

Lipoic acid alleviates L-DOPA-induced dyskinesia in 6-OHDA parkinsonian rats via anti-oxidative stress

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Abstract. Levodopa (L-DOPA) is the gold standard for symptomatic treatment of Parkinson's disease (PD); however, long-term therapy is associated with the emergence of L-DOPA-induced dyskinesia (LID). Nigral dopaminergic cell loss determines the degree of drug exposure and time required for the initial onset of LID. Accumulating evidence indicates that α -lipoic acid (ALA) decreases this nigral dopaminergic cell loss. However, until now, the precise mechanisms of ALA have only been partially understood in LID. Chronic L-DOPA treatment was demonstrated to develop intense AIM scores to assess dyskinetic symptoms. Rats in the LID group were administrated twice daily with L-DOPA + benserazide for 3 weeks to induce a rat model of dyskinesia. Moreover, other 6-OHDA-lesioned rats were treatment with ALA (31.5 mg/kg or 63 mg/kg) in combination with L-DOPA treatment. Furthermore, the authors investigated the level of malondialdehyde (MDA) and glutathione (GSH) activity, as well as IBA-1, caspase-3 and poly (ADP-ribose) polymerase (PARP) in substantia nigra by the way of western blotting and immunofluorescence. ALA reduced LID in a dose-dependent manner without compromising the anti-PD effect of L-DOPA. Moreover, ALA reduced the level of MDA and upregulated the GSH activity, as well as ameliorated IBA-1 positive neurons in the substantia nigra. Finally, it was identified that ALA could reduce L-DOPA-induced cleaved-caspase-3 and PARP overexpression in the substantia nigra. Based on the present findings, ALA could be recommended as a promising disease-modifying therapy when administered with L-DOPA early in the course of PD. The exact mechanism for this

action, although incompletely understood, appears to relate to anti-oxidative stress and anti-apoptosis.

Introduction

Levodopa (L-DOPA), the precursor of dopamine (DA), has provided obvious effective treatment for Parkinson's disease (PD) by replacing DA neurotransmission following the death of substantia nigra neurons (1). Its high efficacy is due to the ability to restore synaptic DA levels, an effect thought to be mediated by the spared DA neurons. Following chronic administration of L-DOPA, however, leads to motor side effects, L-DOPA-induced dyskinesias (LIDs), limiting the utility of the drug (2). LIDs are perceived as debilitating by affected patients and continue to be a major problem to the clinical management of PD patients. For this reason, there is a great interest in developing non-dopaminergic treatments that can be added to L-DOPA to reduce these untoward effects (3).

Together with previous findings in LID models, this led to the overall impression that the development of LID is caused by a complex interaction of both pre- and postsynaptic changes, taking place not only in the DA system, but also involving a variety of other mechanisms (4). There are literature reports indicating that especially long-time L-DOPA therapy may cause side effects in the form of increased toxicity and inflammatory response, as well as disturbances in biothiol metabolism (5). Spencer *et al* (6) reported that the augmented oxidative stress in patients treated with L-DOPA may have resulted from lowered levels of antioxidants, disturbed mitochondrial transport, and from excessive oxidation of DA. Moreover, some previous studies indicated that, in PD patients treated with L-DOPA, increased plasma levels of neuroinflammation markers, such as oxidized-low density lipoproteins and soluble intracellular adhesion molecule (7). Therefore, in PD patients treated with L-DOPA, monitoring of oxidative stress markers and inflammatory factors, as well as biothiol compounds is recommended.

α -lipoic acid (ALA), is an antioxidant naturally synthesized in human body with potential therapeutic value against a range of pathophysiological insults (8). Emerging evidence has indicated that ALA has effective antioxidative activities by scavenging reactive oxygen species (ROS) and inhibits free radical formation by chelating various metal types, indicating that it can exert beneficial effects on various disorders

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correlated with oxidative stress (9). In addition, ALA is reported to have anti-inflammatory properties and to increase intracellular glutathione (GSH) formation in a range of cell types and tissues, which may be beneficial in neurodegenerative conditions (10). It has been reported that ALA protected DA neurons against MPPT-induced apoptosis by attenuating ROS formation (11). Moreover, a previous study (12) demonstrated that ALA prevented the damage induced by 6-OHDA or by chronic use of L-DOPA in dopaminergic neurons, suggesting that ALA could be a new therapeutic target for PD prevention and treatment (13). Indeed, emerging evidences *in vivo* and *in vitro* have come to further support the crucial position of ALA in the neuroprotective of PD, especially in the field of anti-oxidative stress and anti-inflammation. However, there is still limited data on ALA in animal models of LID. Therefore, the aim of the current study was to investigate neurochemical and behavioral effects of ALA in combination with L-DOPA using an animal model of LID in 6-OHDA-lesioned parkinsonian rats.

Materials and methods

Animals. Experiments were conducted on 48 female Sprague-Dawley (SD) rats (age, 3–4 months; weight, 180–220 g), which were purchased from the Experimental Animal Center of China Medical University (Beijing, China). Upon their arrival, the animals were housed in clean cages with a maximum of four rats per cage under a 12 h light:12 h dark cycle, relative humidity of 55±10% and temperature 22.0±2.0°C. Animals had unrestricted access to standard chow and water, which were supplemented daily, and animal care was supervised by skilled veterinarians in the health care center (Medical School of Shanghai Jiaotong University, Shanghai, China). All experimental protocols involving the animals were reviewed and approved by the Ethical Committee of the Medical School of Shanghai Jiaotong University (Shanghai, China). Efforts were made to reduce to a minimum the number of animals required for statistically valid analyses and to minimize their suffering. The methods were carried out in accordance with the approved guidelines and regulations of the National Institutes of Health for the Care and Use of Laboratory Animals (Bethesda, MD, USA).

