Effects of Balance and Gait Training on the Recovery of the Motor Function in an Animal Model of Parkinson’s Disease

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Abstract. [Purpose] This study was conducted to investigate the effect of balance and gait training on the recovery of the motor function in a Parkinson’s disease animal model. [Subjects and Methods] A total of 40 mice were randomly classified into four groups with 10 in each group: Group I-Normal; Group II-Parkinson’s disease and no training; Group III-Parkinson’s disease and balance training was performed; and Group IV-Parkinson’s disease and gait training. Parkinson’s disease was induced by administration of MPTP to animals in Groups II–IV. Groups III and IV did training once a day, five days a week, for four weeks. Neurobehavioral evaluation was performed through the pole and open-field tests. Immunological evaluation was performed via TH (tyrosine hydroxylase) protein expression, using western blot analysis. [Results] In the result of the pole test, Groups III and IV showed significantly greater motor function recovery than to Group II. The results of the open-field test also showed that Groups III and IV had significantly greater motor function recovery than to Group II, and Group IV showed significantly greater motor function recovery than to Group III. Using western blot analysis, we determined that the expression of TH protein in the corpus striatum was greatest in group I, followed by Groups III and IV, and that Group II had the lowest TH protein expression in the corpus striatum. [Conclusion] The results of this study showed that balance and gait training were effective at recovering the motor functions of a Parkinson’s disease animal models induced by MPTP, and that gait training was more effective than balance training.

Key words: Parkinson’s disease, Balance training, Gait training

INTRODUCTION

Parkinson’s disease afflicts the diseases of 1% of all people over 60 years old and 3% of all those over the age of 80 in developed countries, and it is caused a decrease in the number of dopamine-generating cells in the substantial nigra. It is known as an idiopathic disease, with no particular cause having been conclusively established thus far, and generally appears in people in their 60s. It has been reported, though, that heavy metals, pesticides, or medicines are the causes of Parkinson-like diseases. In particular, the symptoms shown by young people addicted to drugs containing MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) are similar to those of Parkinson’s disease. Also, farmers exposed to pesticides combined with MPTP show a high incidence of Parkinson's disease. These facts support the possibility that Parkinson’s disease is related to MPTP, a neurotoxin. For A pathophysiological characteristics of Parkinson’s disease is that Lewy bodies are found in dopamine-secreting nerve cells in the substantia nigra. In the early stage of the disease, when the olfactory bulb, medulla oblongata, and pons are affected, no motor abnormality is found. The motor abnormality begins at the stage when the nigra, midbrain, and basal forebrain are affected. The number of dopamine-producing nerve cells decreases by about 25% after middle age, and by about 35% at 65 years old in healthy people. The motor abnormality of Parkinson’s disease begins to appear when the dopamine-producing nerve cells have decreases by more than 80%.

The major motor abnormalities of Parkinson’s disease include resting tremors, bradykinesia, rigidity, and postural instability, as well as its characteristic rapid shuffle step and mask-like facial expression. Parkinson’s disease patients have an increased risk of falls, which leads to a three-fold increased risk of hip fractures compared to healthy people. As the dopamine-producing nerve cells are very sensitive to physical training, the neural plasticity of the substantia nigra increases and the progress of the disease is delayed when patients exercise. There is no definite exercise guideline, however, for patients with Parkinson’s disease. As such, this study aimed to investigate the effects of balance and gait training on motor function recovery in an animal model of Parkinson’s disease using neurobehavioral...
evaluation and western blot analysis.

SUBJECTS AND METHODS

Subjects
All the procedures performed in this study were reviewed by the Animal Ethicals Committee of Dongshin University before the conduct of the study. The animals that were used for this study were C57BL/6 mice weighing 25±5 g (16 weeks, male, Damul Science, South Korea). The temperature of the housing room was 23±2 °C; the humidity, 55±10%; and the light cycle, 12 hr. The animals had free access to water and solid food. The mice were aclimated for one week, and 40 healthy mice were selected for the study. The animals were randomly allocated into four groups with 10 mice per group (Table 1).

Methods
For the induction of Parkinson’s disease, 20 mL MPTP hydrochloride was dissolved in 20 mL sterile saline, and 5 mg/ml was administered intra-peritoneally every 4 hr for 3.5 days (total of 20 times, 100 mg). The Probenecid was administered together with MPTP to maintain the effect of MPTP, and to prevent it from being excreted via urine or the skin9).

