Pressure and Microbubble-Size Dependence of the FUS-Induced Blood Brain Barrier Opening Reversibility In Vivo

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Introduction

Focused Ultrasound (FUS) in conjunction with systemic administration of microbubbles has been previously shown to open the Blood-Brain Barrier (BBB) locally, non-invasively and reversibly[1]-[2]. In this study, we investigate the dependence of BBB closing on the peak rarefractional pressure (PRP), as well on the microbubble size in mice *in vivo*. Volumetric quantification of the BBB opening region, on the day of sonication and up to five consecutive days after, was assessed through contrast-enhanced T1-weighted (CE-T1) high resolution MRI. Gadodiamide's (Gd-DTPA) diffusion into the brain parenchyma was used as a tracer to depict the location and extent of the BBB opened region. Also, residual gadodiamide that remained in the sonicated region after opening was detected in the pre-contrast T1-weighted images in a few cases more than one day after BBB opening, and was correlated with histological findings, to determine whether it can be used as an indication of damage.

Methods

A total of thirty two (n=32) wild-type mice were sonicated transcranially with the targeted region overlapping with the right hippocampus using FUS (1.5 MHz frequency; 100 cycle pulse length; 10 Hz pulse repetition frequency; 1 min sonication duration) immediately after IV monodispersed [3] microbubble administration ($8x10^5$ /g of body mass). PRPs varied between 0.30MPa and 0.60MPa, and three different diameter microbubbles were used at each pressure: 1-2μm, 4-5μm and 6-8μm. After completion of BBB opening [2], pre- and post- contrast enhanced MR T1-weighted FLASH images (TR/TE: 230/3.3ms, flip angle 70°, NEX=18, resolution 86μmx86μm, slice thickness: 500μm) were respectively acquired before and after intraperitoneal injection of gadodiamide (Gd-DTPA, 6.25 mmol/kg). 3D MR imaging was repeated up to five days. All brain images were segmented and reconstructed in 3D for qualitative spatial assessment of the BBB opening *in vivo*. The volume of BBB opening was quantified by counting voxels after thresholding the T1 signal intensity (SI) that was at least 2.5 standard deviations above the average SI in a contralateral small region. Contrast-enhanced voxels due to vasculature were excluded. Histological examination with hematoxylin and eosin (H&E) was performed seven days after sonication, to assess tissue integrity.

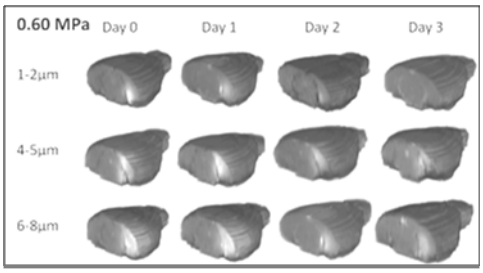

Fig. 1. 3D CE-T1 contoured brain reconstructions, sonicated with a PRP of 0.60MPa, sliced coronally at the depth of the hippocampi

Results

For all cases of microbubble sizes and pressures studied, the BBB was reinstated by day 5, and the time required for closing varied depending on microbubble size and PRP used. Fig.1 shows examples of 3D CE-T1 images and the extent of BBB opening at 0.60MPa up to three days after opening. Closing is detected on day 3 only in the 1-2μm case at that pressure. Fig.2 summarizes the results at all pressures and microbubbles studied. At 0.30MPa, opening was induced only in the 4-5μm and 6-8μm cases, and closing occured on day 2 or 3 after sonication. At 0.45MPa and 1-2μm BBB closed as soon as one day after FUS (Fig.2 (a)), but took longer (3-5 days) in the cases of the 4-5μm and 6-8μm bubbles, without any significant difference between the two. The PRP of 0.60MPa induced BBB opening that required 3 days to close for the 1-2μm, and five days for the 4-5μm and 6-8μm bubbles (Fig.1 and 2). Damage was observed upon histological examination only in four animals, all of which were at higher PRPs. Also, the BBB opening volume increased significantly ($p<0.05$) with pressure for the smaller microbubbles, i.e. 1-2μm and 4-5μm in diameter, but not for the 6-8μm. In all histological damage cases residual gadodiamide was detected on the pre-contrast T1-weighted images; such an example is shown in Fig.3.

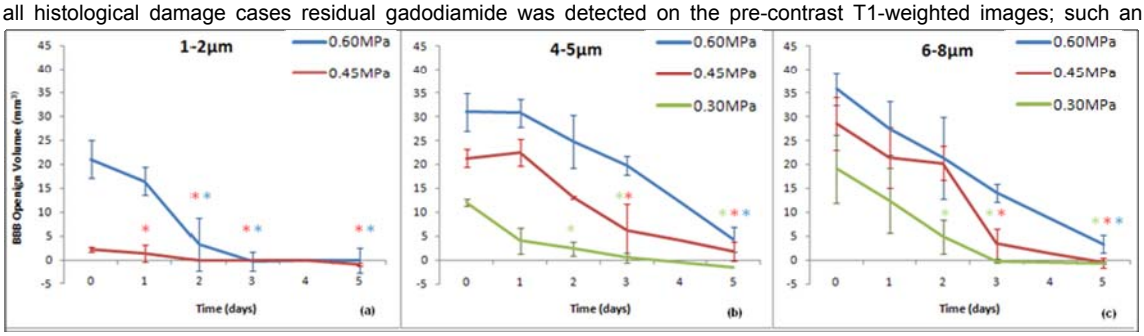

Fig.2. Volume of BBB opening for PRP of 0.30MPa, 0.45MPa and 0.60MPa with microbubbles of (a) 1-2μm, (b) 4-5μm and (c) 6-8μm in diameter. Asterisks denote BBB closing, i.e. not statistically significant difference ($p>0.05$) compared to the control group.

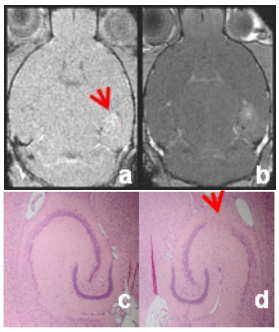

Fig. 3. Horizontal (a) pre- and (b) post- contrast T1 images acquired on day 5, PRP: 0.45MPa, 6-8μm case. H&E stained sections revealed cell loss in the right hippocampus (d), compared to the control contralateral side(c)

Discussion and Conclusion

In this study, the dependence of the duration and volume of BBB opening as induced by FUS on the microbubble diameter and the acoustic pressure was investigated using CE-T1 MR imaging. Our findings indicated that the BBB opening volume increases and lasts longer with the acoustic pressure and microbubble size. In all cases where damage was noted, T1 pre-contrast images showed presence of residual gadolinium. Monitoring of BBB self-repair may provide previously unknown insight on its physiology as well as allow more efficient trans-BBB brain drug delivery.

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References

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