Induction of parkinsonism and L-DOPA-induced dyskinesia. 6-OHDA-lesioned PD rats were induced by the methods mentioned above (14). Briefly, rats were deeply anesthetized by 10% chloral hydrate (0.35 ml/100 g; Beyotime Institute of Biotechnology, Shanghai, China) and mounted in a stereotaxic apparatus equipped with a rat adaptor. Using a syringe, 4 µl 6-OHDA (4 µg/µl) in 0.2% ascorbic acid were injected into the right medial forebrain bundle (MFB) of rats in two deposits at the following stereotaxic coordinates as follows: 1) anterior-posterior (AP), -4.4 mm, medial-lateral (ML), -1.2 mm, dorsal-ventral (DV), -7.8 mm; 2) AP, -3.7 mm, ML, -1.7, DV, -7.8. The tooth bar was set to -2.4 mm. At 3 weeks following surgery, the lesioned rats were screened behaviorally using an apomorphine hydrochloride-induced [0.5 mg/kg, intraperitoneally (i.p.)] rotation test and all animals exhibited >7 full body turns/min toward the side of the unlesioned side were selected for the next experiment.

Once parkinsonism was stable, they were then treated with twice-daily administration of L-DOPA (25 mg/kg/d, i.p.) plus benserazide (6.25 mg/kg/d, i.p.) for 3 weeks to induce a rat model of dyskinesia.

Drugs and treatment. Validated PD rats received vehicle or levodopa injection for 21 d. Apomorphine hydrochloride (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was administered (0.5 mg/kg). L-DOPA (Sigma-Aldrich; Merck KGaA, 25 mg/kg) with a fixed dose of the peripheral DOPA-decarboxylase inhibitor benserazide (Sigma-Aldrich; Merck KGaA, 6.25 mg/kg) were administered twice-daily (9:00 and 16:00). ALA was dissolved in normal saline and was administered i.p. (ALA-L group, 31.5 mg/kg; ALA-H group, 63 mg/kg, respectively) 30 min prior to L-DOPA intake for 3 weeks.

AIM ratings and forelimb functional test. On testing days, rats were placed individually in transparent plastic cages 10 min prior to drug treatment. As described previously (15), abnormal involuntary movements (AIM) were classified into four subtypes: 1) axial AIM: dystonic posturing or choreiform twisting of the neck and upper body towards the side contralateral to the lesion; 2) limb AIM: abnormal, purposeless movements of the forelimb and digits contralateral to the lesion; 3) orolingual AIM: empty jaw movements and contralateral tongue protrusion; and 4) locomotion AIM: increased locomotion with contralateral side bias. The AIM scores were tested at 2, 7, 14 and 21 days during levodopa treatment. Each of these subtypes was scored on a severity scale from 0 to 4. Forelimb functional test was performed five times at 5, 9, 13, 16 and 20 day during L-DOPA treatment, which could as an index of parkinsonian disability score. The rats were placed in a glass cylinder (22x35 cm) to record forelimb use during vertical exploration for 60 min. During a period of 60 min following L-DOPA treatment, forelimb functional test was assessed every 20 min (3 min monitoring period for each). The final value was expressed in terms of the percentage use of the impaired forelimb (contralateral) compared with the total number of limb use movements.

Measurement of GSH and lipid peroxide. To assess the enzymatic activity of GSH and lipid peroxide in striatum, the tissues were homogenized in 0.1 mol/l PBS containing 0.05 mmol/l EDTA. The homogenate was centrifuged at 12,000 x g for 15 min at 4°C. The supernatants were kept for the measurement. Total GSH was assayed by 5,5-dithiobis (2-nitrobenzoic) acid (DTNB)-GSSG reductase recycling. GSSG was obtained by determining the absorbance of 5-thio-2-nitrobenzoic acid produced from the reaction of the reduced GSH with DTNB according to the manufacturer's protocols. The reduced GSH was obtained by subtracting GSSG from the total GSH. Absorbance was determined at 412 nm by using the microplate reader. GSH activity was assessed with GSH Assay kit (Beyotime Institute of Biotechnology, Haimen, China) by the mays of GSH/GSSG. The level of MDA, a product of lipid peroxidation, was measured with MDA Assay kit (Beyotime Institute of Biotechnology) based on modified thiobarbituric acid method.

