Evaluating Novel Adeno-associated viruses (AAVs) as a Potential Gene Delivery Platform for Pediatric Rare Disorders

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Introduction

Neurotropic viruses such as adeno-associated viruses (AAVs) are promising platforms for gene delivery to the nervous system. However, their safety and efficacy can be compromised by the low efficiency of gene transfer and adverse immunological responses by the host. Extracellular vesicles (EVs), small lipid vesicles secreted by nearly all cells, have the potential to improve AAV-mediated gene delivery because of their intrinsic facility for cell-cell communication. We have created new AAV vectors, called exo-AAV vectors that combine the strengths of AAVs with those of EVs. Here we evaluate the advantages of exo-AAV vectors over traditional AAVs for gene transfer to the nervous system in preclinical models.

Methods

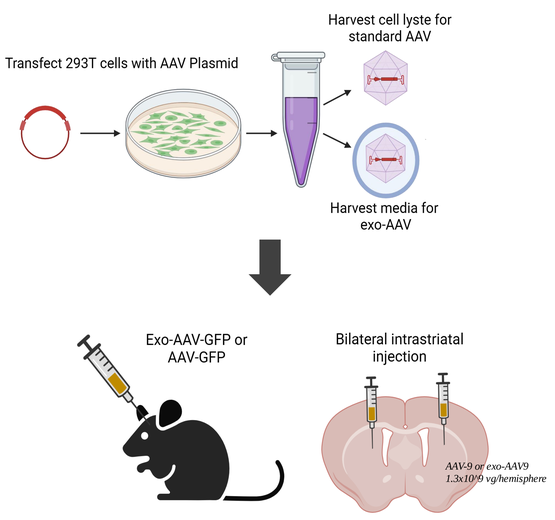

Figure 1: Adeno-associated virus (AAV) production and introduction into the mouse brain. 293T cells (human embryonic kidney cells) were transfected with an AAV plasmid carrying the GFP reporter gene. Cells were harvested for standard AAV isolation and the medium for exo-AAV isolation. Each type of AAV vector was injected bilaterally into the striatum of adult male C57BL/6 mice. Four weeks later, the mice were euthanized and brains harvested for analysis of GFP transduction.

Results

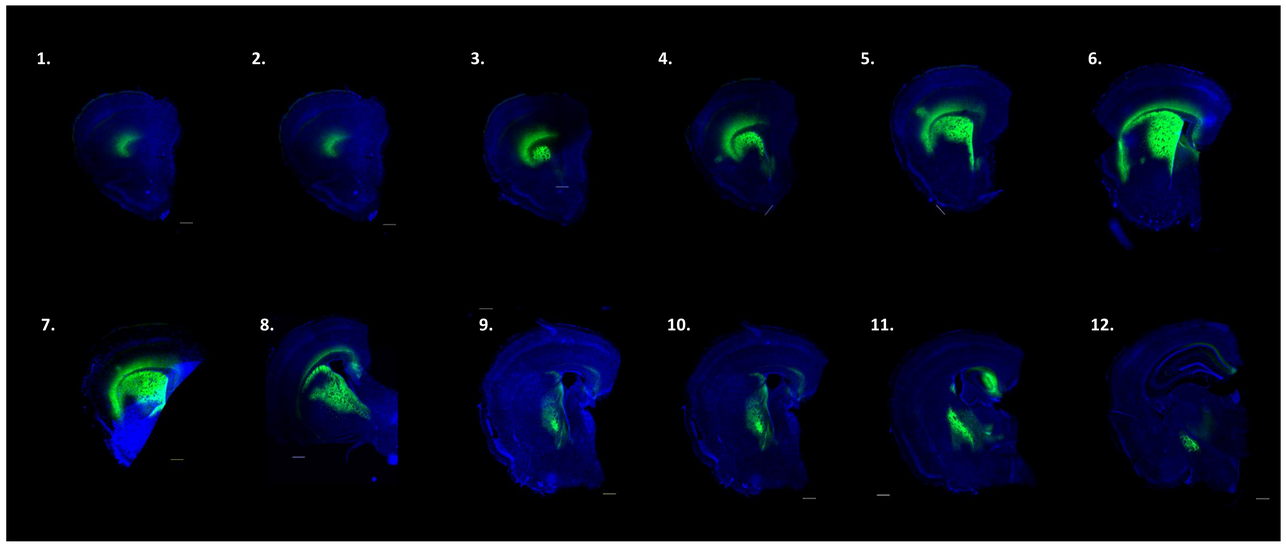

Figure 2: Robust GFP reporter expression in the mouse brain after intrastriatal injection of standard AAV-GFP and exo-AAV-GFP. Images of coronal sections (50 µm-thick) of the brains along the rostral to caudal axis displaying GFP fluorescence in the striatum and adjacent structures. The 12 images represent images of every 8th section from a consecutive series along the rostral-caudal extent. Each section was stained with DAPI (dark blue) to label cell nuclei.

