



Original Article

Effects of skilled reach training with affected forelimb and treadmill exercise on the expression of neurotrophic factor following ischemia-induced brain injury in rats

MIN-SIK YONG, PT, PhD¹⁾, SEONG-GIL KIM, PT, PhD²⁾, SONG-HEE CHEON, PT, PhD^{1)*}

¹⁾ Department of Physical Therapy, Youngsan University: 288 Joonam-ro, Yangsan, Gyeongsangnam-do, Republic of Korea

²⁾ Department of Physical Therapy, Uiduk University, Republic of Korea

Abstract. [Purpose] The aim of the present study was to investigate effects of skilled reach training with affected forelimb and treadmill exercise on the expression of neurotrophic factor following ischemia-induced brain injury in rats. [Subjects and Methods] Thirty male Sprague-Dawley rats were divided into 3 groups randomly: namely, the control sacrificed 2 weeks after surgery, skilled reach training with forepaw contralateral to brain injury for 2 weeks, and treadmill exercise for 2 weeks. Transient focal cerebral ischemia was induced by intraluminal occlusion of the left middle cerebral artery. After that, skilled reach training and treadmill exercise were conducted. Western blot analysis was performed to investigate expressions of neurotrophic factors. [Results] There were significant differences in brain-derived neurotrophic factor and nerve growth factor expression between the control group and the experimental group. There were no significant differences in brain-derived neurotrophic factor and nerve growth factor expression between the skilled reach training group and the treadmill exercise group. [Conclusion] Skilled reach training and treadmill exercise can affect the expression of neurotrophic factors.

Key words: Stroke, Skilled reach training, Neurotrophic factor

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INTRODUCTION

Stroke is one of the leading causes of death. It also gives rise to long-term handicap giving negative influences on activities of daily living in stroke patients^{1, 2)}. There are a lot of stroke sequelae which have bad effects on quality of life in stroke patients. Among these aftereffects, hemiparesis defined as motor impairments of the limb contralateral to unilateral brain damage^{3, 4)}. The increased activation in unaffected hemisphere could be a possible cause of a pathological inhibition in affected hemisphere as well as motor impairments in affected side of the body^{5, 6)}. A number of researches on a physical activity reported that there is a close relationship between a performance of exercise and an improvement of brain plasticity⁷⁾. Skilled reaching can be considered as task-specific training via its purposeful characteristics, so it is used as a training program to rehabilitate stroke patients with a motor disability in their upper limb^{8–10)}. Treadmill exercise has been studied as one of conventional treatments for a long time. In general, treadmill exercise is commonly applied to stroke patients thanks to its beneficial effect on neuroprotection^{11, 12)}. To date, the only therapy approved by FDA has been thrombolytic tissue plasminogen activator (tPA) although approximately 80% of human strokes are classified into ischemic stroke^{13–15)}. It is suggested that continuous researches via experimental ischemic stroke models should be performed to develop novel, proper, and helpful solutions for patients with ischemic stroke. In this regards, the present study investigated effects of skilled reach

*Corresponding author. Song-Hee Cheon (E-mail: 1000sh@ysu.ac.kr)

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training with affected forelimb and treadmill exercise on the expression of neurotrophic factor following ischemia-induced brain injury in rats.

