

# The Effects of Antecedent Exercise on Motor Function Recovery and Brain-derived Neurotrophic Factor Expression after Focal Cerebral Ischemia in Rats

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**Abstract.** [Purpose] In the present study, we investigated the effect of antecedent exercise on functional recovery and brain-derived neurotrophic factor (BDNF) expression following focal cerebral ischemia injury. [Subjects] The rat middle cerebral artery occlusion (MCAO) model was employed. Adult male Sprague-Dawley rats were randomly divided into 4 groups. Group I included untreated normal rats (n=10); Group II included untreated rats with focal cerebral ischemia (n=10); Group III included rats that performed treadmill exercise (20 m/min) training after focal cerebral ischemia (n=10); and Group IV included rats that performed antecedent treadmill exercise (20 m/min) training before focal cerebral ischemia (n=10) as well as treadmill exercise after ischemia. At different time points (1, 7, 14, and 21 days) Garcia's score, and the hippocampal expressions level of BDNF were examined. [Results] In the antecedent exercise group, improvements in the motor behavior index (Garcia's score) were observed and hippocampal BDNF protein expression levels increased. [Conclusion] These results indicate that antecedent treadmill exercise, before permanent brain ischemia exerts a neuroprotective effect against ischemia brain injury by improving motor performance and increasing the level of BDNF expression. Furthermore, the antecedent treadmill exercise of appropriate intensity is critical for post-stroke rehabilitation.

**Key words:** Antecedent exercise, Brain-derived neurotrophic factor, Cerebral ischemia

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## INTRODUCTION

Stroke is an acute and progressive neurodegenerative disorder that has become one of the leading causes of mortality and various disabilities<sup>1)</sup>. Ischemic stroke is the result of a transient or permanent reduction in cerebral arterial blood flow caused by residual tissue infarction via an embolus or local thrombosis<sup>2)</sup>. The major pathobiological mechanisms of cerebral ischemic injury include intracellular excitotoxicity, oxidative stress, apoptosis, and inflammation<sup>3)</sup>. Cerebral ischemia triggers a complex series of biochemical and molecular mechanisms that impairs the neurological functions through breakdown of cellular integrity. These alterations are by mediated by excitotoxic mechanisms, glutamatergic signalling, free-radical reactions, ionic imbalance, and other processes<sup>3)</sup>.

Physical exercise within a relatively short period of time (weeks to months) is able to increase the expression of a number of neurotrophic genes involved in any enhancement of a physical performance<sup>4)</sup>. Among several exercise paradigms, voluntary wheel running, forced treadmill running, and involuntary muscle contraction caused by electri-

cal stimulation are the commonly adopted exercise models. Apart from their physical benefits, these exercises have been separately demonstrated to improve cognitive function and facilitate neural rehabilitation after brain injury<sup>5-7)</sup>. It is important to know which rehabilitation intervention is more effective in facilitating motor function recovery and brain-derived neurotrophic factor (BDNF) upregulation, which is a leading factor responsible for motor learning and memory following brain ischemia<sup>8)</sup>. BDNF is the most abundant protein in the growth factor family<sup>9, 10)</sup>. It is widely expressed in the rodent brain, and is particularly abundant and widely distributed in the hippocampus, cerebral cortex, cerebellum, striatum, and amygdala<sup>11, 12)</sup>. BDNF expression was also reported in various parts of the human brain, including the hippocampus, claustrum, amygdala, bed nucleus of the stria terminalis, septum and the nucleus of the solitary tract<sup>13)</sup>. BDNF is involved in memory formation, including learning and behavior, synaptic plasticity, synaptic efficacy and neuronal connectivity. It also promotes the development of enhances the survival of adult neurons<sup>9, 14, 15)</sup>.

Although extensive studies have been performed to investigate the role of exercise in promoting stroke rehabilitation. It remains unclear whether the endogenous production of neurotrophins induced by physical exercise have neuroprotective effects after stroke. In the present study, we examined rats after transient middle cerebral artery

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occlusion (MCAO) to determine whether regular motor exercise on a treadmill immediately after ischaemia. Thus, we hypothesized that antecedent exercise treadmill training promotes motor function and changes the expressions of BDNF in focal cerebral ischemic injury in rats.

