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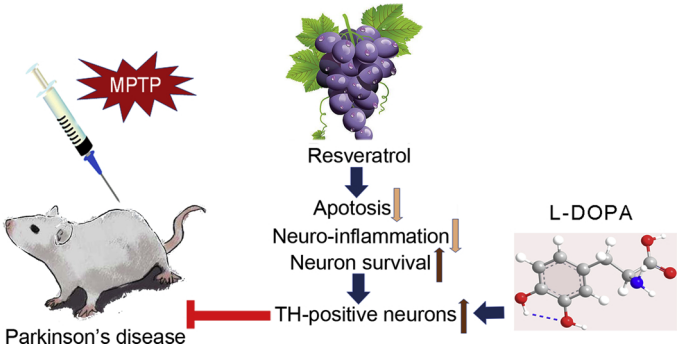
Research report

Resveratrol synergizes with low doses of L-DOPA to improve MPTP-induced Parkinson disease in mice

Qianqian Liu[a], Dashuai Zhu[b], Peien Jiang[a], Xinyu Tang[a], Qiuhan Lang[a], Qinyi Yu[a], Shaozhi Zhang[a], Yongzhe Che[b], Xizeng Feng[a,*]

[a] State Key Laboratory of Medicinal Chemical Biology, Key Laboratory of Bioactive Materials, Ministry of Education, College of Life Sciences, Nankai University, Tianjin, 300071, China
[b] Department of Anatomy, School of Medicine, Nankai University, Tianjin, 300071, China

GRAPHICAL ABSTRACT



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ABSTRACT

L-DOPA (L-3,4-dihydroxyphenylalanine) relieves symptoms of Parkinson disease (PD), but long-term use can cause serious side effects. Resveratrol (3,5,4′-trihydroxy-trans-stilbene, RV), a polyphenolic compound derived from grapes and red wine that has antioxidant activity, has been shown to have neuroprotective effects. RV was investigated to enhance the therapeutic effect of L-DOPA in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mouse model of Parkinson disease. Mice received a saline or RV injection (10 mg/kg/day), then 2 h later, saline or MPTP (15 mg/kg/day) was administered for 7 consecutive days. Saline or L-DOPA (5 or 8 mg/kg/day) was injected post-administration of MPTP for the last 2 consecutive days. Our results indicated that RV alleviated MPTP-induced loss of dopaminergic neurons and attenuated astroglial activation in the nigrostriatal pathway. In parallel, RV reduced the expression of α-synuclein in the striatum. In addition, RV also increased levels of the anti-apoptotic signalling molecule Bcl-2, reduced levels of the pro-apoptotic signalling molecule Bax, and reduced activation of caspase-3 in the striatum. Specifically, RV significantly reduced motor dysfunction in MPTP-treated mice. Furthermore, the RV-treated group showed less IL-1β and an enhanced pAkt/Akt ratio, which promoted dopamine neuron survival in the striatum. We found that the effects of co-administration of RV with L-DOPA (5 mg/kg) were equivalent to those of administration of 8 mg/kg L-DOPA in MPTP-induced PD mice. Therefore, with fewer side effects, L-DOPA can be effectively used in the treatment of PD over a long period of time.

* Corresponding author.
E-mail address: xzfeng@nankai.edu.cn (X. Feng).

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1. Introduction

Parkinson disease (PD) is a common progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc), which reduces dopamine levels in the striatum [1,2]. This change leads to characteristic motor symptoms such as akinesia, resting tremor, slow movement, rigidity, gait disturbances and unstable posture [3]. In addition, the degeneration of dopaminergic neurons is associated with the presence of Lewy bodies in neurons. α-synuclein, which is a causative agent of PD [4], is an agglomerated protein that has been identified as the major protein component of Lewy bodies. MPTP is a neurotoxin precursor to MPP+, which can selectively damage neurons in the nigrostriatal dopaminergic pathway [5,6]. MPTP is a widely used chemical for inducing a state similar to PD in animals to test new drug candidates [7], and MPTP-injected mice have been widely accepted as a model for PD [8].

L-DOPA is a direct precursor of dopamine that was originally introduced as an effective drug for the treatment of PD [9], and it remains the gold standard for PD treatment. Long-term drug intake can cause motor complications, such as L-DOPA-induced dyskinesia (LID) [10]. In addition, a previous study has reported that the use of high doses of L-DOPA results in the production of neurotoxic molecules such as 6-OHDA [6,11]. With the reduction of beneficial effects of L-DOPA, a periodic increase in L-DOPA dose is necessary to achieve a stable therapeutic effect [12]. To counteract the side effects caused by L-DOPA, there is an urgent need to design measures that can reduce the dose of L-DOPA without affecting the treatment outcome of PD patients [13].

Resveratrol is a natural polyphenolic compound extracted from red grapes, giant knotweed rhizome and peanuts, exerts neuroprotective effects on oxidative damage and neuronal damage through its antioxidant as well as anti-inflammatory properties [14–16]. The role of Resveratrol in degenerative diseases has been confirmed in Alzheimer's disease, ischaemic stroke, Huntington's disease and Parkinson disease [17,18].

In the present study, we found that RV could synergize with low doses of L-DOPA to improve MPTP-induced Parkinson's symptoms in mice by inhibiting apoptosis and neuroinflammation and promoting dopamine neuron survival. In addition, the effects of co-administration of RV with L-DOPA at low doses (5 mg/kg) were equivalent to those of 8 mg/kg L-DOPA administration in mice with MPTP-induced Parkinson disease. Therefore, we found that RV has the potential to reduce the dose of L-DOPA necessary to ameliorate PD.

