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Phytic Acid Mitigates Motor Asymmetry in Male Rat with Unilateral 6-Hydroxydopamine Striatal Lesion

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ABSTRACT

Background and Objective: Parkinson's disease (PD) is a movement disorder with debilitating symptoms. Available treatments for PD mainly include its symptomatic relief with no prevention of its progression. Due to the iron-chelating and antioxidant effect of phytic acid (PA), this study was conducted to assess its protective effect in 6-hydroxydopamine-induced model of PD in rat.

Materials and Methods: Unilateral intrastriatal 6-OHDA-lesioned rats were daily pretreated with PA *p.o.* at a dose of 100 mg/kg for three days till 1 h pre-surgery. Apomorphine-induced rotations were counted at 1st week post-surgery.

Results: A significant contralateral rotational behavior was observed in 6-OHDA-lesioned rats ($p<0.0005$) and PA pretreatment significantly reduced it ($p<0.05$).

Conclusion: These findings imply that pre-lesion PA treatment could attenuate motor asymmetry in experimental model of PD and this may be of benefit in lessening motor derangements in afflicted patients.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative condition with debilitating clinical symptoms like bradykinesia, resting tremor, rigidity, postural imbalance and walking impairment (1). These motor function abnormalities are accompanied with a lower life quality (2). Global incidence of PD is about 0.3% in the general population and 1%-2% in those elder than 60-65 years (2). Behavioral and cognitive problems like dementia, depression, anxiety, and sleep disturbances appear in end-stage PD (3, 4). Available treatment strategies for PD are mainly symptomatic and usually begins with gold standard levodopa and/or dopamine agonists (5). However, after some time, most PD patients experience some complications like motor and non-motor fluctuations and dyskinesia (6). The neurotoxin 6-hydroxydopamine (6-OHDA) is used to damage mesencephalic dopaminergic neurons and to produce animal model of PD (7). Because environmental factors are involved in some cases of PD, it is important to evaluate the effectiveness of natural products in neuroprotective strategies for PD (8). Meanwhile, PD patients often turn to complementary and alternative medicine (9). Phytic acid (PA) is the main phosphorus storage form in plant seeds (10).

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Phytic acid, also known as myo-inositol hexaphosphate, has been shown to lower blood glucose levels and to improve insulin sensitivity in rodents (11). Phytic acid (PA) has been reported to have positive nutritional benefits and prevent cancer formation (12). PA has protective effect against ethanol metabolism-induced oxidative insult in living cells by blocking ROS production and elevating antioxidant potentials (13).

Iron plays an important role in PD because it can leads to oxidative stress-dependent neurodegeneration, the iron chelator PA protects against 6-OHDA-induced apoptosis in immortalized rat mesencephalic dopaminergic cells under normal and iron-excess conditions (14). In addition, protective effect of naturally occurring iron chelator PA on 1-methyl-4-phenylpyridinium-induced cell death in immortalized rat mesencephalic/dopaminergic cells has already been established (15). Meanwhile, PA has been reported as a viable treatment option for Alzheimer's disease (AD) (16). For these reasons, PA may be regarded as a suitable candidate and as an effective treatment against PD. Thus, this study was conducted to evaluate the beneficial effect of pre-lesion PA treatment on motor asymmetry in 6-OHDA-induced early model of PD in rat.

2. Materials and Methods

2.1. Animals

Adult male Wistar rats, weighing 210-260 g (n=32) (procured from Pasteur's Institute, Tehran) were housed in a temperature-controlled colony room under light/dark cycle with free access to food and water. The used procedures for animals and their care were according to NIH guidelines. The animals were held in the colony room for at least one week before being tested. Only rats not showing any rotational behavior (net rotations less than 30/hour) following intraperitoneal injection of apomorphine hydrochloride (2 mg/kg) were selected for the present study. The animals were randomly divided into six groups: sham-operated, PA-treated sham-operated group, lesion group (6-OHDA) and PA-treated lesioned groups. Unilateral intrastriatal 6-OHDA injection (left side) was performed through a 5 microliter Hamilton syringe on anesthetized rats (chloral hydrate 300-350 mg/kg, i.p.) using stereotaxic apparatus (Stoelting, USA) at the coordinates: L –3 mm, AP +9.2 mm, V +5 mm from the center of the interaural line, according to the atlas of Paxinos and Watson (17). At the end of injection, the needle was left in place for an additional 5 min and then withdrawn at a rate of 1 mm/min. The lesion group received a single injection of 5 microliter of 0.9% saline containing 2.5 microg/microliter of 6-hydroxydopamine-HCL and 0.2% ascorbic acid (W/V) at a rate of 1 microliter/min. The sham group received an identical volume of ascorbate-saline solution. The 6-OHDA+PA group received the neurotoxin in addition to PA p.o. dissolved in normal saline at a dose of 100 mg/kg. PA was daily administered, three times before the surgery, with the last injection being 1 h pre-surgery. Dose of PA was chosen according to our pilot study.

2.2. Behavioral evaluation

The animals were tested for rotational behavior by apomorphine hydrochloride (2 mg/kg, i.p.) one week before surgery (baseline) and at 1st week post-surgery. The rotations were measured according to a method as described previously (7). Briefly, the animals were allowed to habituate for 10 min and then 1 min after the injection, full rotations were counted in a cylindrical container (a diameter of 33 cm and a height of 35 cm) at 10-min intervals for 60 min in a dimly-lighted and quiet room. Net number of rotations was defined as the positive scores minus the negative scores.

