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Preferred transduction with AAV8 and AAV9 via thalamic administration in the MPS IIIB model: A comparison of four rAAV serotypes

J.A. Gilkes , M.D. Bloom, C.D. Heldermon *

Department of Medicine, University of Florida, Gainesville, FL, USA

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ABSTRACT

Sanfilippo syndrome type B (MPS IIIB) is a lysosomal storage disease caused by a deficiency of N-acetyl-glucosaminidase (NAGLU) activity. Since early therapeutic intervention is likely to yield the most efficacious results, we sought to determine the possible therapeutic utility of rAAV in early gene therapy based interventions. Currently, the application of recombinant adeno-associated virus (AAV) vectors is one of the most widely used gene transfer systems, and represents a promising approach in the treatment of MPS IIIB. From a translational standpoint, a minimally invasive, yet highly efficient method of vector administration is ideal. The thalamus is thought to be the switchboard for signal relay in the central nervous system (CNS) and therefore represents an attractive target. To identify an optimal AAV vector for early therapeutic intervention, and establish whether thalamic administration represents a feasible therapeutic approach, we performed a comprehensive assessment of transduction and biodistribution profiles of four green fluorescent protein (GFP) bearing rAAV serotypes, -5, -8, -9 and -rh10, administered bilaterally into the thalamus. Of the four serotypes compared, AAV8 and -9 proved superior to AAV5 and -rh10 both in biodistribution and transduction efficiency profiles. Genotype differences in transduction efficiency and biodistribution patterns were also observed. Importantly, we conclude that AAV8 and to a lesser extent, AAV9 represent preferable candidates for early gene therapy based intervention in the treatment of MPS IIIB. We also highlight the feasibility of thalamic rAAV administration, and conclude that this method results in moderate rAAV biodistribution with limited treatment capacity, thus suggesting a need for alternate methods of vector delivery.

1. Introduction

A deficiency in N-acetyl-glucosaminidase activity results in the lysosomal storage disease, mucopolysaccharidosis IIIB (MPS IIIB), commonly referred to as Sanfilippo syndrome type B. Although heterogeneous in clinical presentation, this disease typically manifests around 5 years of age. Over time, progressive mental degeneration results in severe impairment of neurocognitive ability and loss of motor function. Death often occurs between the ages of 15–20 years [1,2]. There is currently no cure for MPS IIIB. The well characterized MPS IIIB mouse model possesses many of the same biochemical, histological and clinical features as the human disease, and is therefore utilized in studies to evaluate therapeutic candidates [3,4].

The recombinant adeno-associated virus (rAAV) vector gene transfer system has emerged as a powerful tool for therapeutic gene delivery and is favored over other viral vectors due to its low immunogenicity, ability to transduce both dividing and non-dividing cells, long lasting gene expression, non-pathogenicity, and importantly, clinical safety [5–7]. The most commonly used AAV vectors are based on AAV serotype 2. Several AAV serotypes allow cross-packaging of the AAV2 vector backbone [8–10]. Since AAV transduction is modulated by the presence and distribution of cell surface receptors, differential cellular tropism and transduction efficiency has been observed [11,12]. This phenomenon is also suggested to occur in an age dependent manner [13–15]. Studies suggest that the greatest therapeutic outcome would stem from early treatment intervention, before disease pathology becomes evident and irreversible [14,16]. It is possible that rAAV efficiency may be differentially modulated depending on the disease model and associated extracellular mileau.

Widespread gene delivery throughout the central nervous system (CNS) remains a major challenge to successful treatment due to the need to overcome the blood brain barrier (BBB). To circumvent this limitation, direct CNS administration of rAAVs is typically performed. We have previously demonstrated improvement in behavioral measures,

Abbreviations: rAAV, recombinant adeno-associated virus; ANOVA, analysis of variance; MPS IIIB, mucopolysaccharidosis type III B; CNS, central nervous system; BBB, blood brain barrier; NeuN, neuronal nuclear protein; GFAP, glial fibrillary acidic protein; hGFP, humanized green fluorescent protein.

* Corresponding author at: 1600 SW Archer Rd, Campus Box 100277, Gainesville, FL 32608, USA.
E-mail address: coy.heldermon@medicine.ufl.edu (C.D. Heldermon).

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lysosomal storage and lifespan of MPS IIIB mice with intracranial delivery of AAV5 pseudotyped vector to the CNS [17] but this treatment did not completely correct the disease. To this end, our goal is to assess several CNS trophic rAAV serotypes in the MPS IIIB neonatal mouse brain to determine which rAAV serotype facilitates the best transduction and biodistribution profiles. To limit the invasiveness of the procedure, we assess the methodological utility of a one-time delivery of rAAV vector bilaterally into the thalamus. We anticipate that these results will contribute to clinical approaches in determining the optimal gene delivery vector and method of delivery for age dependent treatment of MPS IIIB.

2. Results

2.1. Differential biodistribution profiles observed for different AAV serotypes

The thalamus is considered an information hub, with complex networks of connections within the brain (Fig. 1A). To facilitate vector biodistribution, we sought to exploit these thalamic connections. Control and MPS IIIB mice received bilateral intracranial administration of GFP-AAV5, -8, -9 and -rh10 into the thalamus. Evans Blue dye was injected to visualize predicted spread and biodistribution of vectors after administration (Fig. 1B). We initially sought to determine the biodistribution profiles of the different serotypes. Three months after vector administration, brain tissue of euthanized animals was analyzed to determine the presence and location of GFP. Moderate expression of GFP was observed in the cortex, thalamus and hippocampus of animals treated with AAV8 and -9. AAVrh10 treated animals showed minimal GFP staining in the cortex, with some presence in the hippocampus. AAV5 yielded the least favorable results as GFP expression was minimally seen in any of the analyzed regions (Fig. 1C). Furthermore, we noted that the thalamic method of administration does not readily facilitate vector spread into the cerebellum by any serotype. The retardation of motor function is a severe impediment suffered by MPS IIIB patients. The regulation of motor coordination is an important function of the cerebellum, therefore making it an important structural area to target in order to achieve maximal therapeutic success.