2. Materials and methods

2.1. Reagents

MPTP was purchased from Yuanye Biological Technology Company Ltd. (Shanghai, S31504). L-DOPA, Resveratrol, paraformaldehyde, phosphate-buffered saline (PBS) and most of the chemicals and reagents used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). The rabbit anti-TH monoclonal antibody was purchased from affinity. The rabbit anti-Akt (total) antibody and mouse anti-phospho-Akt (Ser473) antibody were purchased from Proteintech. The mouse anti-cleaved caspase-3 antibody, mouse anti-α-synuclein antibody, mouse anti-Bax antibody, mouse anti-Bcl-2, mouse anti- IL-1β antibody, and mouse anti-α-tubulin antibody were purchased from Santa Cruz Biotechnology. Anti-rabbit and anti-mouse HRP-tagged IgG secondary antibodies were purchased from Cell Signaling Technology. Polyvinylidene difluoride membranes, protease inhibitors and ECL kits were procured from Merck Millipore.

2.2. Animals and drug treatment

Adult female Balb/c mice (10 weeks) were used in the experiments. All animals were provided by the Institute of Zoology, Chinese Academy of Sciences. All mice procedures were carried out in accordance with the guidelines of the care and use of laboratory animals of Nankai University (approved by State Key Laboratory of Medicinal Chemical Biology, Nankai University). Before the experiments, mice were housed and bred in a pathogen-free animal facility with a 12 h light/dark cycle (lights on during the period 08:00-20:00) with free access to food and water. The room was maintained at an ambient temperature of 22 ± 2 °C and relative humidity of 60 ± 2%. After one week of acclimatization, the animals were divided into six groups, with each group containing six mice (Table 1). Fig. 1 shows the timeline of the experiment detailing acclimatization, grouping, behavioural training and time points for behavioural tests, drug treatments and tissue processing.

2.3. Behavioural tests

2.3.1. Open field locomotion activity test

The open field test was performed in a quiet and dimly lit (25% ± 5% lux) room according to the following instructions. A transparent open field reaction box (45 cm L x 45 cm W x 25 cm H) was prepared. A camera (> 1 million-pixel resolution) was fixed over the field at a height of 1 m. The mice were placed into the box, and allowed to familiarize themselves with the environment for approximately 1 min. Then, a 5 min video was recorded using the camera connected to the computer. Software tools (for example, MATLAB) were used to analyse videos to obtain movement trace figures, the distribution of static (velocity < 1 cm/s), walking (velocity 1–20 cm/s) and running (velocity > 20 cm/s) time, the total travelled distance, and the average speed for each tested mouse.

2.3.2. Rearing test

Normally, mice explore their surroundings in a new environment. Therefore, when they are placed in a transparent cylinder, they move around and lift their bodies, using their forelimbs to contact the walls of the cylinder; we call this behaviour rearing. Rearing is natural, but mice show reduced movement and rearing when treated with neurotoxic agents such as rotenone, MPTP, and 6-hydroxydopamine. The rearing test was carried out as described in previous studies. Briefly, one mouse was placed in a clear glass cylinder (height = 20 cm, diameter = 12 cm) for 3 min and the process was videotaped for analysis. The number of rearing actions with one or both of the forelimbs was recorded. Such actions were recorded only when the mouse raised the forelimb above its shoulders from the bottom of the cylinder.

Table 1
Experimental groups and treatment.

Groups	Treatment
Control	0.9% Saline (p.o.) + 0.9% Saline (i.p.) + 0.9% Saline (i.p.)
MPTP	0.9% Saline (p.o.) + MPTP 15 mg/kg (i.p.) + 0.9% Saline (i.p.)
MPTP + RV10	Resveratrol 10 mg/kg (p.o.) + MPTP 15 mg/kg (i.p.) + 0.9% Saline (i.p.)
MPTP + LD5	0.9% Saline (p.o.) + MPTP 15 mg/kg (i.p.) + LD 5 mg/kg (i.p.)
MPTP + LD8	0.9% Saline (p.o.) + MPTP 15 mg/kg (i.p.) + LD 8 mg/kg (i.p.)
MPTP + LD5 + RV10	Resveratrol 10 mg/kg (p.o.) + MPTP 15 mg/kg (i.p.) + LD 5 mg/kg (i.p.)

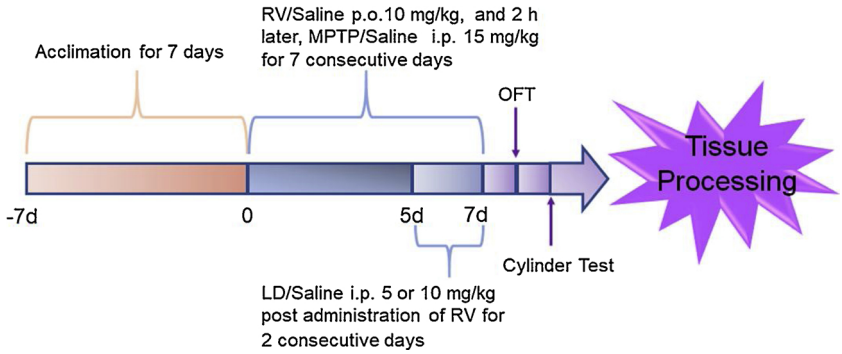

Fig. 1. Schematic representation of the experimental design.

2.4. Immunostaining

At the end of the experiment, mice were anaesthetized with an intraperitoneal injection of 4% chloral hydrate (330 mg/kg), and transcardial perfusion of saline followed by 4% paraformaldehyde (PFA) was performed. Mouse brains were harvested and immersed in PFA for 48 h. Then, the brains were embedded into paraffin blocks, and a series of 5 μm thick sections were cut. Paraffin sections were dewaxed and rehydrated. Antigen retrieval was performed by boiling sections in 0.01 M sodium citrate buffer (pH = 6.0), and the sections were then washed three times with PBS. All sections were incubated with blocking serum for 1 h at room temperature. Rabbit anti-GFAP (Abcam, 1:100) and rabbit anti-TH (1:100) antibodies diluted in blocking serum were applied to cover the brain sections and incubated overnight at 4 °C. After incubation, the sections were washed three times with PBS. Next, HRP-ligated goat anti-rabbit secondary antibodies (Bioworld, 1:500) were applied to the sections and incubated at room temperature for 1 h. The nuclei were counterstained with haematoxylin.

