

MRI-GUIDED DISRUPTION OF THE BLOOD-BRAIN BARRIER USING TRANCRANIAL FOCUSED ULTRASOUND IN A RAT MODEL

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Short Abstract: Microbubble-mediated focused ultrasound disruption of the blood-brain barrier (BBB) is a promising technique for non-invasive targeted drug delivery in the brain¹⁻³. This protocol outlines the experimental procedure for MRI-guided transcranial BBB disruption in a rat model.

Long Abstract: Focused ultrasound (FUS) disruption of the blood-brain barrier (BBB) is an increasingly investigated technique for circumventing the BBB³⁻⁵. The BBB is a great detriment to pharmaceutical treatments of brain disorders as it limits the passage of molecules from the vasculature into the brain tissue to molecules less than approximately 500 kDa in size⁶. FUS induced BBB disruption (BBBD) is temporary and reversible⁴ and has an advantage over chemical means of inducing BBBD by being highly localized. FUS induced BBBD provides a means for investigating the effects of a wide range of therapeutic agents on the brain, which would not otherwise be deliverable to the tissue in sufficient concentration. While a wide range of ultrasound parameters have proven successful at disrupting the BBB^{2,5,7}, there are several critical steps in the experimental procedure to ensure successful disruption with accurate targeting. This protocol outlines how to achieve MRI-guided FUS induced BBBD in a rat model, with a focus on the critical animal preparation and microbubble handling steps of the experiment.

Protocol Text:

1) Ultrasound and MRI setup

An MRI-compatible three-axis focused ultrasound system was used in this study (FUS Instruments, Inc., Toronto, Ontario, Canada). Two different ultrasound transducers were used. An in house constructed 551.5 kHz spherically focused transducer (FN = 0.8, external diameter = 75 mm, internal diameter = 20 mm), and a 1.503 MHz, 8-sector array with integrated PZT hydrophone (Imasonic Inc., Voray-sur-L'Ornon, France) driven as a single element spherically focused transducer (FN=0.8, aperture = 10 cm). An MRI-compatible PVDF receiver⁸ was used to record acoustic emissions when the 551.5 kHz transducer was used. If different equipment is used, the following are suggested:

- 1.1) Select an appropriate, calibrated ultrasound transducer with a center frequency in the range 0.25 MHz -1.5 MHz and an f-number of 1 or less.
- 1.2) Place a water bath filled with degassed, de-ionized water on the bed of a 1.5 T or 3 T MRI. The water bath should have a top-plate which can hold the animals. Mount the ultrasound transducer in the tank on an MRI compatible three-axis positioning stage or system⁹, facing the water surface.
- 1.3) Run the transducer cable to the ultrasound driving equipment through a penetration panel.
- 1.4) Generate the transducer driving signal using a function generator and power amplifier. Use an external matching circuit to minimize the reflected electrical power.

1.5) Knowing the transducer focal length, place the transducer focus at the water surface and sonicate the water surface to generate a visible water fountain.

1.6) Mark the location of the focus using a marker that will be visible on the MR images. This can be done using a plate with a center hole that will fill with water when placed over the focus. The water-filled hole will be visible on the MRI and will provide focal coordinates in two planes, while the coordinate in the axial direction of the transducer can be determined from the water surface.

1.7) Using a 3-plane localizer sequence, image the focus marker and record the location of the focus in the MRI coordinate system.

1.8) To monitor acoustic emissions, mount an MR-compatible hydrophone⁸ that is used as a passive cavitation detector (PCD) in the water bath directed at the transducer focus, or use a transducer with integrated hydrophone.

1.9) Run the PCD cables to a scope card that is sufficiently fast to capture bursts of up to 10 ms at PRFs up to 2 Hz (e.g. ATS460, AlazarTech, Pointe-Claire, Quebec, Canada). Cables must be grounded to the penetration panel or RF shield to minimize noise.

2) Animal preparation

2.1) Anesthetize the animals using isofluorane gas. Since isofluorane has an effect on BBB disruption, the animals should be taken off the gas 10 minutes prior to the start of the experiment.

