Opening the Blood Brain Barrier with Ultrasound for In Vivo Contrast-Enhanced Imaging of the Mouse Brain

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Introduction: In the study of mouse models of neurological diseases, magnetic resonance microscopy (MRM) holds the promise of providing high-resolution, high-throughput, and longitudinal images of the mouse brain. However, the slow T1 relaxation of brain tissue at high field strength has been a significant barrier to fulfilling this promise. This problem has been addressed for fixed *ex vivo* specimens by "active staining" of the brain with T1-shortening contrast agents [1,2]. However, contrast agents do not work well *in vivo*: In live animals, the blood brain barrier (BBB: the specialized endothelium lining the vasculature of the brain) prevents the entry of contrast agents into the brain. To open the BBB non-invasively, focused ultrasound has been co-administered with albumin microspheres [3]; however, this approach only opens a small portion of the BBB. **We present here a novel technique for global BBB Opening with Lipid microspheres and UltraSound (BOLUS); and we show that this technique enables contrast-enhanced MRM of the mouse brain *in vivo*.** Using the BOLUS technique, the mouse brain can be "stained" with T1-shortening contrast agents *in vivo*, thus enabling high-resolution (50x50x100 μm^3) T1-weighted images in less than 1 hour.

Methods: Each mouse was anesthetized with isoflurane and the scalp depilated. Over the head, a thin plastic bag was suspended which contained a 6 cm column of water. Between the bag and the scalp, a thin layer of ultrasound gel was applied. In the water, a single-element ultrasound transducer was positioned 6 cm above the skull. This unfocused transducer had a diameter of 13 mm and a frequency of 2.25 MHz. The BOLUS procedure consisted of the simultaneous administration of 30 µl perflutren lipid microspheres (Definity®) IV and 30 seconds of continuous ultrasound. The ultrasound system was calibrated in water to apply 0.8 MPa at 6 cm. To assay BBB opening, Gd-DTPA (6.3 mMol/kg IP) was given during the BOLUS procedure. After 40 minutes, the animal was scanned with a T1-weighted 3D spoiled gradient recalled echo (SPGR) sequence in a 35 mm volume coil with a 7 T GE EXCITE MRI system.

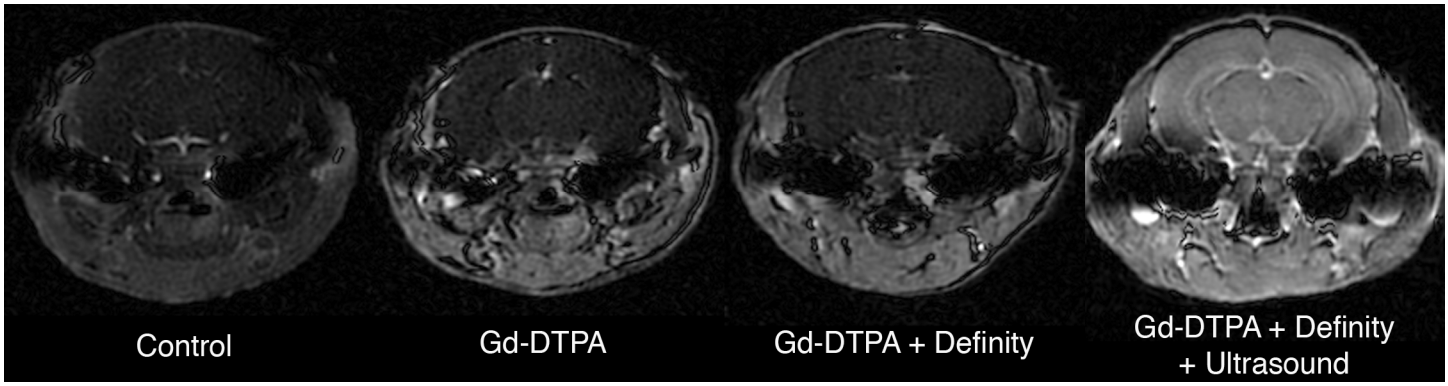

Figure 1: Rapid scans acquired 40 minutes after treatment. Brain enhancement is seen only when ultrasound and Definity are co-administered. (Sequence: 3D SPGR, TR 25 ms, TE 2 ms, FA 30°, matrix 128x128x60, FOV 20x20x12 mm^3, NEX 1)

Results and Discussion: To demonstrate that the BBB is opened by the co-administration of ultrasound and Definity, the BOLUS procedure above was compared (figure 1) with scans from three control groups: no treatment; Gd-DTPA only; and Gd-DTPA and Definity. In all animals receiving Gd-DTPA, enhancement was seen in the non-brain tissues. However, in animals also receiving simultaneous Definity and ultrasound, the brain demonstrated dramatic enhancement. This brain enhancement could be modulated by changing various parameters: ultrasound pressure; ultrasound frequency; and Definity injection timing. Clearly, BOLUS allowed Gd-DTPA to shorten T1 in the brain. This shortened T1 could be harnessed to dramatically improve *in vivo* MRM: T1-weighted images (figure 2) were acquired in 50 minutes *in vivo* at high resolution (50x50x100 μm^3). In conclusion, we have presented a technique, BOLUS, that enables global, non-invasive opening of the BBB; and we have shown how BOLUS can be used to enhance *in vivo* MRM. By increasing the efficacy of *in vivo* brain imaging, this technique may improve the study of mouse models of human neurological disease.

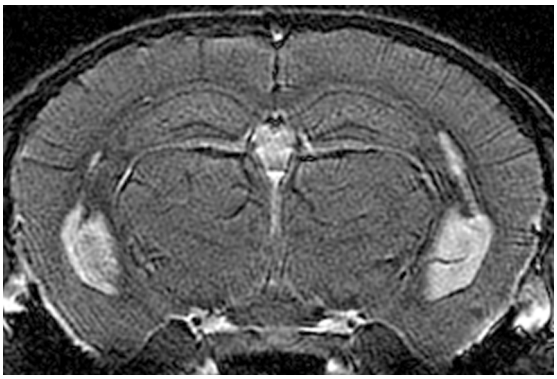

Figure 2: High resolution (50x50x100 μm^3) *in vivo* brain scan 1 hour after Gd-DTPA and BOLUS shows excellent contrast. (Sequence: 3D SPGR, TR 25 ms, TE 3 ms, FA 25°, matrix 384x384x80, FOV 20x20x8 mm^3, NEX 5)

References: [1] Johnson et al. Radiology. 2002;222(3):789-93. [2] Cyr et al. Neuroimage. 2005;26(1):83-90. [3] Choi et al. Ultrasound Med Biol. 2007;33(1):95-104.

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