2.5. Western blot analysis

After the treatment, the striatum of the mouse was separated from the brain and harvested and immediately lysed in a tissue protein extraction reagent (CWBIO, Beijing, China) with PMSF (Sigma-Aldrich). A

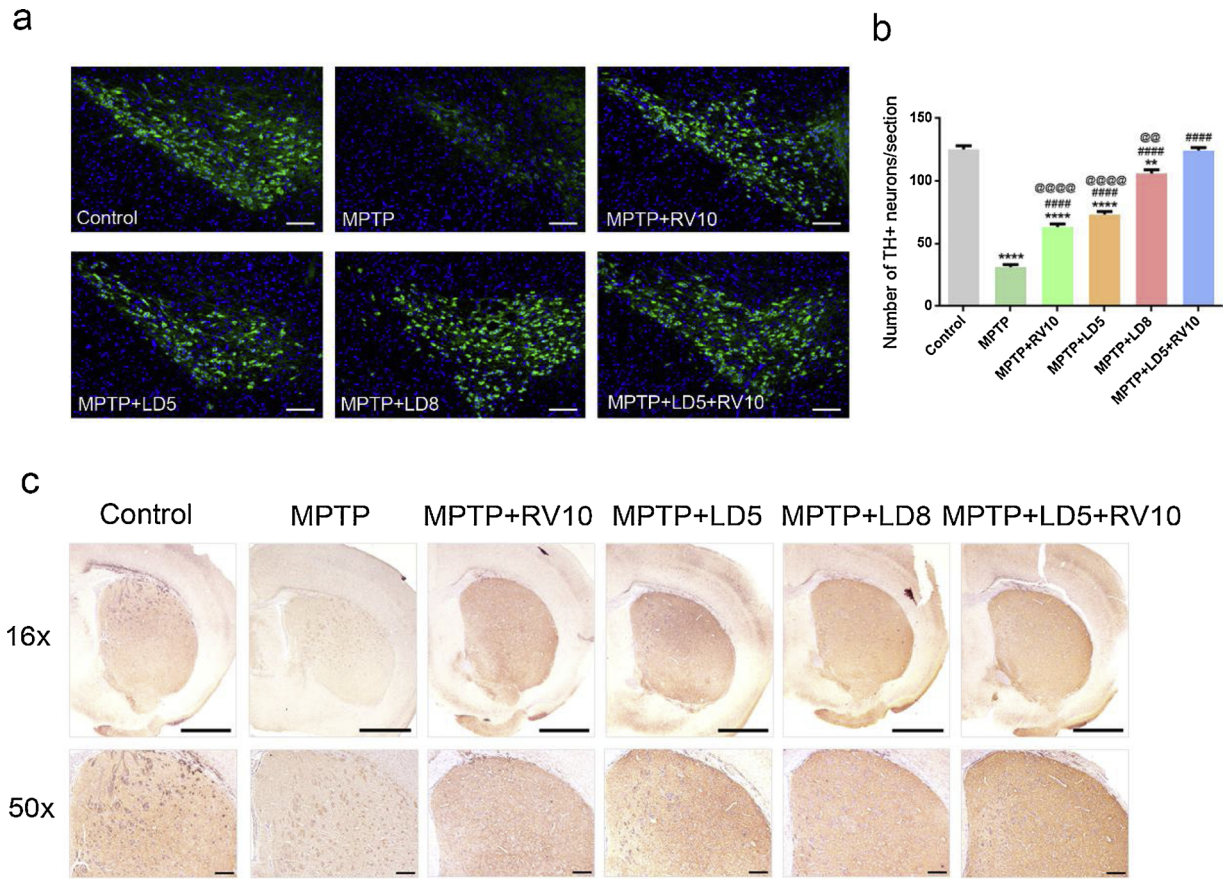

Fig. 2. Effects of L-DOPA with Resveratrol on MPTP-induced dopaminergic neurotoxicity in the nigrostriatal pathway. **(a)** Immunostaining for TH-positive dopaminergic neurons in the nigra. **(b)** Histogram representing TH-positive neuron counts. The images of 3–5 sections were counted to analyse the number of TH-positive neurons. **(c)** Immunostaining of TH-positive fibres in the striatum. Data are expressed as mean values ± S.E.M. (n = 6). Tukey's multiple comparison test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared to the control group. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ compared to the MPTP-treated group. @ $p < 0.05$, @@ $p < 0.01$, @@@ $p < 0.001$, @@@@ $p < 0.0001$ compared to the MPTP + LD 5+ RV group. Scale bars: 40 μm (substantia nigra); 1 mm and 100 μm (striatum).

BCA protein assay kit (CWBIO) was used to quantify the protein concentrations. Then, the proteins were subjected to SDS-PAGE and transferred onto a PVDF membrane. Later, the membrane was blocked with milk, a mixture of 5% non-fat dried milk and 0.05% Tween-20 in Tris-buffered saline for 1 h and then incubated with one of the following primary antibodies: rabbit anti-TH (1:1000), rabbit anti-Akt (total) (1:500), mouse anti-α-synuclein (1:1000), mouse anti-phospho-Akt (Ser473) (1:1000), mouse anti-cleaved caspase-3(1:200), mouse anti-Bax (1:200), mouse anti-Bcl-2 (1:200), mouse anti-α-tubulin (1:1000), or mouse anti- IL-1β (1:200). After washing with TBST (Tris-buffered saline containing 0.05% Tween-20), the membrane was incubated with an anti-mouse or an anti-rabbit peroxidase-conjugated secondary antibody (1:3000; Cell Signaling Technology). Then, the membrane was washed again with TBST, and the Super SignalWest Pico chemiluminescent substrate (Thermo Scientific) was applied to the membrane to reveal the existence and the expression level of target proteins by fluorescence.

