Targeted delivery of antibodies through the blood-brain barrier by MRI-guided focused ultrasound

M. Kinoshita[1], N. McDannold[1], F. A. Jolesz[1], K. Hynynen[1]

[1]Department of Radiology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, United States

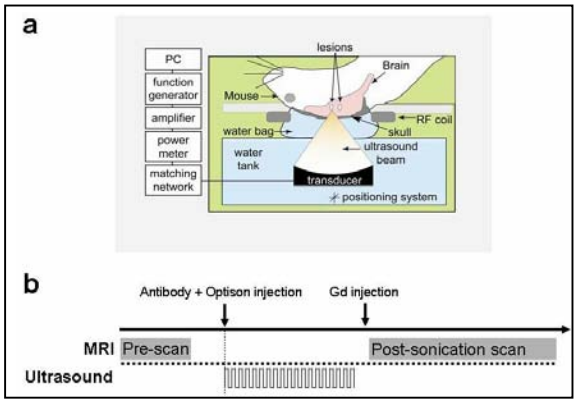
Abstract

The blood-brain-barrier (BBB) is a persistent obstacle for the local delivery of macromolecular therapeutic agents to the central nervous system (CNS). Many drugs that show potential for treating CNS diseases cannot cross the BBB and there is a need for a non-invasive targeted drug delivery method that allows local therapy of the CNS using larger molecules. To overcome this problem, we developed a non-invasive technique that allows the image-guided local disruption of the BBB. Our major aims in this study were to **1)** demonstrate that intravenously administered dopamine D_4 receptor-targeting antibody crossed the BBB and recognized its antigens by using our MRI-guided focused ultrasound BBB disruption technique and **2)** investigate whether or not we can monitor the extent of BBB disruption by the obtained MR images.

Methods

Sonication: The experimental protocol is summarized in **Figure 1b**. T1-weighted images were obtained to aid in the selection of target locations in the brain. After injecting the anti-dopamine D_4 receptor antibody, sonication was performed with simultaneous injection of bolus of microbubble based ultrasound contrast agent OPTSION®. The sonication was delivered to one location in the gray matter with the center of the focal spot by MR guidance using a 0.69 MHz focused ultrasound transducer. The peak acoustic pressure amplitude ranged from 0.6 to 1.1 MPa, depending on the sonication. After the sonication and MR study was completed, trypan blue was injected through the tail vein to mark and confirm the BBB disruption on the tissue blocks.

Magnetic Resonance Imaging (MRI): The MRI scanner was a standard 1.5 T Signa system (General Electric Medical Systems, Milwaukee, WI). A 7.5-cm diameter surface coil was placed under the head. The sonications were performed through the hole of the coil that was filled with the bag containing degassed water (**Fig. 1a**). A gradient echo sequence was used to aim the beam at the brain. Following the sonications, T1-weighted fast spin echo (FSE) images (see Table below) were obtained and repeated after injection of an intravenous (iv) bolus of gadopentetate dimeglumine MR contrast agent (Magnevist, Berlex Laboratories Inc., Wayne, NJ) to detect and evaluate the opening of the blood–brain barrier (BBB).



Sequence	Purpose	Repetition time (ms)	Echo time	Matrix	Flip angle	Bandwidth (kHz)	Number of acquisitions
FSE T1-W	Target Selection and Contrast Enhancement	500	15 ms	256 × 256	90°	16	4

Results and Discussion

When BBB opening was achieved, enhanced signals were detected on the MR images. Successful BBB disruption was confirmed by the presence of a blue spot due to leakage of the dye into the brain parenchyma. In groups where the applied pressure amplitude was lower than 0.8 MPa, we observed no, or minute hemorrhagic foci at the center of the ultrasound focus. On the other hand, in groups where it exceeded 0.8 MPa, we noted major tissue damage in some animals and at an acoustic pressure amplitude of 1.1 MPa, all mice manifested major tissue damage. As all of the animals were injected with rabbit anti-dopamine D_4 receptor antibody before sonication, the localization of the injected anti-dopamine D_4 receptor antibody through out the brain was detectable by immunohistological staining of the specimen with anti-rabbit IgG antibody. Positive signals of the antibody were detected at the locations where the trypan blue staining was observed which correlates with enhanced location on the MRI (**Fig. 2**). Under a microscope, the signals emanated from locations within the sonicated focus such as the hippocampus and small cells at the basal ganglia, sites characteristic for the localization of the dopamine D_4 receptor. On the contralateral side of the brain, no obvious staining was detected. These findings suggest that the anti-dopamine D_4 receptor antibody was delivered only at sites where BBB disruption had been produced by sonication. When we compared the staining intensity and the MR signal change before and after sonication, we noted a good correlation (**Fig. 3**). Although further optimization is necessary to render our method clinically applicable, this investigation represents a major step toward the local and site specific use of antibodies and other macromolecular therapeutic and/or diagnostic agents in the treatment of various CNS disorders by MRI guidance.

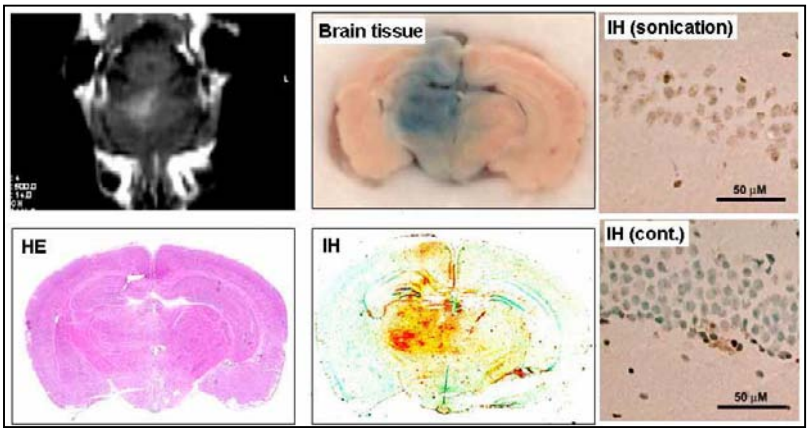


Figure 2 MR monitoring of the BBB disruption and photographs of harvested brain showing the BBB disruption induced by focused ultrasound. The brain of the animal was harvested 3 h after sonication (0.8 MPa with 50 μl OPTISON®). The location of the BBB opening was confirmed by trypan blue staining the affected area. Although no apparent damage was seen in successful cases, the signals from the injected antibody completely overlapped the area stained with trypan blue. A microscopic examination shows the positive signals at the hippocampal region, while no signal can be detected on the control side.

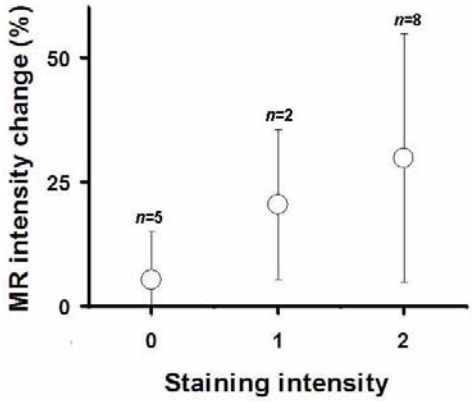


Figure 3 Correlation between antibody staining intensity and the intensity of changes seen by MRI after BBB opening induced by focused ultrasound. The immunohistochemical reaction from the injected antibody was recorded as 0, 1, and 2 according to its intensity and the intensity of the MR changes after BBB opening was plotted as a function of staining intensity. There is a clear correlation.