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Research Report

Alpha-lipoic acid protects against 6-hydroxydopamine-induced neurotoxicity in a rat model of hemi-parkinsonism

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ABSTRACT

Parkinson's disease (PD) is a progressive and debilitating neurodegenerative disorder for which current treatments afford symptomatic relief with no prevention of disease progression. Due to the neuroprotective and anti-apoptotic potential of alpha lipoic acid (LA), this study was undertaken to evaluate whether LA could improve behavioral and cellular abnormalities and markers of oxidative stress in an experimental model of early PD in rat. Unilateral intrastriatal 6-hydroxydopamine (6-OHDA)-lesioned rats were pre-treated p.o. with LA at doses of 50 and/or 100 mg/kg twice at an interval of 24 h. After 1 week, apomorphine caused significant contralateral rotations, a significant reduction in the number of neurons was observed on the left side of the substantia nigra pars compacta (SNC), and malondialdehyde (MDA) and nitrite levels in midbrain homogenate significantly increased and activity of superoxide dismutase significantly reduced in the 6-OHDA group. LA pretreatment at a dose of 100 mg/kg significantly attenuated rotations, prevented loss of SNC neurons, and lowered levels of MDA and nitrite. These results suggest that LA could partially afford neuroprotection against 6-OHDA neurotoxicity that is in part due to the attenuation of oxidative stress burden and this may provide benefits, along with other therapies, in neurodegenerative disorders including PD.

1. Introduction

Parkinson's disease (PD) is the most common movement disorder (Covy and Giasson, 2011) that is characterized by debilitating motor abnormalities including rest tremor, muscle stiffness, paucity of voluntary movements, and postural instability (Garcia Ruiz et al., 2011). Primary neuropathological feature of PD is the progressive degeneration of the nigrostriatal dopaminergic neurons, whose cell bodies are in the substantia nigra pars compacta (SNC) and nerve terminals project to the neostriatum (Covy and Giasson, 2011; Stocchi and Marconi, 2010). The specific neurotoxin 6-hydroxydopamine (6-OHDA) is used to initiate degeneration of dopaminergic neurons and for induction of experimental model of PD in rodents (Roghani et al., 2010). Following 6-OHDA injection, some behavioral, biochemical, and pathological hallmarks of PD develop (Schober, 2004). The toxic

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effects of 6-OHDA are attributed to enhanced oxidative stress burden, inflammatory processes and apoptosis (Mu et al., 2009). Mitochondrial dysfunction and increased oxidative stress are also responsible for neuronal loss in patients with PD (Henchcliffe and Beal, 2008). Although great advances have been made in the development of novel agents to treat PD, to date, no pharmacological agent has convincingly demonstrated the ability to slow the progression of PD (Ybot-Gorrin et al., 2011). Also, some patients with PD receiving dopamine replacement therapy in the form of levodopa develop dyskinesia that becomes a major complication (Iravani and Jenner, 2011). Neuroprotection is an important alternative strategy for slowing PD progression and to be effective if begun at its early stages (Schapira, 2009).

Alpha-lipoic acid (LA) is a strong antioxidant with free radical scavenging activity (Connell and Saleh, 2012) and acting as a neuroprotective agent in some neurological disorders (De Araujo et al., 2011; Holmquist et al., 2007; Maczurek et al., 2008; Soczynska et al., 2008). LA is naturally found in mitochondria, involved in its metabolic machinery and is regarded as a promising candidate for the treatment of neurological diseases whose etiology is related to mitochondrial dysfunction and oxidative stress (Connell and Saleh, 2012; Zaitone et al., 2012). Neuroprotective (Zaitone et al., 2012) and anti-apoptotic (Abdin and Sarhan, 2011) effects of LA in rotenone-induced PD have recently been reported. Considering these impressive array of beneficial effects, the present study attempted to investigate the neuroprotective effect of LA in 6-OHDA rat model of hemi-parkinsonism and some underlying mechanisms.

2. Results

The beneficial effect of LA was evaluated on apomorphine-induced rotations for a period of 1 h (Fig. 1). There were no significant differences among the groups at baseline (before surgery). Statistical analysis of the total net number of rotations 1 week after the surgery showed that apomorphine caused a non-significantly lower contralateral turning in the 6-OHDA+lipoic acid 50 mg group and significantly lower rotations in 6-OHDA+lipoic acid 100 mg group ($p<0.05$) as compared to 6-OHDA+Cremophor group.

The results of histochemical studies (Figs. 2 and 3) showed that a significant reduction in the number of Nissl-stained neurons on the left side of SNC exists in 6-OHDA and 6-OHDA+Cremophor groups ($p<0.01$) versus Sham group and there were high and non-significant neuronal counts for 6-OHDA+lipoic acid 50 mg group and higher and significant neuronal counts for 6-OHDA+lipoic acid 100 mg ($p<0.05$) as compared to 6-OHDA+Cremophor group ($p<0.05$).