2.6. Statistical analysis

All analyses were performed using Statistical Product and Service Solutions (SPSS) version 22.0 (IBM), GraphPad Prism 6.0 (GraphPad software) and ImageJ 1.48v (National Institutes of Health). The data were tested for equality of variance using the F-test. The data from multiple groups were analysed using ANOVA and Tukey's test.

3. Results

3.1. Dopaminergic neuroprotection by co-administration of Resveratrol with L-DOPA in the nigrostriatal pathway

As a marker of dopaminergic neurons, TH (tyrosine hydroxylase) is the rate-limiting enzyme for dopamine synthesis which is present in TH-positive neurons in the nigra and TH-positive fibres in striatum. Therefore, the neuroprotective effect of Resveratrol against MPTP neurotoxicity was determined by immunostaining for TH-positive neurons in the nigrostriatal pathway (Fig. 2). In the nigra, MPTP caused extensive loss of dopaminergic neurons compared with a control group that did not receive MPTP injections. Resveratrol mitigated the MPTP-induced the loss of dopaminergic neurons to a certain degree, and administration of 5 or 8 mg/kg L-DOPA alone led to a dose-dependent and significant attenuation of the loss of the dopa minergic neurons caused by MPTP. The co-administration of RV with 5 mg/kg L-DOPA caused a more significant retrieval of dopaminergic neurons than administration of either L-DOPA or RV alone (Fig. 2**a**,**b**). In parallel with the results in the nigra, we obtained the same result in the striatum. Resveratrol improved MPTP-induced decrease in density of TH-positive fibres in the striatum. We revealed that Resveratrol can enhance L-DOPA therapeutic effects and helped to reduce the dose necessary to achieve those effects (Fig. 2**c**).

To confirm the results, we conducted Western blotting for striatal TH, and the results were consistent with the TH immunostaining results (Fig. 3**a**, **b**). In addition, PD is characterized by misfolded fibrillar a-synuclein known as Lewy bodies, which are associated with the process of nigrostriatal degeneration. Therefore, we also examined the expression of a-synuclein in the striatum (Fig. 3**a**). The co-administration of RV with 5 mg/kg L-DOPA more significantly mitigated the MPTP-induced increase in the expression of a-synuclein than the administration of L-DOPA alone and RV alone (Fig. 3**c**).

3.2. Effects of co-administration of Resveratrol with L-DOPA on MPTP-induced neuro-inflammation and apoptosis

It is well known that neuro-inflammation and apoptosis are the major mechanisms of MPTP-induced neuronal death. We measured the levels of IL-1β, Bcl-2, Bax and cleaved caspase-3 in the striatum, which were involved in neuro-inflammation and apoptosis. The levels of the proinflammatory cytokine IL-1β were higher in the MPTP group than in the control group. The MPTP + RV, MPTP + LD 5, MPTP + LD 8 and MPTP + LD 5 + RV groups showed lower levels of IL-1β than the MPTP group. Specifically, the MPTP + LD 8, MPTP + LD 5 + RV groups had the best therapeutic effect (Fig. 4**b**).

The expression of the anti-apoptotic signalling molecule Bcl-2 was reduced, and the expression of the pro-apoptotic signalling molecule Bax was increased by MPTP treatment compared with the control group. The MPTP + LD 8 and MPTP + LD 5 + RV groups showed no difference from the control group in levels of Bcl-2 and Bax (Fig. 4**c**, **d**). We also measured the activation of caspase-3 in the striatum. We found that the injection of MPTP increased the levels of cleaved caspase-3. Moreover, Resveratrol alone also decreased its levels compared to the MPTP group. The MPTP + LD 8 and MPTP + LD 5 + RV groups had the best therapeutic effect to reduce the levels of cleaved caspase-3 (Fig. 4**e**).

3.3. Recovery from motor dysfunction by co-administration of Resveratrol with L-DOPA

We conducted behavioural tests, including open field tests and rearing tests, to reveal the synergistic effect of Resveratrol and L-DOPA on the motor function of MPTP-induced PD mice. Fig. 5**a** shows representative trajectories and the distribution of static, walking and running times from every group of mice. We observed a decrease in total travel distance, average velocity and running time in the MPTP group compared with the control group, all of which decreases were alleviated in mice treated with L-DOPA alone, RV alone and both. Specifically, both the MPTP + LD 8 and MPTP + LD 5 + RV groups presented behaviours, as measured by the parameters above, that did not significantly differ from the control group (Fig. 5**b–d**). Similar

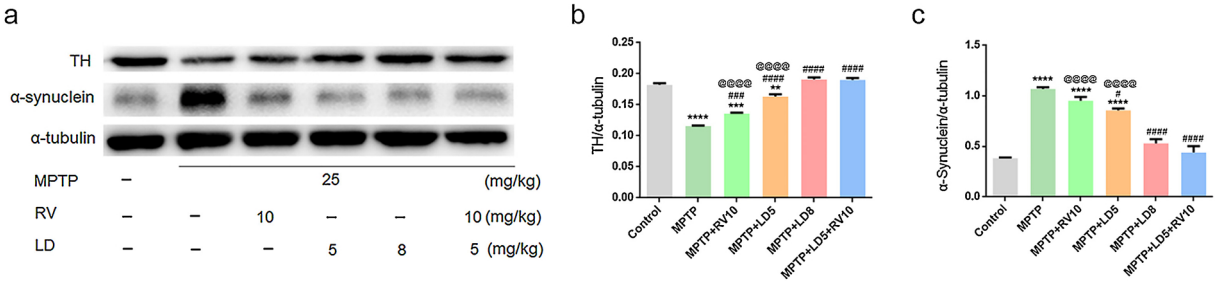

Fig. 3. Western blot analysis showed the expression of tyrosine hydroxylase (TH) and α-synuclein protein levels in the striatum of mice. **(a)** Representative Western blot bands of TH and α-synuclein. **(b, c)** Histograms representing the quantitative analysis of TH and α-synuclein levels, respectively, normalized to α-tubulin protein. Data are expressed as mean values ± S.E.M. (n = 6). Tukey's multiple comparison test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared to the control group. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ compared to the MPTP-treated group. @ $p < 0.05$, @@ $p < 0.01$, @@@ $p < 0.001$, @@@@ $p < 0.0001$ compared to the MPTP + LD 5+ RV group.

