Focused ultrasound–mediated noninvasive blood-brain barrier modulation: preclinical examination of efficacy and safety in various sonication parameters

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OBJECTIVE The application of pharmacological therapeutics in neurological disorders is limited by the ability of these agents to penetrate the blood-brain barrier (BBB). Focused ultrasound (FUS) has recently gained attention for its potential application as a method for locally opening the BBB and thereby facilitating drug delivery into the brain parenchyma. However, this method still requires optimization to maximize its safety and efficacy for clinical use. In the present study, the authors examined several sonication parameters of FUS influencing BBB opening in small animals.

METHODS Changes in BBB permeability were observed during transcranial sonication using low-intensity FUS in 20 adult male Sprague-Dawley rats. The authors examined the effects of FUS sonication with different sonication parameters, varying acoustic pressure, center frequency, burst duration, microbubble (MB) type, MB dose, pulse repetition frequency (PRF), and total exposure time. The focal region of BBB opening was identified by Evans blue dye. Additionally, H & E staining was used to identify blood vessel damage.

RESULTS Acoustic pressure amplitude and burst duration were closely associated with enhancement of BBB opening efficiency, but these parameters were also highly correlated with tissue damage in the sonicated region. In contrast, MB types, MB dose, total exposure time, and PRF had an influence on BBB opening without conspicuous tissue damage after FUS sonication.

CONCLUSIONS The study aimed to identify these influential conditions and provide safety and efficacy values for further studies. Future work based on the current results is anticipated to facilitate the implementation of FUS sonication for drug delivery in various CNS disease states in the near future.

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KEY WORDS focused ultrasound; blood-brain barrier; BBB opening; noninvasive; drug delivery; sonication factor

VARIOUS pharmacological therapeutic agents have been developed for treatment of CNS diseases including neurodegenerative disorders and neuro-oncological disorders; however, the success of these agents principally depends on their ability to cross the blood-brain barrier (BBB). The BBB is a physiological, structural, and functional barrier that limits substance penetration into the CNS. Therefore, modulation of the BBB has

become an important target for therapeutic development in CNS diseases.¹⁵

The BBB is controlled by tight junctions between capillary endothelial cells, which restrict the diffusion of pathogenic microscopic organisms and large, hydrophilic molecules (> 400 D) from the bloodstream into the brain parenchyma.¹ A number of approaches have been developed with the goal of transiently increasing paracellular

ABBREVIATIONS BBB = blood-brain barrier; EB = Evans blue; FUS = focused ultrasound; MB = microbubble; PRF = pulse repetition frequency; UCA = ultrasound contrast agent.

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permeability of the BBB for drug delivery, also known as “opening” of the BBB, including intravascular injection of mannitol and nanoparticles.³⁵ Yet these methods are either invasive or unable to increase BBB paracellular permeability in a specific and localized manner.

Focused ultrasound (FUS) has recently gained attention for its potential application in several neurological disorders. Thermal ablation with high-intensity focused ultrasound (HIFU), in particular, has shown clinical efficacy for the treatment of Parkinson’s disease, essential tremor, and obsessive-compulsive disorder.^{7,19,20,29} In addition to the ablative uses of FUS, current research has focused on the application of FUS to open the BBB in preclinical therapeutic studies and clinical trials.

The combination of contrast agent microbubble (MB) infusion and FUS sonication has been proposed as a method for localized and transient BBB impairment. FUS sonication produces MB oscillations in blood vessels through stable and/or inertial cavitation mechanisms. Stable MB oscillations are usually accompanied by shear stress and microstreaming, whereas inertial cavitation produces unstable oscillations as well as the violent and rapid collapse of MBs proximal to blood vessel walls.³⁰ Both types of cavitations ultimately result in decreased tight junction integrity and improved drug penetration into the brain parenchyma.¹³

Transcranial FUS has been shown to open the BBB in a focalized and reversible manner and has thus far been applied for the treatment of brain tumors and for the delivery of Alzheimer’s disease therapy in nonhuman primates.^{2,5} Additionally, several reports have examined the influence of various sonication parameters on BBB opening efficiency in nonhuman primates, acoustic pressure amplitude, including pulse repetition frequency (PRF), total sonication duration, frequency, pulse duration, MB type, and MB concentration.^{3,11,26,28} However, comparison among these studies is limited due to the use of different systems, animal models, and MBs. Thus, additional work is required to validate the biological effects of FUS and optimize the abovementioned parameters in a comprehensive manner.

