Increased expression of P2X7 receptor during the blood-brain barrier opening by low-intensity focused ultrasound

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Directed by Professor Jin Woo Chang

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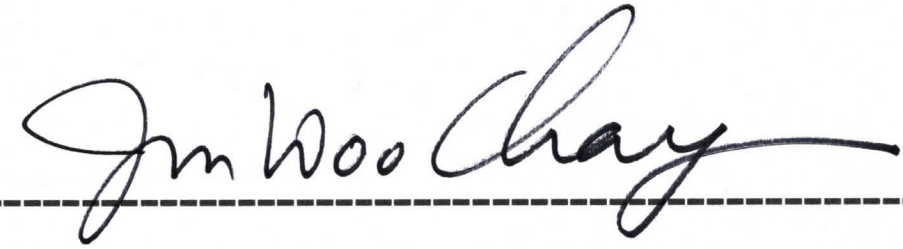
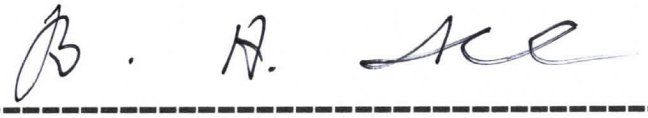
This certifies that Master's Thesis of
Jiyeon Sim is approved.



Thesis Supervisor: Jin Woo Chang



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마지막으로 늘 같은 자리에서 응원해 주시고 사랑해 주신 부모님께 깊고 깊은 감사의 말씀을 드리며 이 글을 마칩니다.

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심지연 올림

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ABSTRACT

Increased expression of P2X7 receptor during the blood-brain barrier opening by low-intensity focused ultrasound

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(Directed by Professor Jin Woo Chang)

The combination of focused ultrasound (FUS) and the microbubbles allows non-invasive and localized opening of the blood-brain barrier (BBB), which is transient and potentially safe. This study aimed to understand the mechanism underlying the focused ultrasound mediated opening of the BBB. In this regard, I hypothesized that the BBB opening process induced by FUS may involve IL-1β signaling through the P2X7 receptor. The extravasation of Evans Blue was performed and analyzed to quantify the BBB opening by FUS. Since some of the detailed changes that take place in the tight junction proteins of the cerebral microvascular endothelium could result because of the sonication combined with microbubbles, I evaluated the time-dependent expression levels of the tight junction proteins. Also, I examined the expression level of P2X7 receptor after FUS in time-dependent manner. Any cellular damage that could occur in a series of BBB opening by FUS was examined by expression levels of NF-κB and determination of early apoptotic population by TUNEL assay.

This study makes a contribution to the literature because it shows that increased expression of P2X7 and activation of P2X7 signaling pathway by FUS may be related to the BBB opening mechanism. Also, I can provide insights into the mechanism involved in the modulation of BBB opening.

Key Words: Focused ultrasound, Blood-brain barrier, P2X7 receptor

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I. INTRODUCTION

The blood-brain barrier (BBB) is composed of endothelial tight junctions, basal lamina, and glial processes. It is a closed membrane structure that selectively restricts the movement of substances between blood and brain. [1, 2] This characteristic is essential in maintaining the brain homeostasis, but the closed membrane structure of BBB reduces the drug delivery efficiency. [3] In fact, BBB blocks all the large molecule neurotherapeutic agents, such as monoclonal antibodies (mAbs) and recombinant proteins, which makes it difficult to treat many central nervous system related diseases. [4] Several methods have been devised to enhance the BBB permeability, and recently, many studies using focused ultrasound (FUS) have been performed. The combination of focused ultrasound and the microbubbles allows non-invasive and localized opening of the BBB, which is transient and potentially safe. [5, 6] However, the studies on FUS induced opening of the BBB are still ongoing, and the underlying mechanism has not yet been fully elucidated. Previously,

Sheikov et al confirmed the ultrastructural changes in the endothelial cell fine morphology and brain microvessels, which indicated that the macromolecules could pass through the ultrasound induced BBB opening *via* several routes. This study also suggested three methods for foreign material to pass through BBB after FUS sonication: transcytosis, transendothelial opening (fenestration and channel formation) widening of interendothelial clefts, and opening of parts of tight junctions. [7] However, the mechanism is still not clear. If the mechanism of BBB opening is identified, modulation of BBB opening can be performed more efficiently.

P2X7, one of the purinergic receptors, is a non-selective cation channel permeable to sodium, potassium, and calcium. P2X7 triggers non-selective pore formation, which allows the passage of molecules of up to 900 Da. The extracellular ATP (eATP) is a ligand of the P2X7 receptor that induces the opening of P2X7 channels. The eATP action *via* P2X7 receptor is the second signal to the inflammasome activation, inducing both maturation and release of pro-inflammatory cytokines.[8-10] For instance, this signal receptor induces caspase-1 dependent release of Interleukin-1β (IL-1β), and is dependent upon the formation of the NLRP3 inflammasome. [11]

Several studies have reported the role of IL-1β under pathological conditions; IL-1β, which is produced by the microglia and astrocytes, is the most representative pro-inflammatory cytokine, and its expression results in increased BBB permeability. [12] Considering this, I hypothesized that the BBB opening process induced by FUS may involve IL-1β signaling through the P2X7 receptor under normal conditions. In the previous study, FUS gradually upregulated mRNA expression of P2X7 in a time-dependent manner *in vitro* in the mouse calvarial cell line. [13] Although, the study was not performed in the *in vivo* brain, it showed that the expression levels of P2X7 receptor could be modulated by focused ultrasound.

In the present study, I investigated the role of P2X7 receptor in the BBB opening mechanism, modulated by FUS sonication. The extravasation of Evans Blue was performed and analyzed to

quantify the BBB opening by FUS. Evans Blue (MW, 961 Da) is a blue dye that cannot penetrate BBB under normal conditions, but increased permeability of BBB after FUS treatment, can allow it to penetrate the brain parenchyma. [14] Some of the detailed changes that take place in the tight junction proteins of the cerebral microvascular endothelium could be a result of sonication combined with a microbubble. [15] In order to measure BBB opening indirectly, I evaluated the time-dependent expression levels of tight junction proteins and P2X7 receptor after FUS sonication, that are correlated to BBB opening.