Regarding midbrain oxidative stress markers (Fig. 4), 6-OHDA injection resulted in significant elevation of MDA (as a marker of lipid peroxidation) ($p<0.01$) and nitrite content ($p<0.05$) and significant reduction of SOD activity ($p<0.05$) and treatment of 6-OHDA-lesioned rats with LA at a dose of 100 mg/kg significantly lowered MDA and nitrite content ($p<0.05$) with no significant improvement of SOD activity and pretreatment of 6-OHDA group with LA at a dose of 50 mg/kg did not have such a significant effect versus 6-OHDA+Cremophor group.

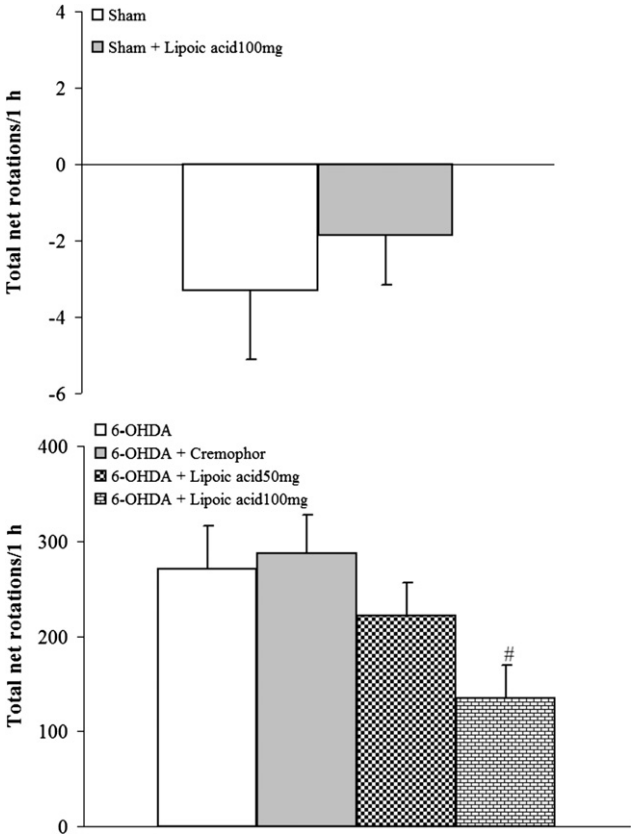

Fig. 1 – Total net number of rotations (mean ± S.E.M.) induced by apomorphine (2 mg/Kg, i.p.) after 1 week over a period of 60 min in Sham (upper panel) and 6-OHDA-lesioned (lower panel) groups. Note that the positive values indicate contralateral rotations. 6-OHDA stands for the neurotoxin 6-hydroxydopamine.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

3. Discussion

In this study, we demonstrated that LA at a dose of 100 mg/kg significantly decreases apomorphine-induced rotations, attenuates loss of SNC neurons and lowers midbrain levels of MDA and nitrite in 6-OHDA-lesioned rats.

The selective loss of dopaminergic neurons in the SNC appears to be the direct cause of neurodegeneration in patients with PD (Hattori, 2004; Schapira and Jenner, 2011). Also, 6-OHDA, which is commonly used for the induction of PD in animals, is believed to cause degeneration of dopaminergic neurons (Schober, 2004). 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 (Schwarting and Huston, 1997). These rotations are considered as reliable indicators of nigrostriatal dopamine depletion (Shapiro et al., 1987). In this study, a significant attenuation of the apomorphine-induced rotational behavior was observed in LA-pretreated 6-OHDA-lesioned group after 1 week.

