

# Ultrasound Attenuates Blood-Brain Barrier Disruption and Cerebral Edema after Traumatic Brain Injury in Mice

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**Abstract:** Traumatic brain injury (TBI) is often caused by accidents that disrupt the normal brain function. The blood-brain barrier disruption (BBBD) is considered to be the major cause of vasogenic brain edema and subsequent brain injury. Cerebral edema is a common life-threatening neurological complication after TBI. In this study, we explored whether ultrasound had a protective effect against BBBD and cerebral edema following TBI. A TBI animal model was established by controlled cortical impact (CCI) injury in mice. The degree of BBBD was assessed by the water content and Evans blue (EB) extravasation in the brain following ultrasound exposure. The results showed that low-intensity pulsed ultrasound (LIPUS) elevated the protein levels of BDNF and tight junction protein ZO-1 following TBI. Furthermore, ultrasound treatment attenuated brain edema and BBBD. Therefore, ultrasound may serve as a potential treatment modality for brain edema after TBI.

**Key-Words:** traumatic brain injury, ultrasound, blood-brain barrier, brain edema, BDNF

## 1 Introduction

Traumatic brain injury (TBI) is a leading cause of death and disability with no definitive treatment options. Cerebral edema is one of the major factors resulting in the high mortality and morbidity in affected patients [1]. As such, attenuating blood-brain barrier disruption (BBBD) has become a promising approach to managing brain edema [2].

Brain-derived neurotrophic factor (BDNF) has an important role in neuronal survival and synaptic plasticity [3, 4]. Previous study demonstrated that increased levels of BDNF in the brain have beneficial effects for TBI [5]. Our studies suggested that low-intensity pulsed ultrasound (LIPUS) can promote the protein levels of BDNF in the sonicated brain [6, 7]. Thus, this study was to investigate whether the occurrence of brain edema exhibited during TBI can be reduced by ultrasound treatment.

## 2 Methodology

### 2.1 Animal model

Male C57BL/6J mice (8 weeks old, about 22-25 g) were intraperitoneally anesthetized with sodium pentobarbital (65 mg/kg; Rhone Merieux, Harlow, UK) and placed in a stereotaxic frame. TBI model was induced by CCI injury in mice as described previously.

### 2.2 Ultrasound system

The pulsed ultrasound setup was similar to our previous study. LIPUS exposures were generated by a 1.0-MHz, single-element focused transducer (A392S, Panametrics, Waltham, MA, USA) with a diameter of 38 mm and a radius of curvature of 63.5 mm. LIPUS was applied for a sonication time of 5 min at an acoustic power of 0.51 W 5 mins after TBI and subsequently daily for a period of 1 or 4 days.

### 2.3 Cerebral water content

Mice were sacrificed at day 1, one time point associated with maximum appearance of edema after TBI. Brain edema was evaluated by measuring brain water content using the formula of (wet weight-dry weight)/wet weight  $\times 100\%$ .

### 2.4 Blood-Brain Barrier Permeability

BBB permeability was measured by EB extravasation at day 1 after TBI. EB (Sigma, St. Louis, MO) with concentration of 100 mg/kg was injected via the tail vein and allowed to circulate for 1 h. After perfusion and brain removal, the ipsilateral hemispheres were cut into 4-mm-thick sections (2 mm from the frontal pole) before measuring the amount of EB extravasated.

## 2.5 Western blot

One, and 4 days following TBI, a 4-mm coronal section was taken from the injured area over the parietal cortex, and then homogenized by T-Per extraction reagent supplemented with the Halt Protease Inhibitor Cocktail (Pierce Biotechnology, Inc.). After blotting, the membranes were blocked for at least 1 h in blocking buffer (Hycell, Taipei, Taiwan), and then the blots were incubated overnight at 4°C in a solution with antibodies against zonula occludens-1 (ZO-1, 1:200, 61-7300) from Invitrogen (Camarillo, CA, USA) and BDNF (1:250, sc-546) from Santa Cruz.

## 3 Results

The intensity of the ultrasound exposures was selected based on data from Fig. 1. The cerebral water content was significantly increased in the mice brain following TBI. A sonication time of 5 min at an acoustic power of 0.51 W of ultrasound attenuated brain water content, however, there was no significant difference between the other two ultrasound-treated TBI groups and the non-treated TBI group.

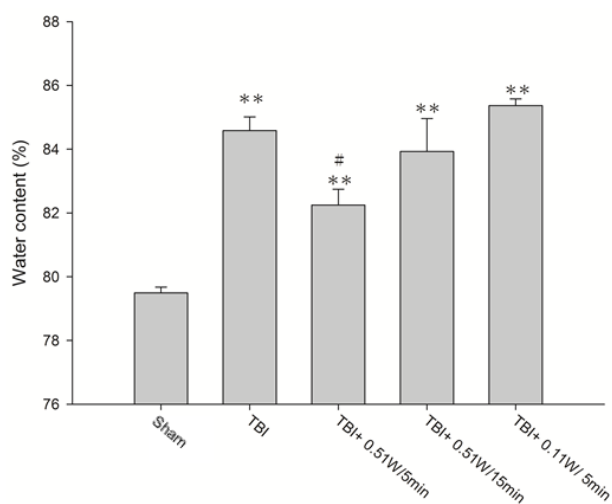


Fig. 1: Effects of different ultrasound parameters on brain water content in TBI mice at 1 day.

TBI resulted in a significant decrease in ZO-1 protein expression at day 1 after TBI (Fig. 2). The ZO-1 protein expression was significantly increased following LIPUS treatment. However, no significant difference was found in the normal brain treated with LIPUS as compared to the sham group.