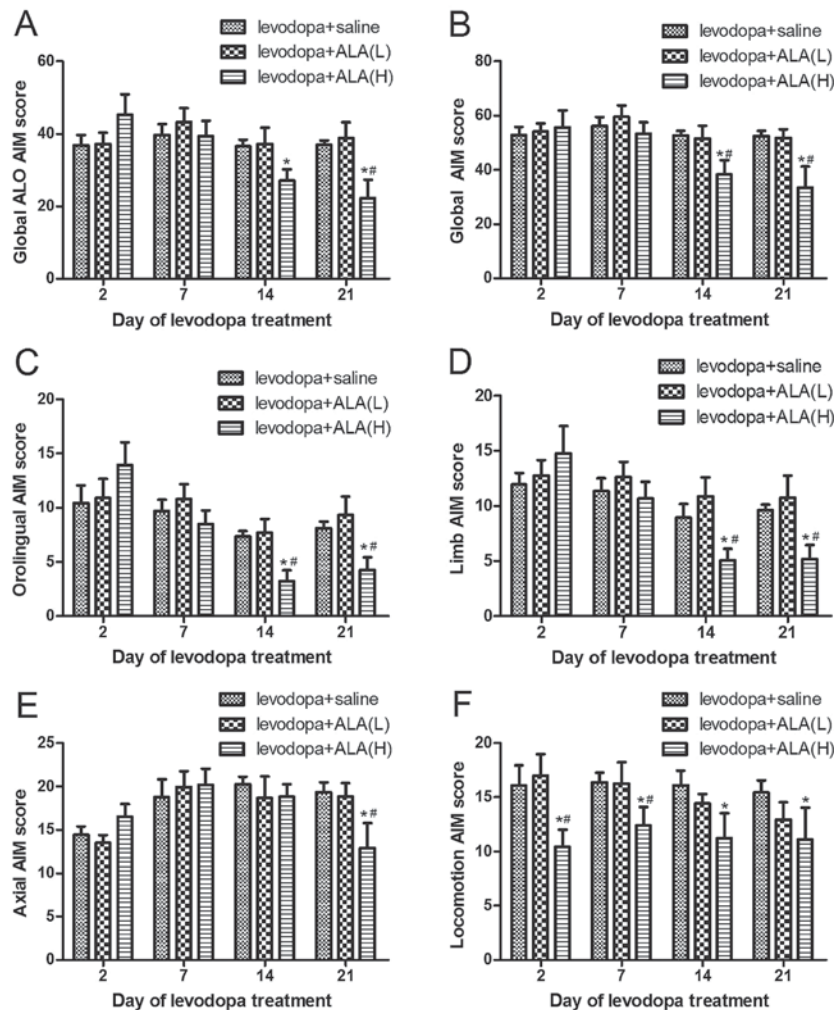


Figure 1. Time course of ALO AIMs development in 6-OHDA-lesioned rats during a 3 week treatment period with L-DOPA, ALA-L and ALA-H, respectively. For each day, a total AIM score was calculated as the sum of the basic score multiplied by the amplitude score for each AIM subtype. (A) global ALO AIM score (including ALO AIM score); (B) total AIM score (including ALO AIM score and locomotive score); (C) orolingual AIM score; (D) limb AIM score; (E) axial AIM score and (F) orolingual AIM score. Data are presented as mean \pm standard deviation. * $P < 0.05$ vs. LID group; # $P < 0.05$ vs. ALA-L group (Kruskal Wallis followed by Dunn's test for multiple comparisons or a Mann-Whitney U test). ALO, axial, limb and orolingual; AIM, abnormal involuntary movements; L-DOPA, levodopa; ALA, α -lipoic acid; LID, L-DOPA-induced dyskinesia.

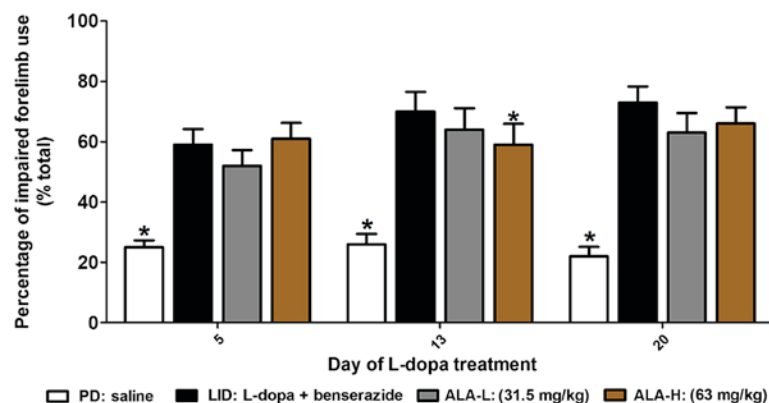


Figure 2. Unilaterally lesioned rats were tested for the percentage of impaired forelimb use compared with the total numbers. Animals were injected with either saline, L-DOPA + benserazide, ALA-L and ALA-H, respectively. The effective ALA dose did not interfere with the therapeutic motor effects of L-DOPA. Experiment consisted of three different sessions, namely 5, 13 and 20 days in the treatment period. Data are presented as mean \pm standard deviation ($n=8$). * $P < 0.05$ vs. LID group. L-DOPA, levodopa; ALA, α -lipoic acid; LID, L-DOPA-induced dyskinesia; PD, Parkinson's disease.

Immunofluorescence (IFC). IFC was carried out in free-floating sections using a standard avidin-biotin immunocytochemical

protocol. Rats were rapidly anesthetized with 10% chloral hydrate (350 mg/kg, i.p.) and transcardially perfused with 4%

paraformaldehyde. Whole brains were post-fixed overnight in the 4% paraformaldehyde, stored at 4°C and then stored in a solution containing 30% sucrose. Sections (30 μ m) were cut with a slicing machine and blocked for 10 min at room temperature in 5% normal donkey serum (Beyotime Institute of Biotechnology), and then incubated overnight at 4°C in the primary antibody solution (monoclonal rabbit anti-IBA; 1:200; cat. no. ab178680; Abcam, Cambridge, UK). Sections were rinsed in PBS and incubated with fluorescein isothiocyanate-conjugated donkey anti-rabbit antibody (cat. no. A0453; 1:200; Beyotime Institute of Biotechnology) for 1 h at room temperature. Subsequently, sections were again rinsed in PBS, mounted on slides, cover slipped, and examined with confocal microscopy. Digitized images were analyzed for distribution of immunoreactive cells in the lesioned hemisphere striatum and substantia nigra of rats.