Gait training Groups were performed on an auto wheel at a speed of 2 m/min for the initial 5 min and 8 m/min during the next 25 min. The training was performed for 30 min per day, five times a week, for four weeks. For balance training, mice were placed inside a 19-cm-diameter transparent acrylic ball. The ball was placed on a 15-cm-wide, 15-cm-long, 10-cm-tall wooden plate with no slide. The mice were trained to maintain their balance while the ball was moving left, right, forward, and backward. The training was performed for 30 min a day, five times a week, for four weeks.

The two edges of a rough-surfaced, cylindrical wood stick (length: 50 cm, diameter: 0.8 cm) were tied to two pillars 15 cm in height (one edge was tied to one pillar). The time that it took each mouse to go from one edge of the stick to the other was measured. If it took a mouse more than 300 sec to do so, the time was recorded as 300 sec.

Squares (13 × 13 cm) were drawn of the floor of a 78 cm-wide, 78 cm-long, 30 cm-tall black box, and the numbers 1 to 36 were written in the boxes, on number on each box, in order (from 1 to 36). A light bulb was placed over the middle of the ceiling of a transparent acrylic plate. The mice were acclimatized in a small (15 × 15 cm) box connected to the black box for 1 min, and were than allowed to enter the black box. The time that it took each animal to make its first movement when passing the boxes numbered 15, 16, 21 and 22, where food was placed under the light, was measured. If it took a mouse more than 300 sec, the time was recorded as 300 sec.

Selected animals from each group were sacrificed under general anesthesia via inhalation on day 28 of the training. The thoracic cavity was opened, and the heart was perfused with 0.9% saline. A pre-fix was performed using 4% paraformaldehyde, and the brain was taken out after opening the skull. The excised brain was fixed in 10% paraformaldehyde solution for two days at 4°C (post-fix), after which it was processed using an automated tissue-processing machine (4640B, Sakura, Japan) for 14 hr and followed by embedding in paraffin wax. The brain was cut into 4- to 5-µm-thick slices using a rotary microtome (Rotary Microtome 2040, Sakura, Japan), with 180 µm gaps between the slices. The specimens were washed using PBS, and were homogenized using a homogenizer at 4 °C. They were then reacted with the in a solution buffer [50 mM HEPES (pH7.5), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM PMSF, 1 mM EGTA, 1.5 mM MgCl2, 6H2O2, sodium orthovanadate, and 100 mM sodium fluoride] for 30 min at 4 °C. The samples were centrifuged for 20 min at 15,000 rpm to obtain supernatant, and the protein concentration in the solution was quantified using Bradford method (Bio-Rad protein assay). The extracted protein was loaded onto the same amount of SDS polyacrylamide gel and for electrophoresis. Thereafter, the gel was transferred to a nitrocellulose membrane (Whatman GmbH, Dassel, Germany) and underwent reacted with TBX and 5% non fat dried milk for 1 hr at 4°C to prevent non-specific binding with the antibody. Then the gel was washed using TBS and underwent reacted with rabbit polyclonal anti-TH (1: 1,000, Santa Cruz Biotechnology, USA) for 24 hr at 4°C. To check the specificity of the of the reaction, a secondary antibody underwent reaction with anti-rabbit IgG (1:1,000, Santa Cruz Biotechnology, USA) for 1 hr was also performed, and the bands were visualized in the radiation film when with to an enhanced chemical-luminescence kit (RPN 2106, Amersham Life Science Inc., USA). To confirm if the equivalent protein, monoclonal anti-β actin (A-5316, 1:5,000, Sigma, USA), had been loaded, goat anti-mouse IgG (BD Bioscience, Franklin Lakes, NJ, USA) was used for comparison.

Data analysis was performed using PASW version 18.0. All the data are presented as means ± SD. The significance of differences between the groups’ neurobehavioral scores and the change in TH Protein expression were analyzed using one-way ANOVA, and Tukey’s multiple-range test was used as a post-hoc test. The level of significance for all the statistical tests was set at α=0.05.

RESULTS

The results of the pole tests before and on the training at days 7, 14, 21 and 28 are shown in Table 2. The ability to balance was decreased with time apart from in Group I. There were a significant differences between before and after training in all the one-way ANOVA results (p<0.05).

The results of the open-field test showed that there were
Using Western blot, The amount of expressed in the brains of the TH protein was observed in mice after their four-week training, to was assayed to determine the increase or decrease of dopamine in their corpus striatum. As shown in Fig. 1, the amount of expressed TH protein was greatest in Group I, followed by Groups III and IV. Group II had the lowest amount of expressed TH protein.

