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Research report

Neuroprotective effects of endurance exercise against neuroinflammation in MPTP-induced Parkinson's disease mice

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ABSTRACT

Parkinson's disease (PD) is one of the main degenerative neurological disorders accompanying death of dopaminergic neurons prevalent in aged population. Endurance exercise (EE) has been suggested to confer neurogenesis and mitigate the degree of seriousness of PD. However, underlying molecular mechanisms responsible for exercise-mediated neuroprotection against PD remain largely unknown. Given the relevant interplay between elevated α-synuclein and neuroinflammation in a poor prognosis and vicious progression of PD and anti-inflammatory effects of EE, we hypothesized that EE would reverse motor dysfunction and cell death caused by PD. To this end, we chose a pharmacological model of PD (e.g., chronic injection of neurotoxin MPTP). Young adult male mice (7 weeks old) were randomly divided into three groups: sedentary control (C, n=10), MPTP (M, n=10), and MPTP + endurance exercise (ME, n=10). Our data showed that EE restored motor function impaired by MPTP in parallel with reduced cell death. Strikingly, EE exhibited a significant reduction in α-synuclein protein along with diminished pro-inflammatory cytokines (i.e., TNF-α and IL-1β). Supporting this, EE prevented activation of Toll like receptor 2 (TLR2) downstream signaling cascades such as MyD88, TRAF6 and TAK-1 incurred by in MPTP administration in the striatum. Moreover, EE reestablished tyrosine hydroxylase at levels similar to C group. Taken together, our data suggest that an EE-mediated neuroprotective mechanism against PD underlies anti-neuroinflammation conferred by reduced levels of α-synuclein. Our data provides an important insight into developing a non-pharmacological countermeasure against neuronal degeneration caused by PD.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder affecting 1~2% of the elderly population and is characterized by cognitive deficits and impaired motor functions (Petzinger et al., 2013). These impairments stem from the progressive loss of dopaminergic neuronal cells in the substantia nigra par compacta, resulting in the depletion of dopamine in the striatum. The decreased levels of dopamine in the striatum are responsible for a motor impairment observed in PD patients (Bezard et al., 2013). Currently, five genes (α-synuclein, parkin, dj-1, pink1, and lrrk2) have been identified to cause PD (Thomas and Beal, 2007). Among these is α-synuclein (α-Syn) a key factor in the pathogenesis of PD based on genetic, neuropathologic, and cellular/molecular lines of evidence, suggesting that elevated levels of α-Syn exert deleterious effects on dopaminergic neurons (Lim et al., 2003). Also, postmortem investigations of PD and other α-synopathy studies have demonstrated the relevance of oligomeric α-Syn aggregates to PD (Spillantini et al., 1997; Spillantini and Goedert, 2000).

Consequence of accumulation of oligomeric α-Syn in neurons has been implicated in activating a serious of Toll-like receptor-2 (TLR2)-mediated signaling cascades through myeloid differentiation factor-88 and nuclear transcription factor-κB (NF-κB), triggering the cerebral inflammation (Codolo et al., 2013; Drouin-Ouellet et al., 2011; Hayward and Lee, 2014; Kim et al., 2013). Indeed, multiple lines of studies have confirmed that the recognition of α-Syn by TLR2 results in production of pro-inflammatory cytokines TNF-α and IL-1β that are toxic and thus contribute to cell death of dopaminergic neurons (Eikelenboom et al., 2006; Hensley et al., 2006). These studies suggest

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that a potential strategy to promote anti-inflammatory countermeasure may significantly contribute to prevention of PD.

Endurance exercise has emerged as a potent, non-pharmacological intervention in the management of patients with PD. A large number of studies in neuroscience have shown that physical exercise improves the symptoms and progression of PD, suggesting exercises as a potential preventive countermeasure against PD (Al-Jarrah et al., 2013; Petzinger et al., 2007; Ridgel et al., 2009). Relating to this, growing evidence indicates that exercise-mediated neuroprotective effects against PD associate with reduced cerebral inflammation (Spielman et al., 2016). Currently, however, how exactly endurance exercise induces anti-inflammatory effects at molecular levels. In the present study, we induced PD-like symptoms in the mice brain via the administration of MPTP, after which 8 weeks of treadmill endurance running exercise were incorporated to test our research hypothesis that endurance exercise-induced decline in α-Syn would prevent activation of TLR2-mediated neuroinflammation.

We report here that endurance exercise restores MPTP-induced motor impairments in parallel with reestablishment of dopamine and prevention of cell death and appeases the production of proinflammatory cytokines by modulating TLR2/MyD88/NF-κB activation. Taken together, our data suggest that endurance exercise-induced neuroprotection against PD may be implicated in downregulation of α-Syn, subsequently leading to anti-inflammation and anti-cell death.