SUBJECTS AND METHODS

Thirty male Sprague-Dawley rats were divided into 3 groups randomly: namely, the control sacrificed 2 weeks after surgery (CON), skilled reach training with forepaw contralateral to brain injury for 2 weeks (SC), and treadmill exercise for 2 weeks (TE). All the experiments were performed in accordance with protocols approved by the Animal Experiment Committee in Daegu University, based on the NIH Guidelines for the Care and Use of Laboratory Animals. Transient focal cerebral ischemia was induced by intraluminal occlusion of the left middle cerebral artery (MCA). Briefly, right common carotid artery (CCA) was exposed and the external carotid artery (ECA) and the CCA were ligated. Middle cerebral artery is occluded by a 4–0 nylon monofilament coated with a silicone tip, the monofilament was inserted into the internal carotid artery from the external carotid artery until mild resistance was felt. Reperfusion was established by completely withdrawing the nylon monofilament after 120 min of occlusion. Skilled reach training was conducted in a Plexiglas chamber (45 cm in height, 15 cm in width, 40 cm in length) containing of a 1 cm by 10 cm window in front wall allowed for one of the rat's paw to reach via for a pellet. Animals were acclimated to the chamber with 4.5 mg of sugar-flavored food pellets (Research Diets, New Brunswick, NJ, USA) placed on the shelf placed in a small indentation 3 cm from the inside wall of the chamber and trained in 30 minutes sessions administrated 6 days per week for 2 weeks based on previous reports. When a pellet was removed by a rat, the shelf was refilled for a time period of 30 minutes continuously. A motor-driven treadmill was used for the treadmill exercise. Before treadmill exercise, rats in TE experienced 1 days of adaptive running exercise at a speed of 6–9 m/min for 5 min. The running speed and duration was determined according to previously protocol with modification. The brains of each group were collected, washed twice in PBS, and then homogenized and lysed with buffer (137 mM NaCl, 8.1 mM Na₂HPO₄, 2.7 mM KCl, 1.5 mM KH₂PO₄, 2.5 mM EDTA, 1 mM dithiothreitol, 0.1 mM PMSF, 10 µg/ml leupeptin [pH 7.5]) for 30 min on ice. The lysates were centrifuged for 10 min at 15,000 rpm at 4 °C, and the protein concentration was determined as described previously. Equal amounts of protein (40 µg) were resolved via 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. The blots were washed with TBST (10 mM Tris · HCl [pH 7.6], 150 mM NaCl, 0.05% Tween 20), blocked with 5% skim milk for 1 hour, then incubated with the appropriate primary antibodies at the dilutions recommended by the suppliers. The membranes were washed, and the primary antibodies were detected using horseradish peroxidase-conjugated goat anti-rabbit IgG or goat-anti mouse IgG. The bands were then visualized via enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

RESULTS

The expression of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in CON, SC, and TE were also investigated to identify the effect of skilled reach training with contralesional forelimb and treadmill exercise on BDNF expression. There were significant differences in BDNF and NGF expression between CON and SC, CON and TE ($p < 0.05$). There were no significant differences in BDNF and NGF expression between SC and TE ($p > 0.05$).

DISCUSSION

Motor function in upper-extremity is closely related to quality of life in stroke patients via having influences on activity and participation, and this is the reason to focus on the recovery of upper limb motor function in stroke patients^{16–18}. Skilled reach training, one of task-specific exercises, has frequently been mentioned as a treatment strategy for stroke patients in many researches^{19, 20}. In many studies, skilled reach training was applied to animal models in order to investigate its effect on neurological diseases. Pagnussat et al. displayed an effect of skilled reaching with affected forelimb on functional recovery in their research²¹. Above this, several studies also reported that recovery of motor function can be induced by applying skilled reach training with contralesional forelimb^{9, 22}. In addition, effects of treadmill exercise have been studied in many researches for a long time. It has several beneficial effects such as motor function recovery and neuroprotection¹².

In many studies investigating recovery of a damaged brain, plasticity is often mentioned²³. Particularly, it is reported that the activation of neurotrophic factors is able to facilitate plasticity via inducing generation and differentiation of neuronal progenitor cell^{24–26}. Park et al. reported that neurotrophic factors have an influence on neuroprotective function after brain injury²⁷.

For these reasons, the present study investigated the expression of BDNF and NGF via western blot analysis to examine the effect of skilled reach training and treadmill exercise on neurotrophic factor expression. From results, there were significant differences in BDNF and NGF expression between CON and SC, CON and TE ($p < 0.05$). Although expression of BDNF and NGF in SC slightly increased compared with in TE, the difference was not statistically significant. ($p > 0.05$) (Table 1). These results imply that both skilled reach training with affected forelimb and treadmill exercise can induce expression of neurotrophic factors. It is suggested that skilled reach training may have more influence on them than treadmill exercise because of its repetitive and task-specific characteristics.

Table 1. Expression of BDNF and NGF in each group (unit: pixel)

	CON	SC	TE
BDNF	2,046.5 ± 108.7	4,240.1 ± 72.5*	4,173.3 ± 65.1*
NGF	2,211.3 ± 87.9	3,881.6 ± 91.4*	3,830.8 ± 88.7*

Values are reported as the Mean ± SD.

*p<0.05 vs. CON

There is the limitation of the present study. Since the results of the study only showed expression of BDNF and NGF, it is not enough to explain the relationship between expression of neurotrophic factors and recovery of function. Further study applying tests for evaluating motor function is necessary to make the results of the present study more obvious.

In consequence, skilled reach training and treadmill exercise could affect expression of neurotrophic factors. Especially, skilled reach training with affected forelimb showed more beneficial effects on neurotrophic factor expression than treadmill exercise with a moderate intensity.

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