

The Effect of Sesamin on Motor Asymmetry in Intrastratial 6-Hydroxydopamine Rat Model of Parkinson's Disease

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Article info

Received: 1 July 2015
Revised: 8 Aug 2015
Accepted: 15 Aug 2015

Key Words:

Sesamin
6-hydroxydopamine
Rotational behavior
Motor asymmetry

ABSTRACT

Background and Objective: Parkinson's disease (PD) is an age-related neurodegenerative disease with selective damage of dopaminergic neurons of the mesencephalon. L-dihydroxyphenylamine (L-DOPA) therapy is currently the gold standard maneuver for PD. Due to the protective, anti-inflammatory, and antioxidant effect of sesamin, this study was undertaken to assess dose-dependent effect of this agent on motor asymmetry induced by intrastratial injection of 6-hydroxydopamine in the rat.

Materials and Methods: In this experimental research, male Wistar rats (n=48) were equally divided into sham, sesamin20-treated sham, 6-hydroxydopamine (OHDA)-lesioned, sesamin10 and sesamin20-treated lesion groups, and sesamin20-treated lesion group receiving the estrogenic antagonist fulvestrant. Model of PD was induced by microinjecting 12.5 microgram of 6-OHDA dissolved in normal saline-ascorbate solution into the left striatum. Treated lesion groups received sesamin at doses of 10 or 20 mg/kg/day started one week till 1 h before the surgery. After 1 week, ipsilateral and contralateral rotations induced by apomorphine were counted and net scores were obtained.

Results: In the 6-OHDA-lesioned group, the dopaminergic agonist apomorphine induced contralateral rotational behavior ($P<0.001$) as compared to sham. In addition, administration of sesamin at both doses (10 and 20 mg/kg) significantly reduced the number of contralateral rotations ($P<0.01$) versus 6-OHDA group and fulvestrant attenuated this effect.

Conclusion: Sesamin administration at doses of 10 and 20 mg/kg could reduce motor asymmetry and attenuate forced biased rotational behavior in 6-OHDA-induced model of PD and part of this effect is possibly via an estrogenic pathway.

1. Introduction

Parkinson's disease (PD) is a rather common neurodegenerative disorder with progressive nature and the most common movement disorder characterized with degeneration of nigrostriatal dopaminergic neurons within basal ganglia leading to movement

abnormalities like tremor, bradykinesia, rigidity, and postural imbalance (1). The main neuropathological hallmark of this disease is the selective degeneration of the nigrostriatal dopaminergic neurons within substantia nigra pars compacta (SNc) (2, 3).

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The neurotoxin 6-hydroxydopamine (6-OHDA) is generally used for induction of the degeneration of dopaminergic neurons and modeling of PD in rodents like rat (4).

Following 6-OHDA injection, some behavioral, biochemical, and pathological hallmarks of PD are observed (5). The toxic and deteriorating effect of 6-OHDA are due to enhanced oxidative stress, inflammatory processes and induction of apoptosis (6). Mitochondrial dysfunction and increased oxidative stress burden are also responsible for neuronal loss in patients with PD (7). Although great achievements have been made in the development and innovation of novel agents for treatment of PD, until now, no pharmacological agent has satisfactorily had the ability to stop or slow the progression of PD pathogenic mechanism (8).

Sesamin is a major lignan constituent of *Asiasari Radix* (*Asiasarum heterotropoides* F. Maekawa var. *mandshuricum* F. Maekawa, *Aristolochiaceae*), which is currently used as a herbal medicine for antipyretics, antitussives, and analgesics. Sesamin has shown cholesterol-lowering, lipid-lowering, anti-inflammatory and anti-cancer effects (9). It has also a protective effect on hypoxia-induced cell death in PC12 cells and BV-2 microglia cells (10). Sesamin has also been shown to prevent the loss of dopaminergic neuronal cells in the striatal regions of an MPTP-induced parkinsonian rat model (11). However, the pharmacological effects of sesamin on neurodegenerative disorders is not understood. The present study tried to investigate dose-dependent effect of sesamin on motor asymmetry induced by intrastriatal injection of 6-hydroxydopamine in the rat.

