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Original Article

Skilled Reach Training Influences Brain Recovery Following Intracerebral Hemorrhage in Rats

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Abstract. [Purpose] The present study investigated how skilled reach training influences functional and neurological brain recovery via a rat model with intracerebral hemorrhage. [Subjects] Thirty rats with intracerebral hemorrhage were divided into 2 groups randomly: the control group (CON) that did not receive any treatment, and the experimental group (SRT) that received skilled reach training. [Methods] The experimental group was trained through skilled reaching training with the affected upper limb in 15-minute sessions administered 6 days per week for 4 weeks. [Results] In the behavioral test, the results showed that motor function was significantly improved in the skilled reach training group compared with the control group. In the neurological teat, the expression level of brain-derived neurotrophic factor (BDNF) was significantly increased in the skilled reach training group compared with the control group. [Conclusion] Skilled reach training is able to facilitate both the expression of neurotrophic factor in the motor cortex and motor function recovery following intracerebral hemorrhage.
Key words: Intracerebral hemorrhage, Brain-derived neurotrophic factor, Skilled reach training

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INTRODUCTION

Stroke, the major cause of motor impairment throughout the world, results in the problems such as motor, sensory, and cognitive disability[1, 2)]. Because there are many subtypes of stroke, it could be classified into two main categories: ischemic and hemorrhagic stroke[3)]. Although research regarding ischemic stroke has been lively studied, pathophysiological mechanisms of hemorrhagic stroke such as intracerebral hemorrhage (ICH) have rarely been disclosed[4)].

Most patients surviving stroke have many significant impairments including social, cognitive, and sensorimotor aftereffects, and these are considered factors having great influence on quality of life[1, 5)]. Patients with left or right hemiparesis resulting from stroke have difficulties in performing activities of daily living due to paresis of the limb contralateral to the injured site of the brain[6)]. In particular, motor function of the upper limbs is considered one of the most important factors because it is closely related to patients' quality of life. For this reason, therapists working in the field of rehabilitation should concentrate on recovery of function in the more affected upper limb[1, 7)].

Learning new motor skills can affect cortical reorganization in a damaged site of the brain, thereby causing neural plasticity. New motor skills can be acquired by Task-specific treatments[8, 9)]. Neuronal recovery of function as well as structure can be promoted by task-specific training such as skilled reach training[10)]. Neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and vascular endothelial growth factor (VEGF), play an important role in generation and differentiation of neuronal progenitor cells and regulation of neuronal survival[11–14)]. BDNF, one of the neurotrophic factors most extensively distributed throughout the brain, is well known to have effects in relation to the growth, survival, and proliferation of neurons[15–17)]. The especially high expression of BDNF usually observed in the hippocampus implies that BDNF influences learning and memory as well as neurogenesis in the hippocampus[15, 18)].

Less research about ICH has been conducted than about ischemic stroke, although ICH patients have higher mortality as well as lower functional recovery than patients with ischemic stroke[19)]. Because of the lack of research, the pathophysiology of ICH has been unclearly revealed[4)]. For these reasons, the present study was performed to investigate effects of skilled reach training on brain recovery in rats with ICH.

SUBJECTS AND METHODS

Subjects

Twenty male Sprague-Dawley rats weighing between 250 g and 300 g were used and maintained with a 12 hour on/12 hour off light/dark cycle and ad libitum access to food

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and water. The animals were divided into 2 groups randomly: a control group (CON) that did not receive any treatment and an experimental group (SRT) the received skilled reach training. Each group consisted of 2 subgroups: a group sacrificed 1 week after surgery for brain injury (CON1, SRT1) and a group sacrificed 4 weeks after surgery (CON4, SRT4). All surgical procedures followed Daegu University's guidelines and were approved by the Institution of Animal Care and Use Committee (IACUC).

Methods

All rats were anesthetized by intraperitoneal injection with 2 mL/kg of a 50% Zoletil and 50% xylazine hydrochloride mixture and placed in a stereotaxic frame for induction of brain injury. Animals were subjected to a 3.0-mm-diameter craniotomy centered on the left side, 3.0 mm lateral to the sagittal suture and 0.2 mm posterior to the bregma, and a 30-gauge Hamilton syringe needle was inserted through the hole into the striatum 6.0 mm ventrally; 1 μL containing 0.23 collagen digestion units of collagenase type VII (Sigma, St. Louis, MO, USA) was administered for 5 minutes to induce the intracerebral hemorrhagic injury. Skilled reach training was conducted in a plexiglass chamber (45 cm in height, 15 cm in width, 40 cm in length) containing a 1 cm by 10 cm window in the front wall, which allowed for one of the rat's paw to reach through 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 a shelf placed in a small indentation 3 cm from the inside wall of the chamber and trained in 15-minute sessions administered 6 days per week for 1 or 4 weeks. To investigate the effect of skilled reach training on brain recovery in rats with ICH, a skilled ladder rung walking test was performed. The ladder walk device consisted of a 1-m-long wooden-rung walkway with the distance between rungs varying by 1.5 cm. Rats were video recorded as they traversed the walkway 3 times. In a single testing session, the animals crossed the walkway three times and received a score for the number of fore and hind limb foot faults per each 10 steps. Only affected limbs were analyzed, and a foot fault was characterized as a total miss or slip from the rung, or a misplacement of a paw on the rung. To analyze the difference in BDNF expression between groups, Western blot analysis was performed. 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 and 4 °C. 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, and 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.