The purpose of the present study was to investigate the efficacy and safety of FUS sonication in a single system and to comprehensively optimize parameters including acoustic pressure, center frequency, burst duration, MB type, MB dose, PRF, and total exposure time to minimize collateral tissue damage while maximizing therapeutic utility.

Methods

Animals

All animal experimental procedures were conducted in compliance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee (IACUC) of Yonsei University. The study used a total of 20 adult male Sprague-Dawley rats, each weighing 250–300 g. Animals were housed in groups of 3 in laboratory cages with food and water available *ad libitum* on a 12-hour light/dark cycle (lights on at 07:00) in a

room with controlled temperature ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and relative humidity ($55\% \pm 5\%$). For experiments, animals were anesthetized with a mixture of ketamine (75 mg/kg) and xylazine (4 mg/kg) and fixed on a stereotaxic apparatus.

Experimental Setup and Procedure

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 Concepts, Inc.]) was driven by a waveform generator (33220A, Agilent) and RF amplifier (240 L, Electronics & Innovation, Ltd.). The transducer electrical impedance was matched to the output impedance of the amplifier (50 Ω) with an external matching network (Sonic Concepts, Inc.) as shown in Fig. 1. A cone filled with distilled and degassed water was mounted onto the transducer assembly. A needle-type hydrophone (HNA-0400, Onda) was used for transducer calibration, which measured the acoustic beam profile in the tank filled with degassed water. Sonication parameter variations are summarized in Table 1 and included the following: acoustic pressure (0.2, 0.3, 0.6, and 1.5 MPa), center frequency (515 kHz and 1.6 MHz), burst duration (1, 10, and 100 msec), MB type (SonoVue and Definity), MB doses, PRF (1, 2, and 5 Hz), and total exposure time (30, 60, 120, and 300 seconds).

The experimental procedure is shown Fig. 1. Rats were deeply anesthetized with mixture of ketamine (75 mg/kg) and xylazine (4 mg/kg) and fixed with a stereotaxic frame (Narishige). The FUS beam was targeted to the right somatosensory cortex by the 3D positioning system. Definity (mean diameter range 1.1–3.3 μm ; Lantheus Medical Imaging) and SonoVue (mean diameter 2–5 μm ; Bracco Diagnostics Inc.) MBs were diluted in saline and injected intravenously into the tail vein 10 seconds prior to ultrasound sonication. Thereafter, Evans blue (EB) dye (2%, 100 mg/kg) was injected intravenously and examined *ex vivo* to observe BBB opening.⁴¹

Histological Evaluation and Damage Scoring

Animals were sacrificed at 4 hours after sonication for histological examination. Rats were anesthetized with a mixture of ketamine (75 mg/kg) and xylazine (4 mg/kg) and subsequently underwent transcardial perfusion with normal saline and 4% paraformaldehyde. Brains were removed, postfixed, transferred to 30% sucrose, and stored for 3 days. Brains were sectioned into 30- μm slices using a freezing microtome.

