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Research article

Post-lesion administration of 7-NI attenuated motor and non-motor deficits in 6-OHDA induced bilaterally lesioned female rat model of Parkinson's disease

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HIGHLIGHTS

- Postoperative administration of 7-NI restores the damage caused by 6-OHDA in bilateral lesioned rats.
- 7-Nitroindazole treatment appears to enhance the cognition in female Sprague–Dwaley.
- The present work showed potential of 7-NI in the pharmacotherapy of PD in females.

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ABSTRACT

The preoperative neuroprotective effect of the 7-nitroindazole (7-NI) in 6-hydroxydopamine (6-OHDA) induced unilateral male animal models of Parkinson's disease (PD) has been widely reported. However, the therapeutic approach to PD pathology would be closely associated with the post-lesion treatment by 7-NI in 6-OHDA–induced bilateral model. Also, there is a scarcity of data on neuroprotective effect of 7-NI in PD in females. We have studied the neuroprotective effects of 7-NI in 6-OHDA-induced bilaterally lesioned female rats after short-term post-lesion treatment.

Sprague–Dawley female rats with bilateral intraventricular injection of either 6-OHDA (10.5 μg) (n = 8–11/group) or saline (sham; n = 8/group) at substantia nigra (SN) were provided with 7-NI (30 mg/kg/day) intraperitoneal, once a day during the 3 consecutive days of short term treatment. 6-OHDA lesioned animals developed the motor and non-motor deficits, which were evaluated by behavioral and neuro-biochemical tests from the substantia nigra. Post-lesion administration of 7-NI reduced the motor deficits induced by 6-OHDA in the behavioral tasks such as Rota rod, open field test and forced swim test. Simultaneously, the dopamine levels were restored by 7-NI in post lesion animals up to 76% in comparison to 6-OHDA lesioned animals (23%). Furthermore, antioxidant-like effect of 7-NI was observed in lipid peroxidation, catalase, superoxide dismutase, and reduced glutathione tests. Conclusively, the present study showed that early postoperative administration of 7-NI attenuates the motor deficits induced by 6-OHDA in bilaterally lesioned female rat model of PD.

1. Introduction

Nitric oxide synthase (NOS) plays important role in the pathophysiology of Parkinson disease (PD) by generating reactive nitrogen species [1]. 7-NI, a neuronal isoforms specific NOS inhibitor showed neuroprotective effect against 4-methyl-4-phenylpyridinium (MPP+) and 6-OHDA mediated toxicity [2]. Mostly, the neuroprotective studies of 7-NI have been carried either as preoperative in unilateral 6-OHDA/MPP+ induced animal models or post-lesion with 7-NI as adjuvant [3–5]. In both ways, it is imprecise pharmacological representation of 7-NI, since the unlesioned part of the animal brain in unilateral animal model tends to compensate the neuronal loss and being used as adjuvant masks its therapeutic potential [6]. Regardless the types of lesion, female animals have been sporadically used to study the female pathology of PD. In the present paper, 6-OHDA-induced bilaterally lesioned female rat model has been used to study the post-lesion neuroprotective effect of 7-NI.

During the course of present study, the behavioral and neuro-biochemical modulations leading to motor and non-motor

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functional deficits were evaluated. The forced swim test (FST) has been routinely utilized to evaluate the anti-depressant effect of new drug molecule. However, we had reported previously that the swimming disability could be used to study motor deficit in PD [7]; therefore, in present work, the swim test, Rota rod and open field tests were used to assess the motor dysfunction. Non-motor deficits like cognition, was assessed by the olfactory discrimination test. The behavioral studies were correlated with neuro-biochemical tests such as levels of dopamine, lipid peroxidation and reduced glutathione, and the activities of catalase, superoxide dismutase.

2. Experimental procedures

2.1. Animals

Thirty five female Sprague–Dawley rats weighing 200–350 g were procured from National Brain Research Center, Manesar, Haryana, India. Rats were housed in Individually Ventilated Cage system, and all the surgical procedure were according to the institutional animal ethical committee guidelines. The rats were divided into following four groups:

Group I ($n = 8$; sham + control) – Control animals (sham surgery) ($n = 4$) received 1 μl of vehicle (normal saline supplemented with 0.02% ascorbic acid) by stereotaxic injection into the substantia nigra or animals ($n = 4$) received no treatment (without surgery). Initially, sham lesioned and control (no surgery) groups were kept separately; however, no behavioral difference was observed in the animals of both the groups, therefore, were grouped together.

Group II (($n = 11$; 6-OHDA lesioned) – The animals in the group received 6-OHDA (10.5 μg) in vehicle per injection in the substantia nigra.

