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SHORT- AND LONG-TERM EFFECTS INDUCED BY REPEATED 6-OHDA INTRAVENTRICULAR ADMINISTRATION: A NEW PROGRESSIVE AND BILATERAL RODENT MODEL OF PARKINSON'S DISEASE

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Abstract—The pathological hallmark of Parkinson's disease (PD) is the progressive degeneration of dopaminergic neurons in the substantia nigra *pars compacta* (SNpc), and the resulting striatal dopamine deficiency, which are responsible for the classic motor features. Although a diagnosis of PD relies on the clinical effects of dopamine deficiency, this disease is also associated with other neurotransmitter deficits that are recognized as causing various motor and non-motor symptoms. However, the cause of dopaminergic nigral neurodegeneration in PD and the underlying mechanisms remain unknown. While animal models are considered valuable tools with which to investigate dopaminergic cell vulnerability, rodent models usually fail to mimic the neurodegeneration progression that occurs in human PD. To find a convenient rat model for studying the progression of dopaminergic cell degeneration and motor signs, we have developed a progressive rodent model using a repeated daily, intraventricular administration of the neurotoxin 6-hydroxydopamine (6-OHDA) (100 μg/day) in awakened rats for 1 to 10 consecutive days. The short- (6-day) and long-term (32-day) progression of motor alterations was studied. This model leads to a bilateral and progressive increase in catalepsy (evident from the 3rd infusion in the short-term groups ($p < 0.01$) and from the 7th infusion in the long-term groups ($p < 0.01$), which was associated with a progressive nigrostriatal dopaminergic deficit. All together this makes the new model an interesting experimental tool to investigate the mechanisms involved in the progression of dopaminergic neurodegeneration.

Key words: Parkinson's disease, 6-hydroxydopamine, intracerebroventricular, neurodegeneration.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disease characterized mainly by nigro-striatal degeneration at onset leading to the classic triad (resting tremor, rigidity, akinesia), but also by non-motor symptoms (depression, sleep disorders, hiposmia...) (Obeso et al., 2014; Marras and Chaudhuri, 2016). The motor manifestations are mainly caused by the degeneration of the dopaminergic neurons in the substantia nigra *pars compacta* (SNpc) and, to a lesser extent, in the midbrain ventral tegmental area (VTA). The nigral dopaminergic neuronal loss depletes dopamine (DA) in the striatum, particularly in the dorsolateral and posterior putamen which is the region involved in the motor circuit (Otsuka et al., 1996; Rodriguez-Oroz et al., 2009). PD is also associated with deficits in other neurotransmitters, such as the cholinergic, serotonergic and noradrenergic systems possibly involved in various motor and non-motor symptoms (Buddhala et al., 2015).

Animal models have been developed as useful tools to allow pathogenic mechanisms, motor and non-motor manifestations of PD, and potential therapeutic agents to be studied (Schneider et al., 2013; Bonito-Oliva et al., 2014; Marin et al., 2014; Tamtè et al., 2016). The most characteristic features of the disease have been, more or less, faithfully mimicked in animals by administering different neurotoxic agents such as 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or rotenone (Jackson-Lewis and Smeyne, 2005; Iderberg et al., 2015; Johnson et al., 2015; Marin et al., 2015), or drugs that alter dopaminergic neurotransmission, such as reserpine (Shireen et al., 2014; Leão et al., 2015), or by genetic manipulation (Chen et al., 2012; Hinkle et al., 2012; Oaks et al., 2013; Paumier et al., 2013).

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Abbreviations: 6-OHDA, 6-hydroxydopamine; ICV, intracerebroventricular; MFB, medial forebrain bundle; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PBS, phosphate buffer saline; PD, Parkinson's disease; SNpc, substantia nigra *pars compacta*; VTA, ventral tegmental area.

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The most widely used PD model corresponds to administrating the neurotoxin 6-OHDA which produces a degeneration of the nigral dopaminergic neurons (Ungerstedt, 1968; Blandini and Armentero, 2012; Blesa and Przedborski, 2014; Marin et al., 2014; Ramsey and Tansey, 2014). The 6-OHDA administration is usually performed unilaterally in the nigral (Lee et al., 2012; Kim et al., 2016), the striatal (Bové et al., 2005; Marin et al., 2005; Penttinen et al., 2016) or the medial forebrain bundle (MFB) (Marin et al., 2014; Bagga et al., 2015; Boix et al., 2015). In trying to mimic the bilateral motor manifestation of PD, several studies have described the effects of the lesions induced by bilateral nigral (Paillé et al., 2007; Ariza et al., 2015; El Arfani et al., 2015), striatal (Eagle et al., 2015; Silva et al., 2015), or MFB (Smith et al., 2002) 6-OHDA administration in rats. An extensive bilateral MFB 6-OHDA-induced lesion may provoke significant bradykinesia, aphagia or adipsia (Cenci et al., 2002; Deumens et al., 2002; Cass et al., 2005; Ferro et al., 2005), thus forcing extreme care. However, a single intracerebroventricular (ICV) 6-OHDA administration has shown a better tolerability by rats (Rodríguez Díaz et al., 2001; Rodríguez et al., 2001). In addition, a sequential bilateral 6-OHDA MFB lesion model has recently been developed, showing good tolerability and allowing for the behavioral and molecular mechanisms involved in the progression from unilateral to bilateral Parkinsonism to be studied (Marin et al., 2015).