To detect BBB opening, histological analysis of EB extravasation area was performed using Image J software (National Institutes of Health). The area of the sonication region was selected by a lasso tool and measured as number of pixels displaying positive signal. The center slide of focused regions was selected, and then the EB extravasation area was expressed as percent area (EB extravasation area/total brain area $\times$ 100). Measurement of the EB extravasation area was quantified using the method modified from that proposed by Tang et al.³⁷

H & E staining was conducted to examine brain tissue damage. A damage score was assigned to each sonication segment based on macroscopically observable local hem-

FIG. 1. Methodology. **A:** The focused ultrasound experimental setup. **B:** A 515-kHz axial focal beam. **C:** A 515-kHz 3D scan focal beam. **D:** A 1.6-MHz axial focal beam. **E:** A 1.6-MHz 3D scan focal beam. **F:** Ultrasound wave parameter factors including acoustic pressure amplitude, frequency, burst duration, and total exposure time are shown. **G:** Diagram of the experimental procedure.

orrhage. The scoring was as follows: 0, no detected damage; 1, a few tiny red blood cell extravasations; 2, petechial hemorrhages; and 3, hemorrhagic local lesions.¹⁷

Results

EB Extravasation

EB extravasation and tissue damage were identified at the focal region of FUS sonication in all rats. Figures 2–5 show the relationships between EB extravasation and the acoustic pressure amplitude, frequency, burst duration, total exposure time, PRF, MB type, and MB concentration.

Effects of Acoustic Pressure Amplitude and Frequency

To determine BBB opening efficiency and safety according to changes in sonication acoustic pressure amplitude, both EB extravasation and histological tissue damage were confirmed in response to 0.2-, 0.3-, 0.6-, and 1.5-MPa acoustic pressures. EB extravasation was hardly observed for 0.2 MPa (EB 1.80%; damage score 0), whereas BBB opening was identified but only for 0.3 MPa (EB 13.72%; damage score 0) and higher acoustic pressures. Acoustic pressures of 0.6 MPa (EB 21.59%; damage score 2) and 1.5 MPa (EB 41.61%; damage score 3) produced tissue damage, whereas an acoustic pressure of 0.3 MPa facilitated BBB opening of 13.72% in the absence of apparent tissue damage (damage score 0; Fig. 2).

Next, transducer frequencies of 0.5 MHz and 1.6 MHz were compared to evaluate the effects on BBB opening for acoustic pressures of 0.3 and 0.6 MPa. A frequency

of 1.6 MHz facilitated finer BBB opening compared with that of 0.5 MHz. Furthermore, for a 0.6-MPa (EB 9.02%; damage score 1) acoustic pressure, a frequency of 1.6 MHz produced less tissue damage than that of 0.5 MHz (EB 21.59%; damage score 2; Fig. 3).

Effects of Burst Duration and PRF

Burst durations of 1, 10, and 100 msec (0.1%, 1%, and 10% duty cycle, respectively) were tested. The degree of BBB opening appeared to be positively correlated with increases in duty cycle. The 10% duty cycle (EB 29.72%) produced the greatest amount of EB extravasation but also caused considerable tissue damage (damage score 3; Fig. 4).

TABLE 1. Variables of parameter

Parameter	Value
Acoustic pressure amplitude (MPa)	0.2, 0.3, 0.6, 1.5
Central frequency of transducer (MHz)	0.5, 1.6
Burst duration (msec)	1, 10, 100
PRF (Hz)	1, 2, 5
Total exposure time (sec)	30, 60, 120, 300
Type of MB ($\mu\text{l/kg}$)	
SonoVue	30
Definity	20
Definity	100

FIG. 2. BBB opening and tissue damage with increasing acoustic pressure amplitude. **A:** Evans blue extravasation in brain tissues. **B:** Coronal sections of brain tissues. **C:** H & E staining for the identification of tissue damage. Original magnification $\times 2$. **D:** H & E staining. Original magnification $\times 10$. Parameters were as follows: acoustic pressure, 0.2, 0.3, 0.6, or 1.5 MPa; frequency, 0.5 MHz; burst duration, 10 msec; PRF, 1 Hz; total exposure time, 60 seconds; and MB type and dose, Definity 20 $\mu\text{l/kg}$.

Next, we investigated the influence of PRF on BBB opening, examining PRF values of 1, 2, and 5 Hz. None produced apparent tissue damage (score 0), but increasing PRF enhanced the degree of BBB opening (1 Hz 13.72%; 2 Hz 15.88%; and 5 Hz 27.07%; Fig. 4).