## SUBJECTS AND METHODS

Forty 8-week-old male Sprague-Dawley rats, weighing 250–260 g were used in this study following a 1-week acclimatization period. The rats were housed at a temperature of  $25.0\text{ }^{\circ}\text{C} \pm 1.0\text{ }^{\circ}\text{C}$  and a humidity level of  $50 \pm 5\%$  under a 12-h light-dark cycle; they had free access to food and water. The rats were randomly divided into 4 groups. Group I included untreated normal rats ( $n=10$ ); Group II included untreated focal cerebral ischemia rats ( $n=10$ ); Group III included rats that performed treadmill exercise (20 m/min) training after focal cerebral ischemia ( $n=10$ ); and Group IV included rats that performed antecedent treadmill exercise (20 m/min, 14 days) training before focal cerebral ischemia ( $n=10$ ) as well as treadmill exercise after ischemia. All animal experimental protocols were performed in accordance with the guidelines of the Dongshin University Animal Care and Use Committee. Focal cerebral ischemia was induced using a modified intraluminal suture method as described previously<sup>16</sup>. Briefly, the left common internal and external carotid arteries were exposed through a midline incision in the neck and then carefully dissected from the surrounding tissues under an operating microscope. After electro-coagulation of the external and common carotid arteries, a 3–0 silicon rubber-coated monofilament was inserted through the common carotid artery into the internal carotid artery to 18–20 mm beyond the carotid bifurcation at the base of the middle cerebral artery. An atraumatic aneurysm clip was placed on the internal carotid artery to prevent bleeding. The clip and the monofilament were removed 1 h later for transient ischemia and left in place for 24 h for permanent ischemia. The incision was then sutured.

Treadmill exercise was performed according to a previously described method<sup>17</sup>. The treadmill velocity was set at 20 m/min, and 20 min/day antecedent treadmill exercise was performed during the 14 days period at a  $0^{\circ}$  degree incline. During this time, the rats in group III were allowed to move freely in their cages, but no additional treadmill running was performed. The rats in both group III and group IV performed treadmill exercise for 21 days, which began 24 h after the surgery. After 3 weeks of treadmill exercise, the animals were sacrificed by decapitation the morning following the last treatment day and their hippocampi were removed immediately placed on dry ice, and stored at  $-70\text{ }^{\circ}\text{C}$  until used for protein measurements. The motor behavior index of the rats was measured blindly using Garcia's index at 10 a.m. on postoperative days 1, 7, 14, and 21 to account for diurnal variation. Six items were measured and the total score ranged from 3 to 18; the higher the score, the better the motor performance. Items 1–4 (spontaneous activity, symmetry of movements, symmetry of the forelimbs, and climbing the wall of wire cage) measure motor performance, and items 5–6 (reaction to touch

on and response to vibrissae touch) measure sensory function<sup>18</sup>. The hippocampus was homogenized in lysis buffer B (137 mM NaCl, 20 mM Tris, 1% NP40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride [PMSF], 10  $\mu\text{g}/\text{ml}$  aprotinin, 1  $\mu\text{g}/\text{ml}$  leupeptin, 0.5 mM sodium vanadate, pH8.0) for western blot analysis. Tissue was homogenized in freshly prepared lysis buffer (1:10 w/v) and centrifuged at  $12,000 \times g$  for 30 min. The supernatants were collected, divided into aliquots and stored at  $-70\text{ }^{\circ}\text{C}$ . The total protein concentration of the hippocampal homogenates were determined with a MicroBCA kit, using bovine serum albumin as the standard. Aliquots containing an equal amount of hippocampal protein extracts (20  $\mu\text{g}$ ) were mixed with gel loading buffer and separated on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels. After electrophoresis, the proteins were transferred onto polyvinylidene fluoride (PVDF) membranes and nonspecific binding was blocked with 5% nonfat dry milk in tris-buffered saline and Tween 20. Membranes were incubated with the following primary antibodies. After incubation with the anti-BDNF primary antibody (sc-546, Santa Cruz, USA), monoclonal anti- $\beta$  actin (A-5316, 1:5,000, Sigma, USA) antibodies membranes were washed with TBST and incubated with the appropriate horse radish peroxidase-conjugated secondary antibody (1:4,000 dilution). Immuno-complexes were visualized by chemiluminescence using the ECL kit according to the manufacturer's instructions. The film signals were digitally scanned and then quantified using NIH image J software.

Data analysis was performed using SPSS for Windows version 18.0 (SPSS Inc., Chicago, IL, USA). All the data are expressed as mean  $\pm$  SD of 3 replications. Differences between groups were tested by one-way ANOVA, followed by the Student–Newman–Keuls multiple comparisons test when differences were detected. P values less than 0.05 at the 95% confidence level were considered significant.

## RESULTS

The Garcia's motor behavior scores were 18 for all of the rats in Group I (normal) at 1 day postoperatively. These scores for Group II (MCAO) at 1 day postoperatively ( $9.7 \pm 0.9$ ) were significantly different from those of the normal group ( $18.0 \pm 0.0$ ). On day 21, there were apparently significant differences in the Garcia scores between the ischemia group and the exercise groups. After antecedent treadmill exercise, the Garcia's scores increased from 9.3 to 14.5 in the antecedent treadmill exercise group (Group III), while the Garcia's scores increased from 9.1 to 12.5 in the exercise group (Table 1).

We analyzed each animal's brain protein extract for BDNF protein expression. There was a trend toward a higher expression of BDNF protein in the exercise groups (Group III and Group IV) compared to the control group (Group I). The antecedent treadmill exercise group showed higher expression of BDNF than the treadmill exercise (Group III) group (Table 2).

