Effects of Treadmill Training on Limb Motor Function and Acetylcholinesterase Activity in Rats with Stroke

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Abstract. [Purpose] In the present study, we investigated the effects of treadmill training on limb motor function and acetylcholinesterase activity following focal cerebral ischemia injury. [Methods] Focal cerebral ischemia was examined in adult male Sprague-Dawley rats by using a middle cerebral artery occlusion model. Rats were randomly divided into 3 groups. Group I included untreated normal rats (n=12), Group II included untreated rats with focal cerebral ischemia (n=12), and Group III included rats that performed treadmill exercise (20 m/min) training after focal cerebral ischemia (n=12). We determined the limb placement test score for each rat on days 1, 7, 14, and 21; acetylcholinesterase activity in the hippocampus was examined at the end of the experiment. [Results] We observed that the motor behavior index improved in the treadmill group, and hippocampal acetylcholinesterase activity was decreased. [Conclusion] These results indicated that treadmill training after focal cerebral ischemia exerts a neuroprotective effects against ischemic brain injury by improving motor performance and decreasing the levels of acetylcholinesterase activity. Furthermore, these results suggest that treadmill training at an appropriate intensity is critical for post-stroke rehabilitation.

Key words: Acetylcholinesterase, Stroke, Treadmill exercise

INTRODUCTION

Stroke is the most common progressive neurodegenerative disorder and is one of the leading causes of dysfunction in upper extremity movements and walking\textsuperscript{11).} Ischemic stroke occurs when cerebral arterial blood flow is either transiently or permanently reduced, and includes both embolic and thrombotic subtypes\textsuperscript{33).} Stroke is a major cause of morbidity and mortality worldwide\textsuperscript{33)}, and is the third leading cause of death in the United States. It is also a common cause of long-term disability\textsuperscript{9).}

Approximately 85% of patients with stroke experience ischemic strokes, which are mainly caused primarily by local thrombosis or acute thromboembolic occlusion of intracranial arteries\textsuperscript{33).} The major pathological mechanisms that follow cerebral ischemic injury include inflammation, protease activation, oxidative stress, excessive production of reactive oxygen species, and intracellular excitotoxicity\textsuperscript{6, 7).} In the human brain, about 25% of the energy is expended on synaptic transmission, protein synthesis, and intracellular transport\textsuperscript{8-9).} Typically, the first consequence of cerebral ischemia is attenuated synaptic activity and transmission\textsuperscript{10).} Changes in synaptic function are generally considered reversible, but persistent neuronal damage can cause membrane failure and cell death\textsuperscript{11).} Thus, an improved understanding of the pathological mechanisms that underlie neuronal cell death in ischemic stroke may help improve neuroprotective strategies after stroke.

Traditional stroke treatments may include neurodevelopmental training (NDT), proprioceptive neuromuscular facilitation (PNF), a motor relearning program, the Rood approach, and exercise\textsuperscript{12-16).} The most commonly used exercise programs include wheel running, voluntary exercise, involuntary exercise, and forced treadmill running\textsuperscript{17-19).} Considering the many exercise programs available, it is important to know which of these rehabilitation strategies is most effective in facilitating motor function recovery. Recent studies indicate that exercise training protects neural cells from inflammation, apoptosis, and oxidative stress and can enhance choline acetyltransferase activity and inhibit acetylcholinesterase (AchE)\textsuperscript{20-23).} Acetylcholine (Ach) is considered to be the most important neurotransmitter for cognition and motor function\textsuperscript{24, 25).} Nevertheless, it remains unclear whether the endogenous inhibition of AchE that occurs with physical exercise can provide neuroprotection after stroke. We therefore hypothesized that, in rats with focal cerebral ischemic injury, treadmill training would improve motor function and increase AchE activity.

SUBJECTS AND METHODS

Thirty-six male Sprague-Dawley rats (age = 8 weeks; weight = 250–260 g) were used in the study. Rat were...
housed at a temperature of 25.0 °C ± 1.0 °C and 50 ± 5% humidity, with a 12-h light-dark cycle, and had free access to food and water. The rats were introduced to the study after a 1-week acclimatization period, and were divided randomly into 3 groups. Group I included untreated normal rats (n=12), Group II included untreated focal cerebral ischemia rats (n=12), and Group III included rats that performed treadmill exercise training (20 min/min) training after focal cerebral ischemia (n=12). All animal experimental protocols were performed in accordance with the guidelines of the institutional animal care and use committee of Dongshin University.

Focal cerebral ischemia was induced with a modified intraluminal suture method, which was previously described in detail. 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 electrocoagulation 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 a depth of 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 in order to prevent bleeding. The clip and the monofilament were removed after 1 h to model transient ischemia and after 24 h to model permanent ischemia. Finally, the incision was sutured once

Treadmill exercise was performed according to a previously described method. The treadmill exercise group (Group III) trained for 20 min/day with a 0° degree incline for the entire 21-day period. Group II rats were allowed to move freely in their cages during the same 21-day period, and did not participate in treadmill training. Rats were sacrificed by decapitation the morning following the last treatment day. The hippocampi were removed immediately, placed on dry ice, and stored at −70 °C for protein measurements. In the limb-placement test, rats were graded from 0 to 2 in each of the 8 subtests as follows: score 0, unable to place limb; score 1, partial placement or placement delayed over 2-s; and score 2, immediate placement.