Western blot analysis. Striatal tissues and substantia nigra were homogenized in 20 mM Tris-HCl (pH 7.4), containing 1 mM NaF, 150 mM NaCl, 1% Triton X-100 and freshly-added protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland), and 100 μ M phenylmethylsulfonyl fluoride. Cytosols were prepared by centrifugation at 12,000 \times g for 10 min at 4°C. Proteins were separated by SDS-PAGE electrophoresis, using different percentages of gels based on the different protein weights (range, 6-12%) and transferred overnight to polyvinylidene difluoride membranes. Then, the membrane was incubated with polyclonal rabbit anti-caspase-3 (cat. no. 9661S; 1:1,000; Cell Signaling Technology, Inc., Danvers, MA, USA) and polyclonal rabbit anti-poly (ADP-ribose) polymerase (PARP; cat. no. 9542S; 1:1,000; Cell Signaling Technology) overnight at 4°C, respectively, and then incubated in horseradish peroxidase conjugated secondary anti-rabbit β -actin IgG (cat. no. A0208; 1:1,000; Beyotime Institute of Biotechnology). The signal was visualized by enhanced chemiluminescence reagent (EMD Millipore, Billerica, MA, USA) and quantified using Quantity One software (Image Lab).

Statistical analysis. The scores assigned for AIM and parkinsonian disability were non-parametric and were analyzed using a Kruskal Wallis followed by Dunn's test for multiple comparisons in the case of comparing data over multiple days. The western blot analysis and IHC conformed to normal distribution were performed using a one-way analysis of variance (ANOVA) followed by LSD post-hoc comparisons when appropriate as indicated in the figure legends. $P < 0.05$ was considered to indicate a statistically significant difference. All analyses were conducted using SPSS software (version, 16.0; SPSS Inc., Chicago, IL, USA).

Results

Treatment with ALA prevents the development of LID in 6-OHDA-lesioned rat model of PD. A total of 48 SD rats were unilaterally injected with 6-OHDA in the MFB ($n=12$ per group). The anti-dyskinetic potential of ALA against LID was evaluated at two different doses (31.5 and 63 mg/kg). As can be observed in Fig. 1, ALA reduced AIM scores and observed after L-DOPA (25 mg/kg) in a dose-dependent manner. 6-OHDA-lesioned rats treated with L-DOPA for 21 days

developed a progressive increase in LID ($P < 0.05$ compared with PD and ALA groups; Fig. 1A and B). The 6-OHDA-lesioned rats received the saline for 21 days did not develop LID features. Meanwhile, co-administration of ALA with L-DOPA did not develop severe LID over the 21 day treatment period, which differed significantly from the LID group in all testing sessions except at 2 and 7 day time point following administration ($P < 0.05$; Fig. 1A and B). Furthermore, the ALA-H (63 mg/kg) group demonstrated a greater reduction in the AIM scores compared with the rats receiving ALA-L (31.5 mg/kg), but the rats still presented a mild dyskinesia, this effect did not reach completely reversal. Similarly, this seemed to be the same trend in orolingual AIM (Fig. 1C), limb AIM (Fig. 1D), Axial AIM (Fig. 1E), as well as locomotion AIM (Fig. 1F).

Effects of LA on forelimb functional test. Following this, the authors sought to determine whether ALA improvement of LID without ablation of the therapeutic response to L-DOPA. The authors observed that PD rats treated with L-DOPA prefer to use the contralateral forelimb to touch the inner wall of the cylinder compared with the PD group ($P < 0.05$; Fig. 2). If the animals were co-injected with ALA-L (31.5 mg/kg) or ALA-H (63 mg/kg) for 21 days, they also demonstrated preferential to touch the wall with contralateral forelimb at 5, 13 and 20 day time points following administration ($P > 0.05$ vs. LID group, $P < 0.05$ vs. PD group, respectively; Fig. 2). When they continued to measure forelimb preference between ALA-L and ALA-H groups, no significant difference was identified between two groups ($P > 0.05$; Fig. 2).

Effects of LA on MDA and GSH activity. In the current study, the MDA level as a measure of lipid peroxidation was remarkably increased in the LID group compared with the PD group ($P < 0.05$; Fig. 3A). Either the ALA-L group (31.5 mg/kg) or the ALA-H group (31.5 mg/kg) treatment significantly reduced the MDA levels compared to the LID group ($P < 0.05$, Fig. 3A). Similarly, no significant difference was observed between the ALA-L and ALA-H groups in terms of reducing MDA level ($P > 0.05$; Fig. 3A). To investigate the effect of ALA on anti-oxidative stress in an LID model, the authors assessed the biomarkers of oxidative stress, including GSH and GSSG. The level of GSH activity by the means of GSH/GSSG in the LID group was significantly decreased compared with the PD group ($P < 0.05$; Fig. 3B and C). Meanwhile, treatment with ALA remarkably alleviated the GSH activity compared with the LID group ($P < 0.05$; Fig. 3B and C). Furthermore, the ALA-H group demonstrated more effects in the GSH activity compared with the rats receiving ALA-L ($P < 0.05$; Fig. 3B and C).