2.3. Statistical analysis

All data were expressed as means ± standard error. For statistical evaluation of data, the parametric one-way ANOVA test followed by Tukey's *post-hoc* test was used. In all analyses, the null hypothesis was rejected at a level of 0.05.

3. Results

The effect of PA at a dose of 100 mg/kg was evaluated on apomorphine-induced rotations for a period of 1 hour (Fig. 1). There were no significant differences among the groups at

baseline (before surgery). Statistical analysis of the total net number of rotations at 1st week post- surgery showed that apomorphine caused a very significant contralateral turning in the rats of 6-OHDA-lesioned group ($p<0.0005$) and induced less significant rotations in 6-OHDA+PA ($p<0.005$) in comparison with Sham group. Moreover, the group 6-OHDA+PA showed a significant reduction of rotations ($p<0.05$) when compared to 6-OHDA-lesioned rats. Meanwhile, PA pretreatment of sham group did not produce a significant effect.

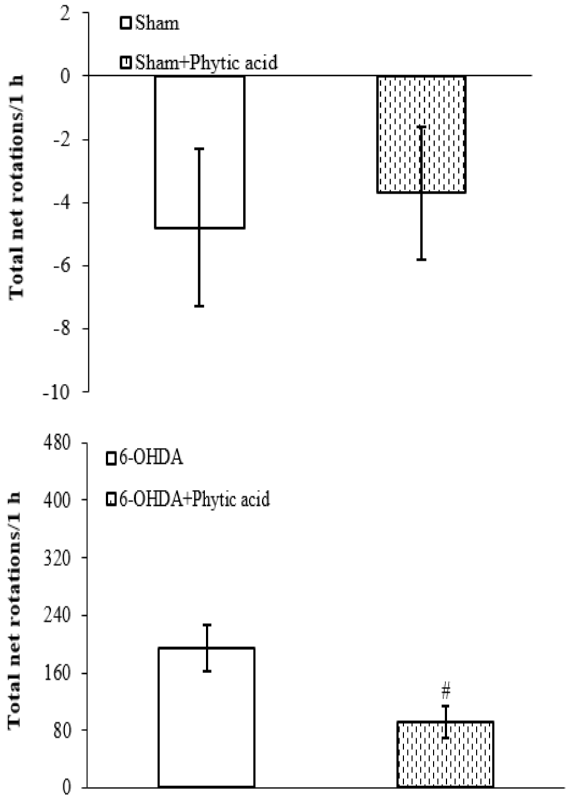

Fig. 1: Total net number of apomorphine-induced rotations/1 h after 1 week in sham (left panel) and 6-OHDA (right panel) groups. The positive values indicate contralateral rotations. 6-OHDA stands for the neurotoxin 6-hydroxydopamine. # $p<0.05$ (versus 6-OHDA)

4. Discussion

In this study, we confirmed that oral PA pretreatment at a dose of 100 mg/kg significantly reduces apomorphine-induced rotations and motor asymmetry in 6-OHDA-lesioned rats.

The selective degeneration of dopaminergic neurons in PD patients is due to a genetically and/or environmentally-induced neurodegenerative process (18). Meanwhile, the neurotoxin 6-OHDA which is usually used for PD induction in rodents, is assumed to cause selective degeneration of dopaminergic neurons of mesencephalon (19). The unilateral damage of the nigrostriatal dopaminergic system through intrastriatal injection of 6-OHDA is followed by a reduction in the striatal dopamine level and an upregulation of dopaminergic postsynaptic receptors at the same side. These changes produce a prominent functional and motor asymmetry that can be evaluated by direct-acting dopaminergic agonists like apomorphine (20). These rotations are considered as reliable indicators of nigrostriatal dopamine depletion (21). In this research study, a significant attenuation of the apomorphine-induced rotational behavior was noted in PA-pretreated 6-OHDA-lesioned rats. The observed attenuation of rotational behavior in this group may be due to the neuroprotective effect of PA against SNC neurodegeneration and maintenance of striatal dopamine at a level that is not accompanied with a marked rotational behavior. In other words, nigrostriatal neurons within SNC were mainly preserved in the presence of PA against neurodegenerative effects induced by the neurotoxin 6-OHDA, which itself needs further investigation.

In addition, overproduction of free radicals, especially reactive oxygen species is also involved in 6-OHDA-induced neurodegeneration (21). Oxidative stress is an important factor that could affect the survival of dopaminergic neurons in PD. Neurons mostly depend on energy produced by mitochondria and are simultaneously faced with high levels of reactive oxygen species as well as increased levels of free iron, which can promote hydroxyl production (22). Overload of the free radical formation may lead to cell death. In addition, auto-oxidation of dopamine may produce dopamine quinine (23). Free radical scavengers may also be helpful in prolonging survival time of dopaminergic neurons (24). In this respect, PA could attenuate neuronal damage and loss through counteracting oxidative stress (13).

In summary, these findings imply that pre-lesion PA treatment could attenuate motor asymmetry in experimental model of PD and this may be of benefit in lessening motor derangements in afflicted patients.

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