A single injection of an optimized AAV vector into cerebrospinal fluid corrects neurological disease in a murine model of GM1 gangliosidosis

Christian Hinderer, Brenden Nosratbakhsh, Nathan Katz and James M. Wilson

Gene Therapy Program, Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Introduction

GM1 gangliosidosis is a rare genetic disorder caused by mutations in the GLB1 gene, which encodes lysosomal beta galactosidase (β-gal). In the absence of β-gal, cells are unable to degrade glycosaminoglycans with terminal galactose residues. Keratan sulfate, a glycosaminoglycan-storage glycosaminoglycan, accumulates in a variety of tissues causing skeletal dysplasia, hepatosplenomegaly, and cardiomyopathy. In the central nervous system (CNS), the inability to catalyze GM1 ganglioside results in marked neuronal GM1 storage and subsequent neurodegeneration. The most common form of GM1 gangliosidosis is the infantile subtype, which occurs in patients with little or no residual β-gal expression. Infantile GM1 gangliosidosis is characterized by a uniformly rapid neurodegenerative course beginning in the first 6 months of life, with survival of less than 5 years.

Previous studies in murine and feline models of GM1 gangliosidosis demonstrated that adeno-associated viral (AAV) vectors can be used to deliver the normal GM1 β-gal coding sequence to neurons, resulting in long-term expression of the β-gal enzyme. β-gal is secreted by transfected cells and can be taken up by surrounding cells via binding to the mannose-β-glucopyranose receptor, resulting in widespread distribution of the enzyme. This phenomenon allows for correction of storage lesions throughout the CNS following a small number of vector injections into the brain parenchyma. Studies in canine and feline models of other lysosomal storage diseases have shown that gene transfer to the brain and spinal cord can alternatively be achieved using AAV vector delivery into the cerebrospinal fluid (CSF). This approach allows for more widespread gene transfer with a single minimally invasive injection and appears to carry less risk of local toxicity at the injection site. AAV delivery into the CSF achieves efficient gene transfer to the brain and spinal cord even in the presence of systemic neutralizing antibodies to the vector capsid, making this approach applicable to patients regardless of the presence of pre-existing AAV antibodies.

Aim

The aim of this study was to develop an optimized AAV vector expressing human β-gal and evaluate the impact of vector administration into the CSF on brain enzyme activity, lysosomal storage lesions, and neurological signs using a murine disease model.

Vector selection

β-gal expression in AAV-treated GLB1−/− mice

Figure 2: β-gal activity in AAV-treated GLB1−/− mice

GLB1−/− mice were treated with a single intracerebroventricular (ICV) injection of an AAV vector expressing a codon-optimized human GLB1 cDNA from a C2B/I7t, or IUC-pretreated (n = 15 per group). Untreated wild-type mice (n = 5) served as controls. Brain (frontal cortex) and CSF were collected 14 days after vector administration, and β-gal activity was measured using a fluorogenic substrate. "p < 0.05, "p < 0.01, "p < 0.001. Kruskal-Wallis test followed by Dunn's test. Error bars = SEM.

• A vector expressing GLB1 from a human ubiquitin C promoter yielded the highest β-gal expression in brain and CSF, and was selected for further studies in a mouse model of GM1 gangliosidosis.

Reduction of brain storage lesions

Figure 3: Brain histology and biochemistry of AAV-treated GLB1−/− mice

50 days after ICV administration of 10^12 GC of AAV9-GLB1 or vehicle, GLB1−/− mice were sacrificed and brain sections were stained with 6-bromo-4-8-dihydroxyfluorescein (BrdH) (A) which binds to GM1 ganglioside and unstained cholesterole, antibodies against LAMP1 (B), a lysosomal marker or GFAP (C) an astrocyte marker. Neuroinflammation activity was measured in samples of frontal cortex (D).

• ICV administration of an AAV vector expressing human GLB1 reduced storage lesions and gliosis in brains of GLB1−/− mice and normalized aberrant overexpression of lysosomal enzymes.

Correction of gait abnormalities

Figure 4: Correction of gait abnormalities in AAV-treated GLB1−/− mice.

Untreated GLB1−/− mice (n = 12) and GLB1−/− controls (n = 22) were evaluated using a CaliWalk system on two consecutive days. Average walking speed and length of the hind paw prints were quantified for each animal across at least 3 trials (A). "p < 0.01 Mann Whitney test. Four-month-old GLB1−/− (n = 15) or GLB1−/− (n = 15) treated with vehicle and AAV-treated GLB1−/− mice (n = 14) were evaluated using the CaliWalk system on two consecutive days. Average walking speed and length of the hind paw prints was quantified for each animal across at least 3 trials on the second day of testing (B). "p < 0.05, "p < 0.01 Kruskal-Wallis test followed by Dunn's test. NS: not significant. Representative hind paw prints for AAV-treated GLB1−/− mice and vehicle-treated GLB1−/− and GLB1−/− controls are shown in C.

• AAV-treated mice exhibited normalization of the gait abnormalities present in untreated GLB1−/− mice.

Conclusion

A single administration of an optimized AAV vector into CSF resulted in systemic β-gal expression and correction of biochemical, histological and functional disease manifestations in a mouse model of GM1 gangliosidosis. These findings support further development of this approach for the treatment of GM1 gangliosidosis.

Acknowledgements

This work was supported by Pasage Bio. We would like to acknowledge the technical assistance of C. Angelica Medina-Jose, Tamara Godde, Julie Hershon, and Victoria Kehoe and support from the Program for Comparative Medicine, Vector Core, and Morphology Core of the Gene Therapy Program, University of Pennsylvania.

Conflict of Interest Statement

JMW is an advisor to, holds equity in, and has a sponsored research agreement with Scout Bio and Passage Bio; he also has a sponsored research agreement with UppRheo, Biogen, Jansen, Precise Biosciences, Moderna Therapeutics, and Amicus Therapeutics who are licensees of Penn technology. JMW is an inventor on patents that have been licensed to various biopharmaceutical companies.