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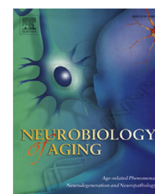

Longitudinal follow-up and characterization of a robust rat model for Parkinson's disease based on overexpression of alpha-synuclein with adeno-associated viral vectors

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ABSTRACT

Testing of new therapeutic strategies for Parkinson's disease (PD) is currently hampered by the lack of relevant and reproducible animal models. Here, we developed a robust rat model for PD by injection of adeno-associated viral vectors (rAAV2/7) encoding α-synuclein into the substantia nigra, resulting in reproducible nigrostriatal pathology and behavioral deficits in a 4-week time period. Progressive dopaminergic dysfunction was corroborated by histopathologic and biochemical analysis, motor behavior testing and in vivo microdialysis. L-DOPA treatment was found to reverse the behavioral phenotype. Non-invasive positron emission tomography imaging and magnetic resonance spectroscopy allowed longitudinal monitoring of neurodegeneration. In addition, insoluble α-synuclein aggregates were formed in this model. This α-synuclein rat model shows improved face and predictive validity, and therefore offers the possibility to reliably test novel therapeutics. Furthermore, it will be of great value for further research into the molecular pathogenesis of PD and the importance of α-synuclein aggregation in the disease process.

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1. Introduction

Parkinson's disease (PD) is a progressive, age-related neurodegenerative movement disorder affecting about 1%–2% of people above 60 years (Wirdefeldt et al., 2011). Both genetic and environmental factors are believed to play a causal role in the onset of PD, but to date, the exact etiology and underlying molecular mechanisms remain unclear. The pathophysiology of PD is characterized by the selective and progressive loss of dopaminergic neurons (DN) in the substantia nigra pars compacta (SNpc) resulting in a striatal depletion of dopamine (DA). The resident neurons contain α-synuclein-positive intracellular inclusions designated as Lewy bodies (LBs) and dystrophic neurites called Lewy neurites (Forno, 1996). α-Synuclein, a protein of 140 amino acids localized in nerve terminals, is the major protein present in fibrillar form in LBs and Lewy neurites in sporadic and inherited PD (Spillantini et al., 1997). Moreover, genetic

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aberrations in the *SNCA* gene encoding human α-synuclein, such as point mutations (A53T, A30P, and E46K), duplications and triplications, are the cause of autosomal dominant forms of PD (Chartier-Harlin et al., 2004; Ibanez et al., 2004; Kruger et al., 1998; Polymeropoulos et al., 1997; Singleton et al., 2003; Zarranz et al., 2004). Very recently, 2 new mutations in human α-synuclein (G51D and H50Q) have been linked to PD (Appel-Cresswell et al., 2013; Kiely et al., 2013; Lesage et al., 2013). The physiological function of α-synuclein and the mechanisms leading to aggregation and selective degeneration of nigral neurons are still not well understood.

To study the pathophysiology of PD and to develop novel therapeutic strategies, there is an urgent need for animal models that more closely resemble the neuropathology, physiology, and motor symptoms of human PD, and therefore might have a higher predictive value to translate new therapies from animal models to patients. Glial cell line-derived neurotrophic factor, for example, is neuroprotective in the commonly used neurotoxin models of PD but was shown to lack neuroprotective efficacy in viral vector-mediated α-synuclein models (Decressac et al., 2011; Lo Bianco et al., 2004); this might explain the poor outcome so far in clinical trials with glial cell line-derived neurotrophic factor in PD patients (Marks et al., 2010). Moreover, new therapeutic avenues targeting α-synuclein aggregation or neurotoxicity require robust α-synuclein-based animal models. Overexpression of both wild type (WT) and several clinical mutants of human α-synuclein in transgenic mice has been shown to induce pathologic accumulation of α-synuclein and neuronal dysfunction (Chesselet et al., 2011; Fleming et al., 2005; Freichel et al., 2007; Kahle et al., 2001; Masliah et al., 2000). However, until now transgenic α-synuclein mouse models failed to display clear age-dependent dopaminergic cell loss and associated-behavioral deficits (reviewed by Magen and Chesselet, 2010).