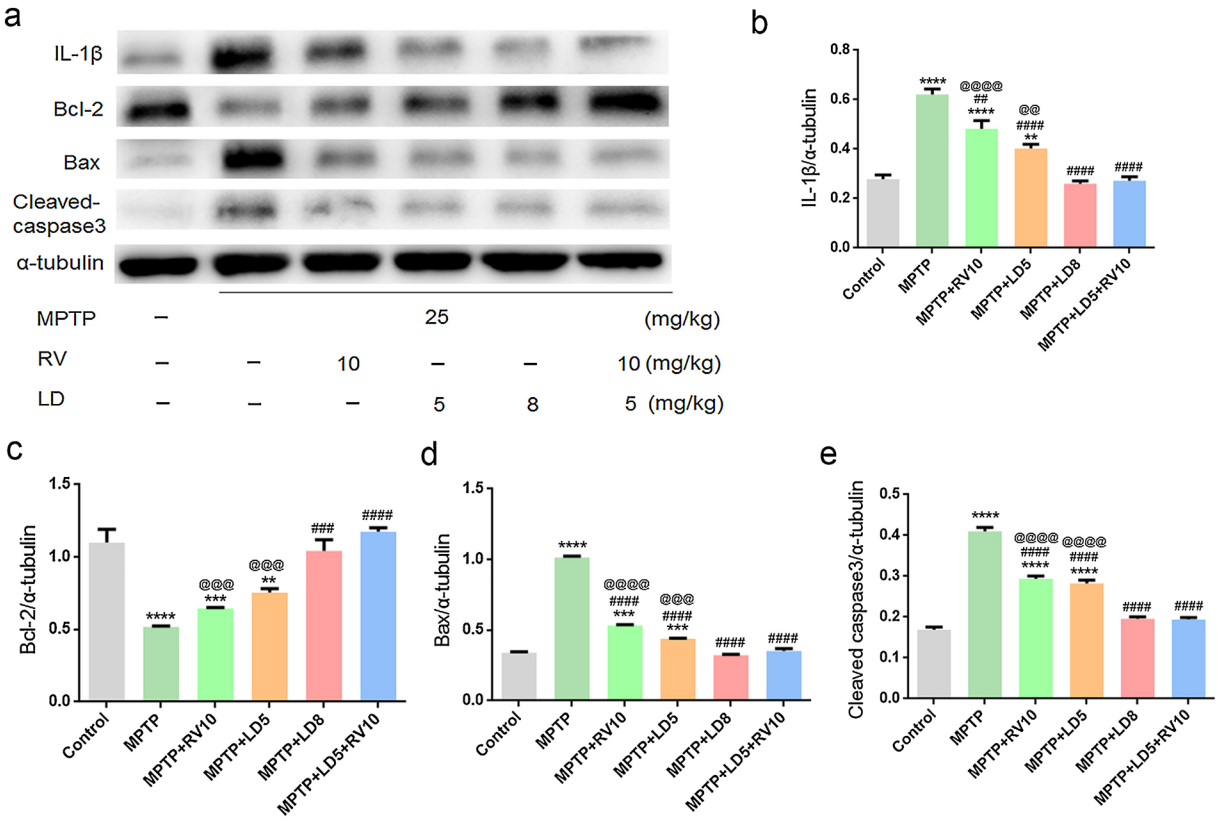

Fig. 4. Western blot quantification of protein levels of IL-1β, Bcl-2, Bax and cleaved caspase-3 in the striatum. **(a)** Representative Western blot bands of IL-1β, Bcl-2, Bax and cleaved caspase-3. **(b,c,d,e)** Histograms representing the quantitative analysis of IL-1β, Bcl-2, Bax and cleaved caspase-3 levels, respectively, normalized to α-tubulin protein. Data are expressed as mean values ± S.E.M. (n = 6). Tukey's multiple comparison test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared to the control group. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ compared to the MPTP-treated group. @ $p < 0.05$, @@ $p < 0.01$, @@@ $p < 0.001$, @@@@ $p < 0.0001$ compared to the MPTP + LD 5+ RV group.

results were obtained with the rearing test (Fig. 5**e**), in that MPTP induced a reduction in rearing when the mice were placed in a cylinder but that co-administration of Resveratrol and L-DOPA recovered the behaviour to levels similar to those of the control group.

3.4. Amelioration of astroglial activation by co-administration of Resveratrol with L-DOPA

Next, we determined the extent of astroglial activation in the substantia nigra and striatum by GFAP immunostaining. We observed a dramatic increase in astroglial activation in the substantia nigra and striatum after MPTP injection. The co-administration of Resveratrol with L-DOPA clearly ameliorated astroglial activation as shown by the reduced number and hypertrophy of astrocytes in the substantia nigra and striatum (Fig. 6). Moreover, administration of Resveratrol alone or L-DOPA alone at 5 or 8 mg/kg doses also slightly ameliorated astroglial activation (Fig. 6).

