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

YUN-HEE SUNG, PT, PhD1)

1) Department of Physical Therapy, College of Natural Sciences, Kyungnam University: Changwon-si, Gyeongsangnam-do 631-701, Republic of Korea

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

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 PD1). 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 PD2).

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 isoforms3). Dopamine receptor subtype 2 (D2DR) has affinity for dopamine and the dopamine receptor agonists used to treat PD3). 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 striatum4). 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 hippocampus5, 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 plasticity7, 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 benefits11, 12). In addition, several studies have demonstrated the beneficial effect of exercise on functional outcomes in
patients with PD. 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 group, 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 an adjuvant, probenecid (250 mg/kg in saline with sodium drochloride (20 mg/kg in saline, s.c.) in combination with probenecid (MPTP/P) group, and MPTP/P-exercise group. 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 the same volume and on the same schedule. Mice in the non-exercise group was placed on the treadmill for 30 min/day, five times/week for 4 weeks at speeds up to the same volume and on the same schedule. 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 lysis 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% H2O2 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% H2O2 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 Cybernetics 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, probenecid exercise, 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/mm², 66.60 ± 7.73/mm², 39.30 ± 5.68/mm², and 61.05 ± 7.31/mm² 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/mm², 48.20 ± 5.40/mm², 26.63 ± 3.21/mm², and 46.33 ± 5.12/mm² 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...
known to benefit patients with movement disorders, such as PD, where D2DR agonists are reported to have effects in MPTP-induced PD mice, while treadmill exercise increased 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. 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. 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. 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. 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.

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 TrkB increases neurogenesis, whereas inhibition of BDNF or TrkB suppresses neurogenesis\(^27, 28\). Suppression on BDNF-TrkB signaling is associated with the inhibition of neurogenesis in the hippocampal DG\(^29\). In the present study, we observed that the administration of MPTP significantly decreased BDNF and TrkB levels in the hippocampus, while treadmill exercise increased BDNF and TrkB 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 TrkB 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.

**ACKNOWLEDGEMENT**

This work was supported by a Kyungnam University Foundation Grant, 2013.

**REFERENCES**

1. Yoon YJ, Lee BH: Effects of balance and gait training on the recovery of the motor function in an animal model of Parkinson's disease. J Phys Ther Sci, 2014, 26: 905–908. [Medline] [CrossRef]

2. Jackson-Lewis V, Przedborski S: Protocol for the MPTP mouse model of Parkinson’s disease. Nat Protoc, 2007, 2: 141–151. [Medline] [CrossRef]

3. Liu IS, George SR, Seeman P: The human dopamine D2(Longer) receptor has a high-affinity state and inhibits adenyl cyclase. Brain Res Mol Brain Res, 2000, 77: 281–284. [Medline] [CrossRef]

4. Toy WA, Petzinger GM, Leyshon BJ, et al.: Treadmill exercise reverses apoptotic neuronal cell death and cell proliferation of maternal-separated rat pups. Brain Dev, 2012, 34: 45–56. [Medline] [CrossRef]

5. Lee NY, Lee DK, Song HS: Effect of virtual reality dance exercise on the balance, activities of daily living, and depressive disorder status of Parkinson's disease patients. J Phys Ther Sci, 2015, 27: 145–147. [Medline] [CrossRef]

6. Baek SS, Jun TW, Kim KJ, et al.: Effects of postnatal treadmill exercise on apoptotic neuronal cell death and cell proliferation of maternal-separated rat pups. Brain Dev, 2012, 34: 45–56. [Medline] [CrossRef]

7. Höglinger GU, Rizk P, Muriel MP, et al.: Dopamine depletion impairs neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci USA, 1999, 96: 13427–13431. [Medline] [CrossRef]

8. Vaynman S, Ying Z, Gomez-Pinilla F: hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur J Neurosci, 2004, 20: 2580–2590. [Medline] [CrossRef]

9. Bauck SE, Jun TZ, Kim KJ, et al.: Effects of postnatal treadmill exercise on apoptotic neuronal cell death and cell proliferation of maternal-separated rat pups. Brain Dev, 2012, 34: 45–56. [Medline] [CrossRef]

10. Lee NY, Lee DK, Song HS: Effect of virtual reality dance exercise on the balance, activities of daily living, and depressive disorder status of Parkinson’s disease patients. J Phys Ther Sci, 2015, 27: 145–147. [Medline] [CrossRef]

11. Fisher BE, Petzinger GM, Nixon K, et al.: Exercise-induced behavioral recovery and neuroplasticity in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse basal ganglia. J Neurosci Res, 2004, 77: 378–390. [Medline] [CrossRef]

12. Rodrigues de Paula F, Teixeira-Salmea LF, Coelho de Morais Faria CD, et al.: Impact of an exercise program on physical, emotional, and social aspects of quality of life of individuals with Parkinson's disease. Mov Disord, 2006, 21: 1073–1077. [Medline] [CrossRef]

13. Lehrner J, Moser D, Klag S, et al.: Subjective memory complaints, depressive symptoms and cognition in Parkinson’s disease patients. Eur J Neurol, 2014, 21: 1276–1284, e77. [Medline] [CrossRef]

14. Castro AA, Ghisini K, Latini A, et al.: Lithium and valproate prevent olfactory discrimination and short-term memory impairments in the intranigral 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) rat model of Parkinson's disease. Behav Brain Res, 2012, 229: 208–215. [Medline] [CrossRef]

15. Prediger RD, Aguiar AS Jr, Rojas-Mayorquin AE, et al.: Single intranasal administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in C57BL/6 mice models early preclinical phase of Parkinson’s disease. Neurotox Res, 2010, 17: 114–129. [Medline] [CrossRef]

16. Pahwa R, Lyons KE, Hauser RA: Ropinirole therapy for Parkinson’s disease. Expert Rev Neurother, 2004, 4: 581–588. [Medline] [CrossRef]

17. Baik JH, Picetti R, Saiardi A, et al.: Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. Nature, 1995, 377: 424–428. [Medline] [CrossRef]

18. Shin MS, Ko IG, Kim SE, et al.: Treadmill exercise ameliorates symptoms of methimazole-induced hypothyroidism through enhancing neurogenesis and suppressing apoptosis in the hippocampus of rat pups. Int J Dev Neurosci, 2013, 50: 116–124. [Medline] [CrossRef]

19. Pahwa R, Lyons KE, Hauser RA: Ropinirole therapy for Parkinson’s disease. Expert Rev Neurother, 2004, 4: 581–588. [Medline] [CrossRef]

20. Baek SS, Jun TW, Kim KJ, et al.: Effects of postnatal treadmill exercise on apoptotic neuronal cell death and cell proliferation of maternal-separated rat pups. Brain Dev, 2012, 34: 45–56. [Medline] [CrossRef]

21. J. Phys. Ther. Sci. Vol. 27, No. 10, 2015