The effect of simvastatin in prevention of histological changes of substantia nigra and behavioral abnormalities in an experimental model of Parkinson's disease in rat

Ladan Habibi-Nikakhlagh [1], **Sima Nasri** [1*], **Mehrdad Roghani** [3]

1. Department of Biology, Payame Noor University,Tehran, Iran.
2. Neurophysiology Research Center, Shahed Univeresity, Tehran, Iran.

Article info:
Received: 26April 2013
Revised: 24 May 2013
Accepted: 05 July 2013

A B S T R A C T

Background and Objective: Parkinson's disease (PD) is a rather common neurological disorder in elders that is due to degeneration of dopaminergic neurons within mesencephalic substantia nigra pars compacta. With regard to protective and antioxidant effect of simvastatin, this study was conducted to evaluate its neuroprotective effect in an experimental model of PD.
Materials and Methods: In this experimental study, male rats (n =40) were divided into 5 groups, i.e. sham-operated, simvastatin20-treated sham-operated, lesioned and simvastatin10 and simvastatin20-treated lesioned groups. The hemi-PD early model was induced by unilateral intrastriatal injection of 5 microliter of saline-ascorbate (left side) containing 12.5 microgram of 6-hydroxydopamine (6-OHDA).Treated sham and lesioned groups received simvastatin i.p. at doses of 10 and 20 mg/kg once a day before surgery for two times at an interval of 24 h. Two weeks after surgery, the animals were tested for rotational behavior by apomorphine for an hour and the number of dopaminergic neurons in the substantia nigra pars compacta (SNC) was counted.
Results: Two weeks after surgery, apomorphine caused a significant contralateral turning ($P<0.0001$) in 6-OHDA-lesioned group and a reduction in the number of neurons on the left side of the SNC in the lesioned group was observed in comparison with sham group ($P<0.01$). In addition, simvastatin pretreatment at both doses significantly decreased the rotational behavior in lesioned rats ($p<0.05$ and $p<0.01$, respectively) and also at a higher dose significantly attenuated the reduction in the number of SNC neurons ($p<0.05$). On the other hand, pretreatment of sham group with simvastatin had no significant effect on the number of apomophine-induced rotations and neurons of SNC.
Conclusion: Intraperitoneal administration of simvastatin exhibits neuroprotective effect against 6-OHDA toxicity in an experimental model of PD, as was shown by a lower rotational behavior and attenuation of neuronal loss.

Key Words:
Simvastatin
Parkinson's disease
6-hydroxydopamine
Rotational behavior
Apomorphine
Substantia nigra

1. Introduction

Parkinson's disease (PD) is regarded as a neurological and debilitating disease, which involves the neurodegeneration of dopaminergic neurons within the substantia nigra pars compacta, with the subsequent loss of their terminals within the striatum. The ensuing loss of the neurotransmitter dopamine leads to the debilitating motor disturbances in PD (1). Oxidative stress and increased lipid peroxidation, low glutathione level, damage to DNA and iron accumulation are reported as the main pathogenic causes of dopaminergic neurons degeneration in PD (2). Oxidative stress not only damages the

[*]**Corresponding Author:**
Sima Nasri
Department of Biology, Payame Noor University, P.O. Box: 19395-3697, Tehran, Iran.
Email: nasri@yahoo.com

dopaminergic neurons, but it also endangers mitochondrial oxidative phosphorylation, leading to decreased energy output by these organelles and eventually to subsequent death of these cells (3). Despite great achievements in innovation of chemicals to treat PD, none yet address the underlying problem associated with it, i.e. the progressive and gradual degeneration of dopaminergic neurons (4).

Statins are known as inhibitors of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase that could inhibit cellular synthesis of cholesterol and isoprenoids. Statins have been known for their potency in reducing serum cholesterol level and prevention of cardiovascular disorders, while growing evidence has shown the efficacy of statins in treating neurodegenerative diseases and in conditions of brain injury. The neuroprotective and beneficial effects of statins is related to their properties such as endothelial protection, anti-oxidant, and anti-inflammatory effects. In this respect, statins could suppress inflammatory processes following brain insult and inhibit cytokines release in neurological disorders (5-7). Simvastatin is a statin with neuroprotective and antioxidant activity in in vitro and in vivo environments (8, 9). Therefore, this study was carried out to investigate the possible neuroprotective potential of simvastatin administration in an early model of Parkinson's disease in rat.

