Exercise in the Early Stage after Stroke Enhances Hippocampal Brain-Derived Neurotrophic Factor Expression and Memory Function Recovery

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Background: Exercise in the early stage after stroke onset has been shown to facilitate the recovery from physical dysfunction. However, the mechanism of recovery has not been clarified. In this study, the effect of exercise on spatial memory function recovery in the early stage was shown, and the mechanism of recovery was discussed using a rat model of brain embolism. *Methods:* Intra-arterial microsphere (MS) injection induced small emboli in the rat brain. Treadmill exercise was started at 24 hours (early group) or 8 days (late group) after MS injection. The non-exercise (NE) and sham-operated groups were included as controls. Memory function was evaluated by the Morris water maze test, and hippocampal levels of brain-derived neurotrophic factor (BDNF) were measured by enzyme-linked immunosorbent assays. To further investigate the effect of BDNF on memory function, BDNF was continuously infused into the hippocampus via implantable osmotic pumps in the early or late stage after stroke. *Results:* Memory function significantly improved only in the early group compared with the late and the NE groups, although hippocampal BDNF concentrations were temporarily elevated after exercise in both the early and the late groups. Rats infused with BDNF in the early stage exhibited significant memory function recovery; however, rats that received BDNF infusion in the late stage showed no improvement. *Conclusion:* Exercise elevates hippocampal BDNF levels in the early stage after cerebral embolism, and this event facilitates memory function recovery. **Key Words:** Rat—memory function recovery—embolism—BDNF—exercise.

Introduction

Stroke causes cognitive dysfunction in addition to motor and sensory dysfunction, and poststroke rehabilitation is effective in helping patients recover. The beneficial effects of rehabilitation on cognitive function are similar to those observed for motor function.[1,2] Findings from basic and clinical studies support these effects; for example, a 6-month aerobic and resistance exercise protocol improved cognition in poststroke patients.[3] Physical exercise in the chronic poststroke stage is effective and widely used. Aerobic exercise has been shown to improve motor[3] and cognitive function[4] poststroke. These improvements are observed following long-term exercise regimen for several months; however, disuse syndrome and atrophy persist

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if a rehabilitation protocol is applied in the chronic stage of poststroke.[5] To overcome these limitations in the chronic poststroke stage, starting rehabilitation as early as possible is desirable. Recent studies have mentioned that initiating an exercise regimen earlier improved recovery and prevented pathological progression.[6] The therapeutic application, and safety and feasibility of a very early rehabilitation trial for stroke were discussed.[7] Furthermore, a meta-analysis discussed the positive effects of acute exercise on cognitive performance.[8] However, Sjöholm et al[9] indicated that the application of early mobilization poststroke would increase the risk of cerebral blood flow changes, and additional studies are required to address this concern. Others have indicated that there were insufficient data to prove the beneficial effects of early mobilization after stroke.[10] Stinear et al also showed that there was a small sample size of early rehabilitation group in all patients with stroke, which was insufficient to discuss the effects of early rehabilitation.[11] Further clarification of the mechanism(s) of early rehabilitation is required. The assumed mechanism of functional recovery due to physical exercise is thought to depend on mitochondrial biogenesis[12] and increased levels of brain-derived neurotrophic factor (BDNF) in the brain.[4,13] Hippocampal BDNF levels are increased by treadmill running both in normal rats[14] and in rats with stroke.[15] Similarly, in healthy human subjects, a 3-month endurance training regimen increased internal jugular venous blood BDNF levels.[16] BDNF expression also correlates with poststroke cognitive function recovery. Zhang et al reported that decreased hippocampal BDNF expression aggravated cognitive impairment in both stroke and depression models.[17] Based on these previous reports, we hypothesized that the exercise-induced elevation of brain BDNF level in the early stage after stroke was important for cognitive functional recovery.

A large number of basic studies describing the effects of poststroke exercise have used a severe stroke model in which rats undergo middle cerebral artery occlusion (MCAo) and reperfusion. It is difficult to induce rats in the MCAo stroke model to exercise (even moderately) in the early poststroke stage because of their severe motor dysfunction.[18] We supposed that the MCAo model is not suitable for the study of the effect of a very early rehabilitation. On the other hand, microsphere (MS) embolism model allows the fine-tuning of motor function impairment that is dependent on the amount injected. The MS embolism stroke model[19] was adopted in this study because the rats develop mild impairment that allows physical exercise in the early poststroke stage. Moreover, the embolisms are found throughout the brain,[20] which induces spatial memory dysfunction, making it a good model of vascular dementia.

In the present study, we tested whether starting a physical exercise protocol at an early stage after stroke induced cognitive function recovery via increased hippocampal BDNF concentration using a rat model of the MS embolism stroke.

Materials and Methods

Animal and Surgery

All animal experiments were approved by the Animal Research Committee of Kawasaki Medical School and conducted according to the "Guide for the Care and Use of Laboratory Animals" of Kawasaki Medical School.

Male Sprague-Dawley rats (9 weeks old, 270-290 g, CLEA Japan Inc., Tokyo, Japan) were housed at 20°C under a 12 hours light-12 hours dark cycle. The rats were anesthetized with 3.0% vaporized sevoflurane (Maruishi Pharmaceutical Co. Ltd., Osaka, Japan) perfused at .5 L/min, and temperature was kept at 37.0°C. The right carotid artery was exposed, and the external carotid and pterygopalatine arteries were temporarily clamped. A total of 3000 particles of polystyrene MS (Polysciences, Inc., Warrington, PA) that were 45 μm in diameter were mixed with saline containing 5.0% gelatin (Sigma, St. Louis, MO) and injected into the common carotid artery when the 2 arteries were clamped. The total number of rats was 133 (MS injected: 112, and sham: 21). In the rats that were employed for the water maze test (sham; n = 7, early and late; n = 14 per group, non-exercise [NE]; n = 12) and BDNF measurement (8 days after MS injection; early and late: n = 7 per group), 3 physical deficits (truncal curvature, forced circling, and lack of movement) at 6 hours after MS injection were observed and scored as 0: no deficit, 1: weak, 2: obvious, and 3: severe. Five rats were injected with fluorescent MS (Polysciences Inc.) as described above, and 24 hours after MS injection, 100 mL 4% paraformaldehyde solution was perfused from the cardiac ventricle. Their brains were separated and were coronal sectioned into 200-μm-thick slices at –18°C by cryostat (CM3050S, Leica, Solms, Deutschland). These slices were sealed with fluoromount (Sigma) and observed under a fluorescent microscope (E600, Nikon Instruments, Tokyo, Japan).

Protocols of Exercise and Water Maze Test

Rats were randomly divided into several groups and forced to exercise by each protocol (Fig 3A). The regimen was started at 24 hours and 8 days after MS injection in the early and in the late exercise groups, respectively (early and late, n = 9 per group). The rats were exercised on a treadmill (Muromachi Kikai Co. Ltd., Tokyo, Japan) at 15 m/min for 30 minutes everyday. The NE group without exercise (n = 7) and the sham-operated group without MS (sham, n = 7) were employed as controls.

The cognitive function was evaluated by the Morris water maze test (MWM), a widely applied test of spatial memory function. A rat was forced to start swimming at the arbitrary edge of the circular pool (diameter: 1.5 m,