In order to identify if any serious cellular damage could occur in a series of BBB openings by FUS, the expression levels of NF-κB, the most represented inflammatory response factor, were analyzed. Activated NF-κB subsequently alters the transcription of a large number of genes, most of which participate in innate immunity, inflammation, and cell survival. [16, 17] To measure additional cell damage in this experiment, a TUNEL assay was performed to label early-stage apoptotic cells.

Consequentially, this study provides important insights required to identify BBB opening mechanism.

II. MATERIALS AND METHODS

1. Animals

All animal experimental procedures in this study were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) of Yonsei University Health System (IACUC #2019-208). A total of 86 male Sprague-Dawley rats (250-300 g) were used in this study. Rats were housed as three per cage under a 12 hr light/ dark cycle (lights on between 07:00 and 19:00) in a room with controlled temperature (22ºC ± 2°C) and were allowed access to food and water *ad libitum*.

2. Focused ultrasound

Sonication was performed using a 515 kHz single-element transducer (H-107MR, Sonic Concept Inc., Bothell, WA, USA) with a diameter of 51.7 mm and radius of curvature of 63.2 mm. A waveform generator (33220A, Agilent, Palo Alto, CA, USA) was connected to a 50-dB Radio Frequency Power Amplifier (240L, ENI Inc., Rochester, NY, USA) to drive the FUS transducer and a power meter (E4419B, Agilent) was used to measure the input electrical power. The acoustic parameters used for each sonication were as follows: pressure amplitude, 0.27 MPa; burst duration, 10 ms; pulse repetition frequency, 1Hz; and duration, 120 s. The rats were deeply anesthetized *via* an intraperitoneal (i.p.) injection of ketamine cocktail and positioned in a stereotaxic frame. Ultrasound transmission gel (ProGel-Dayo Medical Co., Seoul, Korea) was used to cover the area between the animal's skull and the transducer cone tip to maximize the transmission efficiency of the ultrasound. The target region was the hippocampal area at the position of -3.5 mm posterior and 2 mm lateral from the bregma. Before the sonication, microbubble (Definity, 20 ul/kg) was injected into the tail vein of the rats. A normal control group was not subjected to sonication.

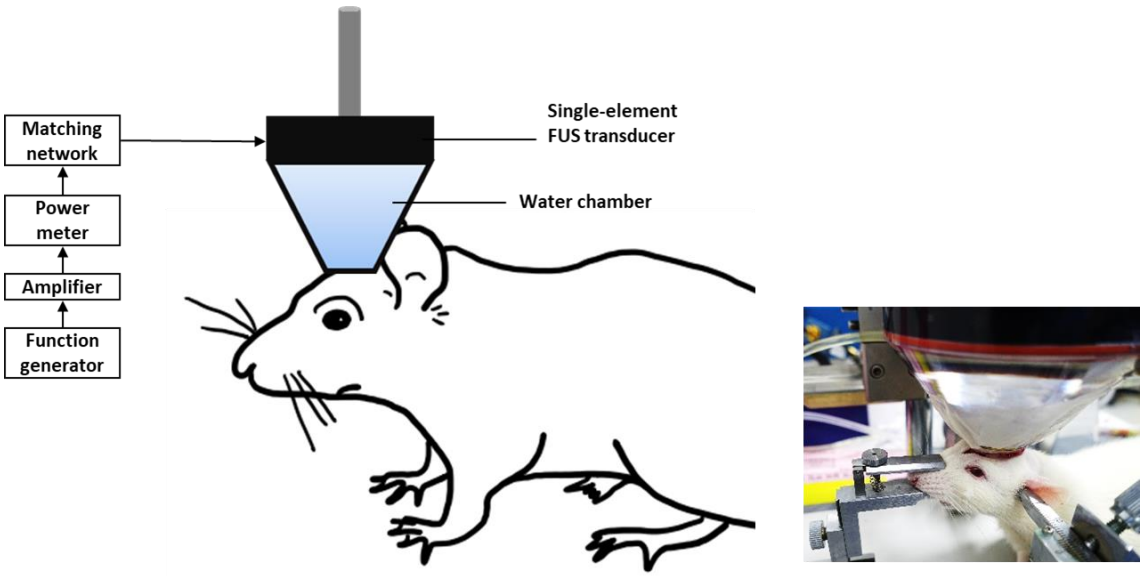


Figure 1. Schematic diagram of focused ultrasound experimental set-up.

3. Blood-brain barrier permeability assay

Evans blue (2% in saline, 100 mg/kg; Sigma-Aldrich, MO, USA) was injected into tail vein before the end time points (0, 1, 4, and 24 hr) after FUS sonication and allowed to circulate for 30 min. After that, the rats were intracardially perfused with phosphate-buffered solution (PBS) and their brains were extract. The samples were divided into the midline of the brain and take the right hemisphere (which was sonicated) for homogenates. The samples were weighted, homogenized with saline (mg/ml), and centrifuged at 15,000 g, 4ºC for 30 min. Then, an equal volume of trichloroacetic acid was added to the resultant supernatant. The samples were incubated overnight at 4ºC and centrifuged at 15,000 g, 4ºC for 30 min. The resultant supernatant (30 µl) of each well with 90 µl of 95% ethanol to increase its optic length, each well was thoroughly mixed by repetitive pipetting, and then spectrophotometrically quantified for the extravasated Evans blue dye at 620 nm.[18]

4. Immunohistochemistry

At 0, 1, 4, 24, and 48 hr after sonication, the rats were re-anesthetized with ketamine cocktail (60 mg/kg, i.p.) and transcardially perfused with saline and fixed with 4% paraformaldehyde (PFA). The brains were then carefully extracted, and post-fixed in 4% PFA at 4ºC. Subsequently, the brain tissue was transferred to a 30% sucrose solution for 72 hr, and prepared for sectioning. The brain was sliced into 30 µm coronal sections using a Leica CM1850 cryostat (Leica Biosystems, Wetzlar, Germany) and preserved in cryoprotectant solution (0.1 M PBS, 30% sucrose, 1% polyvinylpyrrolidone, 30% ethylene glycol). The sections were washed in PBS, blocked with 5% normal goat serum in PBS with 0.3% Tween 20 (PBS-T, pH7.4, CN001-1000, Cellnest) at room temperature for 30 min. The tissues were incubated overnight with the primary

antibody, rabbit anti-P2X7 (1:500, Abcam, Cambridge, UK) at 4ºC. The tissues were then incubated with the secondary antibody, Alexa Fluor 488 IgG goat anti-rabbit (1:1000, Invitrogen, Carlsbad, CA, USA) at room temperature for 2 hr. The tissues were incubated with DAPI (1:1000, Invitrogen) along with the secondary antibody. All sections were mounted with DPX mountant (Sigma-Aldrich), and the fluorescent images were obtained using a M2 microscope.

