodies at the following dilutions overnight at 4℃: ICAM-1 (Alexa Fluor 488; 1:100; Santa Cruz Biotechnology, Dallas, TX, USA), VCAM-1 (Alexa Fluor 647; 1:100; Santa Cruz Biotechnology, USA), microglia (Iba1; 1:300; Wako Chemicals, Richmond, VA, USA), astrocytes (glial fibrillary acidic protein; GFAP; 1:300; Abcam, Cambridge, UK), and endothelial cells (rat endothelial cell antigen-1; RECA-1; 1:300; Serotec, Oxford, UK). After the primary immunoreaction, sections were incubated with secondary antibodies conjugated with Alexa Fluor 647 (AF647; 1:300; Thermo Fisher Scientific, USA) or Alexa Fluor 488 (AF488; 1:300; Thermo Fisher Scientific, USA) for 2 hours at room temperature. The staining intensity of the sections was visualized using a LSM 700 confocal microscope (Carl Zeiss, Jena, Germany).

Hematoxylin (Vector Labs, USA) & eosin (Sigma-Aldrich, USA) (H&E) staining was performed to examine brain tissue damage and red blood cell extravasation. Brains were embedded in paraffin-wax, cut into 4 μm sections, and stained with H&E.

The BBB opening was confirmed in gross tissue sections based on the presence of Evans blue leakage in the FUS-sonicated regions of the brain and on the basis of histological assessment.

6. Western blotting

The brains of the remaining half of the anesthetized rats were quickly excised for protein extraction. The MS and hippocampus were dissected using fine forceps from 1 mm coronal brain slices. The samples were homogenized in lysis buffer (Intron, Seongnam, Korea) and placed on ice for 30 minutes. They were then centrifuged for 20 minutes at 12,000 rpm, and the protein content in the supernatant was measured using the bicinchoninic acid protein assay reagent kit (Pierce, Rockford, IL, USA). The protein samples were stored at -70℃. Proteins were separated by 10-15% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes. The membranes were incubated with blocking buffer (5% non-fat dry milk in phosphate-buffered saline containing 0.05% Tween 20, PBST) for 1hour at room temperature. The membranes were then incubated with the indicated antibodies overnight at 4℃ and washed three times (5 minutes each) with PBST. Subsequently, the membranes were incubated with the corresponding secondary antibodies for 2 hours at room temperature. After washing with PBST, proteins were detected using enhanced chemiluminescence solution (Pierce, USA) and LAS-4,000 (Fujifilm, Tokyo, Japan). The intensity of each band was determined using an analysis system (Multi Gauge version 3.0, Fujifilm, Japan). The membranes were incubated with antibodies against MMP-2 (Matrix metalloproteinase-2; 1:1,000; Santa Cruz Biotechnology, USA), DCX (Doublecortin; 1:1,000; Santa Cruz Biotechnology, USA), NeuN (Neuronal nuclei; 1:1,000; Chemicon, Temecula, CA, USA), BDNF (Brain-derived neurotrophic factor; 1:1,000; Millipore, Temecula, CA, USA), NGF (Nerve growth factor; 1:1,000; Abcam, UK), TrkB (Tropomyosin receptor kinase B; 1:1,000; Abcam, UK), IL-1β (Interleukin 1 beta; 1:1,000; Abcam,

UK), and β-actin (1:10,000; Sigma-Aldrich, USA).

7. Acetylcholinesterase (AChE) assay

The protein sample used for this assay was the same as that used for western blotting. The enzymatic activity of AChE was determined using the method proposed by Ellman et al.[31] with some modifications. In brief, triplicate samples (20 μL) were mixed with the reaction mixture (0.2 mM dithiobisnitrobenzoic acid (Sigma-Aldrich, USA), 0.56 mM acetylthiocholine iodide (Sigma-Aldrich, USA), 10 μM tetraisopropylpyrophosphoramide (Sigma-Aldrich, USA), and 39 mM phosphate buffer (pH 7.2)) at 37℃. After 30 minutes, the optical density was measured at 405 nm.

8. Statistical analyses

A. Analysis of Morris water maze results

All data were expressed as the mean ± standard error of the mean (SEM). SPSS software (v.25; IBM Corp.; Armonk, NY, USA) was used for statistical analysis, and a *p* value <0.05 was considered as statistically significant. Student's t-test and One-way ANOVA were used for the statistical analysis. ANOVA was performed followed by a post hoc least significant difference test or Tukey's honestly significant difference test.

B. Analysis of immunohistochemistry and western blotting results

The numbers of ICAM-1, VCAM-1, and Iba-1 immunopositive cells and PKH26 positive cells were counted in nine coronal sections per group, located 3.0 to 3.6 mm posterior to the bregma. The protein band intensity was normalized to that of β-actin in each sample and expressed as a percentage of the control values. Student's t-test and One-way ANOVA were used for the statistical analysis. ANOVA was performed followed by a post hoc least significant difference test or Tukey's honestly significant difference test. p values <0.05 were considered as statistically significant. All statistical analyses were performed using SPSS software.

III. RESULTS

1. Evans blue extravasation at the time of MSC infusion

To confirm BBB permeability during MSC injection, 5 rats injected with Evans blue 3 hours after FUS sonication were examined. Leakage of Evans blue was visualized in the sonicated hippocampal region. To clearly observe dye leakage, Evans blue fluorescence (excitation at 620 nm, emission at 680 nm) was detected in the cryostat sections of the brain tissue using LSM 700 confocal microscopy imaging (Figure 3).

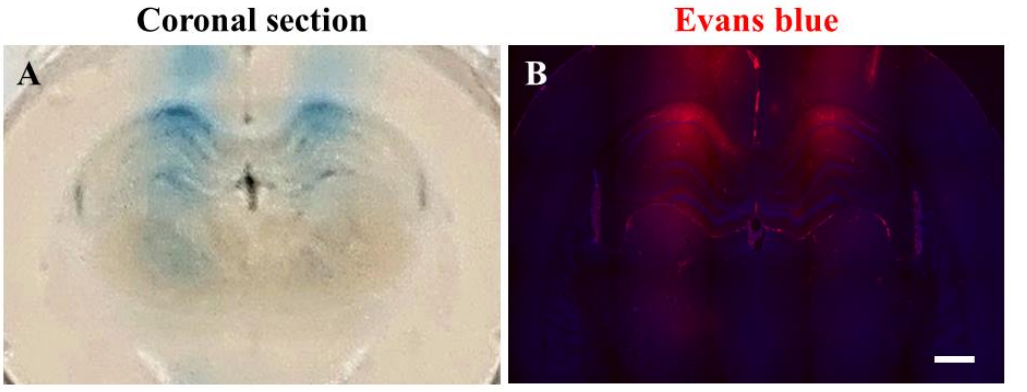

Figure 3. Evans blue extravasation in the brain tissue 3 hours post-FUS sonication. (A) In the sonicated region, Evans blue extravasation was confirmed in the coronal section of the brain tissue, and (B) the fluorescence signal of Evans blue was detected in the sonicated region. Scale bar represents 1 mm.

2. Increased MSC transplantation efficacy by FUS sonication

To analyze the effects of FUS on trans-endothelial MSC migration, we compared five rats not receiving FUS (Cell only group) with seven rats receiving FUS before injection of PKH26-prestained MSCs (FUS + Cell group) via the tail vein. The sonicated hippocampal region, which was the FUS-target area, was transected with a 30 μm width in both the groups, and PKH26-positive cells were counted in nine coronal sections per group, located 3.0 to 3.6 mm posterior to the bregma. The mean number of PHK26-positive cells in the hippocampal region in the Cell only group was 758.8 ± 40.55, while that in the FUS + Cell group was 1701 ± 42.26 (*p <0.0001), indicating that FUS significantly increased the yield of trans-endothelial migrated MSCs (Figure 4).

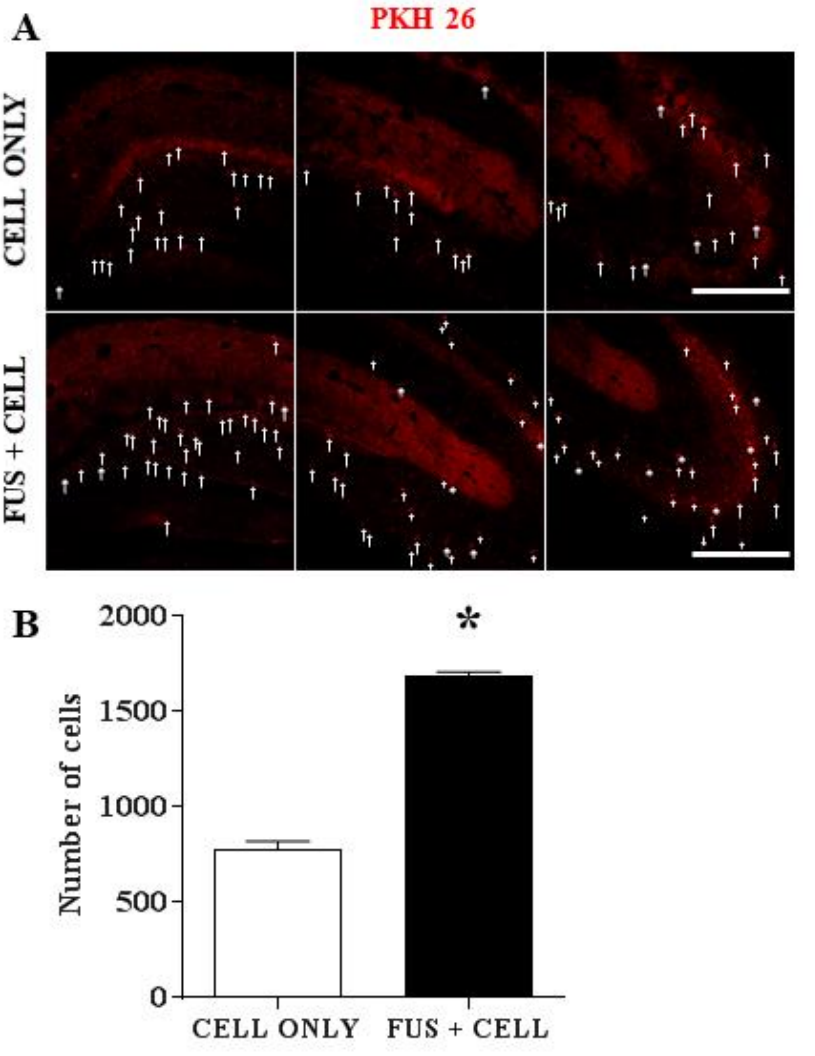

Figure 4. Comparison of transplantation yield between the control and FUS groups. (A) Immunohistochemistry of PKH26-positive cells in the hippocampal region (white arrows) and (B) statistical analysis of the difference between groups. "Cell only" indicates the group receiving cell transplantation without FUS. And FUS + Cell indicates the group receiving cell transplantation with FUS. Scale bar represents 500 μm. Data are expressed as the mean ± SEM.

3. Changes in the expression of proteins related to MSC homing after FUS sonication

Half of the rats without any treatment (normal) and the other half of rats receiving FUS sonication in the hippocampal region (FUS only group) were used to analyze the effect of FUS on ICAM-1, VCAM-1, and MMP-2 expression. The same three hippocampal regions in both groups were selected for immunohistochemical analysis, and cells reactive to antibodies for ICAM-1 and VCAM-1 were counted for comparison. The mean number of ICAM-1-positive cells in the normal group was significantly lower than that in the FUS only group (167.5 ± 13.74 vs. 367.2 ± 18.37, respectively; *p <0.0001). Similarly, the mean number of VCAM-1-positive cells in the normal group was significantly lower than that in the FUS only group (199.9 ± 12.05 vs. 334.9 ± 13.91, respectively; *p <0.0001) (Figure 5).

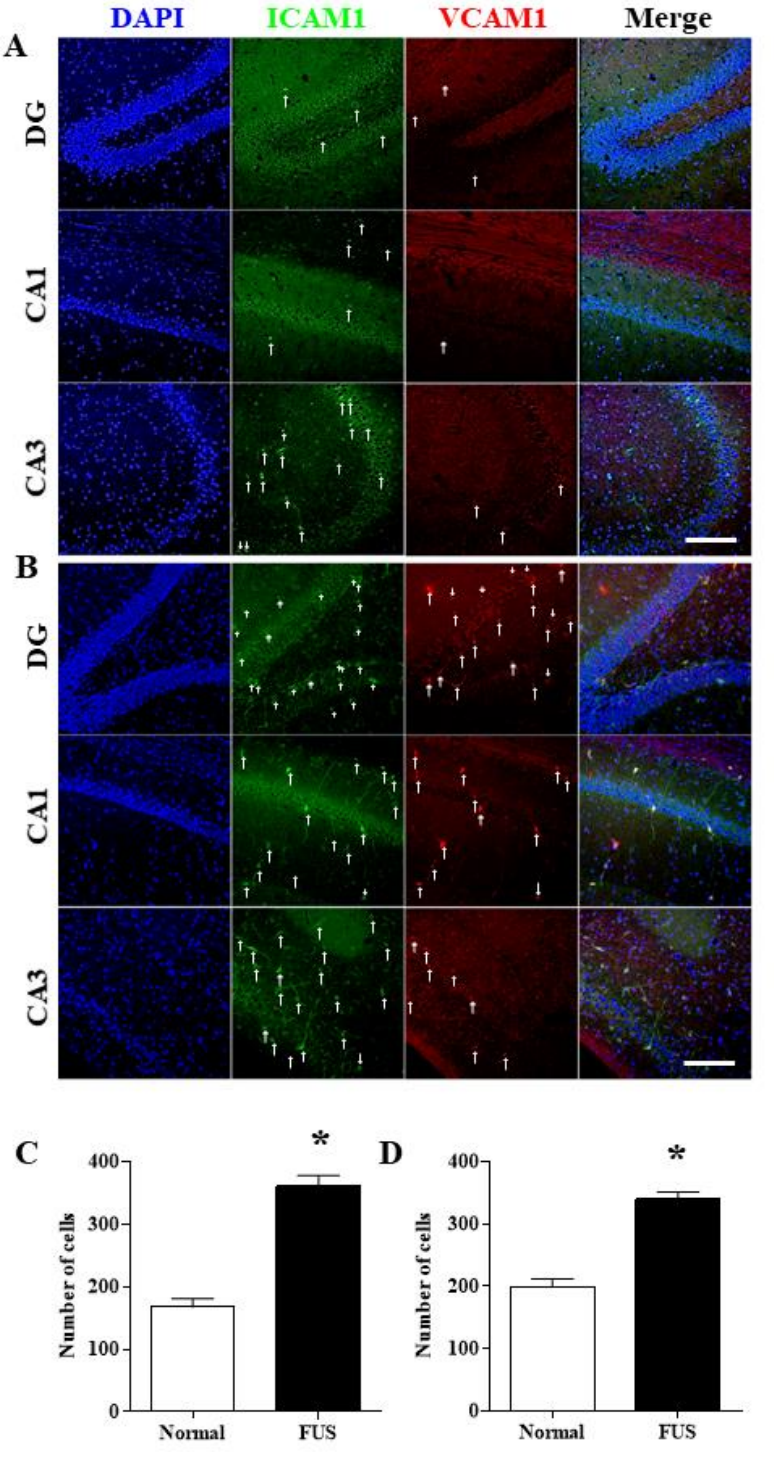

Figure 5. Immunohistochemical analysis of ICAM-1 and VCAM-1. Expression of both ICAM-1 and VCAM-1 was higher in the FUS-treated group (white arrows) (B) than in the untreated group (A). Comparison of ICAM-1(C) and VCAM-1 expression (D) between the control and FUS groups. Scale 200 μm. Data are expressed as the mean ± SEM.