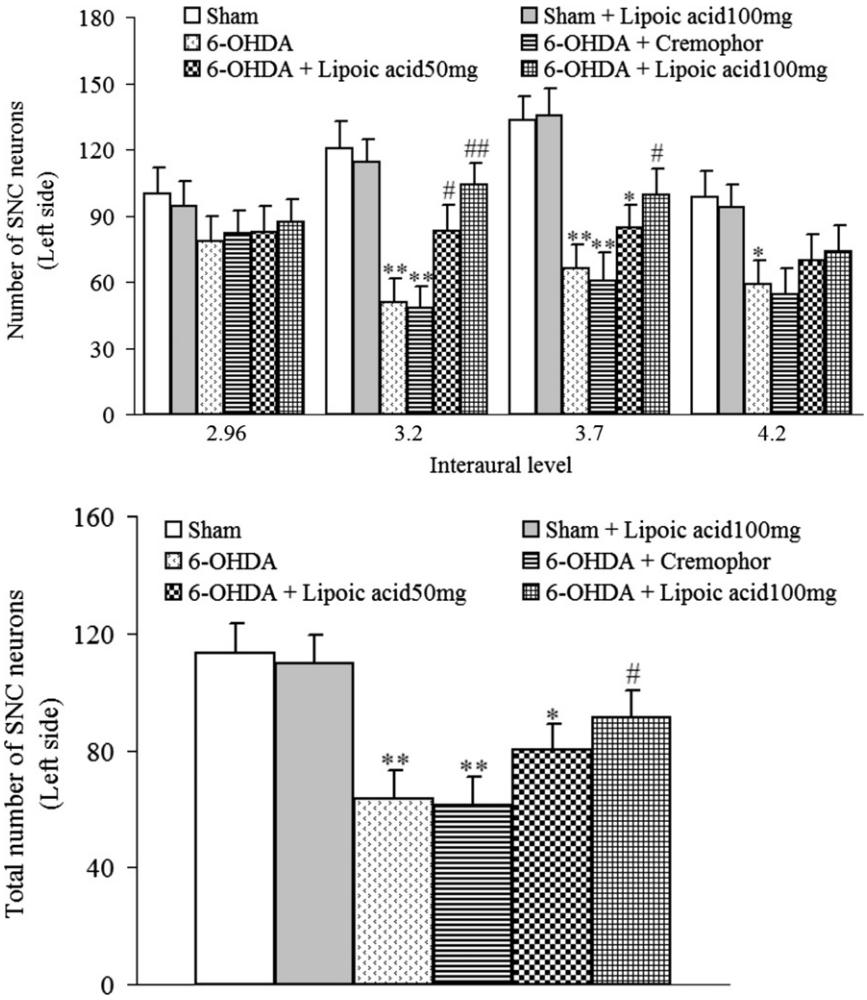

Fig. 2 – Total number of Nissl-stained neurons on the left side of substantia nigra pars compacta (SNC) at different interaural stereotaxic planes (upper panel) and its averaged number at all planes (lower panel) in different groups 1 week post-surgery. 6-OHDA stands for the neurotoxin 6-hydroxydopamine. $^{*}p<0.05$, $^{**}p<0.01$ (in comparison with Sham) $^{\#}p<0.05$, $^{\#\#}p<0.01$ (in comparison with 6-OHDA+Cremophor).

The observed attenuation of rotational behavior in LA-pretreated lesioned group could be attributed to possible protective effect of LA 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 LA against neurodegenerative effects induced by the neurotoxin 6-OHDA.

Free radicals are strongly involved in the toxicity of 6-OHDA-induced nigrostriatal lesions (Guo et al., 2007). Oxidative stress is an important factor that could affect the survival of dopaminergic neurons in PD. Neurons mostly depend on energy produced by the 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 (Foley and Riederer, 2000). Overload of the free radical formation may lead to cell death. In addition, auto-oxidation of dopamine may produce dopamine quinine (Lotharius and Brundin, 2002). Formation of species such as semiquinones and other free radicals could especially damage nucleic acids, proteins, and membrane lipid components (Von Bohlen und Halbach et al., 2004). 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 (Chen and Le, 2006). In this respect, LA could attenuate neuronal damage and loss through counteracting oxidative stress, possibly via regulating antioxidant defense system as well as inhibition of free radical generation (Connell and Saleh, 2012) Inflammation in the brain is another causative factor in the pathogenesis of PD (Miklossy et al., 2006; Zhou et al., 2007). Pro-inflammatory cytokines released from glial cells could stimulate nitric oxide production and exert a deleterious effect on dopaminergic neurons by activating receptors that contain intra-cytoplasmic death domains involved in apoptotic pathway (Sriram and O'Callaghan, 2007). It has been shown that LA has anti-inflammatory activity (Chaudhary et al., 2011) and inhibits lipopolysaccharide-induced inflammatory processes (Aly et al., 2009). It is possible that LA may have decreased the level of these inflammatory mediators within the brain, which itself contributes to neuroprotection in 6-OHDA induced PD in rats, as observed in our study. Apoptosis is another factor