5. Western blot

The rats were anesthetized with ketamine cocktail (60 mg/kg, i.p.) and rapidly decapitated at the indicated time points (0, 1, 4, 24, 48 hr) after sonication. The samples were homogenized in the lysis buffer (PRO-PREP; Intron Biotechnology, Pyeongtaek, Korea) and placed in ice for 30 min, followed by centrifugation at 13,000 rpm for 30 min; the protein concentration in the lysate was measured using the bicinchoninic acid protein assay reagent kit (Thermo Fisher Scientific, Fremont, CA, USA). 10-20 μg of each protein sample was size-separated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membrane using a Bio-rad Trans-Blot apparatus. The membranes were incubated with blocking buffer (5% non-fat dry milk in phosphate buffered saline containing 0.05% Tween 20, TBST) for an hour at room temperature. The membranes were then incubated with primary antibodies overnight at 4ºC, with a rabbit anti-P2X7 (1:1000, Abcam), rabbit anti-occludin (1:1000, Abcam), rabbit anti-ZO-1 (1:1000, Thermo Fisher Scientific), rabbit anti-IL-1β (1:500; Abcam), rabbit anti-NF-κB (1:1000, Cell Signaling Technology, Beverly, MA, USA), mouse anti-β actin (1:10000, Sigma-Aldrich). After that, the membrane was washed three times for 5 min each and incubated with corresponding secondary antibodies, with goat anti-rabbit IgG (H+L)-HRP (GenDEPOT, Katy, TX, USA), goat anti-mouse IgG (H+L)-HRP (GenDEPOT) for 90 min at room temperature. Immunoreactive bands were visualized by enhanced

chemiluminescent solution (WEST-Queen; iNtRON Biotechnology, Seongnam, Korea) and developed with LAS 4000 mini (GE Healthcare Life Sciences, Pittsburgh, PA, USA). The intensity of each band was determined using an analysis system (Multi Gauge version 3.0, Fujifilm, Tokyo, Japan).

6. Labeling of apoptotic cells

TUNEL assay was performed with ApopTag Plus Fluorescein In Situ Apoptosis Detection Kit (S7111, EMD Millipore; Billerica, MA, USA) following the indicated protocol. Briefly, tissues were treated as indicated, fixed in 1% paraformaldehyde, washed in PBS two times, post-fixed in precooled 2:1 ethanol:acetic acid solution for 5 min at -20ºC and incubated with the equilibration buffer for 10 min. After that, the tissues were incubated with the terminal deoxynucleotidyl transferase at 37ºC for 1 hr, washed with stop/ wash buffer for 10 min, and then incubated with anti-digoxygenin conjugate for 30 min at room temperature. The tissues were washed 4 times with PBS, followed by applying the mounting medium with DAPI (Vector Laboratories; Burlingame, CA, USA) to mount under a glass coverslip. The slides were viewed by fluorescein microscopy using standard fluorescein excitation/ emission filters.

7. Statistical analysis

All results were expressed as mean ± standard error of the mean (SEM) and analyzed using GraphPad Prism 7 (Grapad Software, Inc., La Jolla, CA, USA). Data were analyzed using one-way analysis of variance (ANOVA) followed by a Tukey's post hoc test. $P<0.05$ was considered to be statistically significant.

III. RESULTS

1. Quantification of blood-brain barrier opening

To evaluate blood-brain barrier opening induced by FUS, I first performed Evans blue extravasation analysis (Figure 2A-B). The optical density of Evans blue (OD 620) was measured. As compared with the normal group, the Evans blue content was significantly higher at 0 hr (immediately) after FUS sonication. After 24 hr, the Evans blue content was significantly decreased in the FUS group, as compared to the values at 0 hr in the FUS group.

The change in expression of the endothelial tight junction-related proteins was measured to determine the BBB opening in an indirect manner. Occludin was found significantly decreased within 1 h after sonication, as compared to the normal group (Figure 3A). Also, Zonula occludens-1 (ZO-1) was significantly decreased at 4 hr in the FUS group as compared to the normal group (Figure 3B). These effects were temporary, and the rats recovered to approximately normal condition after the BBB closed.

