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Original Article

Effects of treadmill exercise on hippocampal neurogenesis in an MPTP /probenecid-induced Parkinson's disease mouse model

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Abstract. [Purpose] This study aimed to investigate the effect of treadmill exercise on non-motor function, specifically long-term memory, in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid-induced Parkinson's disease mouse model. [Methods] A mouse model of Parkinson's disease was developed by injecting 20 mg/kg of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 250 mg/kg of probenecid (P). We divided in into four groups: probenecid group, probenecid-exercise group, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid group, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid-exercise group. Mice in the exercise groups ran on treadmill for 30 min/day, five times per week for 4 weeks. [Results] Latency in the passive avoidance test increased in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid-exercise group compared with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid group. In addition, the number of 5-bromo-2-deoxyuridine/NeuN-positive cells and 5-bromo-2-deoxyuridine/doublecortin-positive cells in the hippocampal dentate gyrus was higher in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid-exercise group than that in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid group. These changes were associated with the expression of brain-derived neurotrophic factor in the hippocampus. [Conclusion] Our results suggest that treadmill exercise may improve long-term memory in Parkinson's disease mice by facilitating neurogenesis via increased expression of neurotrophic factors.

Key words: Parkinson's disease, Neurogenesis, Long-term memory

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INTRODUCTION

Parkinson's disease (PD) is one of the most common neurodegenerative disorders and is characterized by progressive loss of nigrostriatal dopaminergic neurons leading to dopamine depletion and motor dysfunction deficits. Cognitive dysfunction is a common non-motor complication of PD[1)]. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is known to cause PD-like symptoms by damaging dopaminergic neurons in the substantia nigra (SN), and has been used widely to establish rodent models of PD[2)].

The neurotransmitter dopamine plays a critical role in the cellular signaling processes that underlie information transfer in neurons functioning in the nervous system. Dopamine receptors (DR) belong to the G-protein-coupled receptor superfamily, and have five isoforms[2)]. Dopamine receptor subtype 2 (D2DR) has affinity for dopamine and the dopamine receptor agonists used to treat PD[3)]. It plays a major role in long-term depression (LTD), a form of synaptic plasticity that involves integration of glutamatergic and dopaminergic neurotransmission and leads to motor function encoding in the dorsolateral striatum[4)]. Marked inhibition of the D2DR pathway in the basal ganglia-thalamus-cortical loop leads to parkinsonian features, loss of motor skills, and decreased cell proliferation in the hippocampus[5, 6)].

Adult hippocampal neurogenesis is positively affected by neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurotrophin-3 (NT-3). Among them, BDNF is involved in learning ability, memory function, and synaptic plasticity[7, 8)]. BDNF is a neurotrophin implicated in neuronal survival and plasticity and functions by binding to the high-affinity receptor, tyrosine kinase B (Trk B)[9)]. BDNF-Trk B interaction promotes the survival and differentiation of neurons, increases synaptic plasticity, inhibits apoptosis, and improves cognition deficits. Inhibition of BDNF-Trk B signaling suppresses neurogenesis in the hippocampal dentate gyrus (DG)[10)].

Physical exercise is currently advocated as a behavioral intervention to ameliorate neurological impairment by impeding the neuronal loss that is caused by several neurodegenerative diseases, including PD, and exercise is currently being proposed to have many possible therapeutic benefits[11, 12)]. In addition, several studies have demonstrated the beneficial effect of exercise on functional outcomes in

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patients with PD[13, 14]. In this study, we investigated the effects of treadmill running on long-term memory and hippocampal neurogenesis in an MPTP-induced PD mouse model.

