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TITLE: REAL-TIME CAVITATION-BASED MONITORING TO CONTROL THE DEGREE OF INFLAMMATION AFTER ACOUSTIC CAVITATION-MEDIATED BLOOD-BRAIN BARRIER OPENING

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CURRENT TOPIC: Brain: Blood Brain Barrier

PRESENTATION TYPE: Oral

OBJECTIVES: Acoustic cavitation-mediated blood-brain barrier (BBB) opening has been shown to induce a degree of inflammation in the sonicated region of the brain. Since BBB opening is linked to acoustic cavitation and its interaction with the surrounding blood vessels in the brain, it can be hypothesized that the degree of acoustic cavitation (i.e. cumulative dose) can be used to predict the extent of inflammation. Therefore, the objective of this study was to explore the relationship between acoustic cavitation and the resulting inflammation marker expression after BBB opening in mice.

METHODS: A single-element FUS transducer (center frequency: 1.5MHz, focal depth: 60mm, diameter: 60mm) was used for BBB opening in male C57BL/6 mice. Confocally aligned with the FUS transducer was a single-element, passive cavitation detector (center frequency: 10MHz, focal length 60mm), which was used to passively acquire acoustic cavitation emissions during BBB opening. In-house generated polydispersed lipid-shell microbubbles were used for these experiments and injected as a bolus at ten times the clinical dose of Definity (0.01uL/g). Two sonication schemes were used for these experiments: (1) fixed pressure sonication (0.75 MPa) peak negative pressure), (2) varied pressure (0.005 to 0.75 MPa peak negative pressure) sonication using an acoustic cavitation-based controller. Both sonication schemes used a pulse length of 10 ms and a pulse repetition frequency of 2 Hz. The left caudate putamen was sonicated, while the contralateral side was used as a control. To be able to cover the complete caudate putamen structure, two locations were sonicated for 1 minute each. For the second sonication scheme, an acoustic cavitation-based controller was used slowly ramp the PNP until the radiated acoustic cavitation emissions reached a certain value relative to the control and then held at that pressure until the pre-defined cumulative cavitation dose was reached. For this study, the cumulative cavitation dose was defined as the sum of all acoustic cavitation emissions from 2.5MHz to 13.5MHz.

Post-sonication, contrast-enhanced, T1-weighted magnetic resonance imaging (MRI) was taken to assess BBB opening. The mice were then survived for a 6hr period and then sacrificed through transcardial perfusion. The brains were immediately extracted and left in RNA Stabilizing Reagent (Qiagen, Hilden, Germany) for quantitative real-time polymerase chain reaction (qRT-PCR). RT2 Profiler PCR Array Mouse NFkB Signaling Pathway (Qiagen, Hilden, Germany) was chosen to be capable to assess the array of genes associated to inflammatory response and apoptosis. Quantification of qRT-PCR was done within each animal, where a log2 fold change was calculated relative to the contralateral side.

RESULTS: Following the first sonication scheme with fixed pressure, different cumulative acoustic cavitation doses resulted in a different inflammation response. Table 1 summarizes the results of specific genes that had a greater than 2-fold increase compared to the contralateral side. Specifically, the bolded rows highlight large differences in gene upregulation between the higher relative acoustic cavitation dose (2.47×10^5 V*s) and the lower relative acoustic cavitation dose (1.87×10^5 V*s), leading to a greater inflammatory response in the mouse with greater acoustic cavitation dose. Using the second sonication scheme, the acoustic cavitation-based monitoring system was tested. The left hemisphere was sonicated at a lower cavitation threshold compared to the right hemisphere, however all 4 locations had the same total cavitation dose target. Figure 1 shows the contrast enhanced T1w MR image of the 4 sonicated locations. Using the feedback system at different pressures, it can be observed that even with different pressure thresholds, sonicating for a fixed total cavitation dose (rather than fixed time), BBB openings were very similar in terms of size and enhancement for each respective structure (hippocampus vs caudate putamen). Experiments using the feedback system for different cut off cavitation doses and relating it to the inflammatory response are currently ongoing.