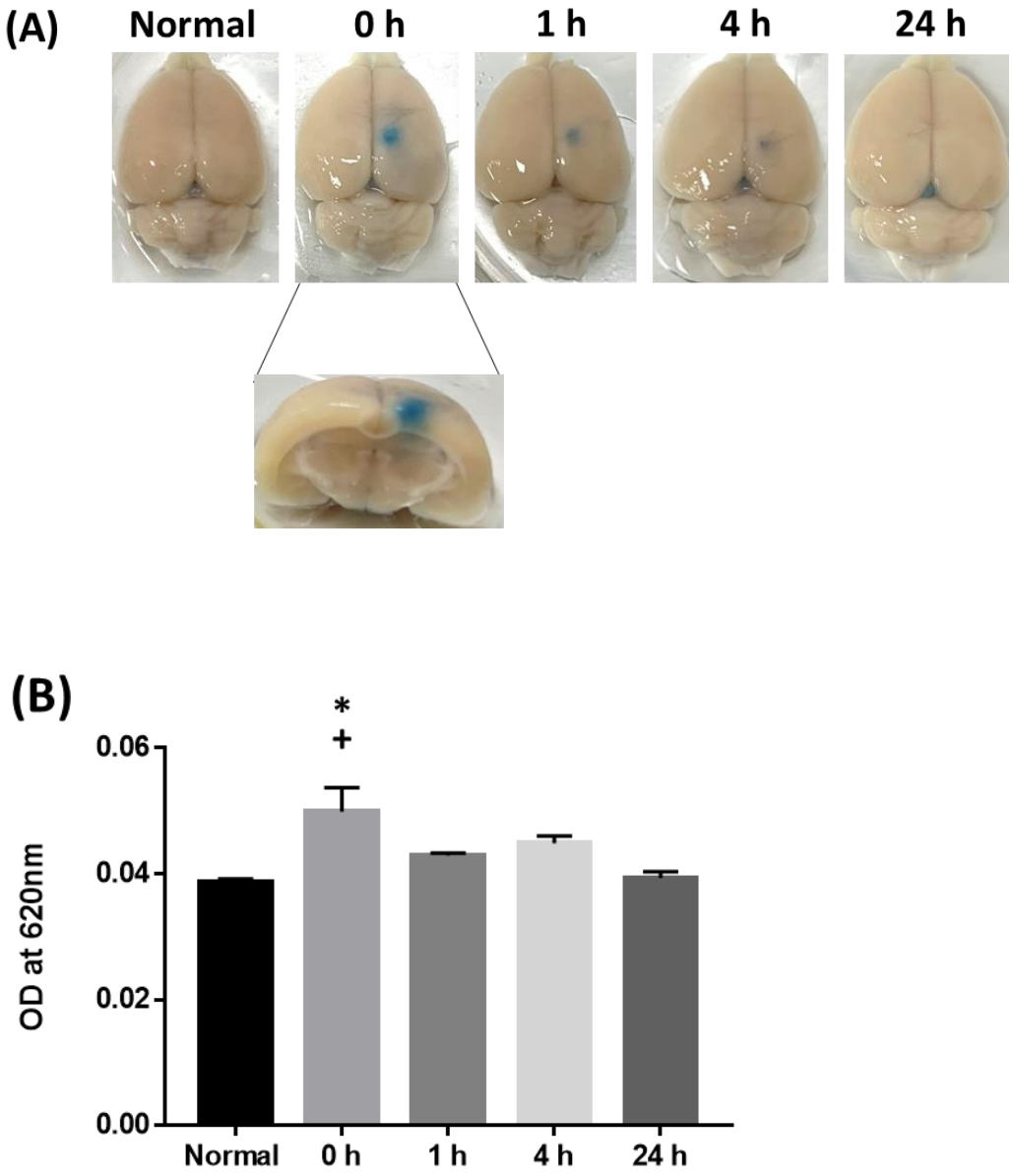
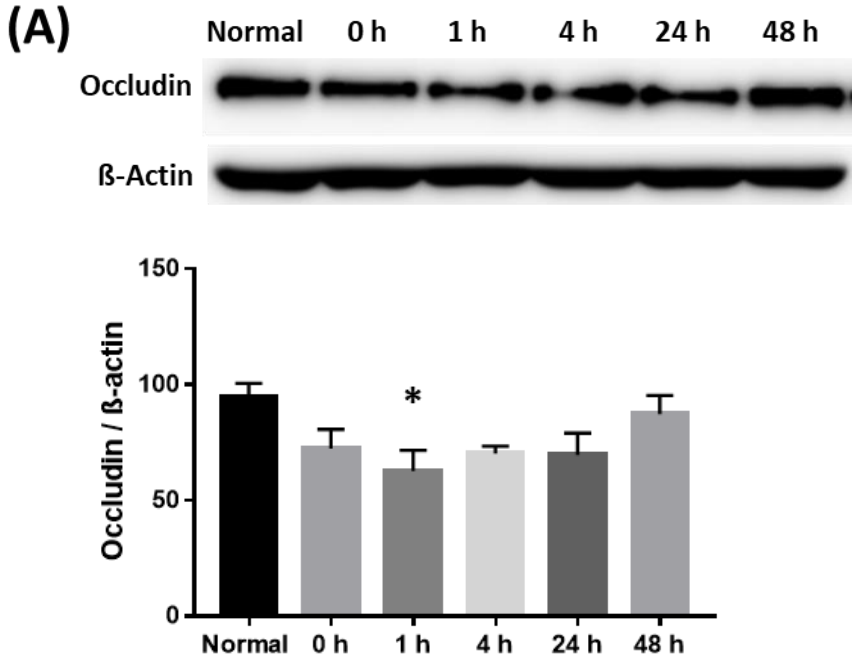


Figure 2. Quantification of the blood-brain barrier opening. (A) Evans blue extravasation in the brain tissue. (B) Evans blue extravasation evaluation at 0, 1, 4, and 24 hr after FUS sonication. * and + denote significant differences compared to normal, 24hr after FUS group, respectively. Bars represent the mean ± SEM One-way ANOVA followed by Tukey post hoc was used to determine statistical significance between the groups (n=3, *$P<0.05$, +$P<0.05$)