Effects of Total Exposure Time, MB Type, and MB Dose

Total exposure times of 30, 60, 120, and 300 seconds were compared. BBB opening was similar for the 30-second (EB 5.87%; damage score 0) and 60-second (EB 13.72%; damage score 0) conditions, and neither value produced apparent tissue damage. Total exposure times of 120 seconds (EB 23.04%; damage score 1) and 300 seconds (EB 25.14%; damage score 2) produced larger degrees of BBB opening than that of the 60 seconds, but it led to some tissue damage (Fig. 5).

MB types and concentrations were also varied to examine effects on BBB opening. Typical doses of SonoVue and Definity MBs used in previous studies (30 $\mu\text{l/kg}$ and 20 $\mu\text{l/kg}$, respectively) and a fivefold dose of Definity MBs (100 $\mu\text{l/kg}$; EB 16.35%; damage score 1) were compared. With typical doses, Definity MBs (20 $\mu\text{l/kg}$) were more effective for BBB opening than SonoVue MBs (30 $\mu\text{l/kg}$; EB: 4.45%; score: 1). Increasing the MB dosage produced greater BBB opening and EB extravasation without conspicuous tissue damage (Fig. 6 and Table 2).

Discussion

The goal of this study was to demonstrate ultrasound sonication parameters for FUS-induced BBB opening in a preclinical rodent model. Previous studies have attempted to optimize these parameters to develop safer and more efficient approaches to implementing ultrasound in clinical settings. Some examples of measurements include contrast-enhanced MRI enhancement and fluorescent luminescence using dextran.^{10,26} However, to our knowledge, the present study is the first to perform a comprehensive optimization of FUS sonication parameters in rodents. In our study, we used the 960-Da EB dye to visualize BBB disruption, which is one of the most popular tools used for study of the BBB.⁴²

Effective Parameters for FUS BBB Modulation

Substances can cross the BBB by 1 of 2 mechanisms: active transport across capillary endothelial cells or paracellular diffusion across tight junctions.³⁴ Inertial cavitation can damage the microvasculature and allow the paracellular extravasation of erythrocytes.²¹ We found that acoustic pressure greater than 0.3 MPa facilitated this type of BBB opening; however, acoustic pressures above 0.6 MPa produced tissue damage, which may have been attributable to inertial cavitation (Fig. 2).

FIG. 3. BBB opening and tissue damage with changes in ultrasound frequency and acoustic pressure. **A:** Evans blue extravasation in brain tissues. **B:** Coronal sections of brain tissues. **C:** H & E staining for the identification of tissue damage. Original magnification $\times 2$. **D:** H & E staining. Original magnification $\times 10$. Parameters were as follows: acoustic pressure, 0.3 or 0.6 MPa; frequency, 0.5 or 1.6 MHz; burst duration, 10 msec; PRF, 1 Hz; total exposure time, 60 seconds; and MB type and dose, Definity 20 μ l/kg.

Previous studies of FUS-induced BBB opening have used a broad range of ultrasound frequency values, from 28 kHz to 8 MHz.^{3,23} While a high-frequency range was reported to produce BBB opening in small animals, the efficacy of the high-frequency range is affected by variations in skull bone thickness; accordingly, the delivered acoustic pressure in vivo is often subject to attenuation and aberrations.³⁸ Recent transcranial applications of ultrasound in nonhumans have used low-range frequencies (0.2–1.6 MHz) to facilitate BBB opening without producing significant tissue damage.³¹ In our study, we compared in vivo applications of 0.5-MHz and 1.6-MHz frequencies after acoustic map scanning and intensity measurement analyses (Fig. 1B–E) and found that a frequency of 1.6 MHz produced a finer focal beam than 0.5 MHz, whereas 0.5 MHz allowed higher energy efficiency. This result may be attributable to higher attenuation and phase aberration characteristics of 1.6-MHz ultrasound compared with 0.5-MHz ultrasound (Fig. 3).²⁴