SUBJECTS AND METHODS

Male ICR mice weighing 45 ± 3 g (10-week-old) were used in this experiment. All animal experimental procedures conformed to National Institutes of Health (NIH) and Korean Academy of Medical Science guidelines. Animals were housed at a controlled room temperature (20 ± 2 °C) and under a 12-hour light/12-hour dark cycle. Animals were allowed access to food and water ad libitum. Animals were randomly divided into four groups (n = 10 in each group): the probenecid group, probenecid-exercise group, MPTP/probenecid (MPTP/P) group, and MPTP/P- exercise group (MPTP/P-ex). To generate a chronic PD mouse model, the mice were injected with a total of 10 doses of MPTP hydrochloride (20 mg/kg in saline, s.c.) in combination with an adjuvant, probenecid (250 mg/kg in saline with sodium hydroxide (NaOH), i.p.), over 5 weeks at 3.5 day intervals. Control mice were treated with the vehicle (probenecid) in the same volume and on the same schedule. Mice in the exercise groups ran on a treadmill after the last drug injection for 30 min/day, five times/week for 4 weeks at speeds up to 10 m/min. Non-exercise group was placed on the treadmill (EXER-6M, Columbus, OH, USA) lane for the same duration as the exercised groups to expose them to similar conditions, but they did not run on the treadmill.

The passive avoidance test was used to evaluate long-term memory after the last exercise session. The apparatus consisted of a dark compartment and a light compartment. The two compartments were separated by a small door. The floor of the dark compartment consisted of a stainless steel grid. During the training trial, each mouse was placed in the light compartment. After 30 s of habituation, the small door was opened. Immediately after the mouse entered the dark compartment, the small door was closed and an electric foot shock was delivered to the floor grids for 3 s. After 72 h, the test trial was performed. The latency was defined as the time taken to enter the dark compartment and place all four paws on the grid. Any latency >300 s was counted as 300 s.

To measure protein expression, the striatum was dissected from deeply anesthetized mice after the passive avoidance test. Sample tissue was stored at −70 °C until analysis. Sample tissue was lysed in ice-cold lysate buffer and then the mixture was incubated for 30 min at 4 °C. Protein aliquots (40 μg) were separated on SDS-polyacrylamide gels and then transferred onto a nitrocellulose membrane (Schleicher & Schuell GmbH, Dassel, Germany) that was subsequently probed with the following primary antibodies: anti-tyrosine hydroxylase (TH), anti-D2DR, anti-BDNF, anti-Trk B (1:1000; Santa Cruz Biotech, CA, USA). Horseradish peroxidase-conjugated anti-rabbit antibody (1:3000; Santa Cruz Biotech) for TH, D2DR, BDNF, and Trk B were used as secondary antibodies. Bands were detected using the enhanced chemiluminescence (ECL) detection system (Santa Cruz Biotech).

To detect the expression of TH in the substantia nigra pars compacta (SNpc) and striatum, floating tissue sections were incubated in 3% H_2O_2 for 30 min and pre-treated with a double detection solution (BrdU/NeuN and BrdU/DCX). After pre-treatment, sections were blocked in solution include 1% BSA and 10% horse serum in 0.05 M PBS. The sections were then incubated with rat anti-mouse TH antibody (1:1000; BD biosciences, San Jose, CA, USA) and BrdU antibody (1:300, Abcam, Biomeda, CA, USA). The sections were incubated with biotinylated anti-mouse IgG (1:200; Vector Laboratories, Burlingame, CA, USA). After BrdU staining, counter-staining was performed on the same sections using NeuN antibody (1:300; Chemicon International, Temecula, CA, USA) and DCX (1:200; Santa Cruz Biotech), respectively. Sections were incubated with a biotinylated anti-mouse IgG (1:200; Vector Laboratories), and processed with the Vector Elite ABC kit® (Vector Laboratories). For visualization, sections were reacted with 0.05% 3,3′-diaminobenzidine (DAB) and 0.01% H_2O_2 in 0.05 M Tris buffer (pH 7.6). Between each step, sections were thoroughly washed three times in 0.05 M PBS. Lastly, sections were mounted on gelatin-coated slides and cover slipped with mounting medium.

All data were analyzed using an Image-Pro® Plus computer-assisted image analysis system (Media Cyberbetics Inc., Silver Spring, MD, USA). Statistical analysis was performed using one-way ANOVA followed by Duncan's post-hoc test, and the results are expressed as the mean ± standard error of the mean (SEM). The threshold for statistical significance was set at $p < 0.05$.