Progression of nigral dopaminergic neuron loss is a relevant feature in PD, but most animal models usually fail to reproduce this, mainly because the neurotoxic degeneration progresses rapidly. Interestingly, an intrastriatal injection of 6-OHDA causes progressive retrograde neuronal degeneration in the SNpc and VTA (Sauer and Oertel, 1994), inducing dopaminergic terminal damage within 1 day of the injection, (while nigral cell loss is minimal at 1 week), and reaching a maximum within 2–3 weeks (Sauer and Oertel, 1994; Przedbroski et al., 1995; Costantini et al., 2001). Unfortunately, the overall extent of nigral cell loss achieved varies between the studies and the degree of degeneration obtained depends on the amount of 6-OHDA injected, the site of the injection, and the species used (Sauer and Oertel, 1994; Przedbroski et al., 1995; Lee et al., 1996; Kirik et al., 1998; Blandini et al., 2007).

The main objective of this study was to develop and characterize a progressive bilateral PD rodent model resulting from the ICV administration of repeated infusions of 6-OHDA on consecutive days. Improving animal models in PD research is of great importance as this will provide more useful tools which, in turn, would allow for more in-depth investigations into the pathophysiological mechanisms underlying the bilateral progression of dopaminergic neurodegeneration in PD and for novel therapies to be tested; both of which are fundamental aspects of pre-clinical research.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (Charles River, France) weighing 250–300 g were housed in a 12-h light/dark cycle, with standard temperature and humidity conditions and free access to food and water. All attempts were made to minimize the number of animals used. Experimental procedures were carried out in accordance with the European Communities Council Directive (EU Directive 2010/63/EU) and the Spanish Animal Welfare Act (Real Decreto 1201/2005) guidelines and were approved by the Ethics Committee for Animal Testing at the Universidad de Navarra.

Repeated 6-OHDA ICV infusion

Animals were anesthetized with a mixture of ketamine/xylazine (0.5 ml/kg Xilagesic VR; Calier Laboratories, Barcelona, Spain; 1 ml/kg Imalgene1000 VR, Merial Laboratories, Barcelona, Spain). The infusions were performed using a single guide cannula (C313G, Plastics One, Roanoke, US) placed in the third ventricle (Stereotaxic coordinates: AP: 0.8 mm posterior to Bregma, L: midline, V: 6.5 mm below the dura, in accordance with Paxinos and Watson rat brain atlas (1998) (Rey et al., 2007). Two small screws were used to firmly fix the guide cannula and the cannula was glued with dental cement. Animals were sutured and a dummy cannula (C313DC, Plastic One, Roanoke, USA) was inserted into the guide cannula and secured in place by screwing it onto the guide to protect the brain from contamination and to prevent any obstructions in the guide cannula. Adequate measures were taken to minimize pain or discomfort (ketoprofen 1%, at a dose of 5 mg/kg, subcutaneous over 3 days).

Two weeks after implanting the guide cannula, the animals were randomly distributed into the following groups: (a) the control group (n = 15): composed of animals with the cannula implant but not being administered the solution to evaluate any possible mechanical effects the cannula implantation may have had, (b) the 1-infusion group (100 μg total 6-OHDA single dose (Sigma-Aldrich®, St. Louis, MO, USA), n = 10), (c) the 3-infusion group (300 μg total 6-OHDA dose, administered over three consecutive days (100 μg/day), n = 10), (d) the 5-infusion group (500 μg total 6-OHDA dose, administered over five consecutive days (100 μg/day), n = 10), (e) the 7-infusion group (700 μg total 6-OHDA dose, administered over seven consecutive days (100 μg/day), n = 18), (f) the 9-infusion group (900 μg total 6-OHDA dose over nine consecutive days (100 μg/day), n = 18), and (g) the 10 infusion group (1 mg total 6-OHDA dose, administered over ten consecutive days (100 μg/day), n = 18). 6-OHDA was diluted in a 0.02% ascorbate solution and was administered via ICV at a volume of 4 μl/infusion. Corresponding sham animals for each experimental group (n = 12 in each group) received ICV infusions of 4 μl saline with 0.02% ascorbate (Sigma-Aldrich®, St. Louis, MO, USA).

The solutions (6-OHDA or vehicle) were injected using an injector cannula (C313I, Plastic One) connected by polyethylene tubing to a Hamilton syringe coupled to a Harvard infusion pump. The rate of infusion was 1 μl/min. Upon completion of the 6-OHDA administration or vehicle, we left the injector in place for five minutes to