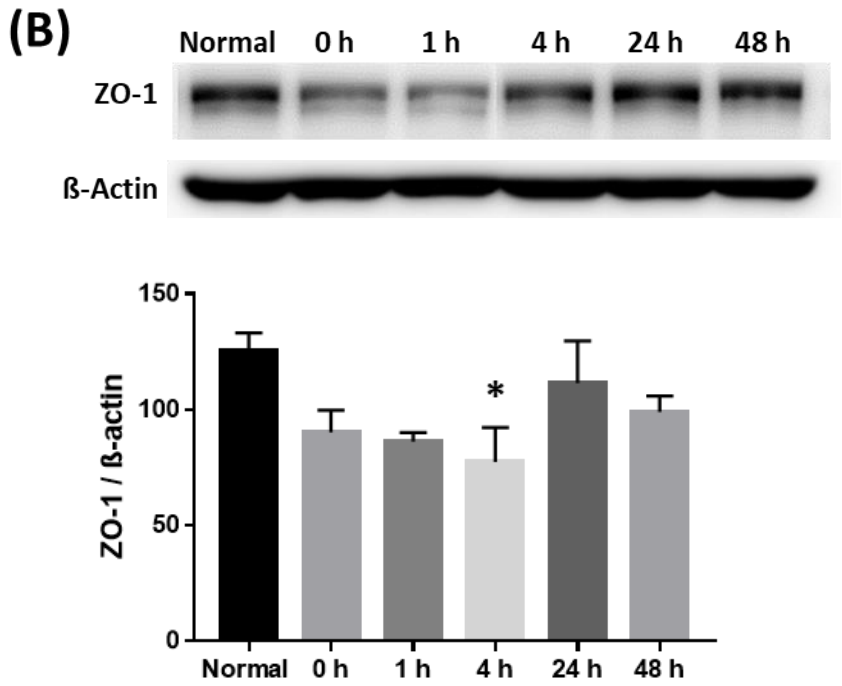
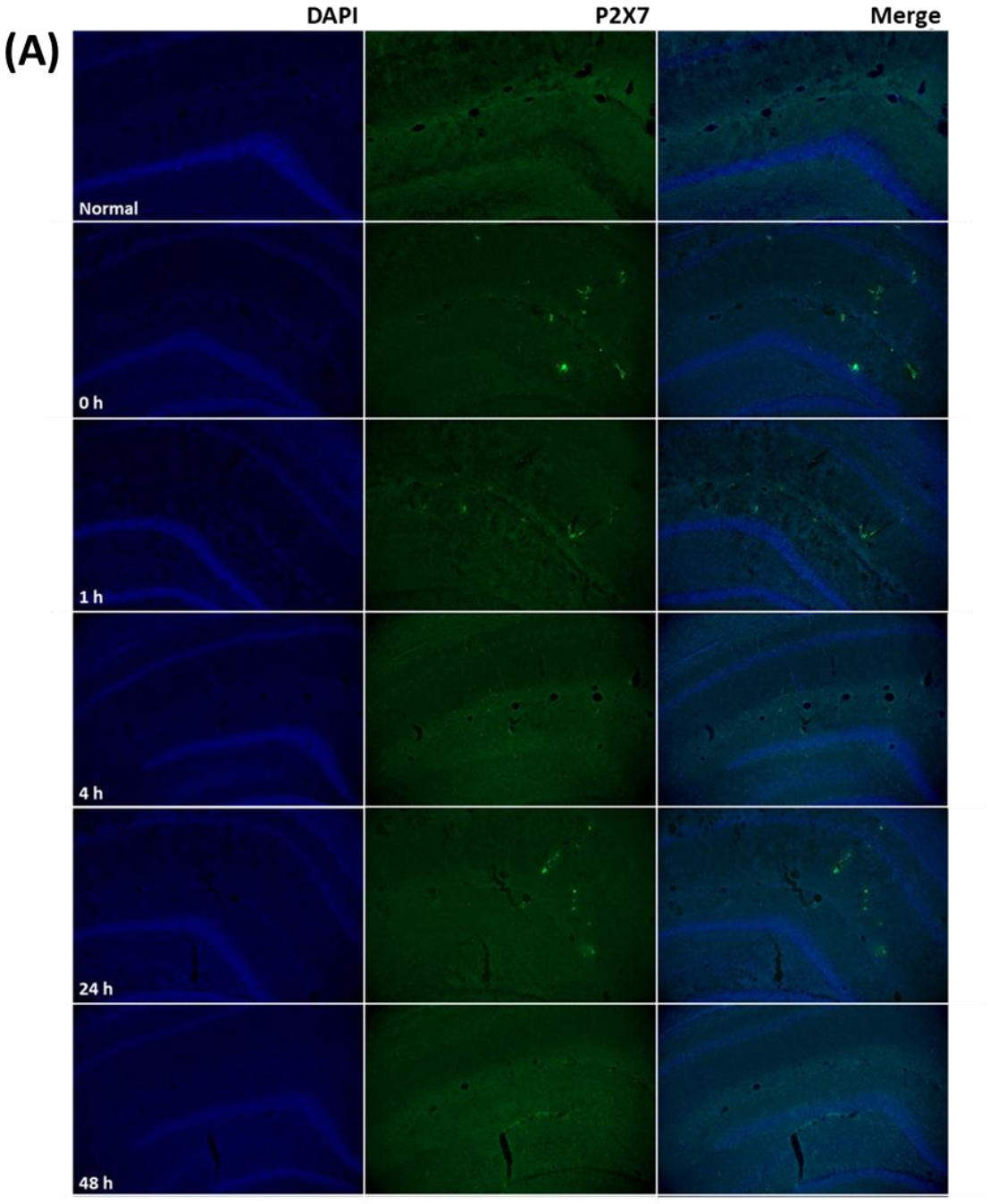


Figure 3. Representative bands and quantitative analysis of occludin, ZO-1, which are related to the endothelial tight junction proteins. (A) 1 hr after FUS group, the expression levels of occludin was found to be significantly decreased as compared to the normal group. (B) Zonula occludens-1 (ZO-1) showed significant reduction within 4 hr after sonication as compared to the normal group. * denotes significant difference compared to normal group. Bars represent the mean ± SEM. One-way ANOVA followed by Tukey post hoc was used to determine statistical significant difference between the groups (n=4~7, $*p<0.05$).

2. Time course analysis of P2X7 receptor after FUS

Immunohistochemistry data (Figure 4A) revealed that P2X7 receptor expression (Green) levels were increased by FUS treatment; however, it was confirmed that the expression levels decreased to the normal levels within 48 hr in the FUS group, when the BBB was completely closed following recovery.

Immunoblotting was also performed to quantify the changes in the expression of P2X7 receptor (Figure 4B). Western blot data shows that the P2X7 receptor expression in FUS treated hippocampus was upregulated until 24 hr after sonication. There was significant increase at 0 hr in the FUS group as compared to the normal group.



