Phagocytic Response to Focused Ultrasound-Mediated Blood-Brain Barrier Opening

Background: More than 5 million people in the United States and nearly 50 million worldwide are suffering from Alzheimer's Disease (AD) or related dementia, making it the most common neurodegenerative disorder. The signature biological markers of AD are an accumulation of amyloid-beta plaques and hyperphosphorylated protein-tau (p-tau) tangles. A reduction in pathology and improvement in associated behavioral deficits is shown in transgenic AD mice when they are treated with focused-ultrasound (FUS) blood brain barrier (BBB) opening.

Pathology reduction has been suggested to be due to the stimulation of microglia, the central nervous system's main phagocytic cell. However, the increased blood-brain barrier permeability due to FUS may also allow the infiltration of macrophages, which hold a similar phagocytic role in the periphery. Macrophage infiltration is the marker used in this study to quantify the immunogenicity of FUS-mediated BBB opening.

Materials and Methods: C57 wild-type mice (n=5/group) were split into two groups – 1) FUS/MB, and 2) MB only, which acted as the negative control. The FUS/MB group was treated with FUS at a frequency of 1.5 MHz, a peak negative pressure of 450 kPa and a PRF of 2 Hz for 120 sec. Prior to sonication, the FUS/MB group was IV injected with in-house, customized polydispersed microbubbles. The MB group was injected with the microbubbles, but were not treated with FUS and so no BBB opening was expected. All mice underwent a contrast-enhanced MRI (Bruker, 9.4T) to evaluate BBB opening within 1 hour post-opening. 24 hours post-treatment, mice were perfused and the targeted area was extracted, homogenized and flow cytometry (BD FACSCANTO II) was used to quantify the macrophage population.

MRIs were post-processed on Matlab using manual area selection followed by 3-Dimensional K-Means filtering, resulting in a 3-dimensional rendering of the Gadolinium distribution in the brain at the time of imaging. The size of opening was quantified from this rendering.

All flow cytometry analysis was performed on FCS Express 7.0. All FCS files were filtered to exclude debris, doublets and dead cells before the macrophages were quantified due to their CD11B and CD45 positivity.

Results:

As expected, all mice in the FUS/MB group had a BBB opening that could be detected with the MRI processing. The openings from this group were quantified to range between 20 and 80 mm^3. The MB only group was used as a negative control and had no detectable opening. The mice in the FUS/MB group had a macrophage population between 0.5 and 1.5% of the alive cells as compared to less than 0.7% of the cells as was seen in the MB group. When the size of the macrophage infiltration was plotted as a function of opening volume for the FUS/MB group, a linear correlation emerges. This correlation is found to be statistically significant with a P-value of 0.03 and an R^2 value of 0.53.

Conclusions: The infiltration of macrophages is evident from these preliminary results. Future work will test time points longer post-sonication to see when (or if) macrophages migrate back out of the brain as well as quantify the reactivity of the infiltrating macrophages in order to fully characterize the effect of these cells on immunogenicity post-BBB opening.