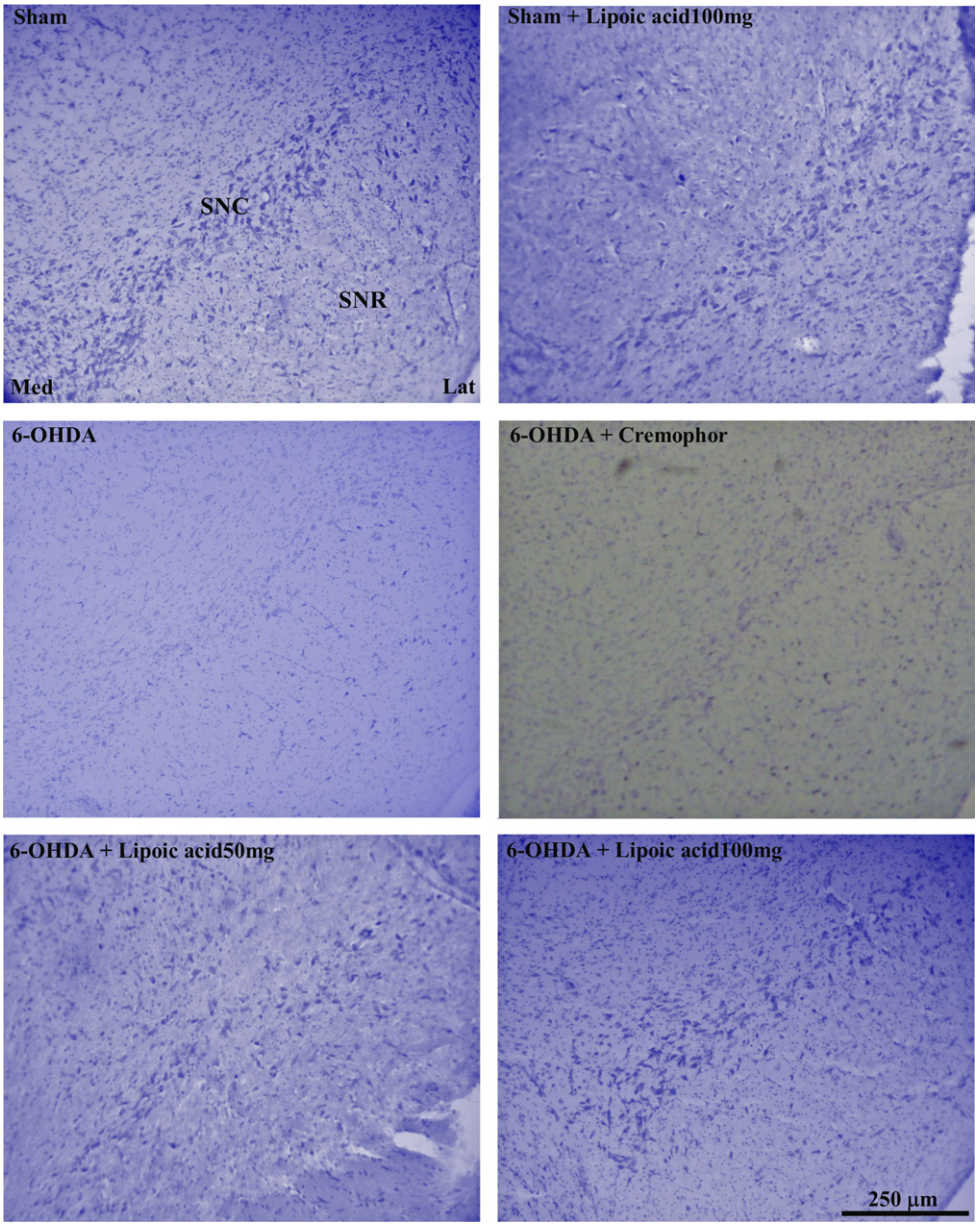

Fig. 3 – Photomicrograph of coronal sections through the midbrain showing Nissl-stained neurons in experimental groups. A severe reduction in the number of neurons in SNC was observed in the 6-OHDA lesioned group, but no such marked reduction was noted in the lipoic acid-treated lesioned groups in comparison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

that plays a critical role when cells are exposed to neurotoxins including 6-OHDA (Hwang and Chun, 2012). LA could suppress 6-OHDA-induced ROS generation and apoptosis through the stimulation of glutathione synthesis (Fujita et al., 2008). The results of another study has also suggested that LA protects PC12 cells from apoptosis induced by 6-OHDA through potentiating the antioxidant pathway (Yuan et al., 2001), and in this way counteracting the enhanced oxidative stress burden following 6-OHDA, as was observed by lower midbrain levels of MDA and nitrite in this study.

In this study, although we did not measure the level of LA in cerebrospinal fluid to ascertain the penetration of LA into the brain tissue, but there is some evidence showing LA could easily cross this barrier and sufficient concentration of LA is attained in the brain tissue following its systemic administration (Astiz et al., 2012; Pederzolli et al., 2010). In addition, it has been shown that LA is easily absorbed from the gastrointestinal tract, is able to cross the blood–brain barrier, and does not exhibit any serious side effects (Malinska and Winiarska, 2005). However, Chng et al. (2009) have shown that oral administration of LA at a dose of 50 mg/kg could barely cross this barrier and perhaps its possible effect at this dose may be due to its indirect effect which itself needs further investigation.