Analyses of ICAM-1 and VCAM-1 co-localization with other cells, such as glial cells, microglia, and endothelial cells, were performed in the dentate gyrus, hilus of hippocampus, cornu ammonis (CA)1, and CA3. The results showed that ICAM-1 was expressed in both glial cells and microglia, whereas VCAM-1 expression was mainly observed in the endothelial cells across all hippocampal structures (Figure 6).

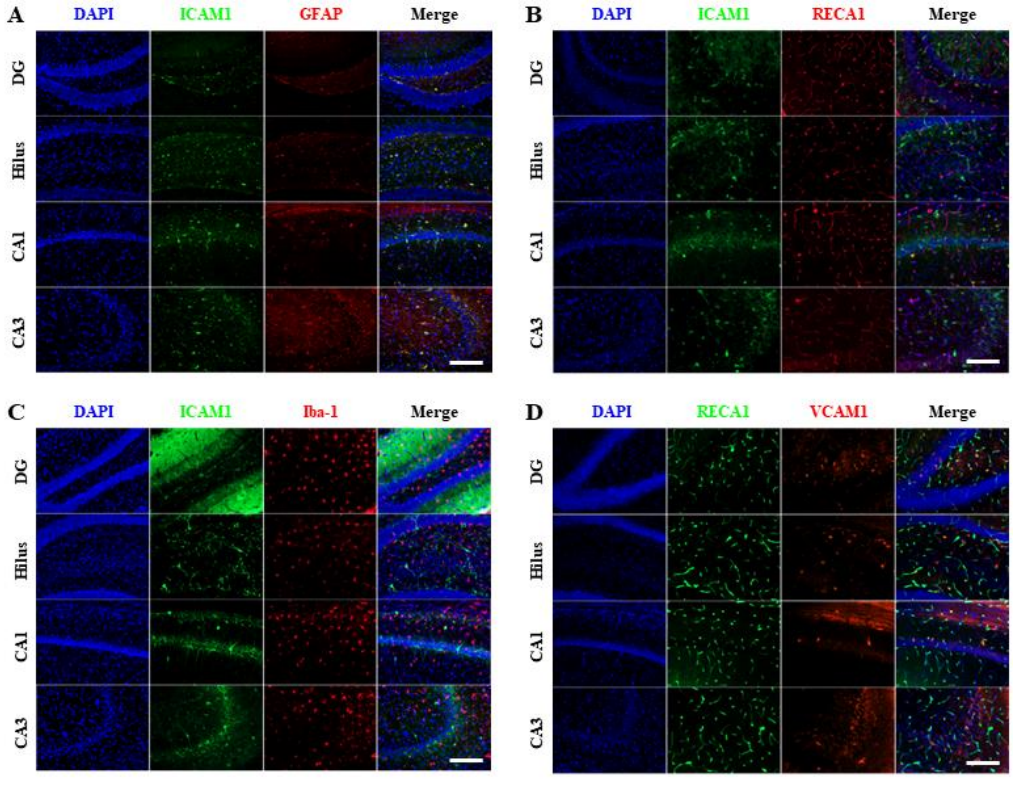

Figure 6. CAM co-localization. Co-localization of ICAM-1-positive cells with astrocytes (A), endothelial cells (B), and microglia (C). Co-localization of VCAM-1 cells with endothelial cells (D). Scale bar represents 200 μm.

Sonicated hippocampal tissues, from 4 rats in the normal group and 12 rats in the FUS only group, were used to determine MMP-2 expression by western blotting. MMP-2 expression was significantly higher in the FUS only group than that in the normal group (2 hours, $*p = 0.0222$ and 3 hours, 0.0347) (Figure 7).

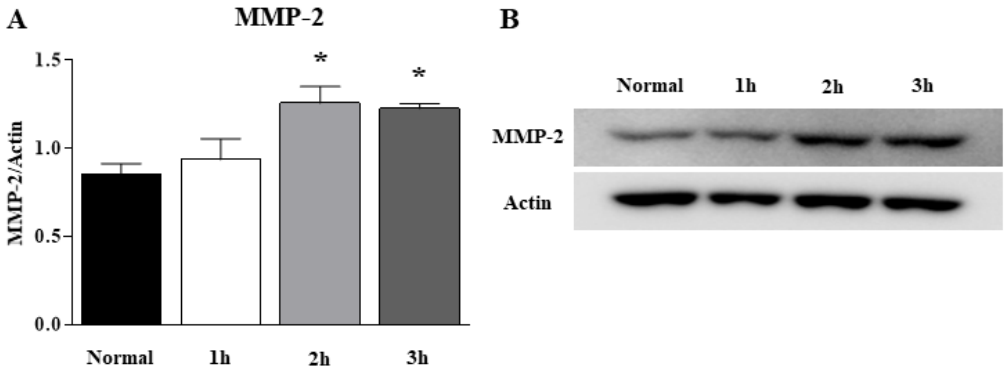

Figure 7. Western blotting analysis of MMP-2 expression. The expression level of MMP-2 was increased after sonication in the hippocampal region. (A) Statistical analysis of the difference in expression at each time point (2 hours, *p = 0.0222 and 3 hours, 0.0347). (B) Representative results of western blotting. Values are represented as the mean ± SEM. p values < 0.05 were considered as statistically significant compared to the normal group.

4. Activation of microglia in the FUS-sonicated region

Microglial cells in the sonicated hippocampal region of both control and FUS group of rats were counted using the microglial cell marker, Iba1. As shown in Figure 8, microglial activities were markedly increased after FUS (*p = 0.0128). Evaluation of damage to the brain region by H&E staining revealed no visible structural injuries or extravasated red blood cells in the sonicated brain regions (Figure 9), indicating that FUS induced an inflammatory reaction without any structural damage.

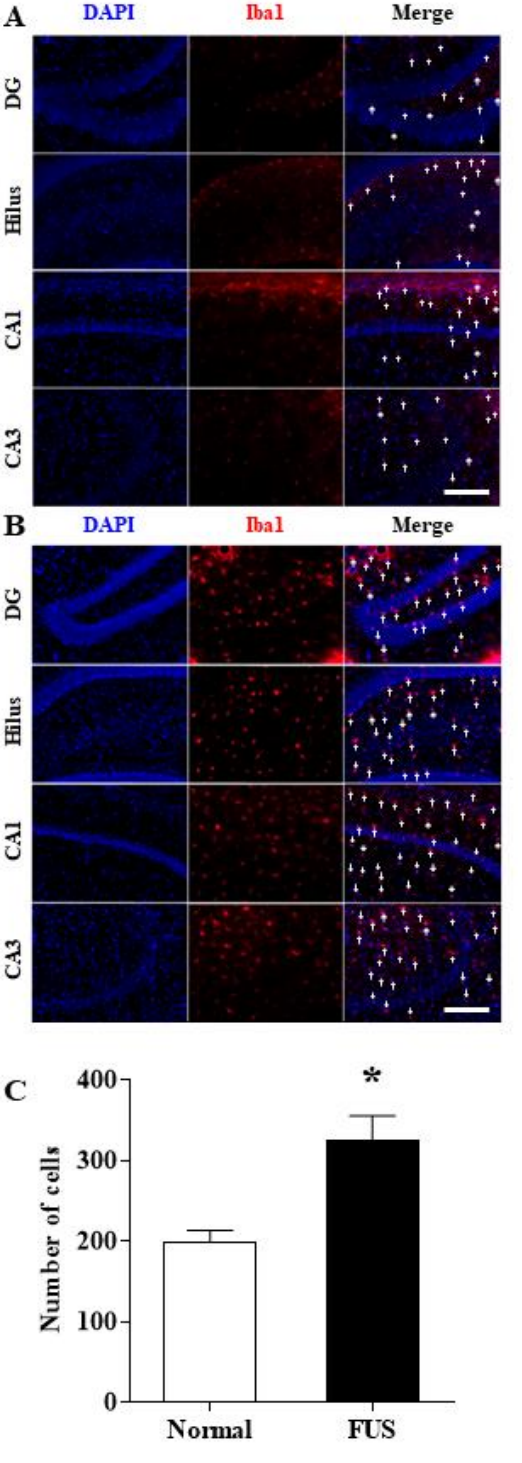

Figure 8. FUS-induced alteration of microglial activity. Changes in microglial activity after FUS (white arrows) (A, B) and statistical analysis of differences between groups (C). Microglial activity was markedly increased in the FUS group (B) relative to that in the control group (*p = 0.0128) (A). Scale bar represents 200 μm. Data are expressed as the mean ± SEM.

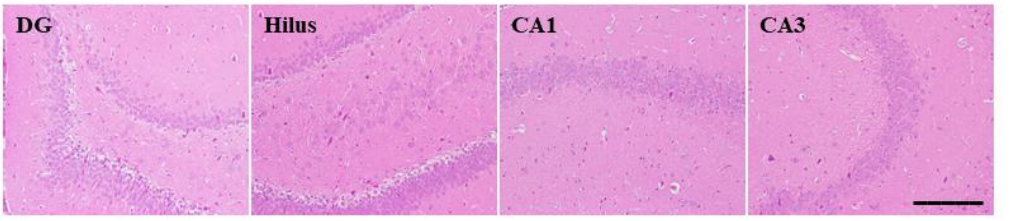

Figure 9. FUS-induced alteration of microglial activity. The sonicated brain sections were histologically evaluated by H&E staining. Scale bar represents 200 μm.

5. Continuance of increased transplantation efficacy of MSCs by FUS sonication

To analyze the robustness of enhanced trans-endothelial MSC existence, we compared 5 rats without FUS (Cell only) with 5 rats receiving FUS before injecting PKH26-prestained MSCs (FUS + Cell group) via the tail vein. The sonicated hippocampal region, which was the FUS-target area, was transected with a 30 μm width in both the groups, and PKH26-positive cells were counted in 9 coronal sections per group, located 3.0 to 3.6 mm posterior to bregma. The mean number of PKH26-positive cells in the hippocampal region in the Cell only group was 850.8 ± 65.63, while that in the FUS + Cell group was 1696 ± 98.95 *(*p* <0.0001). This number was not significantly different from the earlier experimental result (Figure 4), indicating that the FUS-induced increase in MSC number was still significantly high (Figure 10).

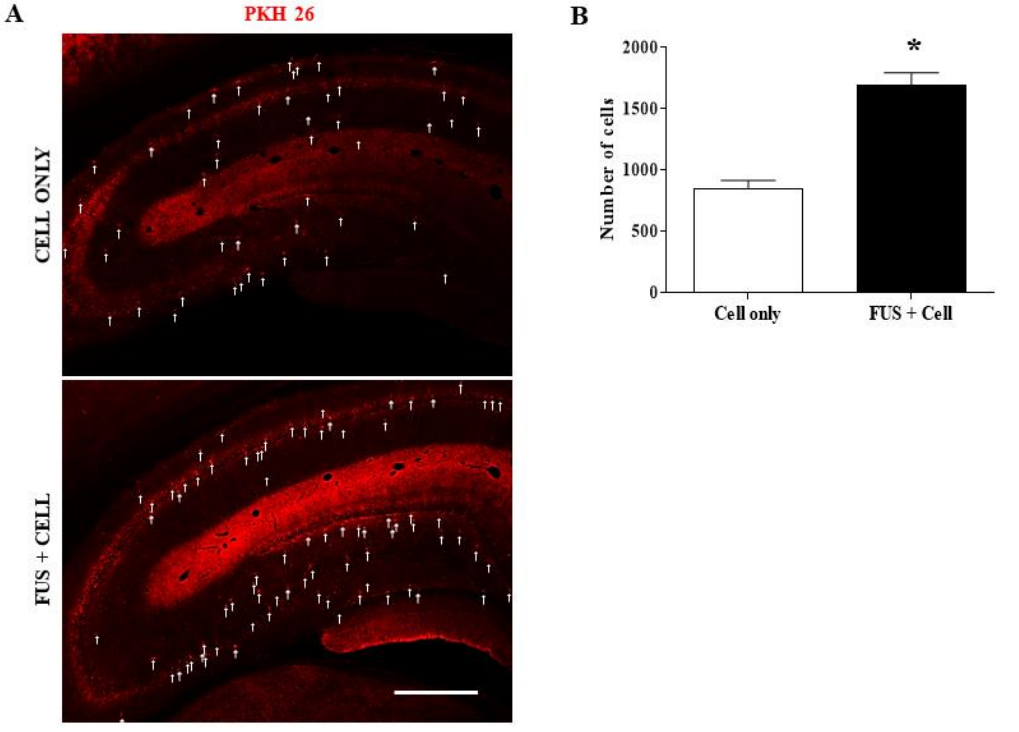

Figure 10. Comparison of transplantation yield between the Cell only and FUS + Cell groups in the 192 IgG-saporin rat model. Immunohistochemistry for PKH26-positive cells in the hippocampal region (white arrows) (A) and statistical analysis of the difference between groups (B). “Cell only” indicates the control group, and “FUS + Cell” indicates the group receiving cell transplantation with FUS. Scale bar represents 500 μm. Data are expressed as the mean ± SEM.