RESULTS

The latency in the passive avoidance task was 282.6 ± 11.68 s, 276.16 ± 16.06 s, 183.0 ± 35.44 s, and 269.0 ± 9.86 s in the probenecid, probenecid exercise, MPTP/P, and MPTP/P-exercise groups, respectively ($p < 0.05$). These results demonstrate that MPTP/P treatment decreases memory function and that treadmill exercise protects against this effect (Table 1).

In the present study, TH and D2DR expression in the ventral midbrain in the probenecid group was set at 1.00. The level of TH protein was 1.05 ± 0.01, 0.79 ± 0.01, and 1.18 ± 0.01 in the probenecid, MPTP/P, and MPTP/P-exercise groups, respectively. The level of D2DR protein was 1.08 ± 0.03, 0.61 ± 0.02, and 0.77 ± 0.02 in the probenecid exercise, MPTP/P, and MPTP/P-exercise groups, respectively ($p < 0.05$) (Table 2).

The number of BrdU/DCX-positive cells in the hippocampus was 67.54 ± 9.38/mm^2, 66.60 ± 7.73/mm^2, 39.30 ± 5.68/mm^2, and 61.05 ± 7.31/mm^2 in the probenecid, probenecid exercise, MPTP/P, and MPTP/P-exercise groups, respectively. The number of BrdU/NeuN-positive cells in the hippocampus was 41.46 ± 5.05/mm^2, 48.20 ± 5.40/mm^2, 26.63 ± 3.21/mm^2, and 46.33 ± 5.12/mm^2 in the probenecid, probenecid exercise, MPTP/P, and MPTP/P-exercise groups, respectively ($p < 0.05$) (Table 3).

In the present study, BDNF and Trk B expression in the ventral midbrain in the probenecid group was set at 1.00. The level of BDNF protein was 0.97 ± 0.02, 0.74 ± 0.01, and 0.87 ± 0.01 in the probenecid exercise, MPTP/P, and MPTP/P-exercise groups, respectively. The level of Trk B

Table 1. Effects of treadmill exercise on long-term memory in the passive avoidance test

Group	Probenecid	Probenecid-Ex	MPTP/P	MPTP/P-Ex
Latency (s)	282.6 ± 11.68$^{\#}$	276.16 ± 16.06$^{\#}$	183.0 ± 35.44*	269.0 ± 9.86$^{\#}$

Ex: exercise; MPTP/P: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid; s: seconds
$^{*}p<0.05$ compared to the probenecid group, $^{\#}p<0.05$ compared to the MPTP/P group

Table 2. Effects of treadmill exercise on protein expression (unit: O.D.)

Group	Probenecid	Probenecid-Ex	MPTP/P	MPTP/P-Ex
TH	1.00±0.00$^{\#}$	1.05±0.01$^{*,\#}$	0.79±0.01*	1.18±0.01$^{*,\#}$
D2DR	1.00±0.00$^{\#}$	1.08±0.03$^{*,\#}$	0.61±0.02*	0.77±0.02$^{*,\#}$
BDNF	1.00±0.00$^{\#}$	0.97±0.02$^{\#}$	0.74±0.01*	0.87±0.01$^{*,\#}$
Trk B	1.00±0.00$^{\#}$	1.10±0.03$^{\#}$	0.52±0.07*	0.71±0.02$^{*,\#}$

BDNF: brain-derived neurotrophic factor; D2DR: dopamine receptors subtype 2; Ex: exercise; MPTP/P: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid; O.D.: optical density; TH: tyrosine hydroxylase; Trk B: tyrosine kinase B
$^{*}p<0.05$ compared to the probenecid group, $^{\#}p<0.05$ compared to the MPTP/P group

Table 3. Effects of treadmill exercise on neurogenesis in the hippocampal dentate gyrus (DG) (unit: mm^2)

Group	Probenecid	Probenecid-Ex	MPTP/P	MPTP/P-Ex
BrdU/DCX	67.54±9.38$^{\#}$	66.60±7.73$^{\#}$	39.30±5.68*	61.05±7.3$^{\#}$
BrdU/NeuN	41.46±5.05$^{\#}$	48.20±5.40$^{\#}$	26.63±3.21*	46.33±5.1$^{\#}$

BrdU: 5-bromo-2-deoxyuridine; DCX: doublecortin; Ex: exercise; MPTP/P: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid
$^{*}p<0.05$ compared to the probenecid group, $^{\#}p<0.05$ compared to the MPTP/P group

protein was 1.10 ± 0.03, 0.52 ± 0.07, and 0.71 ± 0.02 in the probenecid exercise, MPTP/P, and MPTP/P-exercise groups, respectively ($p < 0.05$) (Table 2).