Overall, the results of our study clearly suggest that LA could afford neuroprotection against 6-OHDA neurotoxicity that is partially due to the attenuation of oxidative stress burden and this may provide benefits, along with other

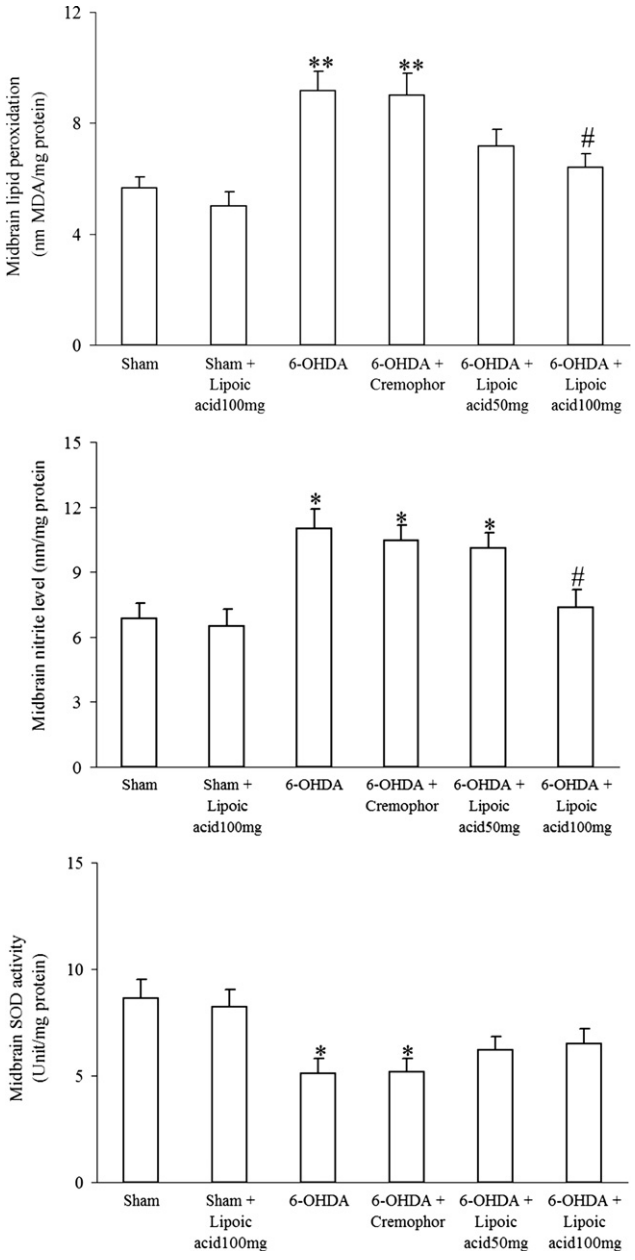

Fig. 4 – Malondialdehyde (MDA) content (top panel), nitrite content (middle panel) and superoxide dismutase (SOD) activity (bottom panel) in midbrain homogenate. 6-OHDA stands for 6-hydroxydopamine. $^{*}p<0.05$, $^{**}p<0.01$ (versus Sham) $^{\#}p<0.05$ (versus 6-OHDA+Cremophor).

therapies, in neurodegenerative disorders including PD. However, further studies are required to understand its basic mechanisms of action.

4. Experimental procedures

4.1. Animals

Adult male Wistar rats (190–255 g; $n=64$) (Pasteur's Institute, Tehran) were housed 3–4 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 h) following intraperitoneal injection of apomorphine hydrochloride (2 mg/kg) (Sigma Chemical, USA) were selected for the present study. The animals were randomly divided into six groups: Sham-operated group ($n=12$), lipoic acid-treated Sham-operated group ($n=8$; Sham+Lipoic acid 100 mg), lesion group ($n=12$; 6-OHDA), Cremophor-treated lesion group ($n=8$; 6-OHDA+Cremophor) and lipoic acid-treated lesion groups (6-OHDA+lipoic acid 50 mg and 6-OHDA+lipoic acid 100 mg, $n=12$ for each of them). Unilateral intrastriatal 6-OHDA (Sigma Chemical, USA) injection (left side) was performed through a 5 μl Hamilton syringe on anesthetized rats (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 +4.5 mm from the center of the interaural line, according to the atlas of Paxinos and Watson (1986). 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% saline containing 2.5 μg/μl of 6-hydroxydopamine-HCl (6-OHDA, Sigma Chemical, USA) and 0.2% ascorbic acid (W/V) at a rate of 1 μl/min. The Sham group received an identical volume of ascorbate–saline solution. The 6-OHDA+lipoic acid 50 mg and 6-OHDA+lipoic acid 100 mg groups received the neurotoxin in addition to LA p.o. (using rodent gavage) dissolved in 30% Cremophor (Sigma Chemical, USA) at doses of 50 and/or 100 mg/kg respectively. LA (Sigma Chemical, USA) was administered one day before and on the day of surgery with an interval of 24 h. The second injection of LA was 1 h before surgery. The following experiments were conducted blind to treatments.