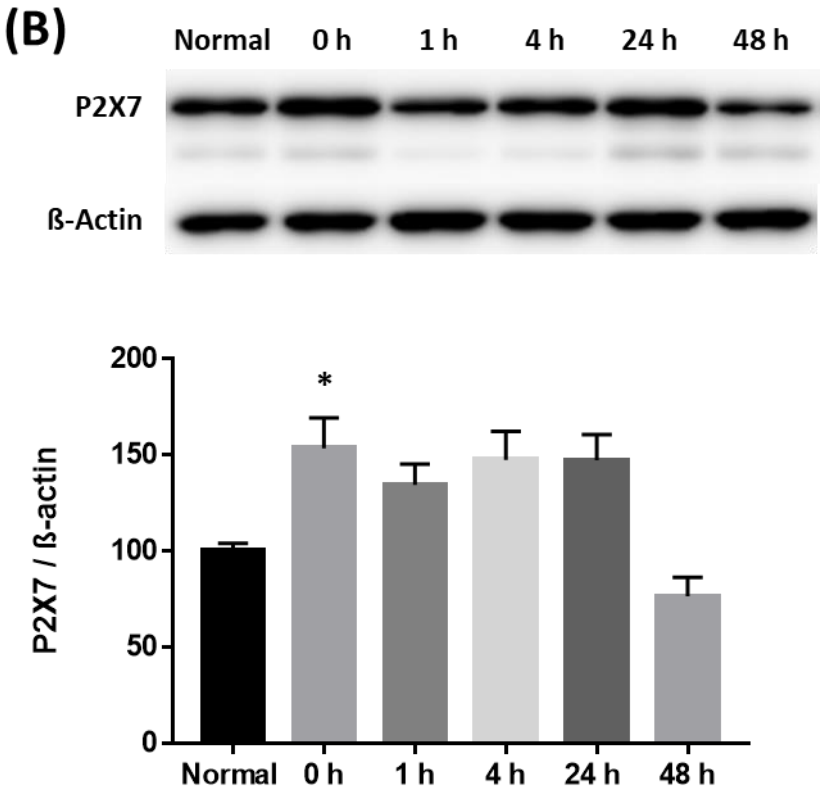
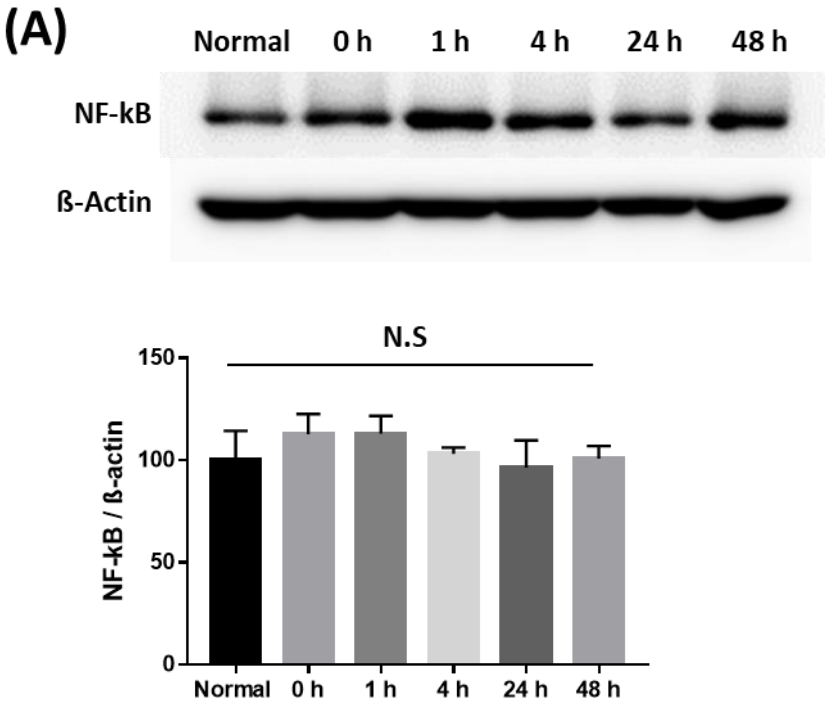


Figure 4. Time course analysis of P2X7 receptor after sonication. (A) Representative immunofluorescence staining slices of P2X7 receptor (Green), magnification: 10x (B) Representative bands and quantitative analysis of P2X7 receptor expression. * denotes significant difference between normal and 0 hr after FUS group. Bars represent the mean ± SEM. One-way ANOVA followed Tukey post hoc was used to determine statistically significant difference between the groups (n=7~8 per group, *$p<0.05$).

3. Effect of FUS on changes in NF-κB and IL-1β

In order to determine if BBB opening induced by FUS caused any severe damage to the cells, such as excessive immune response, the expression levels of NF-κB, one of most representative immune response factors, were examined. The protein levels of NF-κB did not change significantly after FUS treatment (Figure 5A). However, IL-1β, a potent pro-inflammatory cytokine activated by P2X7, was significantly increased at 0 hr in the FUS group as compared to the normal group (Figure 5B).



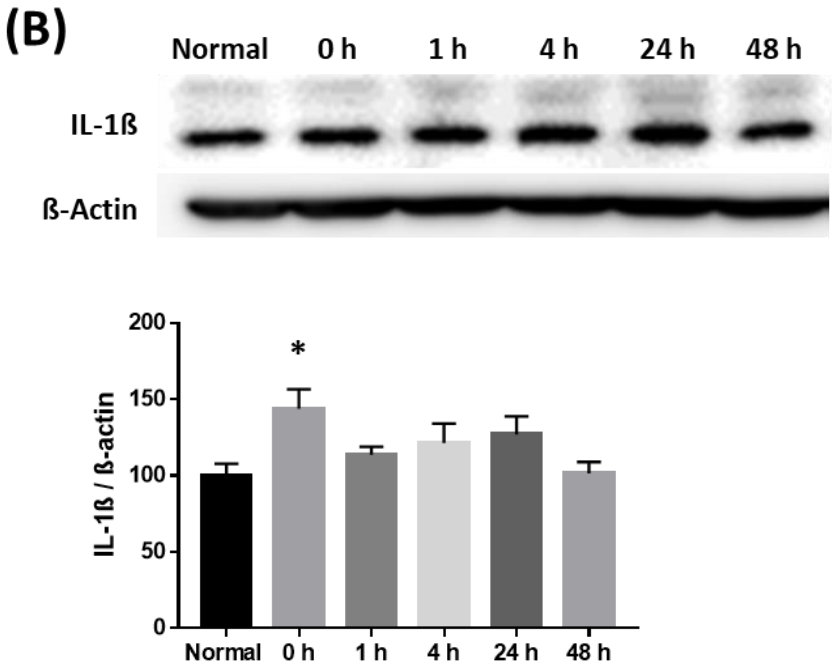


Figure 5. Representative bands and quantitative analysis of NF-κB and IL-1β. (A) There were no significant difference in the expression levels of NF-κB in any time groups after FUS sonication. (B) The expression level of IL-1β was significantly increased 0 hr after FUS group as compared to the normal group. * denotes significant difference between normal and 0 hr after FUS group. Bars represent the mean ± SEM. One-way ANOVA followed Tukey post hoc was used to determine statistically significant difference between the groups (n=4~7 per group, *$p<0.05$).