This hurdle was overcome by direct targeting of the substantia nigra (SN) with viral vectors overexpressing α-synuclein. Both lentiviral (LV) and recombinant adeno-associated viral (rAAV) vectors encoding human α-synuclein have been explored for this purpose (reviewed in Löw and Aebischer, 2012; Van der Perren et al., 2014), but rAAV vectors are particularly attractive to target the SN because of their high transduction efficiency and tropism for DN (Taymans et al., 2007; Van der Perren et al., 2011). Several rAAV serotypes (rAAV2/2, 2/5, 2/6, and 1/2) have been applied to overexpress α-synuclein in the rat, mouse, and primate brain (Azeredo da Silveira et al., 2009; Eslamboli et al., 2007; Gorbatyuk et al., 2008; Kirik et al., 2002; Klein et al., 2002; Koprich et al., 2011; Martin et al., 2007; Yamada et al., 2004). These models exhibit different key features of the human disease such as nigral dopaminergic cell loss, α-synucleinopathy in surviving neurons and DA-related motor deficits. However, the observed nigral cell loss as well as the time course described has been quite variable. Because of to this variation, the lack of overt behavioral impairments is a shortcoming of most current rAAV-based α-synuclein models (reviewed in Löw and Aebischer., 2012; Ulusoy et al., 2010; Van der Perren et al., 2014).

In this study, we aimed to establish a viral vector-based rat model for PD with robust and reproducible nigrostriatal pathology that allowed detection of behavioral deficits in a relatively short-time period. Therefore, we stereotactically injected rAAV2/7 encoding the human A53T α-synuclein mutant in the SN of adult rats. Progressive dopaminergic dysfunction and α-synuclein aggregation were assessed by histopathologic and biochemical analysis, non-invasive positron emission tomography (PET) imaging, magnetic resonance (MR) spectroscopy, microdialysis, and motor behavior.

2. Methods

2.1. Recombinant AAV production and purification

Vector production and purification was performed as previously described (Van der Perren et al., 2011). The plasmids include the constructs for the AAV7 serotype, the AAV transfer plasmid encoding the human A53T mutant or WT α-synuclein or the enhanced green fluorescent protein (eGFP) transgene under the control of the ubiquitous CMVie-enhanced synapsin1 promoter and the pAdvDeltaF6 adenoviral helper plasmid. Real-time polymerase chain reaction analysis was used for genomic copy determination.

2.2. Stereotactic injections

All animal experiments were carried out in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and approved by the Bioethical Committee of the KU Leuven (Belgium). Young, adult, female Wistar rats (Janvier, France) weighing about 200–250 g were housed under a normal 12 hour light and/or dark cycle with free access to pelleted food and tap water. All surgical procedures were performed using aseptic techniques and ketamine (60 mg/kg intraperitoneal [i.p.], Ketalar, Pfizer, Belgium) and medetomidine (0.4 mg/kg, Dormitor, Pfizer) anesthesia. Following anesthesia, the rodents were placed in a stereotactic head frame (Stoelting, IL, USA). Injections were performed with a 30-gauge needle and a 10-μL Hamilton syringe. All animals were injected with 3 μL A53T α-synuclein rAAV2/7 (low: 3.0 E + 10 GC/mL, standard: 3.0 E + 11 GC/mL or high: 1.0 E + 12 GC/mL vector dose). Stereotactic coordinates used for the SN were anteroposterior, −5.3; lateral, −2.0; and dorsoventral, −7.2 calculated from the dura using bregma as reference. The injection rate was 0.25 μL/min, the needle was left in place for an additional 5 minutes before being retracted. Control animals were injected with eGFP rAAV2/7 (standard: 3.0 E + 11 GC/mL vector dose) or 6-OHDA (Sigma; calculated as free base, 25 μg dissolved in 3 μL of 0.05% ascorbate saline) into the SN using the same coordinates. The 6-OHDA solution was kept on ice, used fresh, and protected from light to minimize oxidation.

2.3. Histology

Rats were sacrificed with an overdose of sodium pentobarbital (60 mg/kg, i.p., Nembutal, Ceva Santé, Belgium) followed by intracardial perfusion with 4% paraformaldehyde in phosphate buffered saline (PBS). After postfixation overnight, 50-μm-thick coronal brain sections were made with a vibrating microtome (HM 650V, Microm, Germany). Immunohistochemistry (IHC) was performed on free-floating sections using an antibody against α-synuclein (rabbit polyclonal 1:5000, Chemicon 5038). This antibody can detect both human and rat α-synuclein, but endogenous levels of rat α-synuclein were below detection limits within nigral cell somata, owing to its predominant localization at synaptic membranes. We also used antibodies against tyrosine hydroxylase (TH; rabbit polyclonal 1:1000, Chemicon 152) and vesicular monoamine transporter (VMAT; rabbit polyclonal 1:1000, Abcam 81,855). Sections were pretreated with 3% hydrogen peroxide for 10 minutes and incubated overnight with primary antibody in 10% normal goat or swine serum (DakoCytomation, Belgium). As secondary antibody we used biotinylated anti-rabbit IgG (1:600, α-synuclein; 1:300, other antibodies DakoCytomation), followed by incubation with streptavidin–horseradish peroxidase complex (1:1000, DakoCytomation). α-Synuclein and VMAT2 immunoreactivity were visualized using 3,3-diaminobenzidine (0.4 mg/mL, Sigma-Aldrich) and

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