LA treatment ameliorates IBA-1 positive neurons in substantia nigra. IBA-1 is a protein that is specifically expressed in macrophages/microglia and is upregulated during the activation of these cells (16). Activated macrophages are reported in tissues with inflammation. Therefore, IBA-1 levels have been found to positively correlate with chronic inflammation indicators. IBA-1 positive neurons are induced about two-fold increase in the substantia nigra by chronic L-DOPA treatment compared with other two groups ($P < 0.05$; Fig. 4), the inductive effect of L-DOPA on this marker can be seen in all substantia nigra regions. Meanwhile, animals treated with ALA tended

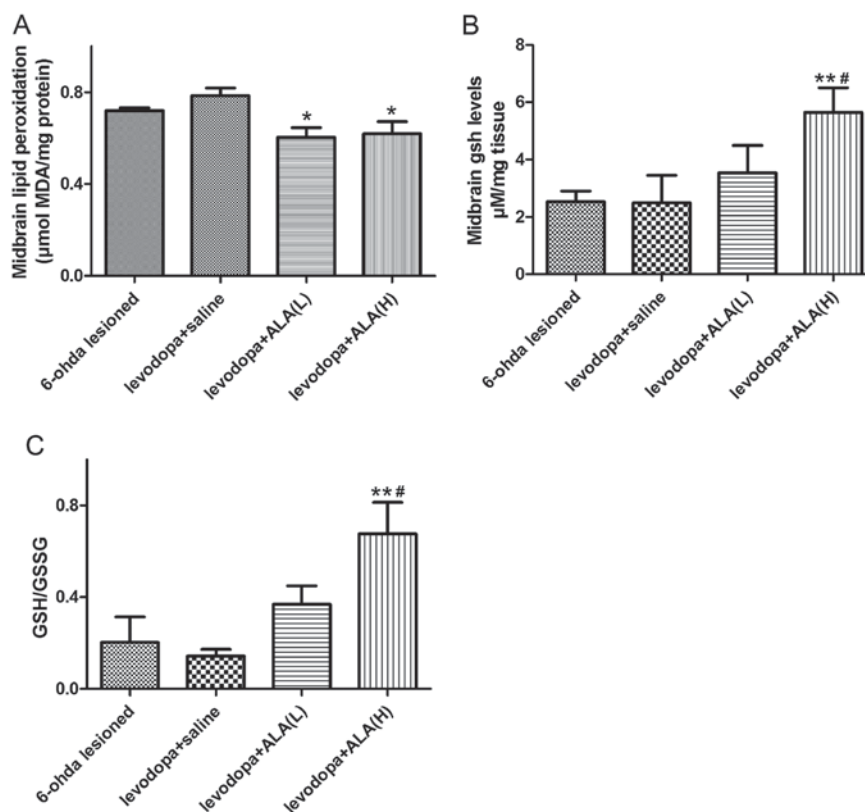


Figure 3. Effects of ALA on MDA and GSH levels. (A) MDA level as a measure of lipid peroxidation; (B) GSH level in striatum; (C) GSH/GSSG level in striatum. Data are presented as mean \pm standard error of the mean. * $P<0.05$, ** $P<0.01$ vs. LID group; # $P<0.05$ vs. ALA-L group, $n=4$ per group (one-way analysis of variance followed by least significant difference post-hoc analysis). ALA, α -lipoic acid; MDA, malondialdehyde; GSH, reduced glutathione; GSSG, oxidized glutathione; LID, levodopa-induced dyskinesia.

to present lower levels of cellular immunostaining for IBA-1 positive neurons than LID group ($P<0.05$; Fig. 4). Note that the ALA-H entailed significantly more effective than the treatment with ALA-L in terms of lower the levels of IBA-1 positive neurons in the substantia nigra ($P<0.05$; Fig. 4).

Treatment with LA prevents activation cleaved-caspase-3 and PARP. Fig. 5 indicated that chronic L-DOPA treatment of hemi-parkinsonian rats increased cleaved-caspase-3 and PARP levels in the lesioned substantia nigra. ALA at 31.5 mg/kg or 63 mg/kg both reduced the induction of cleaved-caspase-3 and PARP levels following chronic L-DOPA treatment in parkinsonian animals. Furthermore, ALA-H demonstrated more reduction in terms of cleaved-caspase-3 and PARP levels compared with ALA-L administration ($P<0.05$; Fig. 5A-F). The degree of dopamine depletion was verified by western blotting with an antibody raised against tyrosine hydroxylase (TH). Significant changes were observed in TH levels in the substantia nigra between the PD group and other four groups ($P<0.05$; Fig. 5G and H), indicating $>90\%$ depletion of nigral dopamine cell bodies in PD, LID and ALA groups.

Discussion

L-DOPA is the most successful approach to manage motor symptoms in PD patients. However, the emergence of LID with long-term use is a severe challenge for PD. The present study has demonstrated that chronic challenges of ALA (31.5 and 63 mg/kg)

