Theranostic ultrasound-mediated gene delivery to the brain of non-human primates through intravenous and intrathecal administration of adeno-associated viruses

Background, Motivation and Objective

Recent studies in mice and non-human primates (NHP) have demonstrated the promise for focused ultrasound (FUS)-enhanced gene delivery through intravenous (IV) administration of adeno-associated viruses (AAV). However, IV injections of AAV (particularly AAV9) may lead to widespread viral delivery throughout the body, potentially inducing off-target transduction, raising safety concerns. Additionally, IV administration of AAV requires larger titers, thereby increasing cost substantially relative to other CNS gene delivery routes of administration such as intra-CSF delivery. Therefore, in this study, we evaluated the effectiveness of FUS-mediated BBB opening for gene delivery to brain regions implicated in frontotemporal dementia (FTD) in NHP through either intrathecal (IT) or IV administration of AAV5, using a theranostic ultrasound (ThUS) linear phased array.

Statement of Contribution/Methods

Four male cynomolgus macaques aged 2-3 years were administered with AAV5 encoding GFP provided by VectorY Therapeutics; two were injected with $1.5x10^{15}$ gene copies (gc) IV, and two were injected with $1.3x10^{14}$ gc IT via lumbar puncture under fluoroscopic X-ray guidance. Immediately after IV AAV injection and ~1 hour post-IT injection, lipid-shelled microbubbles were injected IV while the ThUS array (32 elements, 500 kHz f_c) driven by a Verasonics Vantage system was used to simultaneously transmit focused pulses transcranially and reconstruct cavitation maps (Fig. **A**). A neuronavigation system (Brainsight, Rogue Research) was used to target 3 sonications in the left anterior cingulate cortex (ACC) and one sonication in the insular cortex (IC) of each NHP. Following a two-week survival period, the NHP were euthanized and GFP expression in the brain was evaluated using immunofluorescence staining.

Results/Discussion

Real-time cavitation imaging revealed an average 40.0±7.1 dB rise in cavitation within the brain upon microbubble injection in all NHP (Fig. **B**). Contrast-enhanced T1-weighted MRI confirmed successful BBB opening in each targeted region for all four NHP (Fig. **C-D**). Immunofluorescence staining showed successful AAV transduction for both IV and IT routes of administration (Fig. **E-F**). Our results demonstrate for the first time the potential of ThUS-enhanced AAV5 delivery to FTD-relevant targets in NHP through two distinct routes of AAV administration.