2.2) Using an electric razor shave the fur from the top of the animal's head and neck, then remove the remaining fur using a depilatory cream (e.g Nair, Church & Dwight Co., Princeton, NJ, USA).

2.3) Insert a 22 gauge catheter with 3-way stopcock into the tail vein and flush with a heparin/saline mixture (33 U/mL) to prevent clot formation in the catheter.

2.4) Deliver injectable anesthetics (10 mg/kg ketamine, 40-50 mg/kg xylazine) via intramuscular injection, and remove the animal from the isofluorane.

2.5) Place the anesthetized animal supine on the ultrasound positioning system with the top of the head contacting the waterbath through a hole in the top-plate (Fig.1). A small amount of ultrasound gel maybe applied to the top of the animal's head to minimize the chance of trapped airbubbles.

2.6) Tape the legs to the positioning system. The head may be held in place using either a bite bar, if available, or tape placed firmly across the chin.

2.7) Cover the animal with a towel or blanket. If the room is particularly cold or the animal will be anesthetized for a long time, a circulating water blanket may be used in order to keep it warm.

3) Target selection

3.1) Acquire baseline axial T₂-weighted and T₁-weighted MR images of the brain. If using a 1.5T MRI and dedicated head-sized RF-receive surface coil, suitable scan parameters might be

T₂ weighted: FSE, TE = 60 ms, TR = 2000 ms

T₁ weighted: FSE, TE = 10 ms, TR = 500 ms

3.2) Select the target from the T₂-weighted scans, avoiding the ventricles and the brain midline, and selecting a mid-brain depth.

3.3) Move the transducer focus to the target location.

4) Microbubble preparation

Definity microbubbles (Lantheus Medical Imaging, MA, USA) are used by several groups for microbubble mediated FUS induced BBB^{2,5,7}. Appropriate dosages for other microbubble types can be found in the literature^{10,11}.

4.1) Activate the Definity microbubbles and slowly draw up a small volume into a 1 mL syringe using an 18-gauge needle.

4.2) Remove trapped air from the syringe by gently moving the plunger back and forth. Do not tap the syringe.

4.3) Dilute the Definity in normal saline in a ratio of 10:1 saline to Definity by slowly injecting the required volume of Definity into a syringe of saline. If an infusion delivery is used, the Definity can be further diluted, to 50:1 or 100:1.

4.4) Gently invert the syringe to thoroughly mix the Definity and saline until an even appearance is achieved. Gentle inversion of the syringe immediately prior to injection may also be necessary if the bubbles have started to float out of suspension.

4.5) Calculate the required dose volume based on 0.02 mL/kg of Definity, or 0.2 mL/kg of the solution (at 10:1 dilution).

5) Ultrasound delivery

5.1) Set the sonication parameters, using low duty cycle bursts, not continuous wave sonications. Appropriate sonication parameters at 0.5 MHz are 0.23 MPa *in situ* pressure, 10 ms

bursts at 1 Hz PRF, 2 min. total sonication time. At 1.5 MHz appropriate *in situ* pressures fall around 0.45-0.5 MPa.

Appropriate pressures at different frequencies can be estimated using a mechanical index of 0.46¹².

5.2) Check that the animal's head is still coupled to the water.

5.3) Inject the microbubbles slowly into the tail vein catheter and flush with 0.5 mL normal saline. Begin the sonication simultaneously with the start of injection.

6) MRI Evaluation of treatment outcome

6.1) Following the sonication, inject an MRI contrast agent (e.g. 0.2 ml/kg Omniscan, GE Healthcare) via the tail vein catheter followed by a 0.5 mL saline flush.

6.2) Perform T₁-weighted imaging until the contrast peak has passed. Sonication sites which have been successfully disrupted will show greater enhancement than the surrounding tissue.

6.3) Perform T₂-weighted imaging to check for edema. High signal at the sonication sites is indicative of edema.