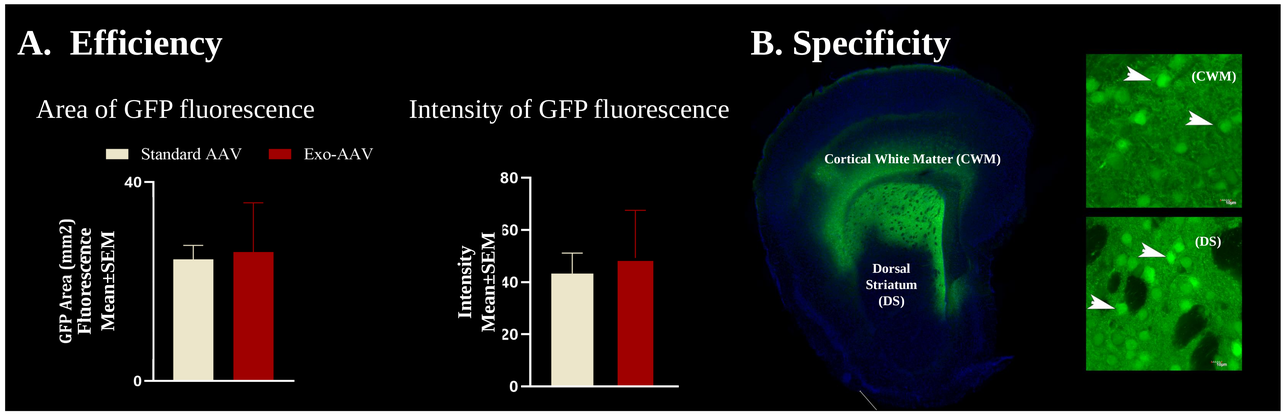

Figure 3: Evaluation of transduction efficiency and specificity of AAV and exo-AAV. Both vectors produced robust GFP labeling. The spatial extent (area) and the intensity of the GFP label were measured in all 12 sections using Image J software. No significant differences were found for either measurement **(A)**. Representative high-power (40x) images of GFP+ cells in the cortical white matter and dorsal striatum **(B)**. The size and morphology of the GFP+ cells are indicative of neurons, which are the intended cell type. To further verify the phenotype of the labeled cells we are performing immunohistochemistry with antibodies specific to neurons and glia. The sample size (N= 3) mice per vector, and an unpaired Student's t-test was used for analysis of statistical significance. The P value was >0.05.

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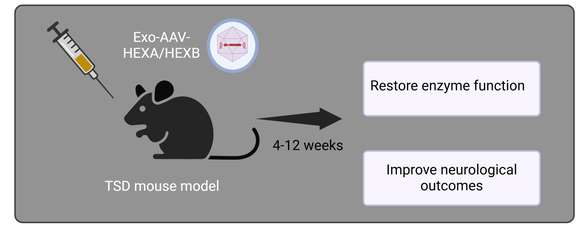
References

Summary

- The transduction efficiency of standard and exo-AAV in the mouse brain was found to be comparable 4 weeks after intracranial injection in this pilot study.
- GFP expression was selective to neurons, our primary target.

Future Applications

- Our research group is developing a gene therapy platform to treat Tay-Sachs Disease, a rare genetic disorder caused by mutations in the *HEXA* gene on chromosome 15. These mutations result in a deficiency of the *HexA* enzyme, which leads to the accumulation of GM2 gangliosides. This causes neuronal damage and loss of function.
- Our long-term goal is to use the exo-AAV platform to deliver wild type *HEXA* gene to neurons in *Hexa* knockout mouse brain to restore normal *HexA* function.



- Our near-term goals are to characterize further the potential advantages of the exo-AAVs over standard AAVs by examining whether

a) The neuronal transduction by exo-AAV persists longer than that produced by the standard AAV

b) The immune response in the host is milder following administration of exo-AAV compared to standard AAV.

Acknowledgments

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