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Research report

Effects of AAV-2-mediated aspartoacylase gene transfer in the tremor rat model of Canavan disease

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Abstract

The tremor rat is a spontaneous epilepsy model with a seizure phenotype caused by a deletion in the aspartoacylase (*ASPA*) gene. The absence of *ASPA* expression in these animals results in undetectable levels of enzyme activity and the accumulation of the substrate *N*-acetyl-aspartate (NAA) in brain, leading to generalized myelin vacuolation and severe motor and cognitive impairment. In support of human gene therapy for CD, recombinant adeno-associated viral vector (AAV-2) expressing *ASPA* was stereotactically delivered to the tremor rat brain and effects on the mutant phenotype were measured. AAV–*ASPA* gene transfer resulted in elevated aspartoacylase bioactivity compared to untreated mutant animals and elicited a significant decrease in the pathologically elevated whole-brain NAA levels. Assessment of motor function via quantitative rotorod testing demonstrated that rats injected with AAV–*ASPA* significantly improved on tests of balance and coordinated locomotion compared to animals receiving control vectors. This study provides evidence that AAV-2-mediated aspartoacylase gene transfer to the brain improves biochemical and behavioral deficits in tremor rat mutants (*tm*/*tm*) and supports the rationale of human gene transfer for Canavan disease.

Theme: Disorders of the nervous system
Topic: Genetic models; degenerative disease

Keywords: Canavan; Leukodystrophy; Tremor rat; Aspartoacylase; NAA; Gene therapy

1. Introduction

Canavan disease is an autosomal recessive neurogenetic disorder caused by mutations or deletions in the aspartoacylase gene (*ASPA*) [17], which results in loss of *ASPA* enzyme function [25,26] and accumulation of its substrate *N*-acetyl-aspartate (NAA) in neural cells, cerebrospinal fluid (CSF), and brain interstitial fluid. The tremor rat has a large inactivating deletion spanning the aspartoacylase (*ASPA*) coding region [18] and thus represents a naturally occurring model of Canavan disease. As with Canavan disease, deletion of *ASPA* and the associated elevation in brain NAA lead to changes in white matter morphology, and behavioral deficits such as akinesia and loss of normal motor coordination and balance. Pharmacological approaches targeted to affected biochemical pathways

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have not been effective in alleviating the neurological deficits associated with CD [20]. Therefore we have focused on the development of gene transfer strategies to safely and efficiently provide functional aspartoacylase activity to the brain of CD patients, thereby targeting the primary causative pathological mechanism of this disease [13,21].

The monogenetic nature of CD combined with the absence of effective treatments supports the rationale of aspartoacylase gene transfer to the brain. NAA is grossly elevated in CD [25] and although its function is not well understood, abnormal accumulation of this metabolite remains the primary symptomatic feature of Canavan disease. NAA causes seizure activity when administered in high doses to normal rats, an effect that is accentuated in tremor rats which have chronically elevated NAA [2]. The loss of normal NAA catabolism in CD has been hypothesized to limit the supply of acetate for lipid synthesis [7,8,32] and is also thought to influence mitochondrial function [11,35] and cellular energetics. The role of NAA catabolism in myelin formation is still unclear. Despite the enigmatic nature of NAA function in normal cell metabolism, production of NAA is known to occur primarily in neurons with a very high intraneuronal–interstitial concentration gradient [40,42]. NAA may be involved in H_2O flux between neurons and oligodendrocytes, with hydrolysis of the osmolyte via aspartoacylase occurring at the cell membrane of oligodendrocytes [6,43]. In CD the intercompartmental cycling of NAA in the absence of breakdown by aspartoacylase is thought to result in a loss of osmotic control and the swelling of astrocytes with formation of spongiform vacuoles [1,44].

A definitive physiological role for *ASPA* in the brain is currently lacking, but the correction of the CD phenotype at a symptomatic stage of disease via gene transfer is a possibility that requires evaluation. AAV-2 is well characterized and widely used as a vector system for the delivery of genetic material to the brain [19,34,36,38,45]. One advantage is the relative absence of immunological responses following its administration and the ability to sustain long-term gene expression in vivo [14,22,24,29]. AAV-2 possesses characteristics of efficacy and safety that offer advantages over other currently available viral vector systems, particularly for clinical applications. Our working hypothesis for the current study was that AAV-2-mediated aspartoacylase gene transfer into neurons in the tremor rat brain results in the breakdown of NAA in the compartment of synthesis, thereby reducing the efflux of metabolite into other compartments and promoting a subsequent decrease in downstream pathology.

Molecular and physiological characterization of the tremor rat (*tm*/*tm*) indicates an absence of aspartoacylase expression as well as accumulation of NAA in brain tissue [2,18]. Rats heterozygous for the aspartoacylase deletion have a relatively normal phenotype, although some go on to develop absence-like seizures as adults. A previous study documented behavioral deficits in tremor rats using the rotorod assay of coordinated locomotion [3], thereby providing a quantitative assessment of phenotype following gene transfer. This assay along with measures of enzyme bioactivity and tissue NAA content were chosen to evaluate the effect of AAV-mediated aspartoacylase gene transfer in tremor rats.

2. Materials and methods

2.1. AAV-2 vector construction

The AAV-2 expression cassette was optimized for neuronal gene transfer with the use of the neuron-specific enolase promoter (*NSE*, provided by J.G. Sutcliffe, Scripps Research Institute) [19,30,31,36,45] and the human aspartoacylase gene (*ASPA*, provided by R. Matalon, University of Texas) [17]. The *ASPA* expression cassette was packaged into recombinant AAV-2 using the pDG helper plasmid system as previously described [12]. Woodchuck post-regulatory elements (WPRE) and bovine growth hormone polyA sequences were utilized to boost gene expression, as previously described [45]. Vectors were DNAse and Proteinase K treated before being titered by quantitative real time PCR (7700, Applied Biosystems) using CYBR Green® to detect linear amplification of a region within the common WPRE sequence, a modified version of the method of Clark et al. [10].

2.2. Gene delivery to tremor mutant cohorts

Three cohorts of rats were used for analyses presented in this study. Cohort 1 was used for biochemical and behavioral analyses, and Cohorts 2 and 3 were used for immunocytochemical analyses. Tremor mutant availability was limited, and both male and female mutants were studied. Cohorts 1 and 3 were males, and Cohort 2 animals were female to both optimize animal use and limit potential variability.

2.3. Gene delivery and behavioral and biochemistry assay time points: Cohort 1

A cohort of 12 male tremor rats received intracranial gene delivery at 30 weeks of age. Behavioral testing was performed on these animals 10 weeks later (40 weeks of age) before tissue collection for biochemical assays. Intracranial gene delivery was performed under 2% isoflurane inhalation anesthesia using standard stereotactic microsurgical techniques [10]. Male tremor rats received bilateral microinjections of either AAV–*ASPA* ($n = 6$) or AAV–*GFP* (control, $n = 6$) in the caudate (AP −0.92 mm (from bregma), ML ± 3.5 mm (from midline), DV −3.8 mm (from dura), and thalamus (AP −2.80 mm (from bregma), ML ± 3.0 mm (from midline), DV −3.8 mm