2. Materials and Methods

Adult male Wistar rats (220-280 g; n = 40) (Pasteur's Institute, Tehran, Iran) were housed three to four per cage in a temperature-controlled colony room under light/dark cycle with food and water available ad libitum. Procedures involving animals and their care were conducted in conformity with NIH guidelines for the care and use of laboratory animals. The animals were held in the colony room for at least one week before being tested. Only rats not showing any biased 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 five groups: sham-operated group, simvastatin-treated sham-operated group (at a dose of 20 mg/kg), lesion group, and two simvastatin-treated lesion groups (at doses of 10 and 20 mg/kg). Unilateral intrastriatal 6-OHDA injection (left side) was performed through a 5 µl Hamilton syringe on anesthetized rats (ketamine 100 mg/kg and xylazine 5 mg/kg, i.p.) using stereotaxic apparatus (Stoelting, USA) at the coordinates: L –3 mm, AP +9.2 mm, V + 4.5 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 5 µl of 0.9% cold saline containing 2.5 µg/µl of 6-hydroxydopamine-HCL (6-OHDA, Sigma) and 0.2% ascorbic acid (W/V). The sham group received an identical volume of ascorbate-saline solution. Simvastatin was administered at doses of 10 and 20 mg/kg for two days presurgery. Simvastatin was dissolved in propylene glycol.

2.1. Behavioral testing

The animals were tested for rotational behavior by apomorphine hydrochloride (2 mg/kg, i.p.) one week before (baseline) and two weeks after the surgery. The rotations were measured according to a method as described previously. Briefly, the animals were allowed to habituate for 10 min and then 1 min after the drug 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 quiet isolated room. Net number of rotations was defined as the positive scores minus the negative scores.

2.2. Histological study

Five animals in each group were used for histological assessment. Following behavioral experiment, the rats were deeply anesthetized with a high dose of ketamine (150 mg/kg) and perfused through the ascending aorta with 50 ml of 0.9% saline followed by 150 ml of fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) followed by 100 ml of 0.1 M PB containing 10% sucrose. Following perfusion, the brains were removed from the skull, the blocks of forebrain and brainstem were prepared, and after final steps of preparation (30% sucrose for 2 days), sections were cut at a thickness of 30 µm on a freezing microtome (Leica) and collected in PB (0.1 M). Every second section was Nissl-stained with

0.1% cresyl violet (Sigma).

2.3. Neuronal counting

For each animal, mesencephalic sections (Interaural 2.9-4.2 mm) were examined by a method as described previously. Briefly, Nissl-stained neurons of the SNC were counted (Light microscopy; X200) using a superimposed grid to facilitate the procedure. At least two sections representative of each of four Paxinos-Watson planes (4.2, 3.8, 3.2, 2.97; Interaural) were examined by scanning the entire extent on each side. Counting was done blind to the treatments received.

2.4. Statistical analysis

All data were expressed as mean ± S.E.M. For rotational behavior, one-way ANOVA followed by Tukey post hoc test was used. For each group, the values of Nissl-stained cells for the injected and non-injected sides were compared using two-tailed student's t-test for paired samples and the inter-group differences were analyzed using one-way ANOVA followed by Tukey's post-hoc test. In all analyses, the null hypothesis was rejected at a level of 0.05.

3. Results

The beneficial effect of simvastatin was evaluated on apomorphine-induced rotations for a period of 1 hour (Table 1). There were no significant differences among the sham groups. Statistical analysis of the total net number of rotations made over a 60-min period (Figure 1) in lesioned groups showed that apomorphine caused a very significant contralateral turning in the rats of 6-OHDA group ($p<0.001$) and induced lower rotations in 6-OHDA+simvastatin10 ($p<0.05$) and 6-OHDA+simvastatin20 ($p<0.01$) in comparison with 6-OHDA group.