Table 1. Results of the skilled ladder walking test in each group (Error ratio (%))

	1 week	4 weeks
CON	43.2 ± 0.56	31.6 ± 0.93*
SRT	43.8 ± 1.28	22.4 ± 0.68$^{#+}$

(Mean ± SE) *p<0.05 vs. CON1; $^{#}$p<0.05 vs. SRT1; $^{+}$p<0.05 vs. CON4

Table 2. Results of Western blot analysis for BDNF expression in each group (Relative optical density (% of CON))

	1 week	4 weeks
CON	100	95.51 ± 1.60
SRT	98.76 ± 2.19	209.04 ± 0.95$^{#+}$

(Mean ± SE) $^{#}$p<0.05 vs. SRT1; $^{+}$p<0.05 vs. CON4

The bands were then visualized through enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The results were expressed as the means ± standard error. All experiments were analyzed via independent t-test. Some experiments were analyzed via comparisons of the treatment mean with the controls using the Bonferroni-Dunn test. Differences were considered statistically significant when $p < 0.05$.

RESULTS

The results showed that BDNF expression in CON4 ($p<0.05$) was decreased compared with in CON1, but this was not significant. BDNF expression in SRT4 ($p<0.05$) was significantly increased compared with in SRT1 (Table 1). There was a significant difference in BDNF expression between CON4 and SRT4 ($p<0.05$). The results of the skilled ladder rung walking test also showed a significant difference between CON4 and SRT4. There was a significant difference not only between CON1 and CON4 but also between SRT1 and SRT4 (Table 2).

DISCUSSION

Stroke patients may recover their functional independence in several months following stroke as a result of spontaneous recovery. However, the rate of patients who regain their motor functions is no more than 20%[7, 20]. One of the factors having a close relation to this recovery is neurotrophic factor, and it can not only enhance neuroplasticity but also influence neuroprotective function following brain injury[16, 21]. Exercise has been well known to provide stroke patients with various beneficial effects related to neural recovery[15, 22]. Previous study showed that voluntary wheel running promotes neuronal plasticity via BDNF expression and demonstrated that increased BDNF expression caused by exercise affects the molecular mechanism leading to changes in neuronal plasticity[23].

Among the many different types of physical exercises, behaviors including reaching and grasping via the forepaws are referred to as prehensions. Although there are various forms of prehensions that may be controlled by similar neural pathways, skilled reaching is considered the behavioral basis for other forms of reaching. Skilled reaching in rat models is extensively used for many different conditions such as spinal cord injury, stroke, and Parkinson's disease because there are close similarities in skilled reaching in rats and primates[24, 25].

In order to investigate the effect of skilled reach training on neurotrophic factor expression, the present study observed BDNF expression through Western blot analysis. BDNF expression in SRT4 was significantly increased compared with in CON4. This increased neurotrophic factor expression corresponded with the results of previous research with regard to effects of voluntary, involuntary, and forced exercises on recovery following ischemic stroke[15].

The skilled ladder rung walking test was also conducted for assessment of forelimb motor function. The results showed more improvement of motor function in the affected limb in SRT4 compared with in CON4. It is thought that task-specific training such as skilled reach training can be helpful to recovery of affected upper limb motor function through repeated stimulation of the damaged motor cortex area. Although a significant difference was found not only between CON1 and CON4 but also between SRT1 and SRT4, the error ratio for SRT4 decreased more than that for CON4. In order to demonstrate the effect of skilled reach training more clearly, it is suggested that a long-term study should be conducted in the future.

In conclusion, skilled reach training may be effective for improvement of motor function and recovery in the brain. However, several things were not considered in the present study. One thing necessary to be examined additionally is identification of the optimal intensity for application of skilled reach training. Also, the quantity of training applied to each rat may not be equal. Therefore, it is suggested that further study will be needed for efficient application of skilled reach training to patients with ICH.

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