Regarding burst duration, Hynynen et al. reported in 2001 that a duration of 100 msec produced tissue damage in rabbits.¹⁸ Alternatively, it was concluded that a duration of 0.01 msec was sufficient to elicit BBB disruption when combined with high acoustic pressure (i.e., 6.3 MPa).¹⁶ A more recent study reported that low-pressure, 0.69-MHz ultrasound sonication of a 10-msec duration led to signifi-

cantly greater BBB disruption than did the same parameters for a 1-msec duration.²⁶ In the present study, we evaluated low-pressure (i.e., 0.3-MPa) ultrasound sonication for 1, 10, and 100 msec and found that the degree of BBB disruption trended upward as burst duration increased (Fig. 4). A burst duration of 100 msec induced tissue damage that was likely related to red blood cell extravasation and inertial cavitation.²⁷

The effect of PRF on BBB opening has been examined in previous studies. McDannold et al. reported in 2008 that increasing PRF from 0.5 to 5 Hz had little effect on BBB disruption,²⁶ whereas others have found that increasing PRF from 0.1 to 1 Hz produced a significant increase in BBB disruption.¹⁰ Our study results are partly consistent with this finding: The degree of BBB opening increased as PRF was increased from 1 to 2 to 5 Hz, and no tissue damage was observed (Fig. 4).

Recently, Choi et al. conducted an experiment in which the total ultrasound exposure time was varied from 0 to 1200 seconds (frequency 1.08 MHz; burst duration 10 msec; PRF 1 Hz; and acoustic pressure amplitude 0.38 MPa), and they identified a significant relationship between exposure time and BBB opening when using contrast-enhanced MRI.¹⁰ Similarly, we observed increases in the degree of BBB disruption with increases in total exposure time from 30 to 300 seconds, although tissue damage

FIG. 4. BBB opening and tissue damage with increasing burst duration and PRF. **A:** Evans blue extravasation in brain tissues. **B:** Coronal sections of brain tissues. **C:** H & E staining for the identification of tissue damage. Original magnification $\times 2$. **D:** H & E staining. Original magnification $\times 10$. Parameters were as follows. **Left:** Acoustic pressure, 0.3 MPa; frequency, 0.5 MHz; burst duration, 1, 10, or 100 ms; PRF, 1 Hz; total exposure time, 60 seconds; and MB type and dose, Definity 20 $\mu\text{l/kg}$. **Right:** Acoustic pressure, 0.3 MPa; frequency, 0.5 MHz; burst duration, 10 msec; PRF, 1, 2, or 5 Hz; total exposure time, 60 seconds; and MB type and dose, Definity 20 $\mu\text{l/kg}$.

FIG. 5. BBB opening and tissue damage with increasing total exposure time and MB type and dose. **A:** Evans blue extravasation in brain tissues. **B:** Coronal sections of brain tissues. **C:** H & E staining for the identification of tissue damage. Original magnification $\times 2$. **D:** H & E staining. Original magnification $\times 10$. Parameters were as follows. **Left:** Acoustic pressure, 0.3 MPa; frequency, 0.5 MHz; burst duration, 10 msec; PRF, 1 Hz; total exposure time, 30, 60, 120, or 300 seconds; and MB type and dose, Definity 20 $\mu\text{l/kg}$. **Right:** Acoustic pressure, 0.3 MPa; frequency, 0.5 MHz; burst duration, 10 msec; PRF, 1 Hz; total exposure time, 60 seconds; and MB type and dose, SonoVue 30 $\mu\text{l/kg}$, Definity 20 $\mu\text{l/kg}$, or Definity 100 $\mu\text{l/kg}$.