4.2. Behavioral testing

The animals were tested for rotational behavior by apomorphine hydrochloride (2 mg/kg, i.p.) one week before surgery (baseline) and after 1 week. The rotations were measured according to a method as described previously (Roghani et al., 2010). 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-lit and quiet room. Net number of rotations was defined as the positive scores minus the negative scores.

4.3. Determination of midbrain MDA concentration

The rats were anesthetized with ketamine (150 mg/kg), decapitated, brains were removed, anterior third block of left midbrain was blotted dry, weighed, then made into 5% tissue homogenate in ice-cold 0.9% saline solution, centrifuged at 4 °C, obtained supernatant was aliquoted, and then stored at −80 °C until assayed. The MDA concentration (thiobarbituric acid reactive substances, TBARS) in the supernatant was measured as described before (Roghani et al., 2010).

Briefly, trichloroacetic acid and TBARS reagent were added to supernatant, then mixed and incubated in boiling water for 80 min. After cooling on ice, samples were centrifuged at 1000*g* for 10 min and the absorbance of the supernatant was read at 532 nm. TBARS results were expressed as MDA equivalents using tetraethoxypropane as standard.

4.4. *Measurement of midbrain SOD activity*

The supernatant of midbrain homogenate was obtained as described earlier. SOD activity measurement was according to previous works (Roghani et al., 2010). Briefly, supernatant was incubated with xanthine and xanthine oxidase in potassium phosphate buffer (pH 7.8, 37 °C) for 40 min and nitroblue tetrazolium was added. Blue formazan was then monitored spectrophotometrically at 550 nm. The amount of protein that inhibited NBT reduction to 50% maximum was defined as 1 nitrite unit (NU) of SOD activity.

4.5. *Assay of midbrain nitrite concentration*

Supernatant nitrite content was assayed by the Griess method according to the previous study (Baluchnejadmojarad and Roghani, 2011). Because NO is a compound with a short half-life and is rapidly converted to the stable end products nitrate (NO_3^-) and nitrite (NO_2^-), the principle of the assay is the conversion of nitrate into nitrite by cadmium and followed by color development with Griess reagent (containing sulfanilamide and N-naphthyl ethylenediamine) in acidic medium. The total nitrite was measured by the Griess reaction. The absorbance was determined at 540 nm with a spectrophotometer.

4.6. *Protein assay*

The protein content of the supernatant was measured with the Bradford method using bovine serum albumin (Sigma Chemical, St. Louis, MO) as the standard (Bradford, 1976).

4.7. *Histological study*

Half of the animals in each group were randomly used for histological assessment. At the end of behavioral experiments, the rats were deeply anesthetized with a high dose of ketamine (150 mg/kg) and perfused through the ascending aorta with 50–100 ml of 0.9% saline followed by 100–200 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, blocks of forebrain and brainstem were prepared, and after final steps of preparation (immersion in 30% sucrose solution for 2–3 days), sections were cut at a thickness of 40 μm on a freezing microtome (Leica, Germany) and collected in PB (0.1 M). Every second section was Nissl-stained with 0.1% cresyl violet (Sigma Chemical, USA).

4.8. *Neuronal counting*

For each animal, mesencephalic sections (Interaural 2.9–4.2 mm) were examined by a method as described previously (Baluchnejadmojarad et al., 2010). Briefly, Nissl-stained neurons of the SNC were counted manually (Light microscopy; X400) using a superimposed grid to facilitate the procedure. At least two sections representative of each of the four Paxinos–Watson planes (4.2, 3.7, 3.2,and 2.97; interaural) were examined by scanning the entire extent on each side. Counting was done blind to the treatments received.

4.9. *Statistical analysis*

All data were expressed as mean±S.E.M. For the drug-induced rotational behavior, the non-parametric Kruskall–Wallis test was used. Inter-group differences for values of Nissl-stained neurons for the injected side and biochemical assays were found out 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.

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REFERENCES

Abdin, A.A., Sarhan, N.I., 2011. Intervention of mitochondrial dysfunction-oxidative stress-dependent apoptosis as a possible neuroprotective mechanism of alpha-lipoic acid against rotenone-induced parkinsonism and L-dopa toxicity. Neurosci. Res. 71, 387–395.

Aly, H.A., Lightfoot, D.A., El-Shemy, H.A., 2009. Modulatory role of lipoic acid on lipopolysaccharide-induced oxidative stress in adult rat Sertoli cells in vitro. Chem. Biol. Interact. 182, 112–118.