Representative Results:

MRI contrast agents can be successfully delivered through the BBB using focused ultrasound and circulating microbubbles. Figure 2 shows typical pre and post-FUS T₁-w images. Figure 2B shows a contrast enhanced (CE) T₁-w image with distinct focal openings in four sonicated locations. Sonication locations 1 and 2 show particularly bright enhancement. In Fig.3 locations 1 and 2 can also be seen to correspond with T₂-w high signal, indicating edema.

The extent of T₂-w edema can sometimes be more easily visualized on sagittal slices. Figure 4 shows CE-T₁-w and T₂-w sagittal slices through sonication locations 1 and 3. Edema is visible at location 1 but not location 3.

Spectral analysis of captured acoustic emissions data (Fig.5) may show harmonic emissions and/or sub/ultra harmonic emissions when stable cavitation is occurring.

The use of higher sonication frequencies results in more localized openings due to the smaller focal spot size. Figure 6 shows that higher frequencies can be used to create smaller regions of opening. This allows investigation of effects mid-brain with fewer near-skull effects.

Tables and Figures (Required):

Figure 1 – Experimental setup

Figure 2 – Pre (left) and post (right) treatment T_1 -w images of a rat brain showing enhancement at four sonication locations.

Figure 3 – Pre (left) and post (right) treatment T_2 -w images of a rat brain (same animal as Fig.2) showing T_2 -w edema at sonication locations 1 and 2

Figure 4 – Post treatment sagittal T_1 -w (left) and T_2 -w (right) images from the same rat brain as Figs. 2 and 3. The opening at location 1 (left) correlates with T_2 -w edema (right). Location 3 shows opening (left) but no T_2 -w edema.

Figure 5 – Frequency spectrum from data captured during a single 10 ms burst at 551.5 kHz. The fundamental frequency (f_0) as well as harmonics ($2f_0$) and sub/ultraharmonics ($0.5f_0$, $1.5f_0$) are visible.

Figure 6 – Post treatment CE- T_1 -w axial (left) and sagittal (right) images from a rat brain sonicated in four locations at 1.503 MHz. BBB openings at this frequency are seen to be more localized.

Discussion: Preparation of the animals and microbubbles are the most critical aspects of this procedure. The hair on the animal's head must be entirely removed to avoid attenuating the ultrasound beam. The BBB can be disrupted under isofluorane anesthetic, however, it becomes more difficult to achieve consistent opening.

The microbubbles should always be handled with care and large gauge needles must be used when drawing up, in order to avoid breaking them. Similarly, the largest gauge catheter which can be reasonably used in the tail vein should be employed (22-gauge is recommended). If a smaller catheter is required to achieve proper placement in the vein then extra care must be taken during the microbubble injection. The microbubble injections should always be done slowly.

Burst mode sonications should always be employed. If continuous wave sonications are used the microbubbles will not replenish into the vessels at the transducer focus and BBBD will not be achieved. If CE- T_1 -w images post-treatment do not show disruption, the treatment can be repeated checking that the water level is topped up so that the animals head is in the water and that there are no air bubbles trapped on the skin surface.

Higher frequencies provide better localization in small animal models but require higher *in situ* pressures to induce opening. It is also important to consider that losses due to the skull are higher at higher pressure and must be accounted for when estimating *in situ* pressures. At 0.5 MHz transmission

through rat skull is approximately 73%⁸, but drops to approximately 50%¹³ at 1.5 MHz. Attenuation can be assumed to be 5 Np m⁻¹ MHz⁻¹ in brain tissue⁴. Higher frequencies are better suited for work in small animal models but are less clinically relevant.

This MRI-guided approach provides advantages over ultrasound-guided techniques by allowing very precise targeting as well as treatment outcome assessment immediately post-treatment.

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Table of specific reagents and equipment:

Name of the reagent	Company	Catalogue number	Comments (optional)
Small Animal Focused Ultrasound System	FUS Instruments, Inc.	RK-100	
Definity	Lantheus Medical Imaging		
Omniscan	GE Healthcare		

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