2. Results

2.1. Endurance exercise restores motor coordination deficits caused by MPTP-induced PD via preventing the accumulation of α-Syn

Key manifestation of PD is a gradual loss of motor function. To examine if EE prevents PD-induced motor coordination impairment, endurance exercise was introduced to MPTP-induced mice for 8 weeks, and retention time assessed via the rota-rod test was measured. MPTP-induced PD mice exhibited significant reduction in the retention time compared with saline-treated control mice; however, endurance exercise-trained MPTP-mice completely abolished the MPTP-induced decline of retention time relative to MPTP-mice (Fig. 1A and B). Given the fact that aberrant accumulation of α-Syn is linked to motor function deficit, we examined if exercise-mediated improvement of motor coordination in MPTP-mice is associated with modulation of α-Syn. Our western blot (Fig. 1C and D) and immunofluorescence microscopy (Fig. 1E) data showed that endurance exercise prevented MPTP-induced elevation of α-Syn.

2.2. Endurance exercise mitigates TLR2 activation in the brain of MPTP-induced PD mice

In order to examine our hypothesis that EE-induced abolition of α-Syn upregulation caused by MPTP-treatment would mitigate neuroinflammation, we assessed levels of a key initiator of inflammation TLR2. Our western blot and immunofluorescence microscopy data showed that EE prevented MPTP-induced elevation of striatum TLR2, whereas MPTP-treated sedentary control mice displayed significant elevation of TLR2 (Fig. 2A, B and F). Similarly, EE prohibited MPTP-induced upregulation of MyD88, a downstream molecule of TLR2 (Fig. 2A and C) and tumor necrosis factor receptor-associated factor 6 (TRAF6), a downstream target of MyD88 (Fig. 2A and D), compared with MPTP treatment. Subsequently, EE inhibited MPTP-mediated activities of transforming growth factor-β-activated protein kinase 1 (TAK1), a downstream signaling molecule of TRAF6 inactivation (phosphorylation) of (Fig. 2A and E).

2.3. Endurance exercise reverses MPTP-induced proinflammatory cytokine overproduction and prevents apoptotic cell death in striatum

Active (phosphorylated) NF-κB via phosphorylated IκBα translocates to nucleus and initiate the expression of proinflammatory cytokines TNF-α and IL-1β. While MPTP-treated mice showed a marked elevation of both IκBα and NF-κB phosphorylation levels, those levels in ME mice remarkably declined to levels similar to control mice (Fig. 3A, B and C). Accordingly, ME mice showed reduced levels of TNFα and IL-1β compared to MPTP-mice (Fig. 3D and E). Finally, our immunohistochemical data confirmed that endurance exercise reversed apoptotic cell death caused by MPTP in the striatum (Fig. 3F).

2.4. Endurance exercise increases levels of striatum TH expression in MPTP-mice

In order to confirm whether opportune recovery of motor function by endurance exercise from MPTP-induced motor function impairment is due to enhanced dopamine production, we measured the levels of tyrosine hydroxylase (TH), an alternative measure of dopamine. TH levels remained repressed in the striatum of MPTP-treated mice whereas endurance exercise reinstated TH levels similar to control. (Fig. 4A and B). Also, an immunostaining analysis confirmed the restoration of TH levels in striatum in response to endurance exercise after MPTP administration (Fig. 4C).

2.5. Endurance exercise augments levels of SNpc TH expression in MPTP-mice

To further confirm neuroprotective effects of endurance exercise in SNpc, we assessed levels of tyrosine hydroxylase (TH). Similar to Striatum, Western blot analysis showed that TH levels in SNpc were suppressed in MPTP-treated mice, but exercise training restored its level up to control group (Fig. 5A and B). Moreover, an immunohistochemical assessment showed an increase in numbers of TH positive neurons in exercise-trained SNpc, compared to MPTP group (Fig. 5C).

3. Discussion

The present study examined how endurance exercise (EE) provides neuroprotection using pharmacological model of Parkinson's disease and demonstrated three key findings. First, EE restored motor performance capacity previously impaired by MPTP administration in parallel with suppressed α-Syn to a level comparable to non-PD control animals. Second, EE reversed PD-mediated neuroinflammation by hindering TLR2 downstream singling cascades. Finally, EE restored tyrosine hydroxylase to a level comparable to those displayed in control animals. Taken together, our results demonstrate that EE-induced neuroprotection against PD is mediated by α-Syn suppression, leading to anti-inflammatory effect via diminished TLR2 signaling. A detailed discussion of these findings follows.

A growing body of evidence has identified EE as a both preventive and therapeutic regimen in the management of patients with PD (Petzinger et al., 2013; Smith et al., 2011; Tuon et al., 2012). Our results that EE improves motor performance assessed by the rota-rod test in MPTP-treated mice are also consistent with the studies. However, exact molecular mechanisms responsible for exercise-mediated neuroprotection against PD are incompletely understood. Nevertheless, recent studies have implicated α-Syn downregulation via EE as a key factor linked to neuroprotection (Kohman et al., 2012; Petzinger et al., 2007; Yoon et al., 2007).

α-Syn is a monomeric protein necessary for proper synaptic process and transmission (Moore et al., 2005). However, aberrant accumulation of α-Syn in the dopamine-generating neurons is linked to neuroinflammation and cell death, leading to PD (Saiki, 2014). In the present study, we observed that long term MPTP administration, which