DISCUSSION

Cognitive and memory impairments are an important symptom of PD[15]. Previous studies have found that MPTP-induced neuronal loss in the SN results in disturbances in memory function and learning impairment[16, 17]. In the present study, long-term memory during the passive avoidance test deteriorated in the MPTP/P-induced PD mice, and treadmill exercise improved short-term memory. The beneficial effects of physical exercise on cognitive functions such as spatial learning ability and memory have been reported previously[11].

D2DR is of clinical interest because of its role in movement disorders, such as PD, where D2DR agonists are known to benefit patients[18]. D2DR gene knockout mice demonstrated hypokinesia, bradykinesia, and decreased spontaneous movement[19]. Dopamine receptor antagonists and dopamine depletion have been reported to reduce cell proliferation in the subventricular zone (SVZ) and hippocampal DG of rodents[5]. In the present study, expression of D2DR in the ventral midbrain significantly decreased in the MPTP-induced PD mice, and treadmill exercise increased D2DR expression in these mice. Höglinger et al.[5] reported that an agonist of dopamine D2-like receptors (D2, D3, and D4 receptors) restored cell proliferation in the SVZ in a PD rodent model. Fisher et al.[13] reported that treadmill exercise reversed the effects of MPTP injection on dopamine D2 receptors in the dorsal striatum.

The present study showed that neurogenesis in the hippocampal DG significantly decreased owing to induction of PD, whereas neurogenesis significantly enhanced in response to treadmill exercise. Increased neurogenesis in the hippocampal DG improves learning ability and memory function[20]. The observed increase in the expression of BrdU-positive and DCX-positive cells in the hippocampal DG indicates that exercise enhanced neuronal plasticity[21]. Expression of DCX can be used as a marker for immature neurons; therefore, the level of DCX expression in the adult brain can be used to measure neurogenesis[22, 23]. In the present study, expression of newborn immature cells in the hippocampal DG was significantly decreased in the MPTP-induced PD mice, while treadmill exercise increased the number of newborn immature cells in these mice. The increase in the number of DCX-positive cells after treadmill exercise could be owing to the addition of newly generated immature cells[22, 23]. These results showed that treadmill exercise is not only capable of promoting cellular proliferation but also differentiation of newborn neuroblasts. Physical exercise increases neurogenesis, long-term potentiation, synaptic plasticity, and improves spatial memory[24, 25].

Adult hippocampal neurogenesis is affected by neurotrophic factors such as BDNF, NGF, and NT-3, which are

implicated in adult neurogenesis[7]. BDNF has been recognized as a key regulator of synaptic development and plasticity in relation to several neurodegenerative and psychiatric disorders[26]. Over-expression of BDNF and its receptor Trk B increases neurogenesis, whereas inhibition of BDNF or Trk B suppresses neurogenesis[27, 28]. Suppression on BDNF-Trk B signaling is associated with the inhibition of neurogenesis in the hippocampal DG[20]. In the present study, we observed that the administration of MPTP significantly decreased BDNF and Trk B levels in the hippocampus, while treadmill exercise increased BDNF and Trk B levels in the hippocampus of MPTP-induced PD mice. Exercise enhances learning and memory, which is accompanied by increased cell proliferation and survival in the hippocampus via increased BDNF and Trk B expression[29, 30]. It has been reported that BDNF significantly increases neurogenesis in the DG[30]. Here, we suggest that exercise can overcome PD-induced hippocampal memory impairment by facilitating neurogenesis through enhancement of BDNF expression and prevention of dopaminergic neuronal damage. Therefore, exercise may be a potential therapeutic strategy for the alleviation of memory dysfunction in PD dementia patients.

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