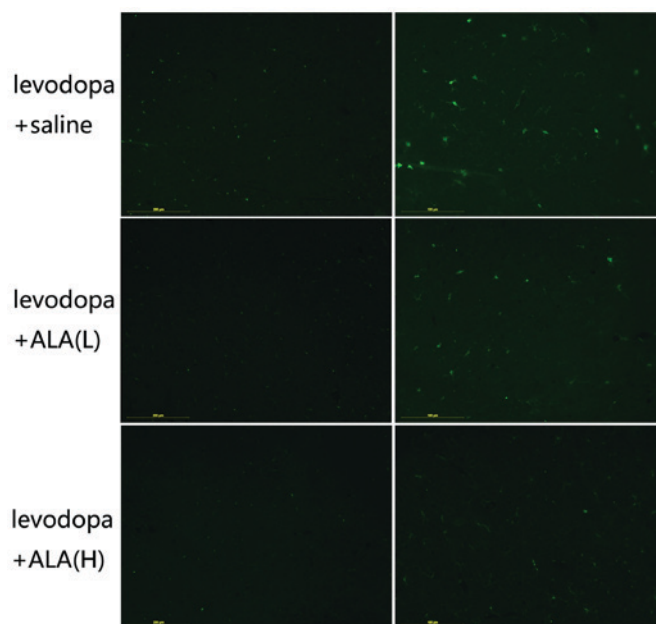


Figure 4. Representative examples of IBA-1 immunofluorescence studies in 6-OHDA-lesioned rats treated with L-DOPA, ALA-L or ALA-H, respectively. In 6-OHDA lesioned rats treated with L-DOPA induced approximately increase in IBA-1-positive neurons compared to other two group. These L-DOPA-induced increases in positive neurons decline in the ALA group. Shown is a coronal section of the striatum from the lesioned hemisphere. * $P<0.05$ vs. LID rats (one-way analysis of variance followed by least significant difference post-hoc analysis). Scale bar= $200\text{ }\mu\text{m}$. L-DOPA, levodopa; ALA, α -lipoic acid; LID, L-DOPA-induced dyskinesia.

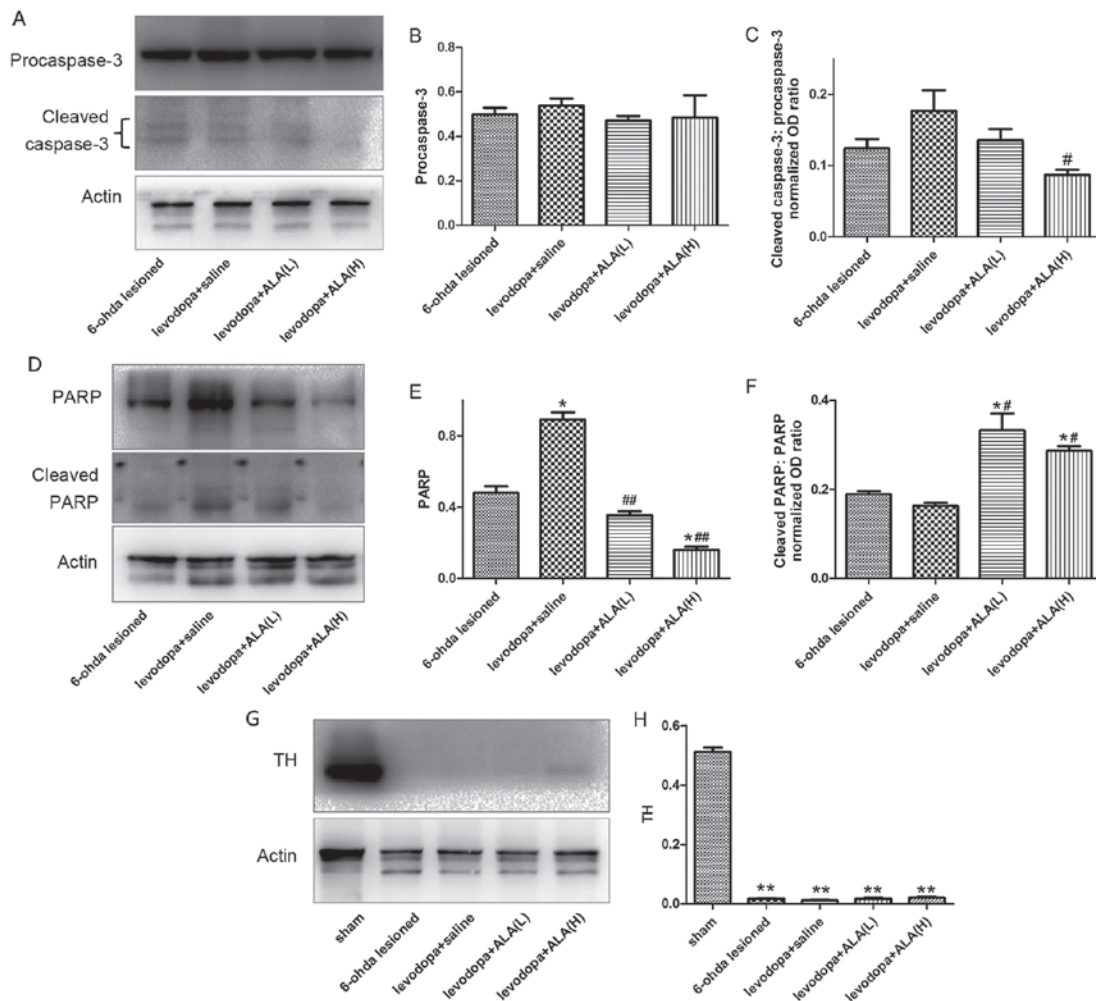


Figure 5. Protein levels were evaluated by western blotting of proteins extracted from the ipsilateral striatum of the rat brain. They were assessed in extracts from 6-OHDA-lesioned rats treated with vehicle, levodopa, ALA-L and ALA-H. (A) Representative image and quantification of (B) pro-caspase-3, and (C) cleaved-caspase-3 level relative to actin level. (D) Representative image and quantification of (E) PARP, and (F) cleaved-PARP relative to actin level. (G) Representative image and (H) quantification of TH level relative to actin level. The data represent the mean of relative optical density \pm standard deviation; * $P < 0.05$, ** $P < 0.01$ vs. 6-OHDA group; [#] $P < 0.05$, ^{##} $P < 0.01$ vs. LID group (one-way analysis of variance followed by least significant difference post-hoc analysis). LID, levodopa-induced dyskinesia; ALA, α -lipoic acid; TH, tyrosine hydroxylase; PARP, poly (ADP-ribose) polymerase.