6. Spatial memory improvement by MSCs transplantation using FUS

The results of the Morris water maze training are shown in Figure 11. In all the groups, the escape latency decreased from the first day until the last day of training (from over 45 seconds to less than 16 seconds). During the acquisition phase, the latency of the rats in the Lesion (second day, $*p = 0.005$), the Cell only (second day, $*p = 0.027$, third day $*p = 0.023$), the FUS + Cell (second day, third day $*p = 0.020$), and the FUS only (second day, $*p = 0.046$) groups was significantly delayed but was recovered within 1 week. These data demonstrate progressive learning of the hidden platform location (Figure 11). In the Morris water maze probe test, rats of all groups showed no differences in motor-related behavior compared to normal rats, as evidenced by similar swim distances and speeds (Figure 12A). These findings suggest that cholinergic lesion and FUS sonication had no effect on motor function. The time spent in the target quadrant (where the platform was placed) was not significantly different between the groups (Figure 12B). However, the amount of time spent in the platform zone was significantly decreased in the Lesion and FUS only groups compared to that in the normal group ($*p = 0.007$ and 0.018, respectively. Figure 12C). It was significantly increased in the FUS + Cell group compared to the Lesion group ($\dagger p = 0.019$, Figure 12C). Additionally, the number of platform crossings was significantly reduced in the Lesion and FUS only groups compared to the normal group ($*p=0.00$ and 0.00, respectively. Figure 12D). However, it was significantly increased in the FUS + Cell group of rats compared to that in the Lesion group of rats ($\dagger p=0.00$, Figure 12D). Moreover, it was significantly increased in the FUS + Cell group compared to that in the Cell only group ($*p = 0.029$)

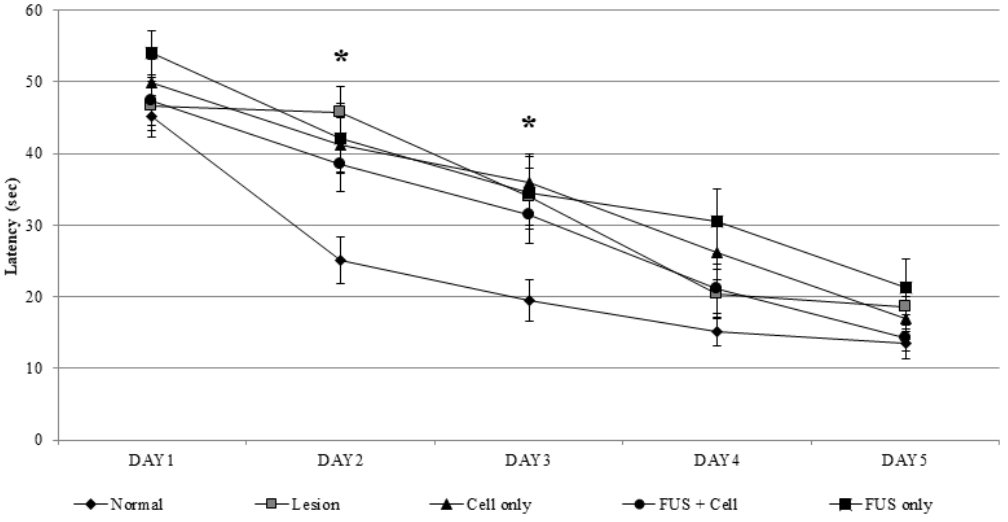

Figure 11. Effect of MSC transplantation using FUS on the spatial memory-training phase. Latency indicates the time required for the rats to find the escape platform during training trials. Rats of all groups gradually reached the platform location (*p = 0.00). During the acquisition phase, the latency of the Lesion (second day, *p = 0.005), the Cell only (second day, *p = 0.027, third day *p = 0.023), the FUS + Cell (second day, third day *p = 0.020), and the FUS only (second day, *p = 0.046) group of rats was significantly delayed but was recovered within 1 week. All data are expressed as the mean ± SEM.

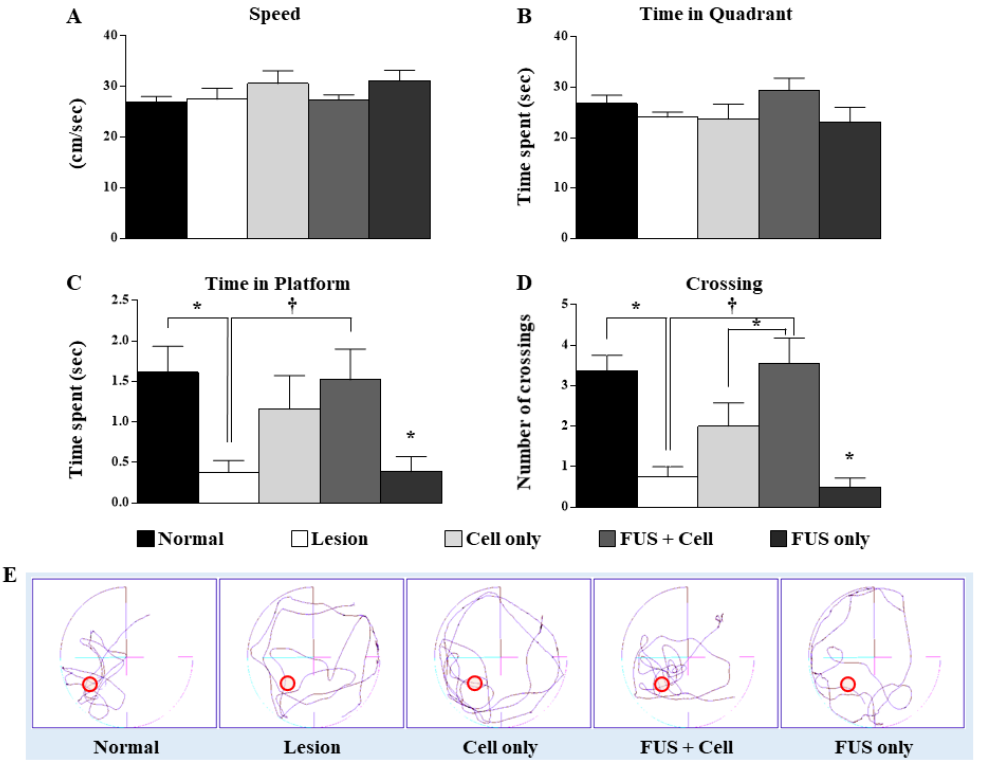

Figure 12. Effect of MSC transplantation using FUS on spatial memory-probe testing. (A) Rats of all groups showed no difference in motor-related behavior compared to normal rats, as evidenced by similar swim distances and speeds. (B) The time spent in the target quadrant (where the platform was placed) was not significantly different between groups. (C) The amount of time spent in the platform zone was significantly decreased in the Lesion and FUS only group of rats compared to that in normal rats (*p = 0.007 and 0.018, respectively). It was significantly increased in the FUS + Cell group compared to that in the Lesion group (†p = 0.019). (D) The number of platform crossings was significantly decreased in the Lesion and FUS only group of rats compared to that in normal rats (*p = 0.00 and 0.00

respectively). However, it was significantly increased in the FUS + Cell group of rats compared to that in the Lesion group of rats (†p = 0.00). And it was significantly increased in the FUS + Cell group compared to that in the Cell only group (*p = 0.029) (E) Trajectories of rats in the Morris water maze probe test. All data are expressed as the mean ± SEM.

7. Effects on cholinergic denervation by MSCs transplantation using FUS

Cholinergic denervation was evaluated by counting the ChAT immunopositive cells (Figure 13) and quantifying AChE activity by Ellman assay in the MS (Figure 14). The number of ChAT immunopositive neurons in normal rats was 97.17 ± 15.6. In contrast, the number of cholinergic neurons in rats injected with 192 IgG-saporin was significantly low (*p = 0.003). The number of cholinergic neurons in the Lesion, Cell only, FUS + Cell and FUS only groups of rats was 24.87 ± 8.462, 43.07 ± 5.84, 72.13 ± 3.813 and 23.78 ± 7.595, respectively. Thus, it was increased in the FUS + Cell group (72.13 ± 3.813) compared to that in lesion rats. And also it was significantly increased in the FUS + Cell group compared to that in the Cell only group (*p = 0.029). AChE activity was significantly reduced in the MS of the Lesion group of rats (*p = 0.013) compared to that in normal rats. However, it was improved in the Cell only and FUS + Cell group of rats compared to the Lesion group (†p = 0.027 and 0.028, respectively). In the hippocampus, unlike MS, the AChE activity was not restored in the Cell only and FUS + Cell group of rats (Figure 14).

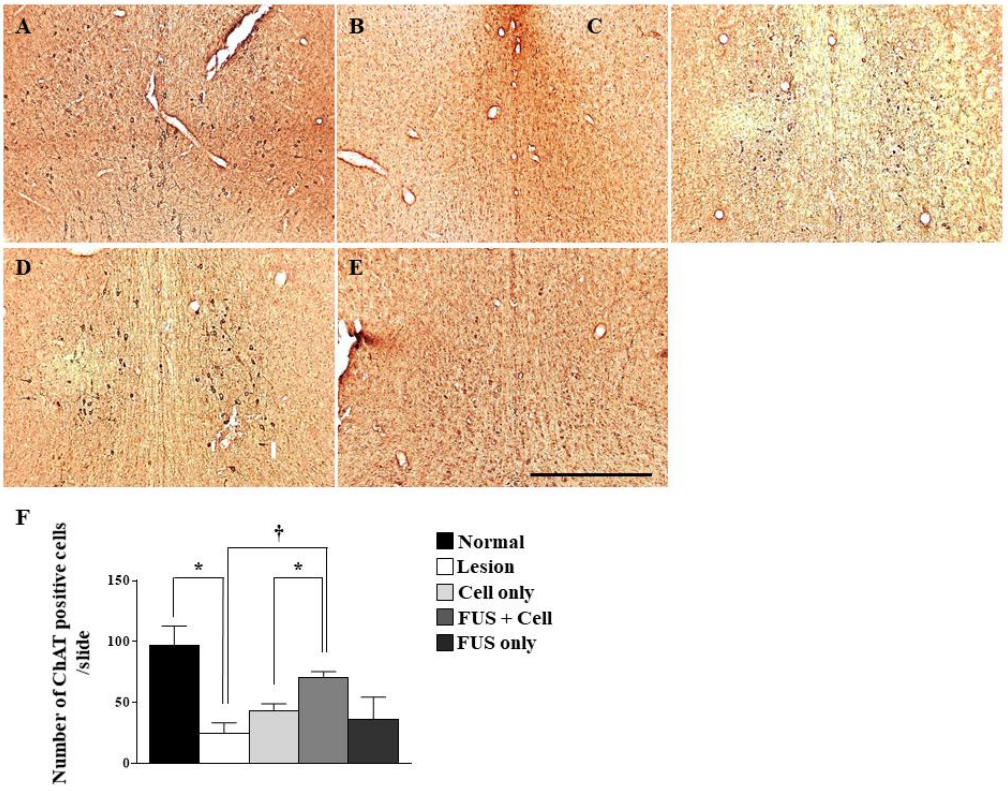

Figure 13. Representative images showing the effects of the cholinergic lesion after intraventricular injection of 192 IgG-saporin. (A) Normal group, (B) Lesion gourp, (C) Cell only group, (D) FUS + Cell group, (E) FUS only group. (F) The number of ChAT immunopositive cells was significantly decreased in the 192 IgG-saporin injected lesion group compared to the normal group (A) in the MS (*p = 0.00). And it was significantly increased in the FUS + Cell group of rats compared to that in the Lesion group of rats (†p = 0.001). And also it was significantly increased in the FUS + Cell group compared to that in the Cell only group (*p = 0.029). Scale bar represents 500 μm. All data are expressed as the mean ± SEM.

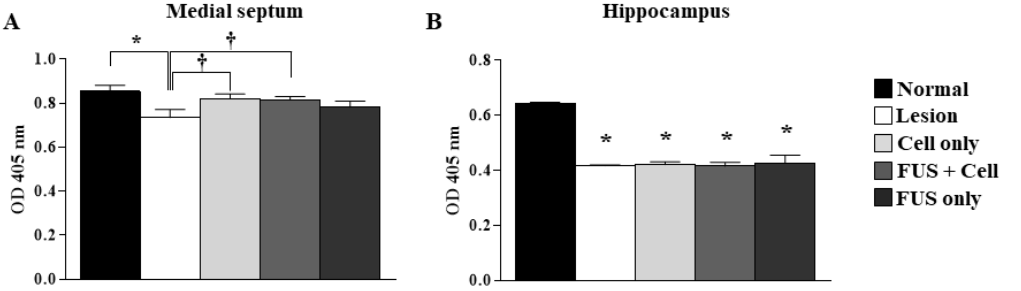

Figure 14. Change in AChE activity in the Cell only and FUS + Cell groups. (A) AChE activity in the MS. AChE activity was significantly decreased in the Lesion group compared to the normal group (*p = 0.013), while it was significantly increased in the Cell only and FUS + Cell groups compared to the Lesion group (†p = 0.027 and †p = 0.028, respectively). (B) AChE activity in the hippocampus. Hippocampal AChE activity in all the groups was significantly lower than that in the normal group (*p = 0.000). The AChE activity was expressed as the optical density at 405 nm. Values are represented as the mean ± SEM. p values <0.05 were considered as statistically significant compared to the normal group.