4. Evaluation of cell damage.

The staining of terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay was performed to further determine whether there was direct cell damage during BBB opening process induced by FUS (Figure 6). The apoptotic cells were labeled with fluorescein (Green). As compared to the normal group, weak TUNEL signal was observed at 0 hr and 1 hr after FUS sonication. However, no TUNEL signal was seen after 4, 24, and 48 hr in the FUS group.

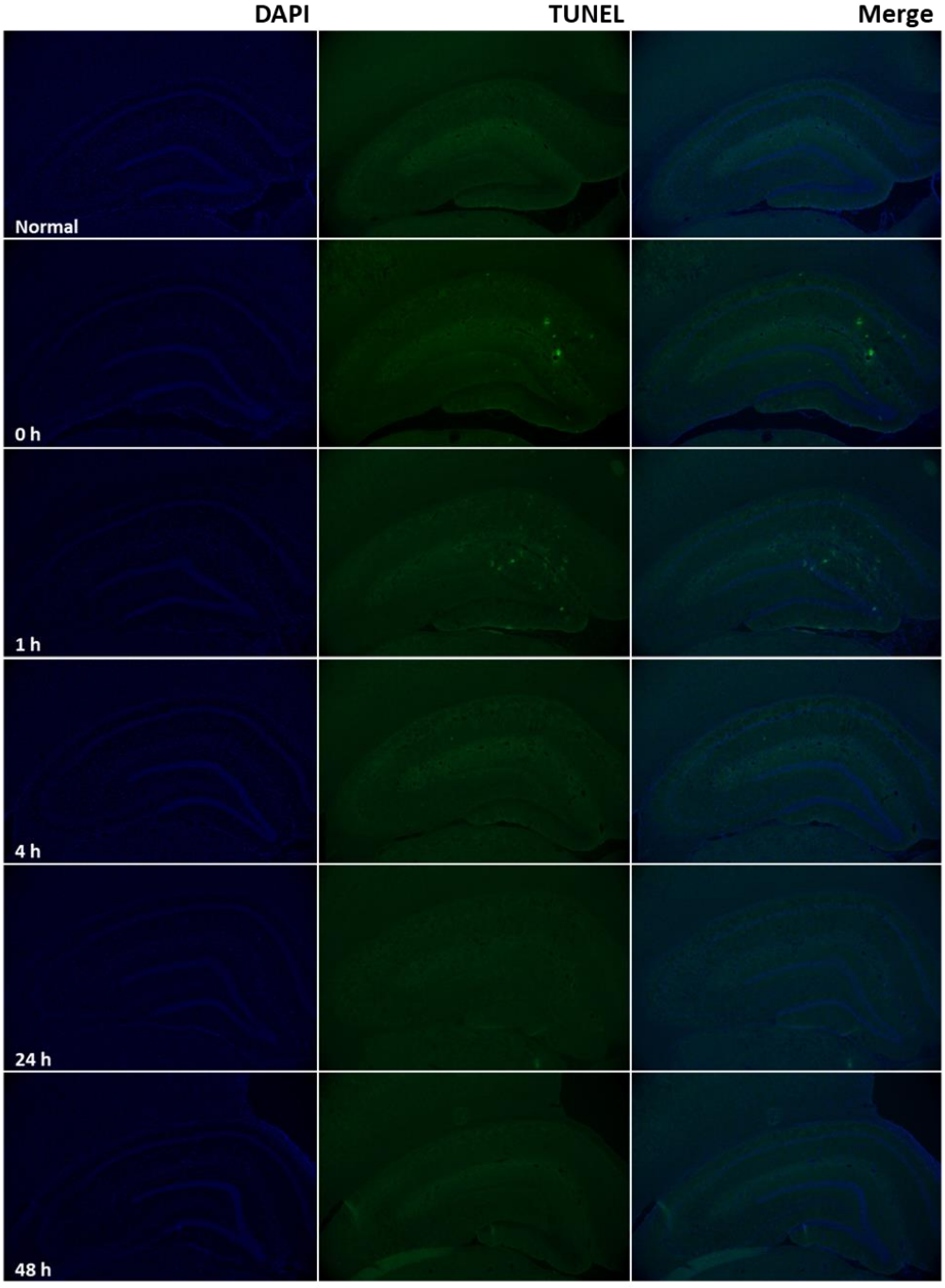


Figure 6. Evaluation of cell damage after FUS sonication. The staining of terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay and the apoptotic cells were labeled with fluorescein intensity (Green).

IV. DISCUSSION

As the need for BBB opening is increased, BBB opening is being studied worldwide. However, most studies have discussed the effects of focused ultrasound and its consequences, but the opening mechanism is not clear. Although some studies have discussed the BBB opening mechanism using focused ultrasound with microbubbles, but those were mainly focused on tight junction proteins. [7, 15, 19] Thus, more research is needed on the BBB opening mechanism induced by focused ultrasound.

In the present study, I found that the protein expression levels of P2X7 receptor were increased after sonication. This effect was not permanent, and the expression levels were restored to the normal range. The change in expression levels of P2X7 receptor due to FUS sonication increased significantly at the time when the BBB remained open by FUS treatment. This result probably indicated that P2X7 receptor might be related to BBB opening induced by FUS. Also, activated P2X7 receptor induced a formation of large pore of up to 900 Da in the membrane leading to the release of the inflammatory cytokines and providing access to various immune cells. [20, 21] The molecular weight of Evans Blue used in this experiment was 961 Da,[14] similar to the pore size formed by activated P2X7 receptor. In other words, it could be that P2X7, whose expression levels are changed by FUS, can increase BBB permeability through direct pore formation instead of going through several signaling pathways.

It is known that many pathologic conditions with central nervous system, such as multiple sclerosis, inflammation, ischemia, and tumors, are typically accompanied by BBB disruption. [22, 23] Also, it was shown that these pathologic conditions are mostly accompanied by upregulation of P2X7 receptor. [8] These phenomenon also provide evidence that P2X7 receptor may affect BBB permeability.