Group III (($n = 8$; 6-OHDA + 7-NI) – After 3 days of 6-OHDA lesion, the animals received 7-NI (30 mg/kg body weight; 7-NI dissolved in 0.05% of DMSO and mixed with groundnut oil) intraperitoneal (i.p.) for 3 consecutive days.

Group IV (($n = 8$); 7-NI) – The animals received 7-NI alone (30 mg/kg body weight) for 3 consecutive days.

After 6 weeks, the post operative neuroprotective potential of 7-NI was assessed by evaluating behavioral and neuro-biochemical tests.

2.2. Surgery and 6-OHDA lesion

All surgical procedures were conducted under aseptic conditions. 6-OHDA (10.5 μg in 1 μl of 0.9% NaCl, supplemented with 0.02% ascorbic acid) was injected by a 26-gauge Hamilton syringe at the rate of 0.2 μl/min over 5 min bilaterally into the SN using stereotaxic head frame (Stoelting, Wood Dale, IL) and following coordinates of the Paxinos and Watson atlas: AP: −5.0 mm, ML: ±2.0 mm and DV: −7.5 mm from bregma. All animals were pretreated with desipramine hydrochloride (25 mg/kg, i.p.) 30 min before surgery to protect noradrenergic neurons from 6-OHDA toxicity. The stereotaxic surgery was performed under ketamine (75 mg/kg)/xylazine (5 mg/kg) anesthesia. Sham-operated rats followed the same protocol except the vehicle was injected instead of 6-OHDA. Three animals from group II died before 3 weeks and rest of the animals were submitted for the behavioral studies between 4th and 6th weeks post surgery. Animals were sacrificed after the behavioral studies under anesthesia.

2.3. Behavioral tests

2.3.1. Assessment of motor function

2.3.1.1. Forced swim test. Previously described method [8] was used with slight modification in the apparatus, a vertical box (17 cm × 17 cm × 40 cm) containing water (water depth 30 cm; 25 °C) was used in the present study instead of 17 cm diameter Plexiglass cylinder. Two swimming sessions were conducted (an initial 15-min pretest followed 24 h later by a 3 min test). The experiment was recorded a period of 3-min using Anymaze software (Stoelting, USA) during 4th to 6th week post lesion. Following parameters were taken into account during data acquisition: the rat was regarded as immobile when floating motionless or making only those movements necessary to keep its head above the water and total immobile time was observed.

2.3.1.2. Rota rod test. Rota rod test was performed to assess the fine motor coordination and balance with some modification in the reported method [9]. The animals were given vigorous pretest training 24 h before the actual test till the animal was able to maintain its balance while walking on top of the rod in two compartment Rota rod instrument at the speed of 25 rpm (Kritij Instruments Pvt. Ltd., India). The animals were subjected to Rota rod test for a maximum of 180 s and the animals completing five trials in 180 s qualified the test. If the animal falls from the Rota rod, the trial was considered over and animal was given the next trial. The time spent on Rota rod was noted, and performance was expressed as the mean of the total time spent per minute on Rota rod.

2.3.1.3. Open field test. In order to evaluate the exploratory behavior as an index of spontaneous motor activity, open-field test was performed. The apparatus, made of wood had a black floor of 100 × 100 cm (divided by imaginary lines drawn in the software protocol into 25 squares of 20 × 20 cm) and 40 cm high white walls. Each rat was placed in the center of the open field and, the numbers of lines crossed were registered for 5 min by Anymaze software.

2.3.2. Assessment of non-motor function

2.3.2.1. Olfactory discrimination test. To assess the possible impairment in the olfactory function of the rat model, the olfactory discrimination task was performed as described by Tadaiesky et al. The time spent by the rat in familiar compartment was recorded by Anymaze software. Total duration of the test was 3 min [10].

2.3.2.2. Dopamine level and biochemical analysis. Total protein (1 mg/ml) isolated from three animals/group (I–IV) was utilized for biochemical analysis and three animals/group for dopamine level estimation. For determining DA content in the brain, the rats were sacrificed under anesthesia after 6th week. Dopamine concentration was determined from the standard curve obtained according to reported method [7]. The concentration of DA was determined by C-18 reverse-phase high performance liquid chromatography (Shimadzu) with Photo diode array (PDA) detector. Thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase were assayed according to the previously described method by Utley et al., Jollow et al., Beers and Sizer, and Marklund et al., respectively (Supplementary material) [11–14].

2.3.2.3. Data analysis and statistics. Statistical analyses were performed with the computer software Graph Pad Prism 6.0 (Graph Pad Software, Inc.). All data are expressed as mean ± S.E.M. Following significant two way ANOVA, post-hoc analysis was done by the Dunnett's test, a P value of $P < 0.05$ was considered significant. $P < 0.05$ is denoted by *, $P < 0.005$ is denoted by **, $P \leq 0.001$ is denoted by ***, and $P < 0.0001$ is denoted by ****.