8. Changes in the expression of BDNF and NGF

Neurotrophic factors, such as BDNF and NGF, are proteins related to neuronal survival and plasticity of cholinergic neurons in the CNS. Especially, cognitive deficit is related to alterations in the levels of neurotrophic factors such as these 2 factors.[32,33] MSCs can produce and secrete neurotrophic factors.[34,35] Western blotting was performed to measure the changes in the expression levels of BDNF and NGF. The expression of NGF was not significantly different in the sonicated hippocampal regions of the Lesion, Cell only, FUS + Cell, and FUS only groups of rats compared to the normal group (Figure 15B). However, BDNF expression in the hippocampus was markedly higher (†p = 0.012) only in the FUS + Cell group of rats (Figure 15A).

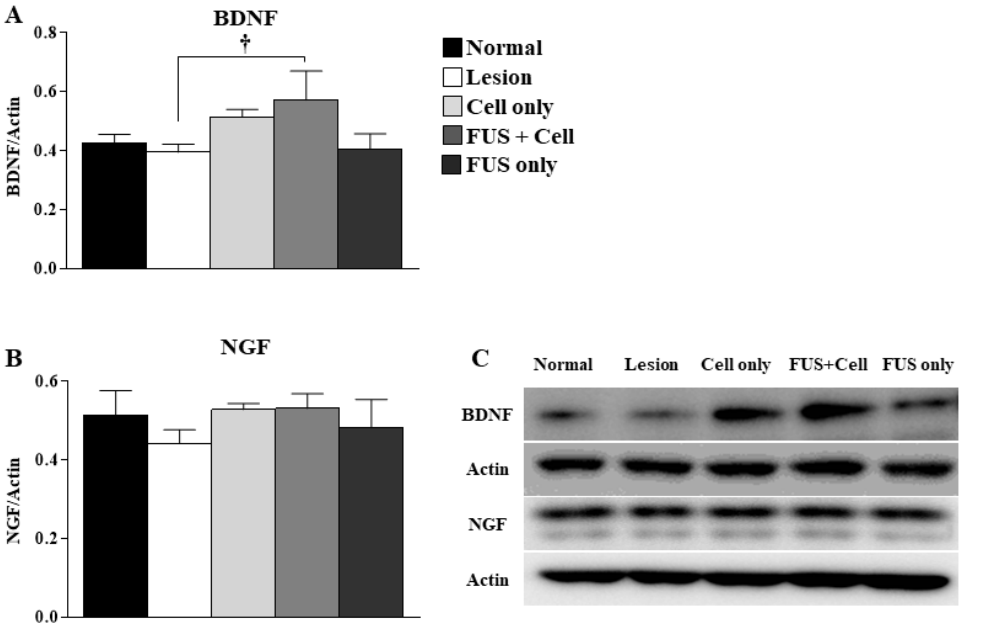

Figure 15. Expression levels of BDNF and NGF associated with MSC transplantation using FUS. (A) The expression level of BDNF was increased only in the FUS + Cell group compared to the Lesion group (†p = 0.012). (B) The NGF expression level showed an increase in the Cell only and FUS + Cell groups but, the increase was not significant. (C) Representative results of western blotting. Values are represented as the mean ± SEM.

9. MSC transplantation by FUS influenced proinflammatory cytokine levels in the 192 IgG-saporin rat model

Some studies have shown the effects of MSCs on immune cells and inflammation-associated cytokines in diseases associated with CNS damage.[36] Hence, to verify the anti-inflammatory and immunomodulatory properties of MSCs transplanted by FUS in the 192 IgG-saporin rat model, the expression of IL-1β, an important mediator of inflammatory response, was evaluated by western blotting. IL-1β expression was significantly increased in the Lesion and FUS only groups of rats (**p*=0.026 and 0.007, respectively). The Cell only group of rats showed a decrease compared to the Lesion group of rats, but there was no significant difference between the levels. The level in the FUS + Cell group of rats was significantly lower than that in the Lesion group of rats (**p*=0.012, Figure 16).

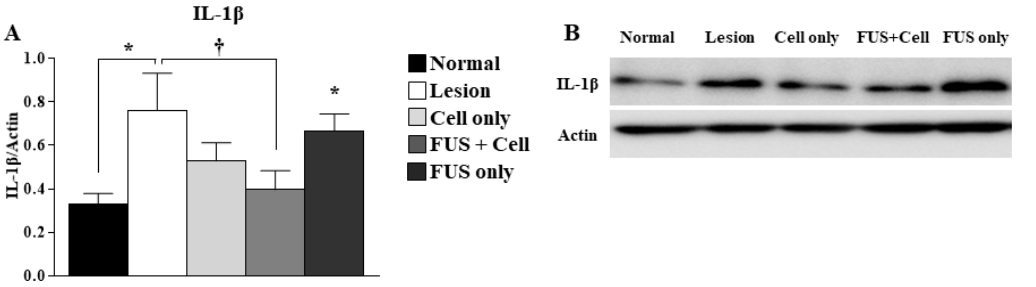

Figure 16. Expression levels of IL-1β associated with MSC transplantation using FUS. (A) Protein levels of IL-1β were significantly elevated in the Lesion and FUS only groups compared to the normal group (*p = 0.026 and 0.007, respectively). However, these high levels were significantly reduced in the FUS + Cell group compared to the Lesion group (†p = 0.012). (B) Representative results of western blotting. Values are represented as the mean ± SEM.

10. Enhanced neurogenesis by MSC transplantation by FUS

It has been reported that MSC transplantation enhances functional recovery and endogenous neurogenesis in neurodegenerative diseases.[37,38] To evaluate the effect of MSC transplantation using FUS on endogenous neurogenesis and differentiation, neuroblasts or post-mitotic immature neuron (DCX) and mature neuron (NeuN) were quantified (Figure 17). DCX and NeuN expression was significantly decreased in the Lesion (DCX, $*p = 0.005$ and NeuN, $*p = 0.047$) and FUS only (DCX, $* p = 0.024$ and NeuN, $*p = 0.047$) groups of rats compared to that in normal rats. However, compared to the Lesion group of rats, levels of both the factors were increased in the FUS + Cell group of rats (DCX, $\dagger p = 0.012$ and NeuN, $\dagger p = 0.003$). They were also increased in the Cell only group of rats compared to the Lesion group of rats; however, it was not a significant increase (Figure 17 A, B). And NeuN expression was significantly increased in the FUS + Cell group compared to that in the Cell only group ($*p = 0.031$). In addition, since it has been reported that the BDNF-TrkB signaling contributes to neurogenesis and TrkB is the high-affinity receptor for BDNF, we confirmed the expression of TrkB.[39,40] The expression of TrkB in the sonicated hippocampal region of the FUS + Cell group of rats was significantly increased ($\dagger p = 0.002$, Figure 17 C).

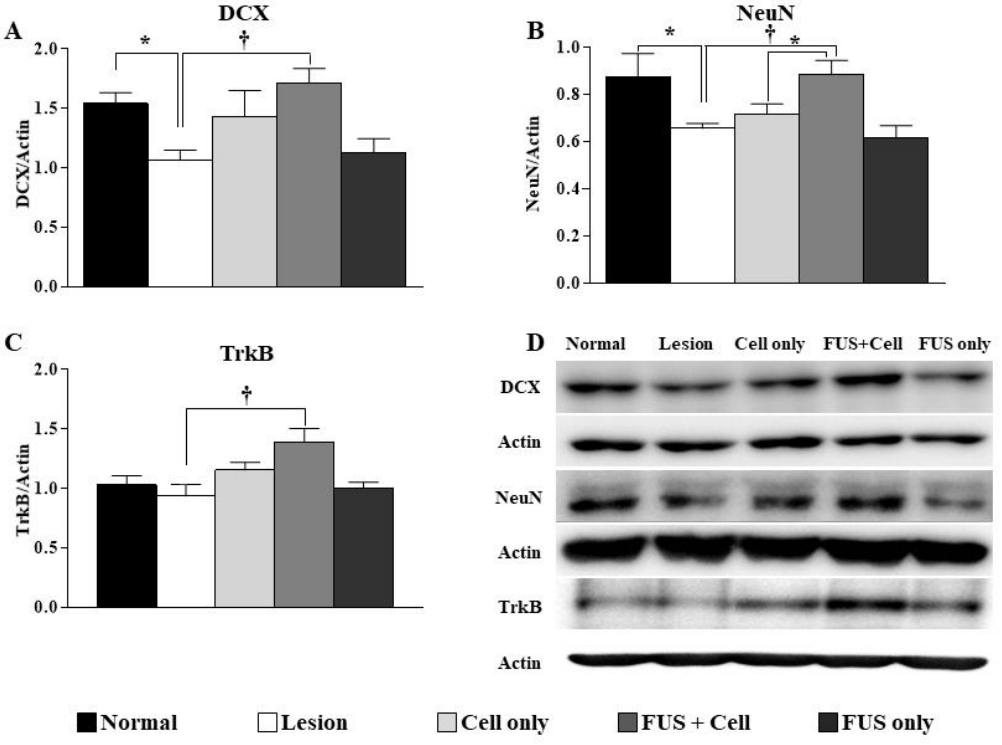

Figure 17. Effects of MSC transplantation using FUS on adult hippocampal neurogenesis. Protein levels of DCX (A) and NeuN (B) were significantly decreased in the Lesion and FUS only groups compared to the normal group (DCX, *p = 0.005 and 0.024, respectively and NeuN, *p = 0.047 and 0.047, respectively) but were elevated in the FUS + Cell group (DCX, †p = 0.012 and NeuN, †p = 0.003). And NeuN expression was significantly increased in the FUS + Cell group compared to that in the Cell only (*p = 0.031). (C) The expression levels of TrkB were significantly increased only in the FUS + Cell group compared to the Lesion group (†p = 0.002). (D) Representative results of western blotting. Values are represented as the mean ± SEM.

IV. DISCUSSION

In this study, first, we focused on the possibility of using FUS for stem cell homing and its biological effect. Therefore, we hypothesized that FUS at a specific area of the brain could non-invasively increase stem cell transplantation efficacy. In the FUS-treated rats, we observed more than 2-fold increase in MSC migration relative to that observed in untreated rats and verified the upregulated expression of cell adhesion molecules and MMP-2 in FUS-treated rats. Interestingly, both ICAM-1 and VCAM-1 were expressed in endothelial cells, whereas only ICAM-1 was expressed in subendothelial cells, such as astrocytes and microglia, even without structural injury and hemorrhage. A previous study has reported that the activation of CAMs and MMPs is related to stimulation by various cytokines and that stem cells migrate to the activated site. Therefore, the results of our first experiment suggest that FUS can enhance the efficacy of stem cell homing, and FUS-induced ICAM-1, VCAM-1, and MMP-2 expression could be associated with the targeted homing of MSCs. However, further studies are required to confirm these findings.

Recently, the possibility of stem cell transplantation using FUS has been studied, but in practice, the effects of transplanted stem cells in CNS diseases have not been evaluated. Therefore, in the second experiment, we confirmed that the effect of transplanted MSCs, was 2-fold more in the specific brain area by FUS, in the 192 IgG-saporin rat model to prove the therapeutic efficacy. The highest restoration of cognitive function was observed in FUS + Cell group of rats in the Morris water maze

test. In addition, we confirmed that the expression levels of endogenous neurogenesis factors and BDNF were increased in the FUS + Cell group of rats. Moreover, we observed that the immuno-modulatory effect of transplanted MSCs was 2-fold more in the FUS + Cell group of rats. Therefore, the results of our second experiment suggest that FUS-induced MSC transplantation can improve cognitive function by stimulating endogenous neurogenesis and upregulating the expression levels of BDNF and TrkB in the 192 IgG-saporin rat model.

1. Non-invasive and targeted MSC transplantation into the brain using FUS

Stem cell-based therapy is positively studied and utilized in all fields of regenerative medicine in the CNS. Furthermore, many clinical trials that focus on treating CNS diseases using stem cells have been conducted; however, few have reported successful outcomes, despite preclinical studies for each disease model showing excellent therapeutic effects. These unfavorable results could be associated with various factors, including the number of transplanted stem cells, biological properties of each stem cell, and the route of transplantation. Since the number of stem cells in the target area is a critical factor for successful treatment, invasive surgical methods including direct transplantation and transplantation using a catheter and reservoir implantation have been used.[41,42] However, because these modalities require direct injection or implantation into the brain parenchyma, tissue damage and surgical complications may occur.

Other strategies to increase the number of transplanted MSCs include modulation of stem cells to exhibit reactivity to migratory stimuli and alteration of target sites to attract stem cells.[43] These strategies include treatment of MSCs with chemical compounds,[44] preconditioning with hypoxia,[45] genetic modifications,[46] and coating with antibodies.[47] Additionally, target site modulation to attract MSCs involves direct injection of chemokines[48] and direct transfection of target tissue with chemokine-encoding genes.[49] However, these methods have limitations. Treatment with various factors or genetic alteration of MSCs can cause unwanted side effects, including degradation of MSC function and angioma formation,[50,51] and modulation of the target site through direct application into the tissue is still invasive.