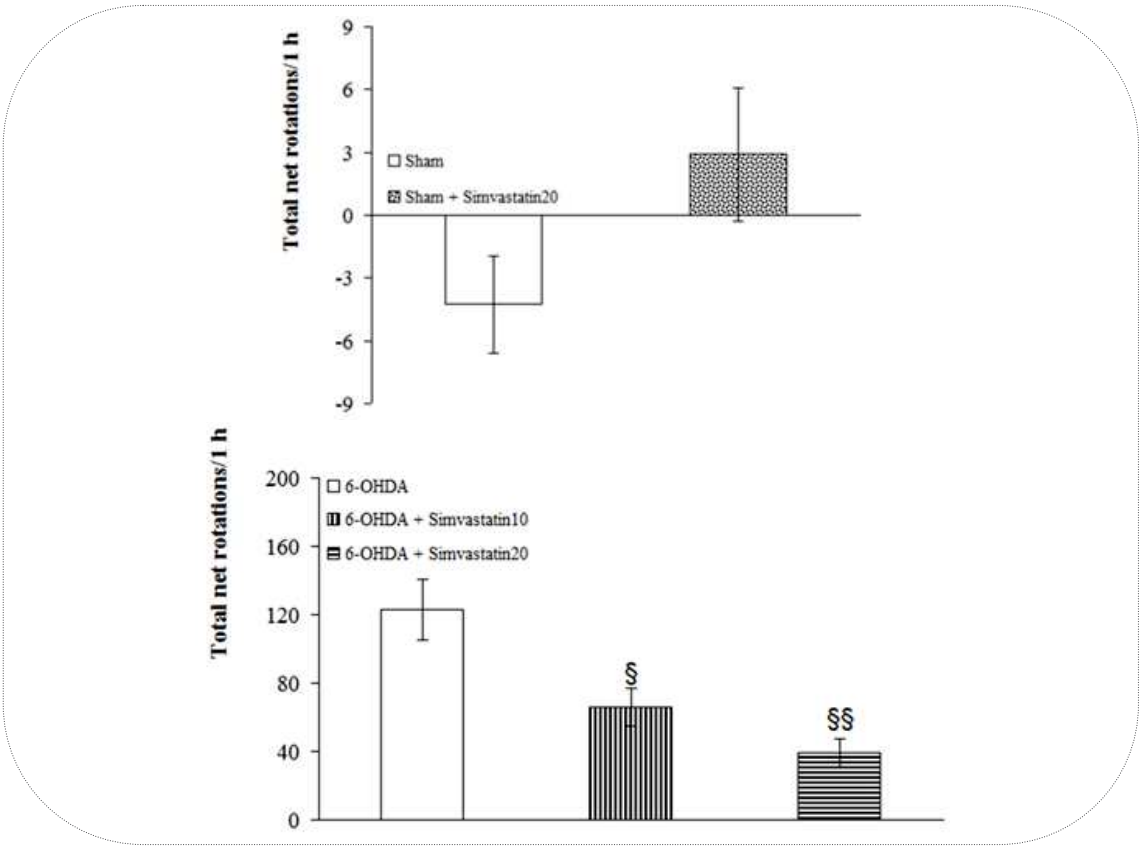

Figure 1. Total net number of rotations (mean ± S.E.M.) induced by apomorphine (2 mg/Kg, i.p.) after 1 week over a period of 60 min in sham (upper panel) and 6-OHDA-lesioned (lower panel) groups. Note that the positive values indicate contralateral rotations. 6-OHDA stands for the neurotoxin 6-hydroxydopamine. $ $p<0.05$, $$ $p<0.01$ (versus 6-OHDA)

The results of histochemical studies (Figure 2) showed that there is no significant difference for the number of Nissl-stained neurons between the two sham groups, a significant reduction was observed for 6-OHDA group (P<0.01) and 6-OHDA+simvastatin10 group (p<0.05), and no such difference was obtained for 6-OHDA+simvastatin20 group. Meanwhile, in the latter group, the number of neurons was significantly higher than 6-OHDA group (p<0.05).

