Non-invasive gene therapy of brain lymphatic system using ultra-short focused ultrasound pulses from an imaging phased array

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Background, Motivation and Objective

Neurodegenerative diseases such as Alzheimer's are characterized by abnormal accumulations of proteins, which is often due to a dysfunctional brain lymphatic system. Most current gene therapy approaches rely on invasive intra-ventricular injections of adeno-associated viruses (AAV), hindering clinical translation. Focused ultrasound (FUS)-mediated blood-brain barrier opening (BBBO) has effectively been used for non-invasive brain AAV delivery. However, no studies to date focus on ventricular delivery for gene therapy of the brain lymphatic system. In this study, we are employing a FUS-BBBO system utilizing a phased array for short pulse AAV delivery to the choroid plexus and periventricular space.

Statement of Contribution/Methods

A phased array (P4-1, ATL Philips) was driven at 1.5 MHz by a Verasonics Vantage system to target the brain of C57BL-6 male mice transcranially. The AAV9-CAG-GFP and AAV2-CAG-GFP constructs were prepared to a titer of $1.1x10^{11}$ gene copies and co-injected with microbubbles through the tail vein while FUS was applied for 2 minutes. For AAV9-treated mice, the P4-1 was axially focused at 35 mm and translated to target 2.8 mm anterior of the lambdoid suture, and over the center of the brain or at 2.8 mm to the right using 10-cycle pulses. For AAV2-treated mice, the beam was electronically steered to target six distinct foci at depths of 35 mm, 36.5 mm, and 37.5 mm, and at the same lateral and elevational targets as AAV9 **(Fig. a)**, using either 10- or 1.5-cycle pulses. The mice were sacrificed after 2 or 3 weeks for AAV2 and AAV9, respectively, to assess GFP expression in the target areas via immunofluorescent staining.

Results/Discussion

GFP expression was observed in cells surrounding ventricles, with astrocytes and neurons manifesting highest gene expression as fluorescence. Notably, a small number of ependymal cells of the choroid plexus (ChP) expressed GFP in mice delivered with AAV9 **(Fig. b)**, despite the AAV promoter being suboptimal for transducing these types of cells. The AAV2 group showed GFP expression as early as 2 weeks post-delivery, with transduced cells located near the ventricles even for mice treated with 1.5-cycle pulses **(Fig. c)**. These results show for the first time a non-invasive method for brain lymphatic system targeting and provide a foundation for targeted gene therapies of brain lymphatics facilitated by therapeutic ultrasound.