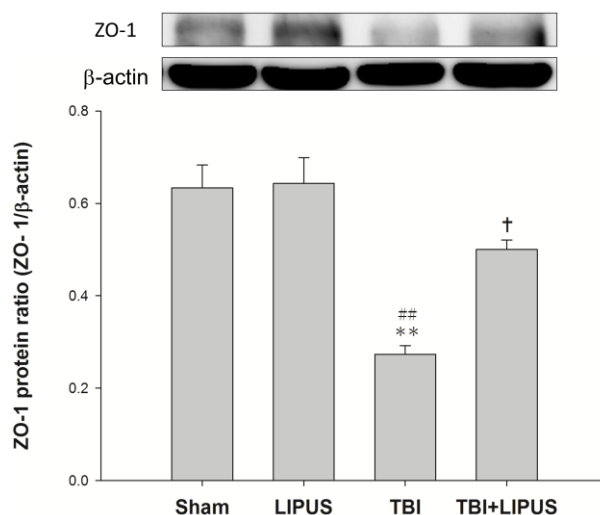


Fig. 2: The protein expression of ZO-1 by LIPUS treatment in TBI mice brain.

The protein levels of BDNF were significantly decreased in the ipsilateral cortex at day 4 after TBI compared with the sham group (Fig. 3). The BDNF protein expression was significantly increased following LIPUS treatment. Additionally, no significant difference was found in the normal brain treated with LIPUS as compared to the sham group.

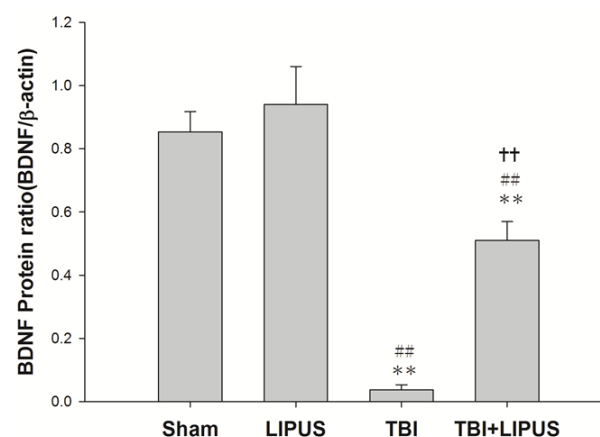


Fig. 3: The protein expression of BDNF by LIPUS treatment in TBI mice brain.

Figure 4A showed that TBI caused a significant increase in the percentage of water content within the ipsilateral hemisphere compared with the Sham group. However, the water content was significantly decreased in the TBI mice brain following LIPUS treatment.

We further evaluated whether LIPUS treatment could attenuate BBBB at day 1 (Fig. 4B). There was a marked increase in EB extravasation in the ipsilateral hemisphere of

the TBI group as compared with the sham group. TBI-induced increases in EB content in the ipsilateral hemisphere were significantly reduced by LIPUS treatment.

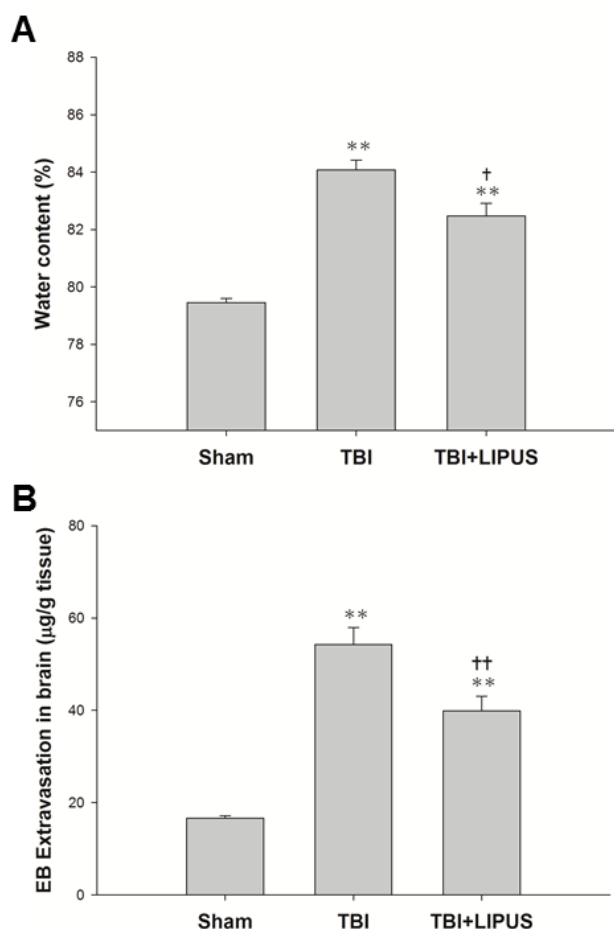


Fig. 4: Effects of LIPUS stimulation on (A) brain edema and (B) BBBB in the TBI mice.

#### 4 Conclusion

In this study, LIPUS treatment produced a dramatic neuroprotection in TBI mice model. This was demonstrated by a reduction in water content and BBBB in head-injured mice.

The importance of BDNF to functional recovery in patients with TBI has been shown in a clinical study [8]. In addition, transcranial LIPUS is capable of promoting protein levels of BDNF in the astrocyte cells and the brain [9, 10]. Thus, LIPUS has become a promising method to treating brain injuries or neurodegenerative diseases. Further researches are necessary to investigate whether TBI followed by LIPUS will improve the functional recovery or repair the brain tissue.

Our preliminary data suggesting that the BBBB and brain edema in the TBI may be alleviated by LIPUS, and this technology might be proposed as a

potential treatment modality for clinical applications in TBI patients.

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