Apart from the previously mentioned somewhat invasive and inefficient methods, magnetic guidance[52] and radiotherapy[53] are 2 relatively non-invasive methods that have been developed. However, these methods also have limitations: magnetic guidance cannot reach deep areas of the brain, and radiotherapy can cause unnecessary tissue injuries as well as various side effects by exposure to radiation.[54] Additionally, when applying stem cell therapies to the brain, the BBB can restrict the entry of stem cells into the CNS.[11] Therefore, several studies have utilized FUS to overcome these limitations.

Recent studies have evaluated the effect of FUS on stem cell application in various organs. Ghanem et al. applied FUS to a rat model of acute myocardial infarction to promote stem cell grafting in a “proof of concept” study involving

intravenous MSC transplantation, resulting in approximately (~) 50% to 60% higher cell count in the myocardium of the FUS group of rats than untreated rats.[55] Furthermore, they found that the activities of cytokines, such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and matrix metalloproteinase (MMP), increased in the FUS-treated rats, which was possibly related to the increased trans-endothelial MSC migration. Another study reported the use of FUS without MBs for targeted homing of stem cells,[56] where intravenous injection of MSCs and endothelial precursor cells along with FUS treatment was applied to the skeletal muscle. The results indicated that maximal stem cell delivery was achieved when FUS was performed before cell infusion, which was possibly due to the increased activities of chemo-attractants. Interestingly, they observed increased stem cell homing when FUS treatment and cell infusion were repeated daily, which indicated that the number of targeted cells can be modulated and substantially increased over short periods of time, regardless of the type of stem cell. It has also been reported that various chemokines and cytokines are activated after ultrasound sonication of the brain.[57] These include IL-1β, TNF-α, and MMPs, which have been reported as FUS-induced stem cell attractant factors.[55]

Recently, simultaneous application of FUS treatment with MB and stem cell transplantation into the brain was investigated.[26] The histological and immunohistochemical results of the brain obtained after FUS treatment and prior to NSC transplantation indicated the presence of live NSCs in the targeted area of the brain. However, limited tissue damage was confirmed by H&E staining that showed red blood cell extravasation. Although the vascular microstructure in the brain differs

from that in other organs due to the unique microstructure of the BBB, this study demonstrated the efficacy of FUS-mediated induction of stem cell homing in the brain.

In the present study, we determined whether FUS treatment with MB could enhance MSC homing to the target area without causing structural brain injury such as red blood cell extravasation. FUS treatment was targeted to the hippocampal region since the hippocampus is a potential target for cognition-related disorders,[58] and represents one of the major indications of MSC treatment.[59] We used BM-MSCs without any pretreatment, such as hypoxic preconditioning or treatment with chemical compounds, and in the absence of any factor that could possibly artificially modulate the microenvironment of the target site. Burgess et al. delivered NSCs to the rat brain using FUS with MB acutely, resulting in NSC migration to the parenchyma.[26] In the present study, we applied MSCs to the rat brain using FUS with MB, 3 hours after sonication via tail vein injection and quantified the transplant efficiency. MSCs were counted in nine coronal sections of the hippocampus in both treated and untreated groups, and we observed more than 2-fold higher average number of MSCs in the hippocampus of the FUS-treated rats than in the untreated rats (Figure 4; $p<0.0001$). This result suggests the possibility that ultrasound application can efficiently transfer other stem cells in addition to NSCs through the BBB to the brain parenchyma. Alkins et al. evaluated whether FUS treatment could increase immune cell migration into the targeted area of the brain region, revealing that FUS treatment enhanced immune cell migration by ~2-fold relative to the control group.[60] This result was comparable to the

transplantation yield of MSCs with FUS treatment in the present study and suggests that FUS treatment can enhance trans-endothelial MSC migration into the brain tissue similar to the immune cell response.

Our findings address the limitations of invasiveness and low delivery efficiency of transplantation methods and demonstrate a strategy for enhancing MSC homing noninvasively with high efficacy to the desired brain regions.

2. Role of CAMs, MMPs, and microglial activation in FUS-mediated targeted MSC transplantation into the brain

Stem cells exhibit a homing response to injured tissues mediated by the increased expression of chemokine receptors or enhanced secretion of chemokines by the injured tissue.[61] FUS can not only selectively focus activity to specific areas of the tissue, but can also target deep structures using magnetic resonance imaging technology without affecting other tissues. At the target region, the mechanical pressure applied by sound waves induces various biological effects. Previous studies have demonstrated that FUS sonication with MBs to the brain can cause a sterile inflammatory response by upregulating a variety of inflammatory and trophic factors;[57,62] however, MSC homing to the brain has not yet been assessed using this method. To induce diapedesis or transmigration through the endothelial cell layer and the underlying basement membrane, lytic enzymes, such as MMPs, are required to cleave the components of the basement membrane. It has been reported that MSC migration is regulated by MMP-2.[63] However, apart from the trans-endothelial

migration induced by lytic enzymes, the tight arrest of MSCs to the endothelium under high-flow conditions is also critical for targeted MSC transplantation. In this study, we determined whether the expression levels of CAMs and MMP-2 were enhanced following FUS treatment. The endothelium of the target tissue can possess a molecular signature that helps determine the specificity of the local immune response through binding to homing receptors, such as VCAM-1 and ICAM-1, when MSCs are delivered systemically.[64] Moreover, coating MSCs with ICAM-1 can promote MSC attachment to endothelial cells *in vitro* under high-flow conditions.[65] Therefore, we confirmed the levels of ICAM-1 and VCAM-1 and observed marked FUS-induced increase in ICAM-1 expression in endothelial cells (Figures 5 and 6 B, respectively), which possibly indicates a mechanism of targeted MSC transplantation, where FUS induces MSC binding to endothelial cells in the target area. Interestingly, ICAM-1 expression in subendothelial cells such as astrocytes and microglia also increased (Figure 6 A, C). ICAM-1 in subendothelial cells plays a role in transporting drugs across the BBB;[66] therefore, this result suggested that increased ICAM-1 levels in subendothelial cells also affect targeted MSC transplantation. Additional studies, however, are needed to confirm this observation.

Regarding ICAM-1 activation by FUS, various cytokine stimulations are associated with ICAM-1 activation, and certain disease-like conditions can result in ICAM-1 overexpression.[66] In the present study, microglia were activated by FUS (Figure 8), even in the absence of structural injury (Figure 9), indicating that FUS induced disease-like conditions without structural damage. Moreover, this might be a

reason for ICAM-1 overexpression and enhanced MSC trans-endothelial migration.

Additionally, VCAM-1 expression in endothelial cells was elevated upon FUS treatment (Figures 5 and 6 D). VCAM-1 enhances stem cell homing due to its propensity to render blood vessels resistant to detachment.[67] It has been confirmed that the overexpression of a subunit of VLA4, which interacts with VCAM-1, resulted in increased BM-MSC homing.[68] Also, another study focusing on the systemic delivery of MSCs in mice with inflammatory bowel disease (IBD) showed that coating MSCs with antibodies against VCAM-1 increased their efficacy to improve IBD.[69]

In the present study, MMP-2 expression in the sonicated region was also increased by FUS (Figure 7). MMPs are proteinases known to have a role in cell migration. Bhoopathi et al. have inhibited MMP-2 expression in tumors *in vivo* to evaluate the role of MMP-2 on tropism of MSC in a human medulloblastoma tumor model and confirmed the repression of MSC migration toward tumors.[70] In this study, MMP-2 expression did not increase significantly 1 hour after FUS sonication but significantly increased after 3 hours, which is the time of MSC injection. Thus, FUS-induced increased expression of ICAM-1, VCAM-1, and MMP-2 might be related to the targeted homing of MSCs, although further studies are required to confirm this.

3. Confirmation of memory impairment model induced by 192 IgG-saporin

In this study, a 192 IgG-saporin-induced memory-impaired rat model was used; this rat model mimics the cholinergic deficit in patients with AD. The 192 IgG-saporin, comprising a monoclonal antibody, has low affinity toward the rat nerve growth factor receptor (NGFr) p75 expressed on cholinergic cell bodies in the basal forebrain and contains a ribosome-inactivating protein called saporin.[71] The 192 IgG-saporin was injected into the cerebroventricle to damage the cholinergic neurons in the basal forebrain. Degeneration of cholinergic neurons in the basal forebrain is one of the characteristic features of AD and vascular dementia and has been correlated with cognitive decline.[27,28] Cholinergic neurons in the basal forebrain express higher levels of NGFr p75 than other cholinergic and non-cholinergic neurons. Injecting 192 IgG-saporin is likely to damage most of the cholinergic projections from the basal forebrain to the cortex or hippocampus.[72] In the present study, injection of 192 IgG-saporin led to a decrease in the number of ChAT positive cholinergic cells in the basal forebrain of the Lesion and FUS only groups of rats (Figures 13 B, E). The rats with cholinergic neuron damage showed impaired spatial memory (Figures 13 C, D) and reduced AChE activity in both groups (Figure 14). These results demonstrated that cholinergic deficit was well-established in this rat model, which was also shown in our previous studies.

4. Application of MSC transplantation by FUS in the 192 IgG-saporin rat model

In the first experiment, we confirmed the enhanced transplant efficacy of the MSCs by FUS (1701 ± 42.26 (*p <0.0001)) compared to the Cell only group of rats (758.8 ± 40.55). Likewise, in the second experiment, we confirmed similar enhanced efficacy compared to the first experiment (FUS + Cell group 1696 ± 98.9, Cell only group 850.8 ± 65.63, *p<0.0001), and the duration of efficacy lasted until 6 weeks after MSC transplantation (Figure 10). These results suggest that FUS-induced MSC transplantation is valid in the 192 IgG-saporin rat model, and the increased efficacy is maintained up to 6 weeks.

5. Behavioral therapeutic efficacy of FUS-induced MSC transplantation compared to that in Cell only and FUS only groups of rats

Although all rats injected with 192 IgG-saporin showed a significant difference compared to normal rats on the second day, and rats with transplanted MSCs showed significant difference on the third day of training, all rats gradually showed improvement in the Morris water maze training phase during the last 5 days of training (Figure 11). This indicates that all rats managed to adopt a certain strategy for escaping the water maze.[73,74] It has been reported that rats with denervation of cholinergic pathways show poor acquisition of spatial memory and alternatively adopt a non-spatial search strategy.[75] Our test results validated such findings. Lesioned rats showed the same improvement in training latency as the normal rats on the final day of training. It is likely that the lesioned rats used a random search approach to reach

the platform rather than relying on learning and spatial cognitive functions. This supposition is also obvious in the water maze probe test results, where the lesioned rats displayed significantly poor performance than the normal rats with respect to the time spent in the platform zone and the number of platform crossings. However, rats with transplanted MSCs using FUS showed improved spatial memory and cognition in the probe test compared to the Lesion group of rats (Figure 12). Interestingly, in our probe test, the Cell only and FUS only groups of rats did not show improved spatial memory and cognitive function compared to the Lesion group of rats. A few points need to be considered to achieve more effective stem cell therapy outcomes. For example, it is critical for transplanted cells to migrate into the impaired brain region after stem cell application. It has been demonstrated that MSC homing toward the injured tissue do not induce an efficient result since very few cells reach the impaired region.[76] Many transplanted cells were trapped in the lung and other organs or cells were lost during the systemic journey.[77] In this case, it is believed that the insufficient number of cells reaching the target will partially account for the decreased number of transplanted MSCs in the target region thereby further decreasing the effects of cell therapy. In the present study, we used 3×10^6 cells, but usually experiments with IV-delivered MSCs in a small animal model require the use of at least 1×10^6 cells and, more frequently, numbers as high as 5×10^6 cells to observe any effect.[78] This suggests that in certain circumstances, increased absolute numbers of cells are needed to ensure that a minimum number of cells reach the injury site distal to the brain. Therefore, we suggest that enhanced number of MSCs transplanted into the

hippocampus by FUS affected the recovery of cognitive function. However, simple IV injection of MSCs may have poor therapeutic effects since relatively few cells reach the hippocampus.

Recently, Shin et al. have reported that FUS treatment increased adult hippocampal neurogenesis and improved spatial memory that was mediated by increased hippocampal BDNF in the 192 IgG-saporin rat model.[79] However, in this study, we could not observe any improvement of hippocampal neurogenesis and spatial memory in the FUS-treated 192 IgG-saporin rat model. In the present study, we confirmed the effects of FUS treatment at 6 weeks after FUS sonication, since it has been reported that stem cells require at least 4-5 months to affect degenerative diseases.[80] However, Shin et al. confirmed the effects 18 days after FUS sonication. Although the animal model is similar, Shin et al. applied FUS 3 days after 192 IgG-saporin infusion, but we applied FUS and MSCs 1 week later. In addition, Shin et al. reported that the mean number of ChAT immunopositive cells in MS of the Lesion group of rats 5 days after sonication was 65.5 ± 13.1, but we confirmed that the mean number of ChAT immunopositive cells in MS was 24.87 ± 8.462 in our study. Although the binding and uptake of 192 IgG-saporin are rapid, the neurons continue to function for 1-3 days after injection. Resolution of the histological reaction to the lesion takes 10-14 days.[81] Taken together, since we applied FUS sonication when the cholinergic denervation was more severe than that in the study by Shin et al. and, the results of the effects of MSCs were confirmed after 6 weeks, it is suggested that the FUS effects in the 192 IgG-saporin rat model did not sustain 6 weeks later.

6. Neuroprotective and neurogenesis effects of FUS-induced MSC transplantation

MSC-based therapies for neurodegenerative diseases aim at impeding the clinical deterioration by regeneration and helping the local improvement of the damaged brain region. MSCs have advantages in cell therapy since they can be efficiently isolated from adult tissue, expanded in culture, and be safely transplanted autologously. MSCs can differentiate into neuronal cells and secrete various factors related to neurotrophic factor-mediated protection, enhanced neurogenesis, and modulation of inflammation. In addition, MSCs also exhibit the property of homing toward lesioned areas. In the present study, we confirmed the increased efficacy of MSC transplantation in the sonicated region of the brain by FUS-induced disease-like environment. These results were associated with the improvement of the spatial memory function.