2. Materials and Methods

2.1. Animals

Adult male Wistar rats (190-240 g; n= 48) were obtained from Pasteur's Institute of Tehran and kept in a temperature-controlled room with food and water freely available. The used protocols were according to NIH guidelines for the care and use of laboratory animals. Only rats not showing any rotational behavior less than 30/hour following intraperitoneal injection of apomorphine (2 mg/kg) were chosen for the present study. The animals were randomly allocated to 6 groups, i.e. sham-operated group

(sham), sesamin20-treated sham, 6-OHDA-lesioned group (6-OHDA), sesamin10- and sesamin20-treated lesioned groups, and sesamin20-treated lesioned groups receiving the estrogenic antagonist fulvestrant (i.c.v. at a dose of 10 microg). Unilateral intrastriatal 6-OHDA (Sigma Chemical, USA) injection (left side) was done via a 5µl Hamilton syringe on anesthetized rats (a combination of ketamine 80 mg/kg and xylazine 10 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. 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 5µl of 0.9% saline containing 2.5µg/µl of 6-hydroxydopamine-HCL (6-OHDA, Sigma Chemical, USA) and 0.2% ascorbic acid. The sham group received an identical volume of ascorbate-saline solution. The treated 6-OHDA groups received the neurotoxin in addition to sesamin dissolved in cremophor at doses of 10 or 20 mg/kg started one week before the surgery till 1 h pre-surgery.

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 after 1 week. Briefly, the animals were allowed to habituate for 10 min and then 1 min after the injection, full rightward and leftward rotations were counted in a cylindrical container (a diameter of 33 cm and a height of 35 cm) for 1 h 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 mean \pm S.E.M. For the drug-induced rotational behavior, one-way ANOVA 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 beneficial effect of sesamin at two doses of 10 and 20 mg/kg was studied on apomorphine-induced rotational bias for 1 hour (Fig. 1). There were no significant differences amongst the groups at baseline (before surgery). Statistical analysis of the total net number of rotations one

week post-surgery showed that apomorphine caused a very significant contralateral turning in the rats of 6-OHDA group ($P < 0.001$) and induced less significant rotations in 6-OHDA+Sesamin10 and 6-OHDA+Sesamin20 groups ($P < 0.005$) in comparison with sham group and the observed response for 6-OHDA+Sesamin10 and 6-OHDA+Sesamin20 groups were significantly attenuated versus 6-OHDA group ($P < 0.01$). In addition, fulvestrant diminished this significant difference.

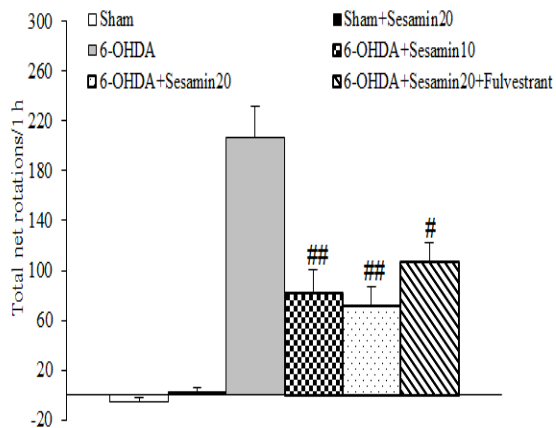


Fig. 1: Total net number of rotations (mean \pm S.E.M.) induced by apomorphine (2 mg/Kg, i.p.) 1 week after surgery over a period of 1 h in 6-OHDA-lesioned group. Note that the positive values indicate contralateral rotations. 6-OHDA stands for the neurotoxin 6-hydroxydopamine. Sesamin was used at doses of 10 or 20 mg/kg. # $p < 0.05$, ## $p < 0.01$ (versus 6-OHDA)

4. Discussion

In this study, we demonstrated that sesamin at doses of 10 and 20 mg/kg could significantly attenuate motor asymmetry in rat PD model.

The selective degeneration of SNC dopaminergic neurons is likely to be due to direct toxicity effect in PD patients (12, 13). In addition, the neurotoxin 6-OHDA is commonly used for the induction of PD in experimental animals and could cause degeneration of dopaminergic neurons (14). 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 dopaminergic agonists like apomorphine (15).

The observed attenuation of rotational behavior in sesamin-treated 6-OHDA group could be due to its possible neuroprotective effect 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 this agent against neurodegenerative effects induced by the neurotoxin 6-OHDA.

Oxidative stress is strongly involved in the toxicity of 6-OHDA-induced nigrostriatal lesions (16). 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 (ROS) as well as increased levels of free iron, which can promote OH generation (17). Overload of the free radical formation may lead to cell death. In addition, auto-oxidation of dopamine may produce dopamine quinone (18). Formation of species such as semiquinones and other free radicals could especially damage nucleic acids, proteins, and membrane lipid components (19). Therefore, the therapeutic approaches are aimed at attenuation of oxidative stress. In addition, free radical scavengers may also be helpful in prolonging survival time of dopaminergic neurons (20). Sesamin has reported to attenuate neuronal damage and loss through counteracting oxidative stress, possibly via regulating antioxidant defense system as well as inhibition of free radical generation (21, 22).

Overall, the results of our study indicate that administration of sesamin at doses of 10 and/or 20 mg/kg could reduce motor asymmetry and attenuate forced biased rotational behavior in 6-OHDA-induced model of PD, possibly via an estrogenic pathway. However, further research studies are warranted to understand its exact mode of action.

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