Astiz, M., de Alaniz, M.J., Marra, C.A., 2012. The oxidative damage and inflammation caused by pesticides are reverted by lipoic acid in rat brain. Neurochem. Int. 61, 1231–1241.

Baluchnejadmojarad, T., Roghani, M., Mafakheri, M., 2010. Neuroprotective effect of silymarin in 6-hydroxydopamine hemi-parkinsonian rat: involvement of estrogen receptors and oxidative stress. Neurosci. Lett. 480, 206–210.

Baluchnejadmojarad, T., Roghani, M., 2011. Chronic epigallocatechin-3-gallate ameliorates learning and memory deficits in diabetic rats via modulation of nitric oxide and oxidative stress. Behav. Brain Res. 224, 305–310.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

Chaudhary, P., Marracci, G., Yu, X., Galipeau, D., Morris, B., Bourdette, D., 2011. Lipoic acid decreases inflammation and confers neuroprotection in experimental autoimmune optic neuritis. J. Neuroimmunol. 233, 90–96.

Chen, S., Le, W., 2006. Neuroprotective therapy in Parkinson disease. Am. J. Ther. 13, 445–457.

Chng, H.T., New, L.S., Neo, A.H., Goh, C.W., Browne, E.R., Chan, E.C., 2009. Distribution study of orally administered lipoic acid in rat brain tissues. Brain Res. 1251, 80–86.

Connell, B.J., Saleh, T.M., 2012. Co-administration of apocynin with lipoic acid enhances neuroprotection in a rat model of ischemia/reperfusion. Neurosci. Lett. 507, 43–46.

Covy, J.P., Giasson, B.I., 2011. alpha-Synuclein, leucine-rich repeat kinase-2, and manganese in the pathogenesis of Parkinson disease. Neurotoxicology 32, 622–629.

De Araujo, D.P., Lobato Rde, F., Cavalcanti, J.R., Sampaio, L.R., Araujo, P.V., Silva, M.C., Neves, K.R., Fonteles, M.M., Sousa, F.C., Vasconcelos, S.M., 2011. The contributions of antioxidant activity of lipoic acid in reducing neurogenerative progression of Parkinson's disease: a review. Int. J. Neurosci. 121, 51–57.

Foley, P., Riederer, P., 2000. Influence of neurotoxins and oxidative stress on the onset and progression of Parkinson's disease. J. Neurol. 247 (Suppl. 2), II82–II94.

Fujita, H., Shiosaka, M., Ogino, T., Okimura, Y., Utsumi, T., Sato, E.F., Akagi, R., Inoue, M., Utsumi, K., Sasaki, J., 2008. Alpha-lipoic acid suppresses 6-hydroxydopamine-induced ROS generation and apoptosis through the stimulation of glutathione synthesis but not by the expression of heme oxygenase-1. Brain Res. 1206, 1–12.

Garcia Ruiz, P.J., Catalan, M.J., Fernandez Carril, J.M., 2011. Initial motor symptoms of Parkinson disease. Neurologist 17, S18–S20.

Guo, S., Yan, J., Yang, T., Yang, X., Bezard, E., Zhao, B., 2007. Protective effects of green tea polyphenols in the 6-OHDA rat model of Parkinson's disease through inhibition of ROS-NO pathway. Biol. Psychiatry 62, 1353–1362.

Hattori, N., 2004. Etiology and pathogenesis of Parkinson's disease: from mitochondrial dysfunctions to familial Parkinson's disease. Rinsho Shinkeigaku 44, 241–262.

Henchcliffe, C., Beal, M.F., 2008. Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. Nat. Clin. Pract. Neurol. 4, 600–609.

Holmquist, L., Stuchbury, G., Berbaum, K., Muscat, S., Young, S., Hager, K., Engel, J., Munch, G., 2007. Lipoic acid as a novel treatment for Alzheimer's disease and related dementias. Pharmacol. Ther. 113, 154–164.

Hwang, C.K., Chun, H.S., 2012. Isoliquiritigenin isolated from licorice Glycyrrhiza uralensis prevents 6-hydroxydopamine-induced apoptosis in dopaminergic neurons. Biosci. Biotechnol. Biochem. 76, 536–543.

Iravani, M.M., Jenner, P., 2011. Mechanisms underlying the onset and expression of levodopa-induced dyskinesia and their pharmacological manipulation. J. Neural Transm. 118, 1661–1690.

Lotharius, J., Brundin, P., 2002. Pathogenesis of Parkinson's disease: dopamine, vesicles and alpha-synuclein. Nat. Rev. Neurosci. 3, 932–942.

