Non-Invasive Adeno-Associated Viral Gene Delivery to the Brain in Non-Human Primates with Cavitation-Guided Focused Ultrasound

Alec J. Batts[1*], Robin Ji[1*], Sua Bae[1], Sergio Jiménez-Gambín[1], Fotis Tsitsos[1], Nancy Kwon[1], Vincent P. Ferrera[2], Jared B. Smith[3], & Elisa E. Konofagou[1,4]

* Authors contributed equally
1. Dept. Biomedical Engineering, Columbia University, New York, NY, USA
2. Dept. Neuroscience, Columbia University, New York, NY, USA
3. REGENXBIO Inc., Rockville, MD, USA
4. Dept. Radiology, Columbia University, New York, NY, USA

Background, Motivation and Objective

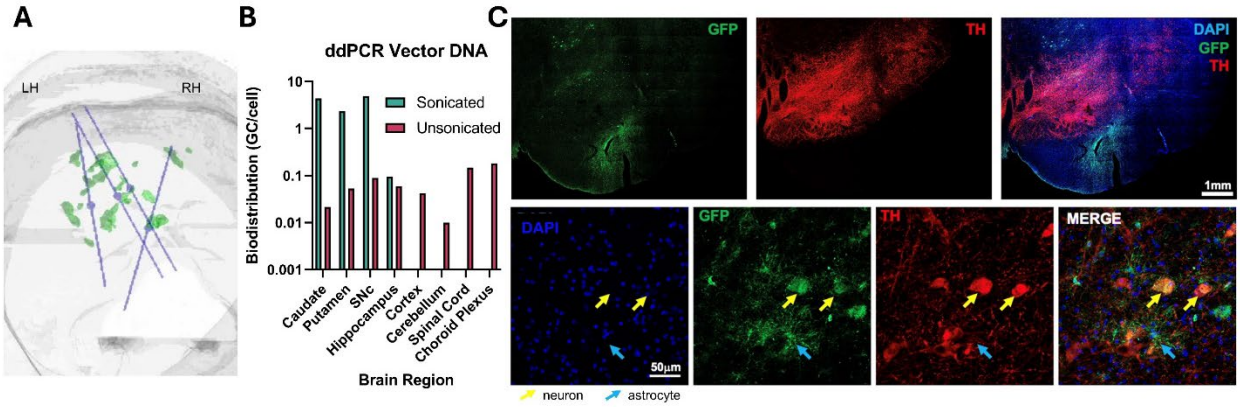
Blood-brain barrier (BBB) opening with focused ultrasound (FUS) in conjunction with systemically administered microbubbles (MBs) and adeno-associated viruses (AAV) has been extensively investigated as a non-invasive strategy for targeted gene delivery in mice, however, only a single study investigating magnetic resonance-guided focused ultrasound (MRgFUS)-facilitated AAV delivery in primates has been published to date. Here, we use a portable, single-element, ultrasound and neuronavigation-guided FUS system to deliver systemically-injected AAV to the brain in rhesus macaques to elucidate the feasibility of non-invasive gene delivery with FUS in primates as evidence for future clinical translation.

Statement of Contribution/Methods

Two ~30 y.o. 13 kg male rhesus macaques, "NHP A" and "NHP B" were used for this terminal study which was approved by our institution's animal care and use committee. BBB opening was induced with a single-element 0.25 MHz USgFUS system (Sonic Concepts) with passive acoustic mapping (PAM) guidance achieved with a P4-2 imaging phased array (Philips), and targeting facilitated with a neuronavigation system (Brainsight). "NHP A" received FUS-mediated BBB opening (2 minutes, 0.4 MPa *in situ* PNP, 10 ms pulse length) along 4 target trajectories: left putamen, left caudate, left hippocampus, and right substantia nigra. A bolus injection of house-made polydisperse microbubbles was injected immediately after starting the FUS BBB opening sequence for the 1st, 3rd, and 4th targets. Due to the rapid transducer repositioning between the 1st and 2nd target, no additional microbubbles were injected prior to the 2nd sonication. A bolus injection of AAV (AAV9-CAG-GFP, 2.0e13 gc/kg) was injected through the right saphenous vein immediately after an observable rise in PAM cavitation dose during sonication of the left putamen. "NHP B" received FUS-mediated BBB opening with the same parameters and AAV construct as "NHP A" for one trajectory targeting the right hippocampus. BBB opening was confirmed with contrast-enhanced T_1-weighted MRI acquired immediately after the last sonication. NHPs were sacrificed 3-4 weeks post-AAV delivery by transcardial perfusion, where tissue punches throughout the sonicated and respective contralateral brain regions, and peripheral tissues were processed for digital-droplet polymerase chain reaction (ddPCR) for quantification of viral vector DNA. Additional brain slabs were prepared for histological readouts of cell-type specific transduction patterns.

Results/Discussion

In "NHP A," BBB opening was confirmed along the 4 target trajectories, and contrast-enhancement on T_1-weighted MRI was observed in the targeted putamen, caudate, and substantia nigra (Fig. A). No BBB opening was confirmed within the targeted hippocampus. ddPCR confirmed significantly increased AAV9 vector DNA in all three regions which exhibited contrast enhancement on MRI, where 200-fold, 44-fold, and 55-fold increases in genome copies (gc) per cell were respectively observed in the sonicated caudate, putamen, and substantia nigra pars compacta, compared to the unsonicated contralateral regions (Fig. B). This yielded an average of ~4 gc/cell in the sonicated brain regions, compared to an average of ~0.07 gc/cell in unsonicated brain regions, corresponding to the approximate level of AAV9 BBB crossing without FUS. Fluorescence confocal microscopy confirmed the majority of transduction in astrocytes (blue arrow), followed by neurons (yellow arrow) and oligodendrocytes in all evaluated brain regions (Fig. C). Most notably, tyrosine hydroxylase (TH) positive dopaminergic neurons were transduced in the substantia nigra, implicating applications for non-invasive gene delivery in NHP models of Parkinson's Disease (Fig. C). Finally, given the intravenous route of injection, quantification of peripheral AAV transduction in the liver and heart revealed vector DNA levels of 181 gc/cell and 3 gc/cell, respectively. Future studies are aimed to enhance brain tropism of AAV while reducing peripheral transgene expression to achieve a translatable FUS-facilitated gene therapy option in the clinic.


A
LH
RH
B
ddPCR Vector DNA
Biodistribution (GC/cell)
10
1
0.1
0.01
0.001
Sonicated
Unsonicated
Caudate
Putamen
SNc
Hippocampus
Cortex
Cerebellum
Spinal Cord
Choroid Plexus
Brain Region
C
GFP
TH
DAPI
GFP
TH
1mm
DAPI
GFP
TH
MERGE
50µm
neuron
astrocyte