Since the MS-hippocampus memory circuit consists of a cholinergic system,[82,83] we first assessed the cholinergic neuronal activities in both these regions of interest using the Ellman assay. Injection of 192 IgG-saporin significantly decreased AChE activity in the MS and hippocampus (Figure 14). MSC administration with or without FUS resulted in a significant increase in AChE activity relative to that in the Lesion group in the MS region, while no functional recovery of AChE activity was observed in the hippocampus. However, the ChAT positive cells were significantly restored only in the MS of FUS + Cell group. Moreover, the Cell only group tended to have increased ChAT positive cell counts, but there was no significant difference compared to that in the Lesion group (Figure 13). Since the hippocampus is a major region of

granule cell neurogenesis and as the contiguous periventricular area also exhibits NSCs, the hippocampal region provides a proper environment for stem cell migration and neurogenesis.[84,85] In fact, the transplanted MSCs regardless of FUS migrated to the hippocampal region and facilitated in restoring the cholinergic activity in the MS but not in the hippocampus. Franz et al. reported that when cholinergic neurons were impaired by partial fimbrial transections, injecting BDNF into the hippocampal ventricles protected the cholinergic neurons in the MS.[86] However, long-term studies are needed to confirm AChE activity in the hippocampus.

In this study, the most effective memory enhancement occurred in the FUS + Cell group. The memory enhancement could be mainly due to the increased BDNF expression owing to the enhanced efficacy of MSC transplantation by FUS. BDNF release associated with MSC transplantation has been reported.[87-89] Our experimental results showed that FUS-induced MSC transplantation increased BDNF expression in the hippocampus. The crucial role of BDNF in hippocampus-dependent learning and memory has been investigated.[90] Memory formation is thought to involve both short-term changes such as variations in electrical properties and long-term structural changes in synapses.[91] A significant role of BDNF in long-term potentiation (LTP) in the hippocampus and cortex has been revealed. The immediate release of BDNF modulates the induction and early maintenance phase of LTP.[92] Additionally, sustained BDNF upregulation and activation of TrkB receptor, which is a neurotrophic receptor tyrosine kinase well known primarily for its function during CNS development, are involved in the formation of stable LTP.[93] TrkB-/- knockout

mice had lower densities of synaptic transmission and important structural alterations such as decreased density of synaptic vesicles.[94] Additionally, BDNF is involved in both spatial memory formation and maintenance.[95] Moreover, enhanced expression of hippocampal BDNF is associated with an increased number of newly generated cells in the granule cell layer,[96] and a lack of endogenous BDNF caused neurodeficiency, cognitive function impairment, and decreased neurogenesis in the hippocampus.[97,98]

Adult neurogenesis is restricted to the hippocampus including the subgranular zone of dentate and new neurons continue to be generated throughout life. Adult hippocampal neurogenesis is considered to be related to hippocampus-dependent spatial memory.[99] BDNF and TrkB signaling pathways are well known to be involved in adult neurogenesis as they mediate neuronal differentiation and survival of newly generated neurons.[100] In our study, MSC transplantation using FUS also enhanced TrkB expression in the hippocampus (Figure 17 C). DCX is a protein that triggers microtubule polymerization and is a molecular marker for adult neurogenesis in late mitotic neuronal precursors and early post-mitotic neurons.[101,102] It is expressed specifically in newly generated neurons and not in GFAP-expressing astrocytes. Additionally, DCX expression is absent during neuronal regeneration or lesion-induced gliogenesis.[103] In this study, the FUS-induced MSC transplantation after cholinergic deficit recovers DCX and NeuN immunopositive cells, suggesting that increased MSC homing promotes neurogenesis in the hippocampus, which in turn, may promote hippocampus-dependent spatial memory function.

Previous studies have shown beneficial effects of MSC transplantation in CNS disease. Most studies have suggested the potential repair mechanisms associated with neuronal differentiation to replace damaged cells, as well as the secretion of neurotrophic and growth factors by MSCs. Few studies have preponderantly reported the effects of MSCs on immune cells and their immunomodulatory functions.[104] IL-1β is a cytokine, which is an important mediator of the inflammatory response, involved in a variety of cellular activities, including apoptosis. MSCs can improve the recovery of neurological functions, decrease apoptosis, and increase endogenous cell proliferation.[105] Run et al. have reported that MSC transplantation in a traumatic brain injury model can modulate inflammation-associated immune reaction by reducing the levels of proinflammatory cytokines such as IL-1β in the injured brain.[36] In our study, MSCs, transplanted by FUS, reduced the levels of IL-1β in the sonicated hippocampal region compared to that in the Lesion group (Figure 16). Therefore, our result suggests that MSCs transplanted by FUS can effectively modulate the immunopathogenic process caused by cholinergic denervation.

7. Safety issues associated with using FUS for MSC delivery and study limitations

There are several possible issues associated with using FUS treatment for MSC transplantation. First, the safety of FUS treatment should be guaranteed before being applied in humans. In this study, we selected the sonication parameters using MBs based on the results of our previous experiment and verified the absence of

FUS-induced injury by microscopic examination.[106,107] A previous study has reported a tendency for MSCs to migrate to injured/inflammatory sites.[76] In the present study, we showed increased levels of ICAM-1 and VCAM-1 in the FUS-sonicated regions, implying that the inflammatory reaction is induced by FUS in the targeted brain area. Additionally, Figure 4 shows the quantitative results indicating increased migration of stem cells to the target site. Therefore, our results represent indirect evidence confirming the enhanced migration of stem cells to the targeted regions induced by FUS using MBs. Although there was no extravasation of red blood cells in our acute model, it is necessary to observe long-term inflammatory responses, since repeated inflammation can cause structural changes in tissues.[108]

Another issue involves the duration of BBB opening related to stem cell homing. Burgess et al. confirmed that NSCs cross the BBB to enter the brain parenchyma during FUS sonication.[26] Previous studies have reported that the FUS-induced BBB opening persists for extended periods of time (1-10 hours).[109,110] We experimentally confirmed through Evans blue leakage that the BBB opening was maintained for 3 hours after FUS sonication (Figure 3). Our data also demonstrated enhanced homing of BM-MSCs to the targeted brain region (Figure 4). We chose an MSC infusion time based on studies describing MSC transplantation into various tissues 3 hours after FUS treatment.[56,111]

In contrast to previously published data,[26] our study parameters using the MBs did not appear to cause red blood cell extravasation (Figure 9). Therefore, we suggest

that FUS successfully promoted MSC delivery without tissue damage. However, as uncontrolled MB cavitation can damage sonicated tissue,[112] the use of acoustic feedback control (e.g., passive cavitation detection) needs to be evaluated in future studies.[107,113] Additional studies with FUS treatment alone, without MBs, are necessary to avoid the uncontrolled effects of cavitation.

V. CONCLUSION

FUS treatment is an emerging modality for treating brain diseases, including neurodegenerative disorders, since the BBB is an important physical barrier, which makes it difficult for therapeutic agents to be permeable, in the treatment of CNS diseases. When applying stem cell therapies to CNS diseases, the BBB can restrict the transmigration of stem cells into the CNS. Recently, various biological effects of FUS treatment have been investigated, and here, we determined whether FUS treatment could increase the efficacy of stem cell homing and have therapeutic effects in the 192 IgG-saporin rat model. Our results demonstrated that FUS increased MSC transplantation into the brain tissue by more than 2-fold, and this effect was possibly related to the activation of ICAM-1, VCAM-1, and MMP-2. Additionally, we confirmed that FUS-induced MSC transplantation restored spatial memory function by increasing endogenous neurogenesis and BDNF expression, thereby suggesting that FUS could enhance spatial memory formation and maintenance. However, the long-term safety and the combined effects of FUS treatment with other strategies for enhancing MSC homing require additional investigations.

REFERENCES

1. Group GBDNDC. Global, regional, and national burden of neurological disorders during 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Neurol 2017;16:877-97.
2. D'Onofrio G, Sancarlo D, Seripa D, Ricciardi F, Giuliani F, Panza F, et al. Non-Pharmacological Approaches in the Treatment of Dementia. Update on Dementia; 2016.
3. Danon JJ, Reekie TA, Kassiou M. Challenges and Opportunities in Central Nervous System Drug Discovery. Trends in Chemistry 2019;1:612-24.
4. Lozano AM, Lipsman N, Bergman H, Brown P, Chabardes S, Chang JW, et al. Deep brain stimulation: current challenges and future directions. Nat Rev Neurol 2019;15:148-60.
5. Jang CK, Jung HH, Chang JH, Chang JW, Park YG, Chang WS. Long-Term Results of Gamma Knife Radiosurgery for Intracranial Meningioma. Brain Tumor Res Treat 2015;3:103-7.
6. McLauchlan D, Robertson NP. Stem cells in the treatment of central nervous system disease. J Neurol 2018;265:984-6.
7. Chang WS, Chang JW. Focused ultrasound treatment for central nervous system disease: neurosurgeon's perspectives. Biomedical Engineering Letters 2017;7:107-14.
8. Kamelska-Sadowska AM, Wojtkiewicz J, Kowalski IM. Review of the Current Knowledge on the Role of Stem Cell Transplantation in Neurorehabilitation. Biomed Res Int 2019;2019:3290894.
9. Zuo F, Xiong F, Wang X, Li X, Wang R, Ge W, et al. Intrastriatal Transplantation of Human Neural Stem Cells Restores the Impaired Subventricular Zone in Parkinsonian Mice. Stem Cells 2017;35:1519-31.
10. Neuman T, Rezak M, Levesque MF. Therapeutic Microinjection of Autologous Adult Human Neural Stem Cells and Differentiated Neurons for Parkinson's Disease: Five-Year Post-Operative Outcome. The Open Stem Cell Journal 2009;1:20-9.
11. de Munter JP, Melamed E, Wolters EC. Stem cell grafting in parkinsonism-why, how and when. Parkinsonism & related disorders 2014;20:S150-S3.
12. Abeysinghe HC, Phillips EL, Chin-Cheng H, Beart PM, Roulston CL. Modulating Astrocyte Transition after Stroke to Promote Brain Rescue and Functional Recovery: Emerging Targets Include Rho Kinase. Int J Mol Sci 2016;17:288.
13. Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SI, et al. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. Stem Cells Dev 2009;18:683-92.

14. Azari MF, Mathias L, Ozturk E, Cram DS, Boyd RL, Petratos S. Mesenchymal stem cells for treatment of CNS injury. Curr Neuropharmacol 2010;8:316-23.
15. Cabanes C, Bonilla S, Tabares L, Martinez S. Neuroprotective effect of adult hematopoietic stem cells in a mouse model of motoneuron degeneration. Neurobiol Dis 2007;26:408-18.
16. Kim JY, Kim DH, Kim JH, Lee D, Jeon HB, Kwon SJ, et al. Soluble intracellular adhesion molecule-1 secreted by human umbilical cord blood-derived mesenchymal stem cell reduces amyloid-β plaques. Cell Death And Differentiation 2011;19:680.
17. Venkataramana NK, Kumar SK, Balaraju S, Radhakrishnan RC, Bansal A, Dixit A, et al. Open-labeled study of unilateral autologous bone-marrow-derived mesenchymal stem cell transplantation in Parkinson's disease. Transl Res 2010;155:62-70.
18. Honmou O, Houkin K, Matsunaga T, Niitsu Y, Ishiai S, Onodera R, et al. Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke. Brain 2011;134:1790-807.
19. Buckner JH. Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. Nat Rev Immunol 2010;10:849-59.
20. Magara A, Buhler R, Moser D, Kowalski M, Pourtehrani P, Jeanmonod D. First experience with MR-guided focused ultrasound in the treatment of Parkinson's disease. J Ther Ultrasound 2014;2:11.
21. Chang WS, Jung HH, Kweon EJ, Zadicario E, Rachmilevitch I, Chang JW. Unilateral magnetic resonance guided focused ultrasound thalamotomy for essential tremor: practices and clinicoradiological outcomes. J Neurol Neurosurg Psychiatry 2015;86:257-64.
22. Jung HH, Kim SJ, Roh D, Chang JG, Chang WS, Kweon EJ, et al. Bilateral thermal capsulotomy with MR-guided focused ultrasound for patients with treatment-refractory obsessive-compulsive disorder: a proof-of-concept study. Mol Psychiatry 2015;20:1205-11.
23. Jaewoo Shin CHK, Jae Sung Cho, Jihyeon Lee, , Chin Su Koh, Min-Sik Yoon, Young Cheol Na, Won Seok Chang, Jin Woo Chang. Focused ultrasound-mediated non-invasive Blood-brain barrier Modulation: Preclinical examination of Efficacy and Safety in various sonication parameters Neurosurgical focus 2018.
24. Kubanek J. Neuromodulation with transcranial focused ultrasound. Neurosurg Focus 2018;44:E14.
25. Curley CT, Sheybani ND, Bullock TN, Price RJ. Focused Ultrasound Immunotherapy for Central Nervous System Pathologies: Challenges and Opportunities. Theranostics 2017;7:3608-23.
26. Burgess A, Ayala-Grosso CA, Ganguly M, Jordao JF, Aubert I, Hynynen

K. Targeted Delivery of Neural Stem Cells to the Brain Using MRI-Guided Focused Ultrasound to Disrupt the Blood-Brain Barrier. Plos One 2011;6:e27877.