3.5. Resveratrol promotes neuronal survival

It is known that the Akt signalling pathway can promote neuronal survival. Thus, we examined the expression of Akt and pAkt in the striatum (Fig. 7). We observed that MPTP treatment considerably decreased the pAkt/Akt ratio compared with the control group. However, the decrease in the pAkt/Akt ratio was markedly suppressed when treated with Resveratrol alone or co-administration of Resveratrol with L-DOPA (Fig. 7**b**).

4. Discussion

In this study, we demonstrated that Resveratrol can synergize with low doses of L-DOPA to improve MPTP-induced Parkinson disease. Resveratrol may reduce the dose of L-DOPA to ameliorate PD by improving the survival of existing dopaminergic neurons in the striatum and substantia nigra. Consistent with our results, Resveratrol has shown neuroprotective effects and been shown to increase the number of TH-positive cells in MPTP-treated mouse striatum and substantia nigra pars compacta [15]. In addition, α-synuclein is the main component of Lewy bodies and is a pathological marker of PD [19]. It has been reported that Resveratrol can prevent α-synuclein aggregation [20]. This confirms our results that RV improved the MPTP-induced increase of α-synuclein levels in the striatum.

The chronic neuroinflammatory response in the brain is an important pathological factor in the development of PD [21]. High levels of inflammatory cytokines such as TNFα and IL-1β have been detected in Parkinson disease patients [22]. In addition, studies have reported the activation of astrocytes in the PD brain [23,24]. Our results suggest that Resveratrol treatment effectively reduced IL-1β levels and astroglial activation in MPTP-treated mice. In agreement with our findings, previous studies demonstrated that Resveratrol plays a neuroprotective role by inhibiting IL-1β production [22] and reducing astrocyte hypertrophy and microglial activation [25,26]. We believe that the anti-inflammatory effect of Resveratrol is a very important factor in its ability to synergize with L-DOPA to treat PD.

Since neurons cannot be replaced, and apoptosis plays an important role in the neurodegenerative process of PD, inhibiting apoptosis is another effective way to prevent the development of parkinsonism [27]. We measured the expression levels of Bcl-2, an anti-apoptotic

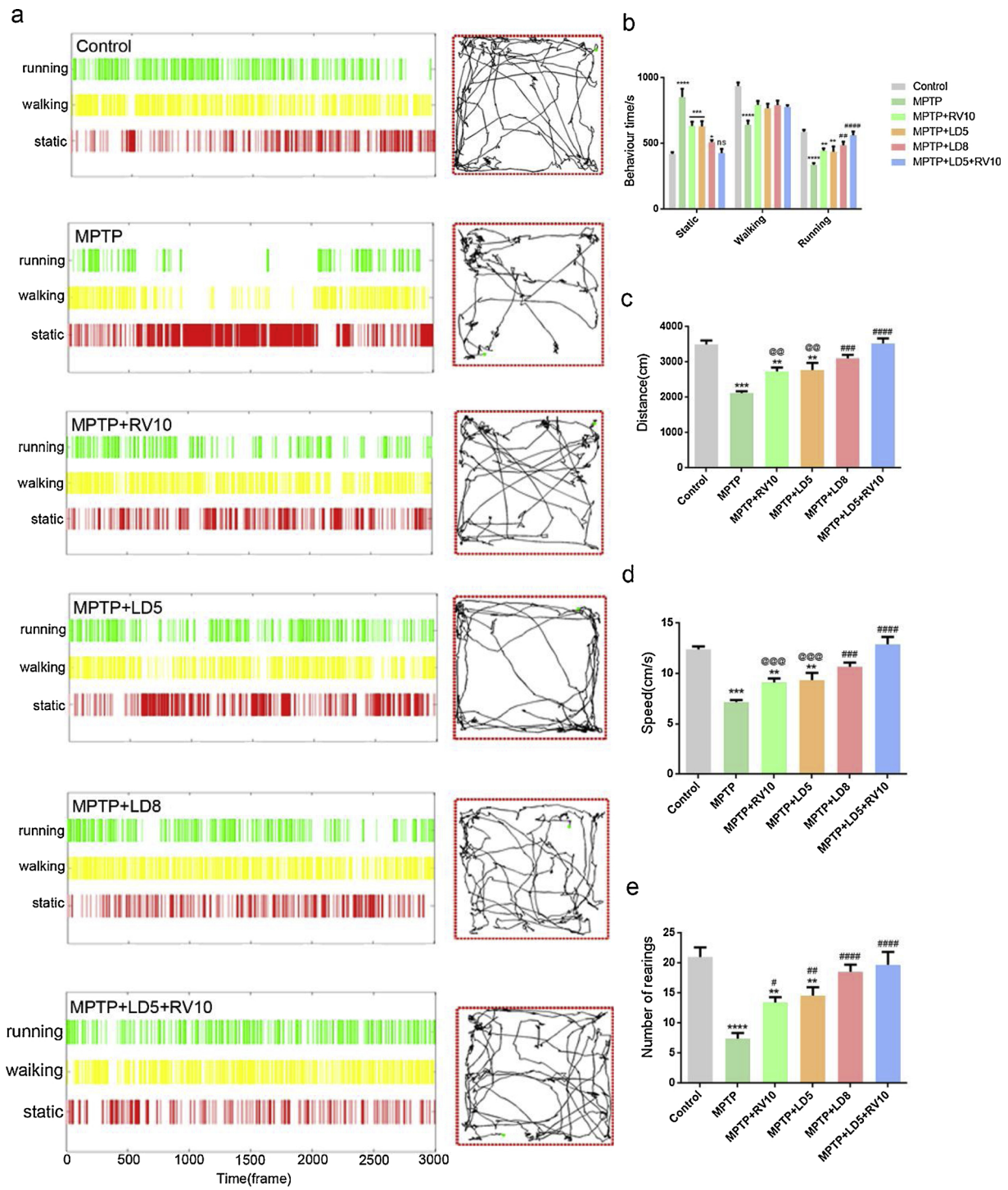

Fig. 5. Examination of locomotor activities. **(a)** An example ethogram of single-mouse locomotion features and trajectories. **(b)** Statistics of behavioural time. **(c)** Total travelled distance. **(d)** Average speed statistics. **(e)** Counts of rearing behaviours. Data are expressed as mean values ± S.E.M. (n = 6). Tukey's multiple comparison test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared to the control group. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ compared to the MPTP-treated group. @ $p < 0.05$, @@ $p < 0.01$, @@@ $p < 0.001$, @@@@ $p < 0.0001$ compared to the MPTP + LD 5+ RV group.

