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Modulation of mitochondrial phenotypes by endurance exercise contributes to neuroprotection against a MPTP-induced animal model of PD

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ABSTRACT

Aim: Endurance exercise (EE) has been reported to confer neuroprotection against Parkinson's disease (PD); however, underlying molecular mechanisms of the protection remain still unclear. Since mitochondrial impairment is commonly observed in the brain of PD patients and animals, this study investigated whether EE-induced neuroprotection is associated with mitochondrial phenotypes, using a mouse model of PD induced by intraperitoneal administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

Main methods: SH-SY5Y cells were cultured with a neurotoxin MPP^+ known to cause PD-like symptoms to examine if modifications of mitochondrial morphology are linked to etiology of PD. For in vivo experiments, C57BL/6 male mice were randomly assigned to four groups: control (CON, n = 12), endurance exercise (EXE, n = 12), MPTP (MPTP, n = 12) and MPTP plus endurance exercise (MPTP + EXE, n = 12). Mice assigned to endurance exercise performed treadmill running at 12 m/min for 60 min/day, 5 days/week for 6 weeks.

Key findings: SH-SY5Y cells exposed to a neurotoxin MPP^+ exhibited mitochondrial fragmentation and diminished mitochondrial proteins, and cell death. Similarly, animals administered with MPTP displayed comparable impairments in the substantia nigra pars compacta (SNpc). In contrast, EE intervention restored motor function to control levels and reduced apoptosis. These propitious effects of EE were associated with mitochondrial phenotypic changes such as upregulated anti-apoptotic proteins (e.g., MCL-1 and BLC-2), reduced a pro-apoptotic protein (e.g., AIF), and improved mitochondrial biogenesis and fusion.

Significance: Our finding that EE-induced mitochondrial phenotypic changes that resist mitochondrial impairment and cell death against PD introduce potential insight into mitochondria as a new therapeutic target for PD.

1. Introduction

Parkinson's disease (PD) is the second most common chronic neurodegenerative disorder after Alzheimer's disease and is caused by deficits of a neurotransmitter, dopamine, due to loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). PD is manifested by motor impairments including bradykinesia, tremor, rigidity and postural instability [56]. Although the exact etiology of PD remains unclear, given that some of the genes involved in familial PD such as PARKIN, DJ-1 and PINK1 are linked to mitochondrial impairment [14], growing evidence indicates that mitochondrial dysfunction is involved in the pathogenesis and progression of PD [7,59]. Supporting this notion, a recent study has observed that activities of mitochondrial complex I are reduced in the SNpc of postmortem patients with PD [70].

A neurotoxin, 1-methyl-1,2,3,6-tetrahydropyridine (MPTP), is commonly used to mimic PD in mice [22,24,65]. MPTP easily crosses the blood-brain barrier due to its lipophilicity and is then converted to its toxic metabolite, 1-methyl-4-phenylpyr-idinium (MPP^+), by monoamine oxidase-B enzyme in astrocytes. MPP^+ is subsequently taken up by dopaminergic neurons and inhibits the activity of mitochondrial electron transport chain complex I, leading to dopaminergic neuron degeneration and behavioral deficits [65].

Mitochondria are essential dynamic organelles responsible for generating large amounts of the cellular energy molecule adenosine triphosphate (ATP). Mitochondria also constantly undergo fission, fusion and biogenesis, and maintain a tubular reticulum under a normal condition. Indeed, these dynamic processes play an important role in

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neuronal survival and homeostasis [49]. For example, recent studies have found that disrupted mitochondrial dynamics (e.g., excessive fission and repressed biogenesis) contribute to mitochondrial dysfunction, triggering neuronal cell death in PD [2,9,72].

Regular endurance exercise (EE) has been known to improve brain function and provide neuroprotection in both human and animal studies [27,81]. Many studies have suggested that EE enhances restoration of dopaminergic neuronal function and promotes neurogenesis, resulting in attenuation of severe progression of PD [64,69]. In our previous studies, we demonstrated that EE-induced neuroprotection against PD is associated with reduced inflammation and oxidative stress, and increased autophagy [23,24]. However, given that the impairment of mitochondrial fission, fusion and biogenesis is associated with pathogenesis of various neurodegenerative diseases including PD [7,33,48], it seems reasonable that EE-mediated neuroprotection could originate from changes in mitochondrial phenotypes that resist PD-induced mitochondrial impairment. While very few studies demonstrating direct effects of EE in alteration of mitochondrial function and phenotypes in the brain of PD are available, multiple layers of evidence have clearly revealed that EE promotes mitochondrial dynamics by altering fusion (e.g., MFN1/2 and OPA1) and fission (e.g., FIS1 and DRP1) [10,12,26,34] in skeletal and cardiac muscle tissues. Also, researches have shown that EE enhances mitochondrial biogenesis [12,18,66] and induces anti-apoptotic responses [1,29,44] in many tissues including the brain. Supporting this concept, a growing number of studies have started to reveal that EE improves mitochondrial function in the brain as well [42,50]. Nevertheless, the cellular and molecular association underlying EE-induced mitochondrial protection and dynamics remains unknown. Thus, in the present study, we examined the effect of MPP^+ upon mitochondrial fragmentation, biogenesis, and cell death, using dopaminergic SH-SY5Y cells. Then, using an in vivo MPTP-induced mouse model of PD, we further investigated if EE-mediated neuroprotection against PD is linked to propitious modulation of mitochondrial dynamics, biogenesis, and apoptosis in the SNpc of the brain of PD mice.