Next, we performed a qualitative assessment of GFP–AAV biodistribution profiles based on serotype (Fig. 2A) and quantified GFP expression in distinct structurally and functionally relevant areas in mid-sagittal brain sections (Fig. 2B). Comparative qualitative assessment of GFP expression patterns revealed that AAV5–GFP expression was heavily localized with ependymal cells, and otherwise exhibited minimal punctate distribution in the cortex (Fig. 2A, *inset*). In MPS IIIB treated animals, thalamic administration of AAV8 vector resulted in appreciable spread of vector into the somatosensory and visual areas of the cortex. Layers II/III of the cortex exhibited light GFP expression, while layers IV and V exhibited moderate to heavy GFP expression. Ventral spread of the AAV8–GFP vector was observed in the hippocampus and proximal areas of thalamus closest to the hippocampus and often along the thalamocortical or fiber tracks surrounding the thalamus. In the hippocampus, moderate staining was witnessed in CA1, CA2 and CA3 regions. Control animals in the AAV8 treated group exhibited a different transduction profile compared to MPS IIIB treated animals, with localized cortical spread and minimal thalamic penetration. This data suggests that there may be an interplay of unknown factors modulating transduction between these two genotypes. Both genotypes in the AAV9 treated groups showed comparable biodistribution profiles. AAVrh10 distribution comparisons were difficult due to low overall expression, although it was primarily localized to the thalamus (Fig. 2A). Quantitative assessment of AAV–GFP biodistribution into the cortex, hippocampus, thalamus and cerebellum revealed that in the Control group, AAV8 and -9 were superior to AAV5 and -rh10 in the cortex ($p < 0.05$ vs -5 and -rh10) and hippocampus (AAV8 vs -5 and -rh10, $p < 0.001$), but not thalamus and cerebellum. Whereas, in the MPS IIIB treated group, the largest GFP positive areas were the cortex with approximately 3% total area, and this was accomplished by AAV8, and to a lesser degree, AAV9. In the cortex, AAV8 was

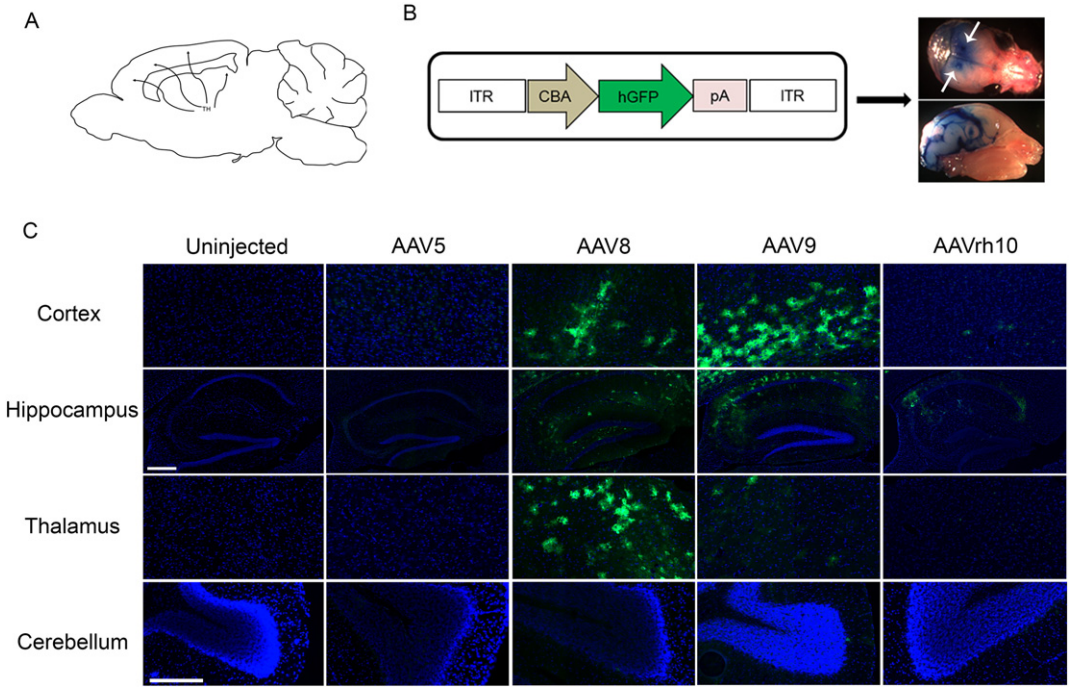

Fig. 1. Regional differences in rAAV biodistribution are observed. (A) Representative images showing thalamic connections within the brain (A). Sites of rAAV vector administration into thalamus (white arrows), and predicted rAAV spread within the CNS using 2% Evans Blue dye are shown (B). Representative tissue sections of interest (C) were analyzed for GFP expression in the cortex, hippocampus, thalamus and cerebellum of three month old MPS IIIB animals after rAAV administration. Scale bar: hippocampus (8×), 300 μm; and cortex, thalamus and hippocampus (20×), 100 μm.