in combination with L-DOPA significantly alleviates dyskinesia. The major findings from this study were: (1) ALA ameliorated L-DOPA induced dyskinesia; (2) the effective ALA dose (31.5 mg/kg or 63 mg/kg) did not interfere with the therapeutic motor effects of L-DOPA; (3) ALA reduced L-DOPA-induced MDA over-expression in substantia nigra and upregulated the level of GSH; (4) ALA reduced L-DOPA-induced cleaved-caspase-3 and PARP overexpression in the substantia nigra; (5) ALA reduced L-DOPA-induced IBA-1 positive neurons in the substantia nigra. The anti-dyskinetic effect of the 31.5 or 63 mg/kg dose of ALA was observed 7 days following L-DOPA administration, when dyskinesias were occurring. Moreover, this effect of ALA was maintained over the entire 21 days without development of tolerance. The current study revealed, for the first time, that the ALA could alleviate LID in 6-OHDA parkinsonian rats via anti-oxidative stress in terms of reduced MDA level and upregulated GSH activity. Moreover, these results further indicate that co-administration with ALA reduced the percentage of apoptotic cells in the substantia nigra observed following chronic L-DOPA treatment. Using western blotting for the distinction of apoptotic event, the authors

demonstrated that ALA could attenuate the apoptotic event induced by L-DOPA. The findings of anti-apoptotic effects of ALA against L-DOPA-induced apoptosis are consistent with those obtained with this compound in previous studies (11,17).

Oxidative stress is a central event in a range of pathological conditions. Such a pathway appears to underlie the pathological processes of PD, in which the inhibition of the mitochondrial complex I elicited by the neuron toxicant increases the formation of ROS that cause the mitochondrial dysfunction finally result in PD occur (18). 6-OHDA has been widely used to study the pathogenesis of PD and is thought to selectively kill dopaminergic neurons and to elicit severe parkinsonism-like symptoms in humans and animals (19). Moreover, the previous data indicated that L-DOPA therapy of PD patients may induce oxidative stress by different mechanisms, and increase the levels of inflammatory markers and led to apoptotic event (7). All of these events induced by L-DOPA probably serve a vital role in the development of LID. Namely, long-term follow-up of PD therapy with L-DOPA improves the parkinsonian symptoms but may lead to fluctuations and dyskinesias and on-off phenomena. Motor fluctuations and LID are common sequelae

of PD that may limit function and quality of life. Meanwhile, in the present study, results indicated that treatment with chronic L-DOPA significantly increased level of MDA and reduced the GSH activity. In addition, the present findings demonstrated that ALA co-administration with L-DOPA could reverse the effect by L-DOPA in the LID models.

Another related issue in the exploration of microglial activation phases is the reliance on Iba-1 immunoreactivity to report on their activation state (20). Based on a previous study, Iba-1 levels were revealed to positively correlate with chronic inflammation indicators (20). The results demonstrated that Iba-1 positive neurons are induced ~two-fold more in the substantia nigra by chronic L-DOPA treatment; the inductive effect of L-DOPA on this marker can be seen in all substantia nigra regions. Meanwhile, co-administration with ALA tended to report lower levels of cellular immunostaining for Iba-1 positive neurons than the LID group. However, the limitation of present study is that only Iba-1 dyeing was used to reflect inflammatory states of glia. Indeed, in the future, studies should show the importance of using multiple approaches when reporting on the level of microglial activation. Moreover, caspase-3 has been identified as a key mediator of neuronal programmed cell death. Caspase-3 activation, a crucial event of neuronal cell death program, is also a feature of many chronic neurodegenerative diseases (21). One previous study demonstrated that PD patients treated with L-DOPA had significantly increased levels of caspase-3 and PARP protein (22). It seems that pharmacological treatment of PD patients with L-DOPA has a major role in modulating the levels of some apoptotic proteins in lymphocytes, which are important for this process, which were consistent with that present results that state that chronic L-DOPA treatment could upregulate the levels of caspase-3 and PARP. In resent research, chronic L-DOPA treatment of hemi-parkinsonian rats increased cleaved-caspase-3 and PARP in the lesioned substantia nigra. ALA at 31.5 mg/kg or 63 mg/kg both reduced the induction of cleaved-caspase-3 and PARP levels following chronic L-DOPA treatment in parkinsonian animals. In conclusion, these data demonstrated that ALA prevents molecular changes from occurring and lends support to the hypothesis that ALA alleviates L-DOPA-induced dyskinesia in 6-OHDA parkinsonian rats via anti-oxidative stress; this may be a promising mode of administration to avoid LID.

Based on the present findings, ALA could be recommended as a promising disease-modifying therapy when administered with L-DOPA early in the course of PD. The exact mechanism for this action, although incompletely understood, appears to relate to anti-oxidative stress and anti-apoptosis.