2. Materials and methods

2.1. Cell culture and treatment

The SH-SY5Y human neuroblastoma cells were purchased from American Type Culture Collection (ATCC, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco) and 1% antibiotic antimycotic solution (Anti-Anti, Gibco) in a 37 °C incubator with 5% CO_2. For MPP^+ treatment, MPP^+ was dissolved with ultra-pure water to make a 33.65 mM solution, and then cells were incubated with 1 mM MPP^+ for 24 h and 48 h. Cells assigned to control experiments were treated with the same amount of the solvent.

2.2. Cell viability assay

After MPP^+ treatment, the cell viability was assessed using Cell Viability Image Kit (Invitrogen, R37609) according to the manufacturer's instructions. Briefly, 2 drops of NucBlue Live reagent (Hoechst 33342) and NucGreen dead reagent were added to each cell culture dish containing cells with 1 ml of cell culture medium and then incubated for 15 min at 37 °C incubator. The viable cells were examined using an immunofluorescence microscope (EVOS, ThermoFisher, USA). The numbers of live and dead cells on the images (100× magnification) were manually counted and calculated to obtain the percentage of cell death.

2.3. Immunofluorescence microscopy for the assessment of mitochondrial morphology

For mitochondrial staining, live cells were incubated with 50 nM Mitotracker red CMXRos (Invitrogen, M7512) for 15 min in 37 °C incubator with 5% CO_2, after which cells were fixed with 4% paraformaldehyde solution for 15 min. Then, cells were rinsed three times with PBS (pH 7.4) solution. The numbers of cells displaying fragmented mitochondria on the images (400× magnification) were manually counted and calculated to obtain the percentage of cells with mitochondrial fragmentation.

2.4. Animals

Male C57BL/6 mice at the age of 7 weeks were obtained from Envigo (USA) and were maintained at 12:12 h dark-light cycle at 22 ± 2 °C with 50% relative humidity with ad libitum access to standard chow diet and water. Upon arrival, the mice were allowed to have one week of the acclimation period to our animal facility, after which the mice were randomly divided into four groups: control (CON, n = 12), endurance exercise (EXE, n = 12), MPTP (MPTP, n = 12) and MPTP + endurance exercise (MPTP + EXE, n = 12). Four mice per cage were housed throughout the experimental period. All procedures in this study were approved by the Institutional Animal Care and Use Committees at the University of West Florida (animal use protocol #: 2015-002).

2.5. MPTP-induced mouse model of Parkinson's disease

As depicted in in Fig. 1, mice assigned to MPTP and MPTP + EXE groups were intraperitoneally injected with 25 mg of MPTP (Sigma-Aldrich, M0896) per kilogram of body weight daily for seven days, while CON and EXE groups were injected with the same amount of saline solution for the same period. After seven days of MPTP administration, the mice were allowed to rest for seven days during which (on the fourth day of the rest period) they performed a wire-hanging test for behavioral (motor) assessment. We confirmed a significant loss of the physical exertion capacity (e.g., reduced scores of a hanging test, which was interpreted as a Parkinson's-like effect) (Fig. 1).

2.6. Endurance exercise protocol

One-week after the last MPTP treatment, animals assigned to endurance exercise groups were familiarized with treadmill exercise at 8 m/min, 30 min/day for five consecutive days. Two days after the familiarization, the mice performed treadmill running exercise at 12 m/min for 60 min/day for 5 days/week for 6 weeks. This exercise protocol was designed to maintain moderate intensity of endurance exercise (about 65–70% VO_2max), according to the study by Schefer et al. [62], and the efficacy of the protocol for PD mice was validated in our previous study [24].

2.7. Hanging wire test

The hanging wire test was performed before and after MPTP treatment and after 6 weeks of endurance exercise to assess neuromuscular coordination and strength. We adopted the protocol of the hanging wire test used by Wang et al. [73] with a slight modification. Briefly, mice were suspended by their forelimbs on a steel wire (50 cm in length, 2 mm in diameter, and 37 cm above the ground covered with a soft sponge panel) and allowed to maintain their hanging. The duration of time was recorded until the mice fell off from the wire and scored their neuromuscular coordination according to the following system: fell off, 0; grasp the wire by forelimbs, 1; grasp the wire by forelimbs with attempts to climb onto the wire, 2; grasp the wire with two fore-paws and one or both hind-paws, 3; grasp the wire with all four paws and with a