**DISCUSSION**

Parkinson's disease patients in their 60s usually show the symptoms of motor disability while resting, such as tremors, bradykinesia, rigidity, and postural instability. Their muscle strength is rapidly weakening, which results in decreased body activity. The decreased muscle strength is not a secondary symptom caused by aging and decreased physical activities, rather it is a primary symptom of Parkinson's disease due to decreased dopamine, which leading to the lack of atory to the motor part of the cortex from the thalamus and basal ganglia. One's Balance and gait are important factors for quality of life in everyday life. As the muscle strength needed to resist gravity forms the basis of the body’s balance and gait, the decreased muscle strength shown in Parkinson’s disease prevents the patients from independently performing daily activities.

In this study, neurobehavioral evaluation and western blot analysis were performed to assess the expression of TH protein, which is related to the number dopamine-producing nerve cells. Our results showed a similar rates of increase in Groups III and IV.

The balance and gait abilities of Parkinson’s disease are related to the occurrence of falls. Improvements in balance and gait thus decrease the risk of fracture due to falls and enables a wider range of independent daily activities on the part of the patients. As balance and gait are organically linked to each other, gait ability improvement can hardly be expected without the improvement of balance ability. In clinical practice, a neurodegenerative process is observed in Parkinson’s disease, which results in a decreased ability of balance and gait as well as in hindering of daily activities. The results of this study, which used balance and gait training as interventions, can be used as an evidence of the need for Parkinson's disease patients to undergo balance and gait training. Our results of this study showed that gait training was more effective than balance training for motor function recovery. It is easier for animals to balance themselves than to humans, as the former use four legs for balance purpose whereas the latter use only two legs, and gait training was shown to be more effective than balance training for the Parkinson's disease animals’ motor function recovery. Fisher et al.\(^\text{12}\) reported in the study that they conducted, intense training was found to be effective at improving the neural plasticity in the brain damage including the basal ganglia.

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**Table 2.** The results of the pole tests in each group (unit: sec)

|         | Pre       | After 7 days | After 14 days | After 21 days | After 28 days |
|---------|-----------|--------------|--------------|--------------|--------------|
| Group I | 2.33±0.51 | 2.35±0.53    | 2.51±0.86    | 2.41±0.74    | 2.39±0.53    |
| Group II| 64.73±3.46 | 59.19±5.90   | 57.56±6.52   | 56.1±5.45    | 55.58±6.69   |
| Group III| 65.53±3.00 | 48.60±5.04   | 29.50±4.95   | 22.95±6.96   | 5.02±1.29    |
| Group IV | 65.35±4.80 | 35.67±5.41   | 28.82±9.30   | 18.62±7.22   | 3.55±1.47    |

Values are expressed as the Mean±SD. Tested by one-way ANOVA, and as a post-hoc test. Tukey’s multiple range test was also performed, *: p<0.05 as compared to Group I; #: p<0.05 as compared to Group II; †: p<0.05 as compared to Group III.

**Table 3.** The results of the open field test in each group (unit: sec)

|         | Pre       | After 7 days | After 14 days | After 21 days | After 28 days |
|---------|-----------|--------------|--------------|--------------|--------------|
| Group I | 20.54±2.52 | 19.45±2.89   | 20.01±2.62   | 16.28±3.25   | 16.10±2.01   |
| Group II| 300.00±0.00 | 297.10±5.04  | 284.30±8.94  | 262.00±8.36  | 245.00±13.27 |
| Group III| 300.00±0.00 | 290.20±9.08  | 274.15±7.21  | 240.05±5.56  | 215.07±5.94  |

Values are expressed as the Mean±SD. Tested by one-way ANOVA, and as a post-hoc test. Tukey’s multiple range test was also performed, *: p<0.05 as compared to Group I; #: p<0.05 as compared to Group II; †: p<0.05 as compared to Group III.
In the present study, gait training was shown to be more effective than balance training. No procedure for stopping the neurodegenerative process of Parkinson’s disease has so far been found. Previous studies have reported that training increases the nerve growth factors required to prevent and regenerate dopamine-producing nerve cells and the supply of oxygen, decreases the severity of Parkinson’s disease, and slows the degenerative process of the nerves.

The present study also showed that balance and gait training were effective at recovering motor function and increasing the expression of TH protein in a Parkinson’s disease animal models, and that gait training was more effective than balance training. A further study using Parkinson’s disease patients, and other studies for developing various training programs depending on the degree of Parkinson’s patients’ motor disability, should be conducted based on the results of this study.

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