Maczurek, A., Hager, K., Kenklies, M., Sharman, M., Martins, R., Engel, J., Carlson, D.A., Munch, G., 2008. Lipoic acid as an anti-inflammatory and neuroprotective treatment for Alzheimer's disease. Adv. Drug Delivery Rev. 60, 1463–1470.

Malinska, D., Winiarska, K., 2005. Lipoic acid: characteristics and therapeutic application. Postepy Hig. Med. Dosw. (Online) 59, 535–543.

Miklossy, J., Doudet, D.D., Schwab, C., Yu, S., McGeer, E.G., McGeer, P.L., 2006. Role of ICAM-1 in persisting inflammation in Parkinson disease and MPTP monkeys. Exp. Neurol. 197, 275–283.

Mu, X., He, G., Cheng, Y., Li, X., Xu, B., Du, G., 2009. Baicalein exerts neuroprotective effects in 6-hydroxydopamine-induced experimental parkinsonism in vivo and in vitro. Pharmacol. Biochem. Behav. 92, 642–648.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, 2nd ed. Academic Press, San Diego.

Pederzolli, C.D., Rosa, A.P., de Oliveira, A.S., Coelho, J.G., Becker Dda, L., Dalazen, G.R., Moraes, T.B., Dutra-Filho, C.S., 2010. Neuroprotective role of lipoic acid against acute toxicity of N-acetylaspartic acid. Mol. Cell. Biochem. 344, 231–239.

Roghani, M., Niknam, A., Jalali-Nadoushan, M.R., Kiasalari, Z., Khalili, M., Baluchnejadmojarad, T., 2010. Oral pelargonidin exerts dose-dependent neuroprotection in 6-hydroxydopamine rat model of hemi-parkinsonism. Brain Res. Bull. 82, 279–283.

Schapira, A.H., 2009. Molecular and clinical pathways to neuroprotection of dopaminergic drugs in Parkinson disease. Neurology 72, S44–S50.

Schapira, A.H., Jenner, P., 2011. Etiology and pathogenesis of Parkinson's disease. Mov. Disord. 26, 1049–1055.

Schober, A., 2004. Classic toxin-induced animal models of Parkinson's disease: 6-OHDA and MPTP. Cell Tissue Res. 318, 215–224.

Schwarting, R.K., Huston, J.P., 1997. Behavioral and neurochemical dynamics of neurotoxic meso-striatal dopamine lesions. Neurotoxicology 18, 689–708.

Shapiro, R.M., Glick, S.D., Camarota, N.A., 1987. A two-population model of rat rotational behavior: effects of unilateral nigrostriatal 6-hydroxydopamine on striatal neurochemistry and amphetamine-induced rotation. Brain Res. 426, 323–331.

Soczynska, J.K., Kennedy, S.H., Chow, C.S., Woldeyohannes, H.O., Konarski, J.Z., McIntyre, R.S., 2008. Acetyl-L-carnitine and alpha-lipoic acid: possible neurotherapeutic agents for mood disorders?. Expert Opin. Invest.Drugs 17, 827–843.

Sriram, K., O'Callaghan, J.P., 2007. Divergent roles for tumor necrosis factor-alpha in the brain. J. Neuroimmune Pharmacol. 2, 140–153.

Stocchi, F., Marconi, S., 2010. Factors associated with motor fluctuations and dyskinesia in Parkinson Disease: potential role of a new melevodopa plus carbidopa formulation (Sirio). Clin. Neuropharmacol. 33, 198–203.

Von Bohlen und Halbach, O., Schober, A., Krieglstein, K., 2004. Genes, proteins, and neurotoxins involved in Parkinson's disease. Prog. Neurobiol. 73, 151–177.

Ybot-Gorrin, I., Vivancos-Matellano, F., Chacon-Pena, J.R., Alonso-Navarro, H., Jimenez-Jimenez, F.J., 2011. Assessment of Parkinson disease: what do we need to show neuroprotection?. Neurologist 17, S21–S29.

Yuan, C.G., He, L., Xue, X.L., Shi, Y.T., Bi, X.H., 2001. Effect of alpha-lipoic acid on the apoptosis of PC12 cells induced by 6-hydroxydopamine. Shi Yan Sheng Wu Xue Bao 34, 65–70.

Zaitone, S.A., Abo-Elmatty, D.M., Shaalan, A.A., 2012. Acetyl-L-carnitine and alpha-lipoic acid affect rotenone-induced damage in nigral dopaminergic neurons of rat brain, implication for Parkinson's disease therapy. Pharmacol. Biochem. Behav. 100, 347–360.

Zhou, F., Wu, J.Y., Sun, X.L., Yao, H.H., Ding, J.H., Hu, G., 2007. Iptakalim alleviates rotenone-induced degeneration of dopaminergic neurons through inhibiting microglia-mediated neuroinflammation. Neuropsychopharmacology 32, 2570–2580.