27. Bierer LM, Haroutunian V, Gabriel S, Knott PJ, Carlin LS, Purohit DP, et al. Neurochemical correlates of dementia severity in Alzheimer's disease: relative importance of the cholinergic deficits. J Neurochem 1995;64:749-60.
28. Baxter MG, Chiba AA. Cognitive functions of the basal forebrain. Curr Opin Neurobiol 1999;9:178-83.
29. Song BW, Kim IK, Lee S, Choi E, Ham O, Lee SY, et al. 1H-pyrrole-2,5-dione-based small molecule-induced generation of mesenchymal stem cell-derived functional endothelial cells that facilitate rapid endothelialization after vascular injury. Stem Cell Res Ther 2015;6:174.
30. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates-The New Coronal Set, 5th Edn; 2004.
31. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 1961;7:88-95.
32. Skaper SD. The neurotrophin family of neurotrophic factors: an overview. Neurotrophic factors: Springer; 2012. p.1-12.
33. Huang EJ, Reichardt LFJAron. Neurotrophins: roles in neuronal development and function. 2001;24:677-736.
34. Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D, et al. Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. J Neurosci Res 2003;73:778-86.
35. Li Y, Chen J, Chen XG, Wang L, Gautam SC, Xu YX, et al. Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. Neurology 2002;59:514-23.
36. Zhang R, Liu Y, Yan K, Chen L, Chen X-R, Li P, et al. Anti-inflammatory and immunomodulatory mechanisms of mesenchymal stem cell transplantation in experimental traumatic brain injury. Journal of Neuroinflammation 2013;10:871.
37. Munoz JR, Stoutenger BR, Robinson AP, Spees JL, Prockop DJ. Human stem/progenitor cells from bone marrow promote neurogenesis of endogenous neural stem cells in the hippocampus of mice. Proc Natl Acad Sci U S A 2005;102:18171-6.
38. Yoo SW, Kim SS, Lee SY, Lee HS, Kim HS, Lee YD, et al. Mesenchymal stem cells promote proliferation of endogenous neural stem cells and survival of newborn cells in a rat stroke model. Exp Mol Med 2008;40:387-97.

39. Wei Z, Liao J, Qi F, Meng Z, Pan S. Evidence for the contribution of BDNF-TrkB signal strength in neurogenesis: An organotypic study. Neurosci Lett 2015;606:48-52.
40. Li Y, Luikart BW, Birnbaum S, Chen J, Kwon CH, Kernie SG, et al. TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment. Neuron 2008;59:399-412.
41. Baek W, Kim YS, Koh SH, Lim SW, Kim HY, Yi HJ, et al. Stem cell transplantation into the intraventricular space via an Ommaya reservoir in a patient with amyotrophic lateral sclerosis. Journal of Neurosurgical Sciences 2012;56:261-3.
42. Park SE, Lee NK, Na DL, Chang JW, Chang JW. Optimal mesenchymal stem cell delivery routes to enhance neurogenesis for the treatment of Alzheimer's disease. Histology and histopathology: cellular and molecular biology 2018;33:533-41.
43. Naderi-Meshkin H, Bahrami AR, Bidkhori HR, Mirahmadi M, Ahmadiankia N. Strategies to improve homing of mesenchymal stem cells for greater efficacy in stem cell therapy. Cell Biol Int 2015;39:23-34.
44. Tsai LK, Wang Z, Munasinghe J, Leng Y, Leeds P, Chuang DM. Mesenchymal stem cells primed with valproate and lithium robustly migrate to infarcted regions and facilitate recovery in a stroke model. Stroke 2011;42:2932-9.
45. Liu H, Liu S, Li Y, Wang X, Xue W, Ge G, et al. The role of SDF-1-CXCR4/CXCR7 axis in the therapeutic effects of hypoxia-preconditioned mesenchymal stem cells for renal ischemia/reperfusion injury. PLoS One 2012;7:e34608.
46. Bobis-Wozowicz S, Miekus K, Wybieralska E, Jarocha D, Zawisz A, Madeja Z, et al. Genetically modified adipose tissue-derived mesenchymal stem cells overexpressing CXCR4 display increased motility, invasiveness, and homing to bone marrow of NOD/SCID mice. Exp Hematol 2011;39:686-96 e4.
47. Gundlach CWt, Caivano A, Cabreira-Hansen Mda G, Gahremanpour A, Brown WS, Zheng Y, et al. Synthesis and evaluation of an anti-MLC1 x anti-CD90 bispecific antibody for targeting and retaining bone-marrow-derived multipotent stromal cells in infarcted myocardium. Bioconjug Chem 2011;22:1706-14.
48. Segers VF, Tokunou T, Higgins LJ, MacGillivray C, Gannon J, Lee RT. Local delivery of protease-resistant stromal cell derived factor-1 for stem cell recruitment after myocardial infarction. Circulation 2007;116:1683-92.
49. Penn MS, Mendelsohn FO, Schaer GL, Sherman W, Farr M, Pastore J, et al. An open-label dose escalation study to evaluate the safety of

administration of nonviral stromal cell-derived factor-1 plasmid to treat symptomatic ischemic heart failure. Circ Res 2013;112:816-25.
50. Udalamaththa VL, Jayasinghe CD, Udagama PV. Potential role of herbal remedies in stem cell therapy: proliferation and differentiation of human mesenchymal stromal cells. Stem Cell Research & Therapy 2016;7:110.
51. Phillips MI, Tang YL. Genetic modification of stem cells for transplantation. Adv Drug Deliv Rev 2008;60:160-72.
52. Yun WS, Choi JS, Ju HM, Kim MH, Choi SJ, Oh ES, et al. Enhanced Homing Technique of Mesenchymal Stem Cells Using Iron Oxide Nanoparticles by Magnetic Attraction in Olfactory-Injured Mouse Models. Int J Mol Sci 2018;19.
53. Mouiseddine M, Francois S, Semont A, Sache A, Allenet B, Mathieu N, et al. Human mesenchymal stem cells home specifically to radiation-injured tissues in a non-obese diabetes/severe combined immunodeficiency mouse model. Br J Radiol 2007;80 Spec No 1:S49-55.
54. Kovalchuk A, Kolb B. Low dose radiation effects on the brain - from mechanisms and behavioral outcomes to mitigation strategies. Cell cycle (Georgetown, Tex.) 2017;16:1266-70.
55. Ghanem A, Steingen C, Brenig F, Funcke F, Bai ZY, Hall C, et al. Focused ultrasound-induced stimulation of microbubbles augments site-targeted engraftment of mesenchymal stem cells after acute myocardial infarction. J Mol Cell Cardiol 2009;47:411-8.
56. Burks SR, Ziadloo A, Kim SJ, Nguyen BA, Frank JA. Noninvasive pulsed focused ultrasound allows spatiotemporal control of targeted homing for multiple stem cell types in murine skeletal muscle and the magnitude of cell homing can be increased through repeated applications. Stem Cells 2013;31:2551-60.
57. Kovacs ZI, Kim S, Jikaria N, Qureshi F, Milo B, Lewis BK, et al. Disrupting the blood-brain barrier by focused ultrasound induces sterile inflammation. Proceedings of the National Academy of Sciences 2017;114:E75.
58. Tandon A, Singh SJ, Chaturvedi RK. Stem Cells as Potential Targets of Polyphenols in Multiple Sclerosis and Alzheimer's Disease. Biomed Res Int 2018;2018:1483791.
59. Cho JS, Lee J, Jeong DU, Kim HW, Chang WS, Moon J, et al. Effect of Placenta-Derived Mesenchymal Stem Cells in a Dementia Rat Model via Microglial Mediation: a Comparison between Stem Cell Transplant Methods. Yonsei Medical Journal 2018;59:406-15.
60. Alkins R, Burgess A, Ganguly M, Francia G, Kerbel R, Wels WS, et al. Focused ultrasound delivers targeted immune cells to metastatic brain tumors. Cancer Res 2013;73:1892-9.
61. Karp JM, Leng Teo GS. Mesenchymal stem cell homing: the devil is in the

details. Cell Stem Cell 2009;4:206-16.
62. McMahon D, Bendayan R, Hynynen K. Acute effects of focused ultrasound-induced increases in blood-brain barrier permeability on rat microvascular transcriptome. Scientific Reports 2017;7:45657.
63. De Becker A, Van Hummelen P, Bakkus M, Vande Broek I, De Wever J, De Waele M, et al. Migration of culture-expanded human mesenchymal stem cells through bone marrow endothelium is regulated by matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-3. Haematologica 2007;92:440-9.
64. Dominguez GA, Anderson NR, Hammer DA. The direction of migration of T-lymphocytes under flow depends upon which adhesion receptors are engaged. Integr Biol (Camb) 2015;7:345-55.
65. Ko IK, Kean TJ, Dennis JE. Targeting mesenchymal stem cells to activated endothelial cells. Biomaterials 2009;30:3702-10.
66. Hsu J, Rappaport J, Muro S. Specific binding, uptake, and transport of ICAM-1-targeted nanocarriers across endothelial and subendothelial cell components of the blood-brain barrier. Pharm Res 2014;31:1855-66.
67. Chigaev A, Sklar LA. Aspects of VLA-4 and LFA-1 regulation that may contribute to rolling and firm adhesion. Front Immunol 2012;3:242.
68. Kumar S, Ponnazhagan S. Bone homing of mesenchymal stem cells by ectopic alpha 4 integrin expression. Faseb j 2007;21:3917-27.
69. Ko IK, Kim BG, Awadallah A, Mikulan J, Lin P, Letterio JJ, et al. Targeting improves MSC treatment of inflammatory bowel disease. Mol Ther 2010;18:1365-72.
70. Bhoopathi P, Chetty C, Gogineni VR, Gujrati M, Dinh DH, Rao JS, et al. MMP-2 mediates mesenchymal stem cell tropism towards medulloblastoma tumors. Gene Therapy 2011;18:692-701.
71. Book AA, Wiley RG, Schweitzer JB. Specificity of 192 IgG-saporin for NGF receptor-positive cholinergic basal forebrain neurons in the rat. Brain Res 1992;590:350-5.
72. Yan Q, Johnson EM, Jr. An immunohistochemical study of the nerve growth factor receptor in developing rats. J Neurosci 1988;8:3481-98.
73. Lee JE, Jeong DU, Lee J, Chang WS, Chang JW. The effect of nucleus basalis magnocellularis deep brain stimulation on memory function in a rat model of dementia. BMC Neurol 2016;16:6.
74. Jeong DU, Lee J, Chang WS, Chang JW. Identifying the appropriate time for deep brain stimulation to achieve spatial memory improvement on the Morris water maze. BMC Neurosci 2017;18:29.
75. Nilsson OG, Strecker RE, Daszuta A, Bjorklund A. Combined cholinergic and serotonergic denervation of the forebrain produces severe deficits in a spatial learning task in the rat. Brain Res 1988;453:235-46.

76. Marquez-Curtis LA, Janowska-Wieczorek A. Enhancing the migration ability of mesenchymal stromal cells by targeting the SDF-1/CXCR4 axis. Biomed Res Int 2013;2013:561098.
77. White SV, Czisch CE, Han MH, Plant CD, Harvey AR, Plant GW. Intravenous Transplantation of Mesenchymal Progenitors Distribute Solely to the Lungs and Improve Outcomes in Cervical Spinal Cord Injury. Stem Cells 2016;34:1812-25.
78. Bali P, Lahiri DK, Banik A, Nehru B, Anand A. Potential for Stem Cells Therapy in Alzheimer's Disease: Do Neurotrophic Factors Play Critical Role? Current Alzheimer research 2017;14:208-20.
79. Shin J, Kong C, Lee J, Choi BY, Sim J, Koh CS, et al. Focused ultrasound-induced blood-brain barrier opening improves adult hippocampal neurogenesis and cognitive function in a cholinergic degeneration dementia rat model. Alzheimer's research & therapy 2019;11:110-.
80. Waldau B. Stem Cell Transplantation for Enhancement of Learning and Memory in Adult Neurocognitive Disorders. Aging and disease 2010;1:60-71.
81. Wiley RG. Toxin-induced death of neurotrophin-sensitive neurons. Methods Mol Biol 2001;169:217-22.
82. Ballinger EC, Ananth M, Talmage DA, Role LW. Basal Forebrain Cholinergic Circuits and Signaling in Cognition and Cognitive Decline. Neuron 2016;91:1199-218.
83. Haam J, Zhou J, Cui G, Yakel JL. Septal cholinergic neurons gate hippocampal output to entorhinal cortex via oriens lacunosum moleculare interneurons. Proceedings of the National Academy of Sciences of the United States of America 2018;115:E1886-E95.
84. Tatebayashi Y, Lee MH, Li L, Iqbal K, Grundke-Iqbal I. The dentate gyrus neurogenesis: a therapeutic target for Alzheimer's disease. Acta Neuropathol 2003;105:225-32.
85. Seaberg RM, van der Kooy D. Adult rodent neurogenic regions: the ventricular subependyma contains neural stem cells, but the dentate gyrus contains restricted progenitors. J Neurosci 2002;22:1784-93.
86. Hefti F, Knusel B, Lapchak PA. Chapter 31: Protective effects of nerve growth factor and brain-derived neurotrophic factor on basal forebrain cholinergic neurons in adult rats with partial fimbrial transections. In: Cuello AC, editor. Progress in Brain Research: Elsevier; 1993. p.257-63.
87. Qi L, Xue X, Sun J, Wu Q, Wang H, Guo Y, et al. The Promising Effects of Transplanted Umbilical Cord Mesenchymal Stem Cells on the Treatment in Traumatic Brain Injury. J Craniofac Surg 2018;29:1689-92.
88. Ji S, Lin S, Chen J, Huang X, Wei CC, Li Z, et al. Neuroprotection of Transplanting Human Umbilical Cord Mesenchymal Stem Cells in a