signalling molecule, and Bax, a pro-apoptotic signalling molecule in the striatum [28,29]. MPTP treatment reduced Bcl-2 levels and increased Bax levels in the striatum, but Resveratrol ameliorated this situation. Furthermore, we found that the activation of caspase-3, which is an important factor in the process of apoptosis [30], was also inhibited in the striatum. Our results suggested that Resveratrol can enhance growth factor signalling and reduce apoptosis, which may be a potential mechanism for its synergy with low doses of L-DOPA to improve Parkinson disease.

PI3K/Akt is an important signalling pathway for neuronal growth and survival [31]. A number of molecular substrates for PI3K have been identified, of which AKT, called protein kinase B (PKB), is responsible for mediating anti-apoptotic signals. PI3K activates the Akt pathway and phosphorylates AKT serine-473 to activate Akt [32]. In this study, we observed that Resveratrol increased pAkt (Ser473) levels in the MPTP-induced mouse striatum. Consistent with our results, a decrease

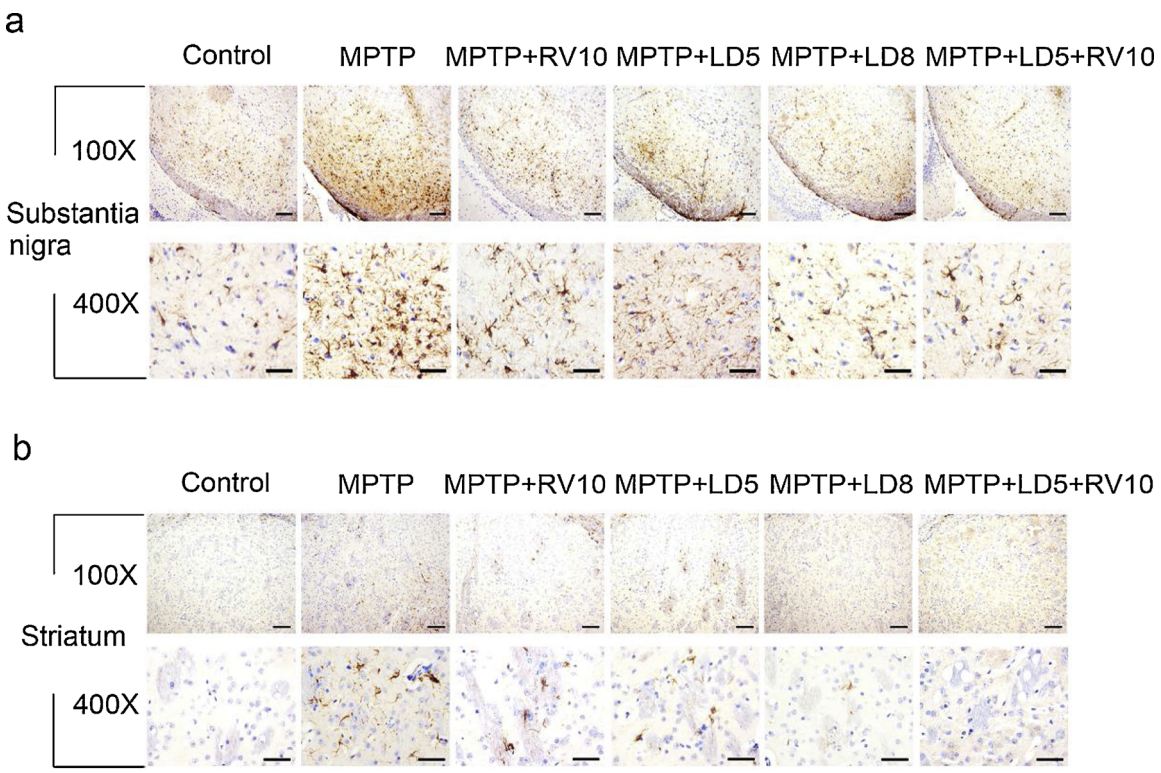

Fig. 6. Effects of co-administration of Resveratrol with L-DOPA on MPTP-induced astroglial activation. **(a)** Immunostaining for GFAP in the substantia nigra. **(b)** Immunostaining for GFAP in the striatum. Scale bars: 100 μm and 40 μm.

in pAkt (Ser473) levels was detected in the brains of MPTP-injected mice [33]. In addition, it has been observed in previously reported studies that the Akt pathway is critical for cell survival in MPP^{+}-treated SH-SY5Y cells [34]. Therefore, Resveratrol can reduce the dose of L-DOPA necessary and improve its therapeutic effect by promoting neuronal survival.

L-DOPA is the most commonly used drug in the clinical treatment of PD because it can effectively improve the symptoms of PD and inhibit akinesia and rigidity in patients [9,13]. However, continued use of L-DOPA for PD comes with considerable side effects [35]. In addition, patients develop tolerance to L-DOPA, eventually leading to LID [36]. Therefore, there is an urgent need to find ways to reduce the dose of L-DOPA needed to effectively treat PD. In previous studies, Resveratrol protected dopaminergic neurons from MPTP-induced cell degeneration to the same extent as PGC-1α overexpression [37]. Our results revealed that co-administration of Resveratrol with L-DOPA attenuated the MPTP-induced increase in static time compared with the control group. In addition, studies have reported that Resveratrol can reduce abnormal rotational behaviour in rats [38] and enhance motor coordination in 6-hydroxydopamine-treated rats [39], which confirms our experimental results.

