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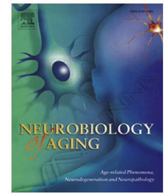

Recombinant adenoassociated virus 2/5-mediated gene transfer is reduced in the aged rat midbrain

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ABSTRACT

Clinical trials are examining the efficacy of viral vector-mediated gene delivery for treating Parkinson's disease. Although viral vector strategies have been successful in preclinical studies, to date clinical trials have disappointed. This may be because of the fact that preclinical studies fail to account for aging. Aging is the single greatest risk factor for developing Parkinson's disease and age alters cellular processes utilized by viral vectors. We hypothesized that the aged brain would be relatively resistant to transduction when compared with the young adult. We examined recombinant adeno-associated virus 2/5-mediated green fluorescent protein (rAAV2/5 GFP) expression in the young adult and aged rat nigrostriatal system. GFP overexpression was produced in both age groups. However, following rAAV2/5 GFP injection to the substantia nigra aged rats displayed 40%–60% less GFP protein in the striatum, regardless of rat strain or duration of expression. Furthermore, aged rats exhibited 40% fewer cells expressing GFP and 4-fold less GFP messenger RNA. rAAV2/5-mediated gene transfer is compromised in the aged rat midbrain, with deficiencies in early steps of transduction leading to significantly less messenger RNA and protein expression.
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1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease and currently impacts approximately 4.6 million individuals worldwide, with the prevalence expected to increase in the coming decades with longer life expectancies (Dorsey et al., 2007). The cardinal symptoms of PD are motor deficits resulting from the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the accompanying loss of dopamine neurotransmission within the striatum. Although it is still unclear what causes PD, aging is known to be a primary risk factor for this disease, with the vast majority of idiopathic cases occurring in patients over the age of 65 years (Collier et al., 2011). Currently, there are no therapies that halt, slow, or reverse the progression of neurodegeneration in PD (Gombash et al., 2014). However, viral vector-mediated gene therapy is a promising therapeutic avenue because of its ability to continuously replenish diminished proteins or overexpress neuroprotective factors that alleviate symptoms or alter disease progression.

Preclinical studies using viral vector-mediated gene transfer have been successful in ameliorating symptoms and rescuing nigral dopamine neuron loss in PD models (Emborg et al., 2009; Gash et al., 1996; Gasmi et al., 2007; Gombash et al., 2012; Herzog et al., 2008; Kordower et al., 2000), yet human clinical trials have not experienced similar success (Bartus et al., 2011; ClinicalTrials.gov, 2013; Marks et al., 2010; Stocchi and Olanow, 2013). This discrepancy may be, in part, attributable to the almost exclusive use of young adult animals in preclinical studies that fail to recapitulate the aged host brain environment typical of most PD patients. For example, previous studies demonstrated that fetal dopamine neuron grafts completely ameliorated motor impairments in young rats yet produced no improvement in aged rats because of dramatically decreased survival and neurite extension in the aged host (Collier et al., 1999; Sortwell et al., 2001). Thus, experimental results derived using young adult animals may have contributed to the overly optimistic expectations of clinical efficacy.

Several groups have reported successful viral vector-mediated gene transfer to the aged brain of rodents and nonhuman primates (Bartus et al., 2011; Emborg et al., 2009; Klein et al., 2010;

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Kordower et al., 2000; Wu et al., 2004). However, only 2 of these reports directly compared transduction efficiency between aged and young animals, with contradicting results (Klein et al., 2010; Wu et al., 2004). Klein et al. (2010) reported equally efficient gene transfer in aged and young adult rat brains utilizing recombinant adeno-associated virus serotype 2/9 (rAAV2/9), whereas Wu et al. (2004) concluded that serotype 2/2 (rAAV2/2) was less efficient in the aged brain as compared with the young adult brain. These results were secondary to the primary findings of the study and, consequently, additional experiments comparing viral vector efficiency between young adult and aged brains were not conducted.

It is well established that certain cellular processes that are normally altered in the aged brain (D'Angelo et al., 2009; Gao et al., 2013; Lee et al., 2000; Ryazanov and Nefsky, 2002; Smith et al., 1995) are also utilized by viral vectors for transduction (Schultz and Chamberlain, 2008). The present study sought to determine whether viral vector-mediated transgene expression was reduced in the aged rat nigrostriatal system as compared with the young adult. We utilized the rAAV2/5 serotype to examine this question because of its efficient transduction of nigral neurons (Burger et al., 2004; Gombash et al., 2013). Intranigral injections of rAAV serotype 2/5 expressing green fluorescent protein (rAAV2/5 GFP) resulted in significantly reduced exogenous protein expression in the aged rat brain as compared with the young adult brain. Significantly, fewer cells throughout the aged rat midbrain expressed the transgene delivered by rAAV2/5 GFP, in addition to producing significantly less GFP protein and GFP messenger RNA (mRNA). Collectively, our results indicate that rAAV2/5-mediated gene transfer is compromised in the aged rat brain environment.