CONCLUSIONS: Cavitation dose has been shown to be possibly linked to the inflammatory response of FUS induced BBB opening. Using a cavitation-based controller, cavitation dose has been shown to be a reliable metric to predict

the size of the opening. Currently, the inflammatory response of FUS induced BBB opening is being assessed using the cavitation-based controller set to different cavitation cuff offs. Real-time cavitation dose feedback is shown capable of preventing inflammatory response will be a critical step translating this technology to the clinic in a safe and efficient manner.

Gene	Function	Lower Relative Cavitation Dose	Higher Relative Cavitation Dose
Bcl2a1a	Apoptosis	1.67 fold UPREG	3.30 fold UPREG
Bcl3	NFkB	No change	3.66 fold UPREG
Birc3	Apoptosis	1.13 fold UPREG	2.38 fold UPREG
Ccl2	**Chemokine**	**9.25 fold UPREG**	**15.37 fold UPREG**
Csf3	**Immunity**	**No change**	**5.21 fold UPREG**
Fos	Inflammation	1.55 fold UPREG	3.21 fold UPREG
Hmox1	Tissue Damage	1.38 fold UPREG	2.32 fold UPREG
Icam1	Immunity	1.55 fold UPREG	2.04 fold UPREG
Il1a	**Immunity**	**1.04 fold DOWNREG**	**7.90 fold UPREG**
Il1b	**Immunity**	**3.60 fold UPREG**	**14.34 fold UPREG**
Lta	Immunity	2.41 fold UPREG	1.37 fold UPREG
Nfkb2	NFkB	1.37 fold UPREG	2.07 fold UPREG
Tlr1	Immunity	1.09 fold UPREG	3.01 fold UPREG
Tlr2	Immunity	1.39 fold UPREG	4.12 fold UPREG
Tnf	**Immunity**	**2.20 fold UPREG**	**32.71 fold UPREG**

Table 1: Genes of interest that showed the greatest amount of change in the qRT-PCR analysis. Relative cavitation dose was used to compare between the two mouse samples, as both were sonicated using sonication scheme 1.

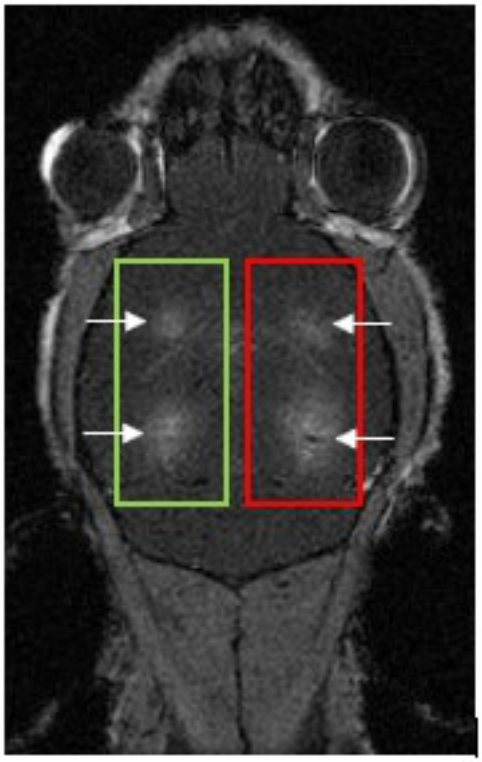

Figure 1: Contrast enhanced T1w MR image showing the BBB opening of the 4 sonicated locations using the cavitation-based controller (indicated by arrows). Each boxed group represents sonications with the same cavitation threshold, while all four sonications had the same overall cumulative cavitation dose. The green box had a cavitation dose that was 12dB above the noise floor, while the red box had a cavitation dose 18dB above the noise floor.

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AWARDS: Student Award Competition|Student Travel Awards