In conclusion, as a neuroprotective compound, RV has the potential to treat PD by inhibiting neuroinflammation, inhibiting apoptosis and promoting neuronal survival (Fig. 8), especially in combination with L-DOPA. In the present study, we showed that the effects of co-administration of Resveratrol with L-DOPA (5 mg/kg) were equivalent to 8 mg/kg L-DOPA administration in parkinsonian mice. Such co-administration could reduce LID and other side effects caused by long-term use of L-DOPA. In summary, this study provided evidence that Resveratrol can reduce the dose of L-DOPA necessary to improve Parkinson disease.

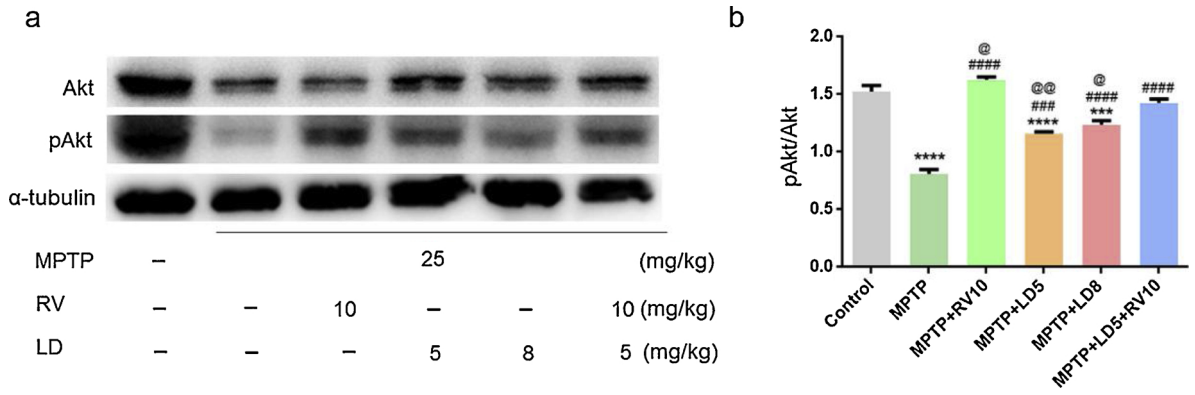

Fig. 7. Western blot analysis protein level of p-Akt/Akt in the striatum. **(a)** Representative protein bands of p-Akt and total Akt. **(b)** The p-Akt/Akt ratio of relative protein levels. Data are expressed as mean values ± S.E.M. (n = 6). Tukey's multiple comparison test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared to the control group. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ compared to the MPTP-treated group. @ $p < 0.05$, @@ $p < 0.01$, @@@ $p < 0.001$, @@@@ $p < 0.0001$ compared to the MPTP + LD 5+ RV group.

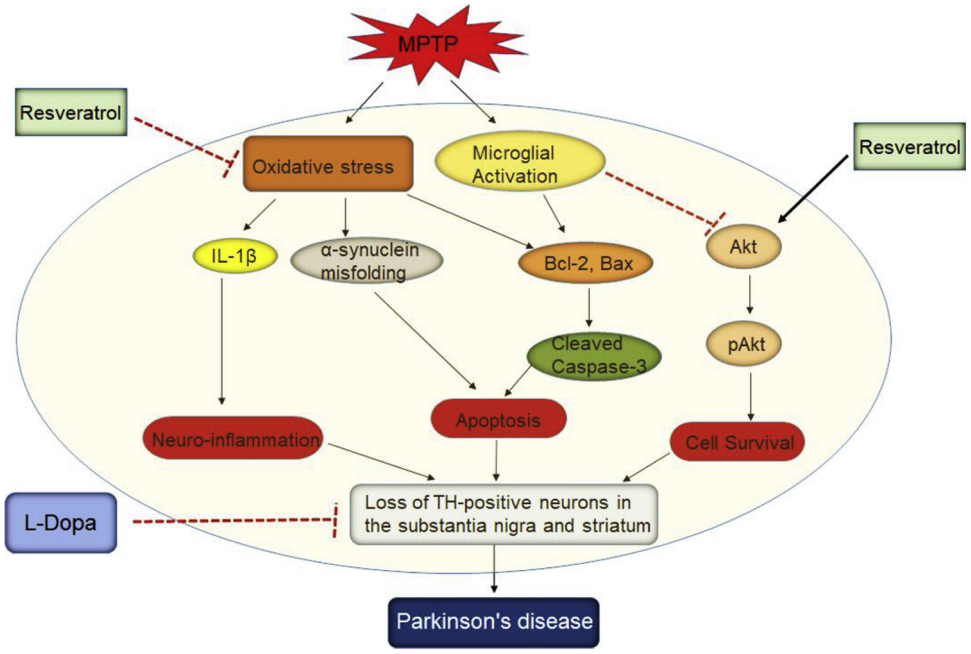

Fig. 8. Resveratrol enhances L-DOPA therapeutic effects, helps to reduce the L-DOPA dose needed, and protects dopaminergic neurons in MPTP-induced parkinsonism by inhibiting neuro-inflammation, apoptosis and promoting neuronal survival.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contributions

X.Z.F. and Q.Q.L. designed the experiments. Behavioural performance data were processed by P.E.J., X.Y.T., Q.H.L., S.Z.Z. and Q.Y.Y., Western blot ananlysis was performed by Q.Q.L., D.S.Z. carried out the immunostaining experiments. X.Z.F. and Y.Z.C. provided suggestions for this study.

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