AChE activity was determined by the colorimetric assay of Ellman et al., as previously described. After rats were euthanized, whole ischemic brains were mixed in 0.1 M phosphate buffer (pH 8.0) and homogenized. Briefly, in the 96 well plates, 25 µL of 15 mM acetylthiocholine iodide, 75 µL of 3 mM DTNB (dithiobisnitrobenzoic acid) and 75 µL of 50 mM Tris–HCl, pH 8.0, containing 0.1% BSA, were combined, and the absorbance was read at 405 nm after a 5-min incubation period at room temperature. Any increase in absorbance due to spontaneous substrate hydrolysis was corrected by subtracting the reaction rate of the enzyme. Next, 25 µL of sample (brain homogenates) was added, and the absorbance was read again after a second 5-min incubation period at room temperature. The AChE activity was expressed as mol/min/g of tissue protein. All determinations were carried out twice and in triplicate.

Data analysis was performed with SPSS for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA). All of the data were expressed as means ± standard deviation (SD). Differences in limb placement test scores were examined with a parametric one-way ANOVA and Scheffe’s post hoc procedure at 95% significance levels. For test-based on scoring (ordinal measures), the nonparametric Kruskal–Wallis test was used with the post hoc multiple-comparison test to determine the number and relation of the group differences at a 95% significance level.

**RESULTS**

All Group I rats scored 18 points in the limb placement test on postoperative day 1. The Group II mean score was 4.4 ± 0.7 points on postoperative day 1, which differed significantly from the Group I mean (17.0±0.0 points). Significant differences in limb placement test scores were observed on day 21 between the focal cerebral ischemia group and the exercise group. After treadmill training, the limb placement behavior score increased significantly in the exercise group from 4.4 ± 0.6 points on day 1 to 8.2 ± 0.6 points on day 21 (p <0.05) (Table 1).

We analyzed each brain fraction extract at the end of the experiment to determine AChE activity levels. Compared with the control group, the treadmill training group showed a trend toward decreased AChE activity (Table 2). After treadmill training, the AChE activity significantly decreased by 1.45±0.5 nmole/mg protein/min in Group II and 0.85±0.17 nmole/mg protein/min in the treadmill training group (p <0.05) (Table 1).

**DISCUSSION**

Patients with stroke experience various symptoms or disabilities that limit their daily activities, which includes physical disability, motor impairment, cognitive impairment, or sensory weakness. These factors have been
shown to influence the quality of life of patients with stroke. Physiotherapists attempt to rehabilitate upper limb function through repetitive practice and other therapeutic interventions that pay special attention to strength, endurance, coordination, speed, and reintegration of specific motor functions into a patient’s everyday routine. A number of previous studies have investigated the role of exercise in promoting stroke rehabilitation. The pathological mechanisms underlying ischemic stroke, at least in part, converge on an impaired cholinergic system, which leads to decreased synaptic transmission. Thus, we hypothesized that treadmill training would promote motor function and change the levels of activation of AchE levels in rats with focal cerebral ischemia rats.

Physical exercise training is a well-established method for protecting neural cells from ischemia-induced brain injury. Exercise shows a tendency to simultaneously promote cell survival mechanisms, while inhibiting neuronal apoptotic pathways. In addition, it increases capillary density by inducing angiogenic factors and protects against ischemic damage. Our results revealed significant motor function recovery, as indicated by improvements in the limb placement test score.

Maintaining a balanced cholinergic system homeostasis is crucial for central and peripheral nervous system function, as well as for a healthy neuromuscular junction. A common mechanism for neuronal cell death following brain ischemia is disruption of the cholinergic system. Neuroinflammation can also impair synaptic function through the synergism of hemocyte activity. Neuronal cell death, like ischemia, triggers a series of events, including neuroinflammation, which can disrupt the cholinergic system. Central cholinergic neurotransmission through muscarinic receptor activation contributes to cognition and memory formation and influences LTP. Ach also plays an important role in regulating synaptic transmission and cellular excitability. Therefore, it is likely that cholinergic factors, such as Ach concentration and AchE inhibition, are important for plasticity in the human motor system.

In the present study, there were significant inhibitory effects on AChE activity and improvements in limb motor function in the treadmill training group. Changes in AChE activity might reflect neuroprotective characteristics that promote the survival of hippocampal neurons, as has been shown in animal experiments modeling focal cerebral ischemia. Based on the findings that AChE is a critical mediator for the effects that treadmill training imposes on synaptic plasticity and motor function, our results showed that changes in AChE are crucial for accomplishing this process. The present results suggest that treadmill training after stroke can enhance cholinergic system activity and thereby improve limb motor function.

Thus, our data clearly show that treadmill training appears to act as a major homeostatic regulator of limb motor function and AchE inhibition, which has important implications for cholinergic system recovery. These findings suggest that treadmill training is important in improving limb motor function. Thus, compared with other poststroke exercise routines, treadmill training may provide further beneficial effects for patients with stroke.

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