**Table 1.** Changes of the Garcia's motor behavior scores after mild cerebral ischemic rats (score)

Groups	1 day	7 days	14 days	21 days
Group I	18.0±0.0	18.0±0.0	18.0±0.0	18.0±0.0
Group II	9.7±0.9 <sup>a</sup>	10.0±0.8 <sup>a</sup>	10.1±0.7 <sup>a</sup>	10.4±0.6 <sup>a</sup>
Group III	9.1±0.9 <sup>a</sup>	10.3±1.4 <sup>a</sup>	12.1±1.0 <sup>b</sup>	12.5±0.5 <sup>b</sup>
Group IV	9.3±0.7 <sup>a</sup>	10.6±1.3 <sup>a</sup>	13.1±1.1 <sup>b</sup>	14.5±1.2 <sup>c</sup>

Data were presented as mean±SD. a:  $p < 0.05$ , compared to group I; b:  $p < 0.05$ , compared to group II; c:  $p < 0.05$ , as compared to group III.

**Table 2.** Effect of antecedent treadmill exercise on brain-derived neurotrophic factor (BDNF) protein expression in focal brain ischemia rat (%)

Groups	Group I	Group II	Group III	Group IV
BDNF	100.00±8.52	124.25±11.24 <sup>a</sup>	148.50±10.25 <sup>b</sup>	180.50±9.54 <sup>c</sup>

Data were presented as mean±SD. a:  $p < 0.05$ , compared to group I; b:  $p < 0.05$ , compared to group II; c:  $p < 0.05$ , as compared to group III.

## DISCUSSION

Stroke patients display various symptoms and disabilities such as motor and sensory weakness, cognitive impairment, perceptual disorders and limitations in daily activities<sup>19</sup>. Several physical therapeutic interventions such as exercise, exposure to an enriched environment and rehabilitation enhance functional recovery after stroke<sup>20</sup>. Therefore, it is necessary to improve the abilities of motor function, cognition, and perception for successful rehabilitation<sup>21, 22</sup>. Beneficial effects of exercise therapies include improved learning and memory, improved motor function, and enhancement of proteins involved in brain plasticity, such as BDNF and transforming tyrosine receptor kinase (Trk)A<sup>23-26</sup>. The purpose of this study was to compare the effect of antecedent treadmill exercise training with that of treadmill exercise training after MCAO on the motor function and changes the expressions of BDNF in focal cerebral ischemia.

In stroke studies, the rat is the most commonly used animal model due to its size, which allows easy monitoring of the physiologic variables and handling of vascular structures. Nonetheless since the rodent is the best-characterized animal in genetics and molecular biology, an increasing number of stroke studies have been performed using mice since the 1990s<sup>27, 28</sup>. Many animal studies have demonstrated the beneficial effects of treadmill exercise, such as a smaller brain infarct volume or improvement in neurological function, either before or after stroke when compared with spontaneous recovery<sup>7, 29</sup>. The action of exercise on the regulation of BDNF, a molecule that plays an important role in rat hippocampal motor learning, might involve epigenetic regulatory mechanisms<sup>20</sup>. Based on information that BDNF is a critical mediator of the effects of antecedent treadmill exercise on synaptic plasticity and motor function, and our results showed that the change in BDNF is crucial to accomplish this process.

Our results revealed significant motor function recovery by Garcia's scores improvement in the treadmill exercise groups. Stroke patients had lower energy expenditure and

improved gross motor efficiency and gained improved ambulatory ability after treadmill exercise<sup>30</sup>. This is consistent with the findings of another study which reported that improved motor performance after exercise was associated with elevated expression of BDNF and TrkB<sup>31</sup>. Therefore, it appears that the brain plasticity mediated by BDNF and TrkB might play a trivial role in motor recovery after cerebral ischemia in mild cerebral ischemic rats compared to moderate-to-severe ischemic rats. According to the study of Cechetti et al.<sup>32</sup>, no significant changes in the BDNF expression were detected in the hippocampi of rats that performed daily moderate intensity exercise (2 weeks of 20 min/day of treadmill training). This result differs from previous studies demonstrating that physical activity, running, and swimming increases the expression of BDNF in the rodent brain<sup>20, 33-35</sup>.

In the present study, there was a significant effect of the antecedent treadmill exercise groups on BDNF expression, and this group of rats showed greater improvement of motor function than the treadmill exercise group. The change in BDNF expression also showed characteristics of neuroprotective characteristics that promoted survival of hippocampal, striatal, and septal neurons in animal experiments against insults such as focal brain ischemia.

Many complex processes contribute to both injury and recovery after stroke<sup>36, 37</sup>. Previous investigations have found that increasing BDNF levels or activating BDNF-associated signaling pathways leads to improved recovery following stroke<sup>24, 38</sup>. However, the present results provide the first direct demonstration that antecedent exercise is crucial for mediating the motor recovery that takes place as a result of post-stroke rehabilitation. Our data clearly show that antecedent treadmill exercise appears to act as a major homeostatic regulator of motor function and BDNF expression, with important implications for brain plasticity. It has been suggested that the antecedent treadmill exercise are important in improving motor function. Thus, antecedent treadmill exercise rather than post-stroke exercises may provide the beneficial effect for the recovery of stroke patients.

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