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References

1. Mosharov EV, Borgkvist A and Sulzer D: Presynaptic effects of levodopa and their possible role in dyskinesia. *Mov Disord* 30: 45-53, 2015.
2. Hely MA, Morris JG, Reid WG and Trafficante R: Sydney multi-center study of Parkinson's disease: Non-L-DOPA-responsive problems dominate at 15 years. *Mov Disord* 20: 190-199, 2005.
3. Finlay C and Duty S: Therapeutic potential of targeting glutamate receptors in Parkinson's disease. *J Neural Transm (Vienna)* 121: 861-880, 2014.
4. Schaeffer E, Pilotto A and Berg D: Pharmacological strategies for the management of levodopa-induced dyskinesia in patients with Parkinson's disease. *CNS Drugs* 28: 1155-1184, 2014.
5. Dorszevska J, Prendecki M, Lianeri M and Kozubski W: Molecular effects of L-dopa therapy in parkinson's disease. *Curr Genomics* 15: 11-17, 2014.
6. Spencer JP, Jenner P and Halliwell B: Superoxide-dependent depletion of reduced glutathione by L-DOPA and dopamine. Relevance to Parkinson's disease. *Neuroreport* 6: 1480-1484, 1995.
7. Andican G, Konukoglu D, Bozluolcay M, Bayulkem K, Firtina S and Burcak G: Plasma oxidative and inflammatory markers in patients with idiopathic Parkinson's disease. *Acta Neurol Belg* 112: 155-159, 2012.
8. Ferreira PM, Militão GC and Freitas RM: Lipoic acid effects on lipid peroxidation level, superoxide dismutase activity and monoamines concentration in rat hippocampus. *Neurosci Lett* 464: 131-134, 2009.
9. Smith AR, Shenvi SV, Widlansky M, Suh JH and Hagen TM: Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress. *Curr Med Chem* 11: 1135-1146, 2004.
10. De Araújo DP, Lobato Rde F, Cavalcanti JR, Sampaio LR, Araújo PV, Silva MC, Neves KR, Fonteles MM, Sousa FC and Vasconcelos SM: The contributions of antioxidant activity of lipoic acid in reducing neurogenerative progression of Parkinson's disease: A review. *Int J Neurosci* 121: 51-57, 2011.
11. Li DW, Li GR, Lu Y, Liu ZQ, Chang M, Yao M, Cheng W and Hu LS: α -lipoic acid protects dopaminergic neurons against MPP+-induced apoptosis by attenuating reactive oxygen species formation. *Int J Mol Med* 32: 108-114, 2013.
12. De Araújo DP, Lobato Rde F, Cavalcanti JR, Sampaio LR, Araújo PV, Silva MC, Neves KR, Fonteles MM, Sousa FC and Vasconcelos SM: The contributions of antioxidant activity of lipoic acid in reducing neurogenerative progression of Parkinson's disease: A review. *Int J Neurosci* 121: 51-57, 2011.
13. de Araújo DP, De Sousa CN, Araújo PV, Menezes CE, Sousa Rodrigues FT, Escudeiro SS, Lima NB, Patrocínio MC, Aguiar LM, Viana GS and Vasconcelos SM: Behavioral and neurochemical effects of alpha-lipoic Acid in the model of Parkinson's disease induced by unilateral stereotaxic injection of 6-ohda in rat. *Evid Based Complement Alternat Med* 2013: 571378, 2013.
14. Xie CL, Wang WW, Zhang SF, Yuan ML, Che JY, Gan J, Song L, Yuan WE and Liu ZG: Levodopa/benserazide microsphere (LBM) prevents L-dopa induced dyskinesia by inactivation of the DR1/PKA/P-tau pathway in 6-OHDA-lesioned Parkinson's rats. *Sci Rep* 4: 7506, 2014.
15. Zhang S, Xie C, Wang Q and Liu Z: Interactions of CaMKII with dopamine D2 receptors: Roles in levodopa-induced dyskinesia in 6-hydroxydopamine lesioned Parkinson's rats. *Sci Rep* 4: 6811, 2014.
16. Deininger MH, Meyermann R and Schluesener HJ: The allograft inflammatory factor-1 family of proteins. *FEBS Lett* 514: 115-121, 2002.
17. Jalali-Nadoushan M and Roghani M: Alpha-lipoic acid protects against 6-hydroxydopamine-induced neurotoxicity in a rat model of hemi-parkinsonism. *Brain Res* 1505: 68-74, 2013.
18. Perfeito R, Cunha-Oliveira T and Rego AC: Reprint of: Revisiting oxidative stress and mitochondrial dysfunction in the pathogenesis of Parkinson disease-resemblance to the effect of amphetamine drugs of abuse. *Free Radic Biol Med* 62: 186-201, 2013.
19. Schober A: Classic toxin-induced animal models of Parkinson's disease: 6-OHDA and MPTP. *Cell Tissue Res* 318: 215-224, 2004.
20. Norden DM, Trojanowski PJ, Villanueva E, Navarro E and Godbout JP: Sequential activation of microglia and astrocyte cytokine expression precedes increased Iba-1 or GFAP immunoreactivity following systemic immune challenge. *Glia* 64: 300-316, 2016.
21. D'Amelio M, Sheng M and Cecconi F: Caspase-3 in the central nervous system: Beyond apoptosis. *Trends Neurosci* 35: 700-709, 2012.
22. Hald A and Lotharius J: Oxidative stress and inflammation in Parkinson's disease: Is there a causal link? *Exp Neurol* 193: 279-290, 2005.