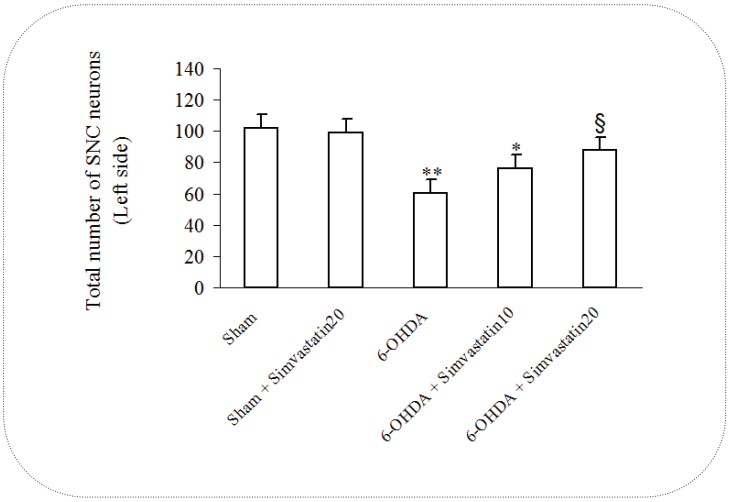

Figure 2. Total number of Nissl-stained neurons on the left side of substantia nigra pars compacta (SNC) in different groups after 1 week post-surgery. 6-OHDA stands for the neurotoxin 6-hydroxydopamine.
* p<0.05, ** p<0.01 (in comparison with Sham)
$ p<0.05 (in comparison with 6-OHDA)

4. Discussion

According to literature, unilateral damage of the nigrostriatal dopaminergic system through intrastriatal injection of neurotoxins like 6-OHDA is associated with 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 (apomorphine) and indirect-acting (amphetamine) dopaminergic agonists (2). These rotations, especially those induced by apomorphine are considered as reliable indicators of nigrostriatal dopamine depletion (10). In the present study, a significant attenuation of the apomorphine-induced rotational behavior was observed in simvastatin-pretreated 6-OHDA-lesioned group after 1 week. The observed attenuation of rotational behavior in simvastatin-pretreated lesioned group in the present study could be attributed to possible protective effect of this agent against nigral neurodegeneration and maintenance of striatal dopamine at a level that is not accompanied with a marked turning behavior. On the other hand, nigrostriatal neurons within SNC were mainly preserved against neurodegenerative effects induced by the neurotoxin 6-OHDA. In this respect, it has been reported that reactive oxygen radicals are involved in the toxicity of 6-OHDA-induced nigrostriatal lesions that is used as an experimental model of unilateral Parkinsonism (2).

Oxidative stress is considered an important pathogenic factor that could affect the survival of dopaminergic neurons in PD. Neuronal cells 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 formation (11). Overload of the free radical formation may lead to cell death. In addition, auto-oxidation of dopamine or levodopa overdosing may produce dopamine quinine (12).

Formation of species such as semiquinones and other free radicals could especially damage nucleic acids, proteins, and membrane lipid components (13). Therefore, the therapeutic approach is aimed at attenuation of oxidative stress. In addition, free radical scavengers may also be helpful in prolonging survival time of dopaminergic neurons (14,15). In this respect, simvastatin may have attenuated neuronal damage and loss through counteracting oxidative stress in this study, as has been reported before (6-8).