FIG. 6. Analysis of BBB opening efficacy and damage with various FUS parameters. **A:** The ratio of BBB disruption and damage with acoustic pressures of 0.2, 0.3, 0.6, and 0.9 MPa. **B:** The ratio of BBB disruption and damage with center frequencies of 0.5, and 1.6 MHz. **C:** The ratio of BBB disruption and damage with burst durations of 1, 10, and 100 msec. **D:** The ratio of BBB disruption and damage with PRFs of 1, 2, and 5 Hz. **E:** The ratios of BBB disruption and damage with total exposure times of 30, 60, 120, and 300 seconds. **F:** The ratio of BBB disruption and damage with MB type and dose.

was produced at the 120- and 300-second durations, as shown Fig. 5. Tissue damage in this context paralleled that shown in previous reports indicating that total exposure times greater than 300 seconds produce irreversible damage in rodents.¹¹

MBs were originally developed as ultrasound contrast agents (UCAs) for imaging purposes. Hynynen et al. first demonstrated the alternative usage of MBs for localized and selective opening of the BBB without damage to the brain.¹⁸ Various MBs are available as contrast agents that differ in terms of their gas core and shell makeup. For example, the UCA Optison has an albumin shell and an octafluoropropane gas core,²⁶ the UCA Definity has a lipid shell and an octafluoropropane gas core,⁴⁰ and the Sono-

oVue UCA has a phospholipid shell and a sulfur hexafluoride gas core;^{33,43} additionally, in-house MBs are currently used to induce BBB opening via cavitation.³² Our study focused on differences between the Definity and SonoVue MBs as well as different dosages of Definity MBs. Definity MBs appeared to produce better BBB opening and to have effects in a dose-dependent manner (Fig. 5). However, previous studies have offered conflicting data regarding the dose dependency of MB effects on BBB opening; while 2 studies indicated that increases in SonoVue MB dose significantly enhanced BBB disruption,^{39,42} another study reported that changes in Optison MB dose in the 50- to 250- μ l/kg range did not significantly alter effects on the BBB.²⁶ Contradictory findings may be related to dif-

TABLE 2. Summary of BBB opening efficacy and damage with various FUS parameters

Group	Acoustic Pressure Amplitude (MPa)	Center Frequency (MHz)	Burst Duration (msec)	Total Exposure Time (sec)	PRF (Hz)	MB Types & Doses (μ l/kg)	EB Extravasation (%)	Damage Score	Effect on Blood Vessel Damage
1	0.2	0.5	10	60	1	Definity (20)	1.8	0	High
	0.3	0.5	10	60	1	Definity (20)	13.72	0	
	0.6	0.5	10	60	1	Definity (20)	21.59	2	
	1.5	0.5	10	60	1	Definity (20)	41.61	3	
2	0.3	1.6	10	60	1	Definity (20)	5.14	1	Moderate
	0.6	1.6	10	60	1	Definity (20)	9.02	1	
3	0.3	0.5	1	60	1	Definity (20)	4.96	0	High
	0.3	0.5	10	60	1	Definity (20)	13.72	0	
	0.3	0.5	100	60	1	Definity (20)	29.72	3	
4	0.3	0.5	10	30	1	Definity (20)	5.87	0	Moderate
	0.3	0.5	10	60	1	Definity (20)	13.72	0	
	0.3	0.5	10	120	1	Definity (20)	23.04	1	
	0.3	0.5	10	300	1	Definity (20)	25.14	2	
5	0.3	0.5	10	60	1	Definity (20)	4.96	0	Low
	0.3	0.5	10	60	2	Definity (20)	13.72	0	
	0.3	0.5	10	60	5	Definity (20)	29.72	0	
6	0.3	0.5	10	60	1	SonoVue (30)	4.45	1	Moderate
	0.3	0.5	10	60	1	Definity (20)	13.72	0	
	0.3	0.5	10	60	1	Definity (100)	16.35	1	

ferences in MB characteristics, complete reperfusion, and sonication parameters that affect cavitation.¹⁴ Additionally, a previous study on MB size and stability properties reported that 1- to 2- μ m MBs were more stable than larger 4- to 5- μ m MBs.¹²