Microbead Induced Ocular Hypertension Rat Model. Curr Eye Res 2018;43:810-20.
89. Gou Y, Liu D, Liu J, Sun H. [Protective effect of transplantation of bone mesenchymal stem cells on demyelination in spinal cord injury]. Sheng Wu Gong Cheng Xue Bao 2018;34:761-76.
90. Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD. From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. Learning & memory (Cold Spring Harbor, N.Y.) 2002;9:224-37.
91. Mayford M, Siegelbaum SA, Kandel ER. Synapses and memory storage. Cold Spring Harbor perspectives in biology 2012;4:a005751.
92. Kossel AH, Cambridge SB, Wagner U, Bonhoeffer T. A caged Ab reveals an immediate/instructive effect of BDNF during hippocampal synaptic potentiation. 2001;98:14702-7.
93. Minichiello LJNRN. TrkB signalling pathways in LTP and learning. 2009;10:850-60.
94. Martinez A, Alcantara S, Borrell V, Del Rio JA, Blasi J, Otal R, et al. TrkB and TrkC signaling are required for maturation and synaptogenesis of hippocampal connections. J Neurosci 1998;18:7336-50.
95. Mizuno M, Yamada K, Olariu A, Nawa H, Nabeshima T. Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. J Neurosci 2000;20:7116-21.
96. Katoh-Semba R, Asano T, Ueda H, Morishita R, Takeuchi IK, Inaguma Y, et al. Riluzole enhances expression of brain-derived neurotrophic factor with consequent proliferation of granule precursor cells in the rat hippocampus. FASEB J 2002;16:1328-30.
97. Taliaz D, Stall N, Dar DE, Zangen A. Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. Mol Psychiatry 2010;15:80-92.
98. Numakawa T, Odaka H, Adachi N. Actions of Brain-Derived Neurotrophin Factor in the Neurogenesis and Neuronal Function, and Its Involvement in the Pathophysiology of Brain Diseases. Int J Mol Sci 2018;19.
99. Abrous DN, Wojtowicz JM. Interaction between Neurogenesis and Hippocampal Memory System: New Vistas. Cold Spring Harbor perspectives in biology 2015;7:a018952.
100. Wurzelmann M, Romeika J, Sun D. Therapeutic potential of brain-derived neurotrophic factor (BDNF) and a small molecular mimics of BDNF for traumatic brain injury. 2017;12:7-12.
101. Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L,

Kuhn HG. Transient expression of doublecortin during adult neurogenesis. J Comp Neurol 2003;467:1-10.
102. Zhang J, Jiao J. Molecular Biomarkers for Embryonic and Adult Neural Stem Cell and Neurogenesis. Biomed Res Int 2015;2015:727542.
103. Couillard-Despres S, Winner B, Schaubeck S, Aigner R, Vroemen M, Weidner N, et al. Doublecortin expression levels in adult brain reflect neurogenesis. Eur J Neurosci 2005;21:1-14.
104. Kim KS, Kim HS, Park JM, Kim HW, Park MK, Lee HS, et al. Long-term immunomodulatory effect of amniotic stem cells in an Alzheimer's disease model. Neurobiol Aging 2013;34:2408-20.
105. Parr AM, Tator CH, Keating A. Bone marrow-derived mesenchymal stromal cells for the repair of central nervous system injury. Bone Marrow Transplant 2007;40:609-19.
106. Muro S, Muzykantov VR. Targeting of antioxidant and anti-thrombotic drugs to endothelial cell adhesion molecules. Curr Pharm Des 2005;11:2383-401.
107. Hua S. Targeting sites of inflammation: intercellular adhesion molecule-1 as a target for novel inflammatory therapies. Front Pharmacol 2013;4:127.
108. Downs ME, Buch A, Sierra C, Karakatsani ME, Chen S, Konofagou EE, et al. Long-term safety of repeated blood-brain barrier opening via focused ultrasound with microbubbles in non-human primates performing a cognitive task. PloS one 2015;10:e0125911.
109. Hynynen K, McDannold N, Sheikov NA, Jolesz FA, Vykhodtseva N. Local and reversible blood-brain barrier disruption by noninvasive focused ultrasound at frequencies suitable for trans-skull sonications. NeuroImage 2005;24:12-20.
110. Wang F, Cheng Y, Mei J, Song Y, Yang Y-q, Liu Y, et al. Focused Ultrasound Microbubble Destruction-Mediated Changes in Blood-Brain Barrier Permeability Assessed by Contrast-Enhanced Magnetic Resonance Imaging. 2009;28:1501-9.
111. Tebebi PA, Burks SR, Kim SJ, Williams RA, Nguyen BA, Venkatesh P, et al. Cyclooxygenase-2 or tumor necrosis factor-alpha inhibitors attenuate the mechanotransductive effects of pulsed focused ultrasound to suppress mesenchymal stromal cell homing to healthy and dystrophic muscle. Stem Cells 2015;33:1173-86.
112. Fan CH, Liu HL, Huang CY, Ma YJ, Yen TC, Yeh CK. Detection of intracerebral hemorrhage and transient blood-supply shortage in focused-ultrasound-induced blood-brain barrier disruption by ultrasound imaging. Ultrasound Med Biol 2012;38:1372-82.
113. Wu SY, Tung YS, Marquet F, Downs M, Sanchez C, Chen C, et al. Transcranial cavitation detection in primates during blood-brain barrier

opening--a performance assessment study. IEEE Trans Ultrason Ferroelectr Freq Control 2014;61:966-78.

ABSTRACT(IN KOREAN)

192 IgG-saporin 을 이용한 흰쥐 기억력 손상 모델에서 초음파를 이용한 비침습적-선택적 중간엽줄기세포 이식과 기전

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이 지 현

인류의 고령화에 따라 치매 환자 수가 점차 증가하고 있으며 이를 치료하기 위한 다양한 약물들이 개발되고 있다. 하지만 인지능력이 퇴행하는 것을 완전하게 막을 수 없으며 약물에 의한 소화기계의 여러 부작용이 동반되거나 간 독성 발생 등의 문제가 발생할 수 있다. 따라서 약물 치료 이외의 치료방법 개발이 절실하다.

또 다른 치료 방법으로 줄기세포 이식법이 시도되고 있고 이는 신경 퇴행성 질환에 대해 효과적인 치료법이라고 보고되고 있다. 하지만 줄기세포를 이용한 뇌신경 질환 치료 기술은 면역거부반응, 종양 형성의 가능성, 신경세포로 분화 유도의 어려움, 대량 증식에 소비되는 고비용의 문제뿐만 아니라, 우선적으로 줄기세포를 이식하는 방법에도 부작용 및 한계점이 따를 수 있다. 예를 들어 기존에 시행되고 있는 대뇌 내 줄기세포 이식법은 크게 2 가지가 있는데, 첫째인 두개 내로 직접 이식하는 방법은 직접 이식에 의해

대뇌 내 2 차 손상과 감염 및 수술에 의한 합병증의 발생 가능성의 문제가 있고, 둘째인 정맥 내로의 주사를 통한 이식은 원하는 부위 즉 뇌의 특정 부위로 줄기세포를 이식하는 것이 어렵고 뇌에 도달하기 전에 이미 간이나 폐 등의 장기에 줄기세포가 불필요하게 생착되어 실제 대뇌 내에는 극히 소량의 세포만이 도달한다는 저효율의 한계점이 있다.

현재 집속 초음파를 통해 약물 치료제를 목표 부위에 전달하는 시도가 전세계적으로 이뤄지고 있으나 우리나라에서는 이러한 연구개발이 부족한 상황이며, 더군다나 세포를 초음파로 뇌에 이식하는 연구는 전세계적으로도 매우 미흡한 상황이다. 더불어 초음파를 이용한 약물 전달과 세포 전달은 다른 기전이기 때문에 본 연구에서는 집속 초음파를 통해 세포를 전달할 수 있는 방법을 제안하여 기존의 이식법 보다 효율적이며 안전성이 보장된 이식법을 수립할 뿐만 아니라 줄기세포를 목표 부위에 안전하게 전달함으로써 기존의 줄기세포 치료에 이용되는 줄기세포 양보다 비교적 적은 양으로도 치료 및 적용이 가능케 하고자 했다. 이로써 기존의 줄기세포 치료법이 갖는 또 다른 단점인 치료제 생산 비용, 즉 줄기세포를 분리 및 증식 시키는데 드는 높은 비용의 문제도 해결하는 연구결과를 제시했다.

최근 뇌에 집속 초음파를 이용한 줄기세포치료의 가능성이 보고되었다. 하지만 집속 초음파를 이용한 줄기세포 이식의 기전과

인지기능회복의 가능성은 알려져 있지 않다. 따라서 본 연구는 기억 손상 흰쥐 모델에서 치료효과와 집속 초음파를 이용한 줄기세포 치료 효과를 확인하기 위해 수행되었다.

먼저 집속 초음파를 이용하여 중간엽줄기세포를 흰쥐 대뇌 내 해마 부위에 효율적으로 전달할 수 있는지 확인하기 위해 흰쥐의 해마에 집속초음파를 조사하고 3 시간 뒤에 중간엽줄기세포를 정맥으로 주사하였다. 그 결과 집속 초음파를 이용하여 중간엽줄기세포를 이식하였을 때 그 이식 효율이 대조군과 비교 시 2 배까지 증가되었다. 그리고 중간엽줄기세포의 이동과 귀환에 관련이 있다고 알려진 ICAM-1 과 VCAM-1 면역 양성 세포의 평균수가 집속 초음파 조사된 실험군에서 유의미하게 높았다. 또한 중간엽줄기세포가 혈관벽을 통과하여 뇌실질 내로 이동하는데 영향을 미친다고 알려진 MMP-2 또한 집속 초음파가 조사된 영역에서 유의미하게 증가되었다. 이 결과는 집속 초음파에 의해 증가된 ICAM-1, VCAM-1, MMP-2 가 위치 선택적으로 중간엽줄기세포를 이동시키는데 영향을 주었음을 시사한다.

둘째로, 콜린성 손상을 유발하기 위해 192 IgG-saporin 을 대뇌 내실에 주입하였다. 1 주 후, 첫번째와 동일한 방법으로 집속 초음파를 해마에 조사 후 3 시간 뒤 중간엽줄기세포를 정맥으로 주사하였고, 줄기세포의 치료효과의 평가를 위해 5 주 뒤에 모리스 수중미로 실험을 통해 공간 기억능력을 평가하였다. 콜린성 활성

저하 그룹에서는 손상 유발로 인한 뇌 내측 중격의 ChAT 세포들의 감소와 해마의 AChE, BDNF, DCX, NeuN 및 공간 기억능력이 정상 그룹과 비교하여 유의미하게 감소되었고 IL-1β 은 유의미하게 증가하였다. 반면 흥미롭게도 집속 초음파를 통해 중간엽줄기세포를 이식 받은 그룹에서는 콜린성 활성 저하 그룹과 비교하여 내측 중격의 ChAT 와 AChE 가 유의미하게 회복됨을 확인하였고, 해마에서 BDNF, DCX, NeuN, TrkB 와 공간 기억능력이 유의미하게 회복되었다. 또한 IL-1β 은 유의미하게 감소하였다.

종합해보면, 집속 초음파로 중간엽줄기세포를 이식 받은 그룹에서의 기억 향상은 BDNF 증가로 인해 향상된 신경 보호 효과와 이식된 중간엽줄기세포의 면역조절효과로 유도된 더 높은 신경 재생과 관련이 있다고 보여진다.

본 연구의 결과는 집속 초음파에 의한 중간엽줄기세포의 이식과 공간 기억 손상 모델에서의 치료효과의 가능성을 제안한다. 하지만 뇌에 이식된 중간엽줄기세포의 직접적 기능과 보다 자세한 이식 및 치료 기전에 대한 추가 연구가 필요하다.

핵심되는 말: 집속 초음파, 중간엽줄기세포, 공간 기억, 해마, 뇌유래 신경영양 인자, 신경 보호, 신경 재생

PUBLICATION LIST

1. Lee J*, Chang WS, Shin J, Seo Y, Kong C, Song BW, et al. Non-invasively enhanced intracranial transplantation of mesenchymal stem cells using focused ultrasound mediated by overexpression of cell-adhesion molecules. Stem Cell Res 2020;43:101726.
2. Shin J, Kong C, Lee J, Choi BY, Sim J, Koh CS, et al. Focused ultrasound-induced blood-brain barrier opening improves adult hippocampal neurogenesis and cognitive function in a cholinergic degeneration dementia rat model. Alzheimers Res Ther 2019;11:110.
3. Kong C, Shin J, Koh CS, Lee J, Yoon MS, Cho YK, et al. Optimization of Medial Forebrain Bundle Stimulation Parameters for Operant Conditioning of Rats. Stereotact Funct Neurosurg 2019;97:1-9.
4. Shin J, Kong C, Cho JS, Lee J, Koh CS, Yoon MS, et al. Focused ultrasound-mediated noninvasive blood-brain barrier modulation: preclinical examination of efficacy and safety in various sonication parameters. Neurosurg Focus 2018;44:E15.
5. Cho JS*, Lee J*, Jeong DU, Kim HW, Chang WS, Moon J, et al. Effect of Placenta-Derived Mesenchymal Stem Cells in a Dementia Rat Model via Microglial Mediation: a Comparison between Stem Cell Transplant Methods. Yonsei Med J 2018;59:406-15.
6. Jeong DU, Lee J, Chang WS, Chang JW. Identifying the appropriate time for deep brain stimulation to achieve spatial memory improvement on the Morris water maze. BMC Neurosci 2017;18:29.
7. Lee JE, Jeong DU, Lee J, Chang WS, Chang JW. The effect of nucleus basalis magnocellularis deep brain stimulation on memory function in a rat model of dementia. BMC Neurol 2016;16:6.
8. Jeong DU, Oh JH, Lee JE, Lee J, Cho ZH, Chang JW, et al. Basal Forebrain Cholinergic Deficits Reduce Glucose Metabolism and Function of Cholinergic and GABAergic Systems in the Cingulate Cortex. Yonsei Med J 2016;57:165-72.