Many studies demonstrated that the time to close BBB, which is opened by ultrasound, is 4 to 24 hr. [5, 15, 24-27] Because of the apparent presence of the FUS parameters used and the differences between the species and individuals of animals, this long interval of BBB closure time is mentioned. Our study used Evans blue dye and confirmed that BBB was kept open by FUS for up to 4 hr. The BBB closed 24 hr after sonication, Evans blue did not penetrate like the normal group. Our results also show the expression of tight junction protein occludin, ZO-1 at different time-intervals after sonication to examine the BBB opening in an indirect manner. Similar to previous studies, occludin and ZO-1 showed significant reduction within 1 hr, 4 hr after sonication respectively, as compared to the normal group, and recovered within 48 hr after sonication when BBB was completely closed. [15] These results are consistent with the fact that the BBB opening by focused ultrasound is transient and reversible.

I also measured the changes in the expression of NK-κB to confirm the immune response during the sonication process. There was no significant difference observed in comparison with the normal group after sonication. This result is in concordance with the data from previous study, which demonstrated that FUS can be used to induce increased BBB permeability without an associated upregulation of NF-κB signaling pathway gene expression. [28] In addition, TUNEL assay also confirmed the early-stage apoptosis. A compared to the normal group, several apoptotic cells were found at 0 and 1 hr after sonication. However, the signal was not found after 4 hr of sonication. This data imply that the apoptotic cells generated rapidly after sonication were removed through the intracellular clearance process. Several studies have addressed the rapid clearance of apoptotic cells by phagocytes, which are the professional engulfers. [29, 30] I should examine the clearance of apoptotic cells by confirming changes in the expression of surrounding engulfer cells, such as phagocytes, after sonication in future studies. IL-1β is a potent pro-inflammatory cytokine that can result in increased BBB permeability by downregulation of tight junction related proteins in most pathological conditions. [31-33] Our results showed that the

expression level of IL-1β was increased at 0 h in the FUS group as compared to the normal group. IL-1β increase by sonication is thought to act for a relatively short time and affect the BBB opening. Many studies have indicated that activated NF-κB induces increased secretion of IL-1β, but our results showed no significant increase in NF-κB. [16, 34, 35] Additional experimentation and discussion will be required to accurately interpret these results.

BBB opening is a special technique, and further insight into the mechanisms underlying BBB opening is required for the management of BBB modulation. As this study suggested, the signaling pathway of P2X7 with increased expression by FUS may be related to the BBB opening mechanism. As mentioned earlier, many studies discuss that P2X7 increases BBB permeability through several immune mediators, such as IL-1β. These are mostly addressed in disease conditions. This study is meaningful in that it is possible to identify these phenomena under conditions that are not pathological enough to cause severe cell damage.

To confirm this hypothesis, future studies will examine the BBB opening induced by FUS after treatment with agonists and antagonists of the P2X7 receptor. Also, I propose a more certain BBB opening mechanism by measuring changes in the expression of P2X7, IL-1β, and substances that are likely to be involved in the expected BBB signaling [36] at different time intervals after ultrasound. In addition, co-staining of P2X7 and several cell markers will confirm which cell have increased expression of P2X7 receptor after sonication. These studies will reveal a more accurate BBB opening mechanism induced by FUS.

V. CONCLUSION

Our study suggested that increased expression of P2X7 and activation of P2X7 signaling pathway by FUS may be related to the BBB opening mechanism. In the present study, I showed that expression levels of P2X7 receptor due to FUS sonication increased significantly at the time when the BBB remained open by FUS. In addition, several inflammatory factors were examined and confirm that any severe cell damages were not observed during this process. Based on our results, I can contribute insights into the mechanism involved in the modulation of BBB opening induced by FUS.

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ABSTRACT (In Korean)

저강도 집속 초음파에 의한 뇌혈관 장벽 개방과 P2X7 발현량의 상관관계 분석

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심 지 연

의료기술의 발달로 인구의 고령화가 진행됨에 따라 치매나 파킨슨병과 같은 퇴행성 뇌 질환 발병률이 동반하여 증가하고 있다. 증가하는 뇌신경계 질환의 치료를 위하여 여러 약물이 개발되어 왔지만 뇌혈관 장벽 (Blood-brain barrier, BBB)이라는 특수한 장벽 때문에 약물의 전단 효율이 감소하여 충분한 치료효과를 내지 못하고 있는 실정이다. 최근 여러 연구를 통해 미세 기포 (microbubble)와 함께 집속 초음파 (Focused ultrasound, FUS)를 이용하여 일시적이고 가역적이며 비침습적으로 뇌혈관 장벽을 개방하여 약물이 더 높은 효율로 뇌에 침투할 수 있음이 입증되었다. 전 세계적으로 집속 초음파를 이용한 뇌혈관 장벽 개방에 대한 연구는 활발하게 진행되고 있으나, 그 개방 기전에 대해서는 명확하게 규명되어 있지 않다. 이에 본 연구는 집속 초음파에 의한 뇌혈관 장벽 개방 메커니즘을 제안하고자,

집속 초음파에 의한 뇌혈관 장벽 개방이 P2X7 수용체의 활성화를 통한 신호 전달 과정을 거쳐 진행될 것이라는 가설을 세웠다.

본 연구에서는 집속 초음파에 의한 뇌혈관 장벽 개방을 에반스 블루 염색약의 혈관 외 유출과, 뇌혈관 장벽의 구성 요소인 밀착 연접 단백질의 발현 변화량을 집속 초음파 조사 후 시간대별로 확인하여 정량화하였다. 또한 집속 초음파에 의해 P2X7 수용체의 발현량이 증가하는 것을 확인하였다. 이러한 P2X7 수용체의 증가 양상은 계속적으로 유지되는 것이 아니라, 집속 초음파에 의해 개방된 뇌혈관 장벽이 완전히 닫혔다고 판단할 수 있는 집속 초음파 조사 후 48시간 이후에는 정상 수치로 발현량이 감소하였다. 더하여 집속 초음파 조사 후 NF-κB의 유의미한 증가가 없음과 TUNEL 분석을 통한 초기과정의 세포 자연사 여부를 검증함으로써 집속 초음파에 의한 뇌혈관 장벽 개방 과정에 심각한 수준의 세포 손상이 없다는 것을 확인하였다.

따라서 본 연구에서는 집속 초음파에 의한 P2X7 수용체의 발현량 증가 및 P2X7 수용체와 연관된 신호 전달 과정의 활성화가 비교적 안전한 범위 내에서의 뇌혈관 장벽 개방 기전에 관여할 수 있다는 가능성을 제시하였다.

핵심되는 말: 집속 초음파, 뇌혈관 장벽, P2X7 수용체