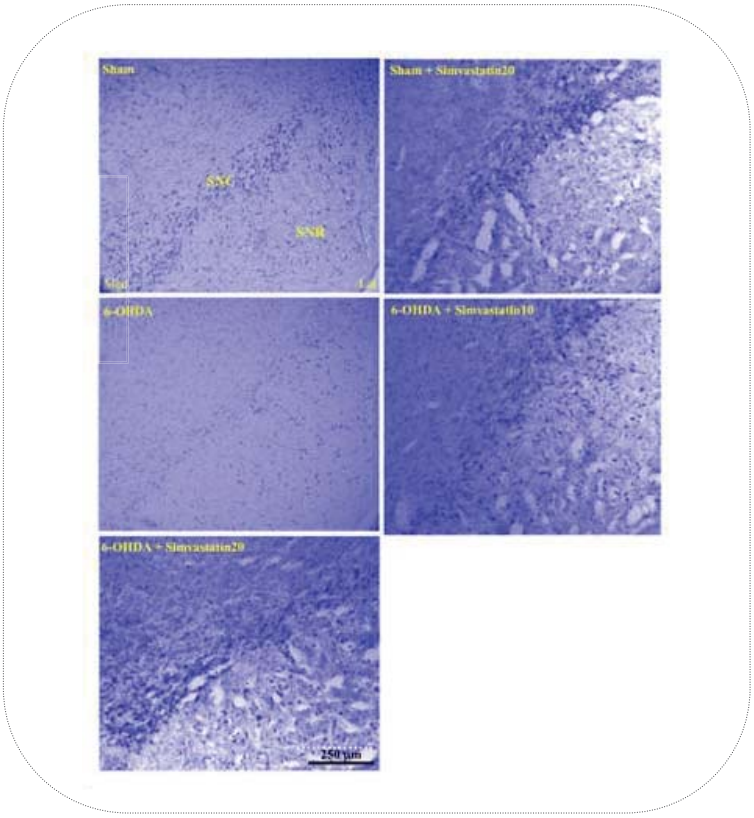

Figure 3. Photomicrograph of coronal sections through the midbrain showing Nissl-stained neurons in experimental groups. A severe reduction in the number of neurons in SNC was observed in the 6-OHDA group, but no such marked decrease was noted in the simvastatin20-treated 6-OHDA group in comparison with 6-OHDA. Scale bar = 250 µm (SNC and SNR = Substantia nigra pars compacta and pars reticulate, respectively)

To conclude, this study showed that intraperitoneal administration of simvastatin exhibits neuroprotective effect against 6-OHDA toxicity in an experimental model of PD, as was shown by a lower rotational behavior and attenuation of neuronal loss and this may be put forward as a novel adjuvant treatment for early PD in clinical settings.

Acknowledgement

This work was supported by a grant from Payame Noor University, Tehran, Iran.

References

1. Sauer H, Oertel WH: Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: a combined retrograde tracing and immunocytochemical study in the rat. Neuroscience 1994; 59: 401-5.

2. Schwarting RKW, Huston JP: The unilateral 6-hydroxydopamine lesion model in behavioral brain research: analysis of functional deficits, recovery and

treatments. Progress in Neurobiology 1996; 50: 275-331.

3. Dauer W, Przedborski S: Parkinson's disease: mechanisms and models. Neuron 2003; 39: 889-909.

4. Wu SS, Frucht SJ: Treatment of Parkinson's disease: what's on the horizon? CNS Drugs 2005; 19: 723-43.

5. Sun J, Xie C, Liu W, Lu D, Qiao W, Huang Q, et al. The effects of simvastatin on hippocampal caspase-3 and Bcl-2 expression following kainate-induced seizures in rats. International Journal of Molecular Medicine 2012; 30: 739-46.

6. Kumar A, Sharma N, Gupta A, Kalonia H, Mishra J. Neuroprotective potential of atorvastatin and simvastatin (HMG-CoA reductase inhibitors) against 6-hydroxydopamine (6-OHDA) induced Parkinson-like symptoms. Brain Research 2012; 1471: 13-22.

7. Xie C, Sun J, Qiao W, Lu D, Wei L, Na M, et al. Administration of simvastatin after kainic acid-induced status epilepticus restrains chronic temporal lobe epilepsy. PLoS One. 2011; 6: e24966.

8. Yan J, Xu Y, Zhu C, Zhang L, Wu A, Yang Y, et al. Simvastatin prevents dopaminergic neurodegeneration in experimental parkinsonian models: the association with anti-inflammatory responses. PLoS One. 2011; 6: e20945.

9. Ghosh A, Roy A, Matras J, Brahmachari S, Gendelman HE, Pahan K. Simvastatin inhibits the activation of p21ras and prevents the loss of dopaminergic neurons in a mouse model of Parkinson's disease. Journal of Neuroscience 2009;29: 13543-56.

10. Shapiro RM, Glick SD, Camarota NA. A two-population model of rat rotational behavior: effects of unilateral nigrostriatal 6-hydroxydopamine on striatal neurochemistry and amphetamine-induced rotation. Brain Research 1987; 426: 323-31.

11. Foley P, Riederer P. Influence of neurotoxins and oxidative stress on the onset and progression of Parkinson's disease. Journal of Neurology 2000; 247: 82-94.

12. Lotharius J, Brundin P. Pathogenesis of Parkinson's disease: dopamine, vesicles and alpha-synuclein. Nature reviews. Neuroscience 2002; 3: 932-42.

13. Von Bohlen und Halbach O, Schober A, Krieglstein K. Genes, proteins, and neurotoxins involved in Parkinson's disease. Progress in Neurobiology 2004; 73: 151-77.

14. Chen S, Le W. Neuroprotective therapy in Parkinson disease. American Journal of Therapeutics 2006; 13: 445-57.

15. Cyr M, Calon F, Morissette M, Di Paolo T. Estrogenic modulation of brain activity: implications for schizophrenia and Parkinson's disease. Journal of Psychiatry and Neuroscience 2002; 27: 12-27.