2. Methods

2.1. Experimental overview

A cohort of 21 male Sprague-Dawley (SD) rats, 11 young adult (3 months old) and 10 aged (20 months old), were unilaterally injected with rAAV2/5 expressing GFP into the substantia nigra (SN). Six young and 5 aged SD rats were sacrificed 11 days postinjection and 5 young and 5 aged SD rats were sacrificed 3 months post-injection. Similarly, a cohort of 12 male Fischer344 (F344) rats, 6 young adult and 6 aged, were unilaterally injected with rAAV2/5 expressing GFP in the SN and sacrificed 12 days post-injection. The striatum was used for Western blot analysis from SD rats sacrificed 11 days and 3 months post-injection, and the SN tissue was used for immunohistochemical staining and in situ hybridization. The striatum from F344 rats sacrificed 12 days post-injection was used for Western blot analyses, and the SN tissue was used for quantitative real-time PCR (qPCR). The overall experimental design is illustrated in Fig. 1.

2.2. Animals

Male Sprague-Dawley rats (Harlan, Indianapolis, IN), 3 months of age (n = 11) and 20 months of age (n = 10), and male Fischer344 rats (National Institute on Aging, Bethesda, MD), 3 months of age (n = 6) and 20 months of age (n = 6), were used in this study. All animals were given food and water *ad libitum* and housed in 12 hours reverse light-dark cycle conditions in the Van Andel Research Institute vivarium, which is fully AAALAC approved. All procedures were conducted in accordance with guidelines set by the Institutional Animal Care and Use Committee of Michigan State University.

2.3. Viral vectors

Plasmid and rAAV vector production were completed as previously described (Gombash et al., 2014). In short, humanized GFP

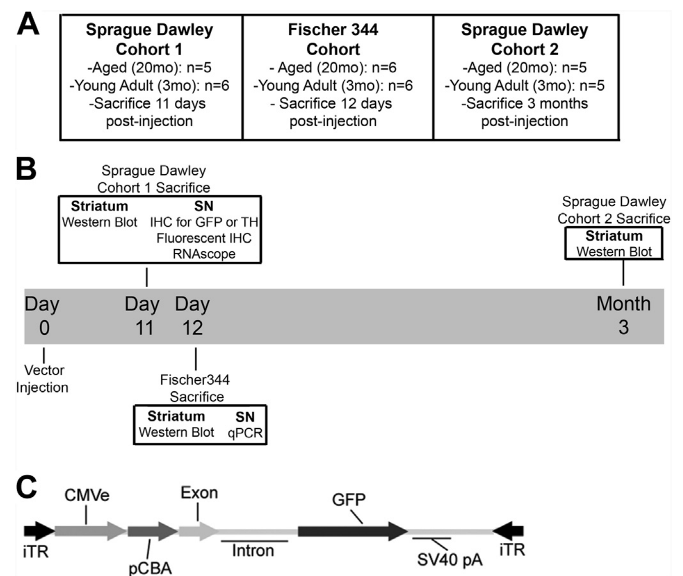

Fig. 1. Schematic of experimental design and rAAV2/5 viral vector construct. (A–B) Overview and timeline of the 3 cohorts of rats used in these studies. Young adult (3 months) and aged (20 months) Sprague-Dawley and Fischer344 rats were all injected in the substantia nigra (SN) with the rAAV2/5 GFP viral vector and were sacrificed at indicated time points to harvest tissue for various outcome measures. (C) Recombinant adeno-associated virus (rAAV) genomic map of rAAV2/5 expressing GFP used in all experiments. Abbreviation: GFP, green fluorescent protein.

was inserted into an AAV plasmid backbone. Expression of the transgene was driven by the chicken beta actin/cytomegalovirus enhancer promoter hybrid. The vector contained AAV2 inverted terminal repeats and was packaged into AAV5 capsids via cotransfection with a plasmid containing *rep* and *cap* genes and adenovirus helper functions. Particles were purified using iodixanol gradients and Q-sepharose chromatography, and dot blot was used to determine vector titer (Zolotukhin et al., 1999). The vector titer used in this study was 5.88×10^{13} genomes/mL (Gombash et al., 2013). To preserve the viral stability and titer, viral preparations were stored at 4 °C and never frozen. All surfaces (syringes, pipettes, and microcentrifuge tubes) were coated in SigmaCote (Sigma-Aldrich, St. Louis, MO; SL2) before coming in contact with the virus to minimize binding of viral particles.

2.4. rAAV2/5 GFP injections

All surgical procedures were performed under isofluorane anesthesia (5% in O_2 for induction and 2% in O_2 for maintenance). Rats were placed in a stereotaxic frame and two 2-μL injections of rAAV2/5 were injected in the left SN at coordinates (from dura) anterior-posterior axis (AP) −5.3 mm, medial-lateral axis (ML) +2.0 mm, dorsal-ventral axis (DV) −7.2 mm, and AP −6.0 mm, ML +2.0 mm, and DV −7.2 mm as previously described (Gombash et al., 2013). A Hamilton syringe fitted with a glass capillary needle (Hamilton Gas Tight syringe 80,000, 26s/2″ needle; Hamilton, Reno, NV; coated in SigmaCote) was used for injection. The glass needle was lowered to the site and vector injection began immediately at a rate of 0.5 μL/min and remained in place after the injection for an additional 5 minutes before retraction.

2.5. Sacrifice and tissue preparation

Six young and 6 aged SD rats were sacrificed 11 days post-injection, all F344 rats were sacrificed 12 days postinjection,

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