Safety Factors for BBB Modulation by FUS

We expect that the relationships between brain tissue damage resulting from FUS sonication and acoustic pressure amplitude, burst duration, and total exposure time were due to the effects of these parameters on MB inertial cavitation thresholds. In their 2008 study, McDannold et al. investigated a correlation between signal intensity on contrast-enhanced MRI and FUS-induced BBB opening mechanical index, and they identified the mechanical index as a possible parameter that explains the ultrasound-induced BBB disruption threshold.²⁵ Moreover, a study that used a passive cavitation detector to analyze different backscattered acoustic emission states characterized the stable and inertial acoustic cavitation states of MBs. Inertial cavitation, which refers to MB collapse and disruption, was characterized by wideband emissions; in contrast, stable cavitation, which refers to the stable contraction/expansion of MBs, was characterized by subharmonic/ultraharmonic emissions.³⁶

In previous studies, stable cavitation has been measured based on the harmony of MB cavitation to infer tissue damage; however, this indirect method has limitations with regard to safety issues. The effects of repeated sonication should be examined in the normal brain and thus intensive histological evaluations are necessary to facilitate the clinical use of FUS-induced BBB opening. Given our observa-

tion that the amplitude of acoustic pressure, burst duration, and total exposure time were most closely related to blood vessel damage, prudent clinical approaches should be optimized in consideration of these parameters. In addition, Chang et al. recently reported in a retrospective clinical study in which they observed that the skull volume of the target area and the density difference between cortical and marrow bone may affect the precision focusing of ultrasound and the efficacy of ultrasonic energy transmission. Additional skull-related factors influencing energy transmission included shape of the skull, marrow composition, and bone health status.^{8,9} Further studies are necessary to optimize FUS sonication treatment with regard to various skull features.

Possible Applications and Future Directions of FUS for BBB Modulation

Low-intensity FUS modulation of the BBB or blood-tumor barrier for drug delivery has gained traction.²² Successful clinical studies in this area are expected to yield the development of a combined FUS-chemotherapy intervention for intractable and malignant brain tumors. Furthermore, active research on stem cell homing and stem cell transplantation therapy will facilitate the use of FUS as an adjunct tool for these treatments as well.⁴ Finally, the integration of gene therapy, nanoparticle delivery, and optogenetics with other beneficial reported effects of FUS sonication such as amyloid beta plaque reduction and neurogenesis induction will provide powerful new strategies for a number of intractable and difficult-to-treat brain diseases.⁶

The present study had several limitations. First, our

sample size was small given the large number of parameters investigated in our research; however, we used rats of similar age and size across all groups to minimize the interference of individual skull factors and improve study power. Therefore, we expect that our results are sufficiently representative. Second, there may be additional mechanical factors that influence FUS sonication results that were not taken into account by our research. Notably, our study placed an exhaustive focus on popular parameters that have been actively researched in previous studies. We thus speculate that other factors not analyzed by our research are of lesser importance for future clinical application.

Conclusions

The BBB is an important physical barrier to the treatment of CNS diseases. While various efforts have been made to overcome this obstacle, FUS is gaining popularity as a promising option and has recently been introduced to clinical medicine. The clinical application of FUS sonication for BBB modulation requires further studies to guarantee its efficacy and safety. To this end, our study aimed to identify the most influential parameters with regard to brain damage. We found that acoustic pressure amplitude and burst duration were closely associated with enhancement of BBB opening efficiency, but these parameters were also highly correlated with tissue damage in the sonicated region. In contrast, MB types, MB dose, total exposure time, and PRF had an influence on BBB opening without conspicuous tissue damage after FUS sonication. Future work is required to verify our results, and to facilitate the use of FUS sonication for CNS drug delivery in the future.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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Conception and design: JW Chang, Shin, Lee, WS Chang. Acquisition of data: Shin, Kong. Analysis and interpretation of data: Shin, Koh. Drafting the article: JW Chang, Shin, Cho, WS Chang. Critically revising the article: Shin, Na, WS Chang. Reviewed submitted version of manuscript: JW Chang, Shin, Cho, Koh, Na, WS Chang. Approved the final version of the manuscript on behalf of all authors: JW Chang. Statistical analysis: Yoon. Study supervision: JW Chang, Na, WS Chang.

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