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Research report

The neuroprotective effects of preconditioning exercise on brain damage and neurotrophic factors after focal brain ischemia in rats

Shotaro Otsuka, Harutoshi Sakakima*, Megumi Sumizono, Seiya Takada, Takuto Terashi, Yoshihiro Yoshida

Course of Physical Therapy, School of Health Sciences, Faculty of Medicine, Kagoshima University, Kagoshima 890-8544, Japan

HIGHLIGHTS

- Preconditioning exercise prior to ischemia reduced infract volume and sensorimotor deficits.
- MK and BDNF expression levels were upregulated by preconditioning exercise after stroke.
- Preconditioning exercise activated astrocytes and promoted angiogenesis after stroke.
- Preconditioning exercise reduced apoptotic activity and oxidative damage in neuronal cells.

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ABSTRACT

Preconditioning exercise can exert neuroprotective effects after stroke. However, the mechanism underlying these neuroprotective effects by preconditioning exercise remains unclear. We investigated the neuroprotective effects of preconditioning exercise on brain damage and the expression levels of the midkine (MK) and brain-derived neurotrophic factor (BDNF) after brain ischemia. Animals were assigned to one of 4 groups: exercise and ischemia (Ex), no exercise and ischemia (No-Ex), exercise and no ischemia (Ex-only), and no exercise and intact (Control). Rats ran on a treadmill for 30 min once a day at a speed of 25 m/min for 5 days a week for 3 weeks. After the exercise program, stroke was induced by a 60 min left middle cerebral artery occlusion using an intraluminal filament. The infarct volume, motor function, neurological deficits, and the cellular expressions levels of MK, BDNF, GFAP, PECAM-1, caspase 3, and nitrotyrosine (NT) were evaluated 48 h after the induction of ischemia. The infarct volume, neurological deficits and motor function in the Ex group were significantly improved compared to that of the No-Ex group. The expression levels of MK, BDNF, GFAP, and PECAM-1 were enhanced in the Ex group compared to the expression levels in the No-Ex group after brain ischemia, while the expression levels of activated caspase 3 and NT were reduced in the area surrounding the necrotic lesion. Our findings suggest that preconditioning exercise reduced the infract volume and ameliorated motor function, enhanced expression levels of MK and BDNF, increased astrocyte proliferation, increased angiogenesis, and reduced neuronal apoptosis and oxidative stress.

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1. Introduction

Ischemic stroke is a major cause of neurological disability and big a significant burden on caregivers and society. Physical exercise can induce neuroprotection in both humans and animal models [1–4]. Generally, physical activity is an important modifiable risk factor, in particular for stroke and cardiovascular disease. Preconditioning exercise prior to ischemia can lead to protection from subsequent serious injury through the promotion of angiogenesis, mediation of inflammatory responses, inhibition of glutamate over-activation, protection of the blood brain barrier and inhibition of apoptosis [5]. Furthermore, animal studies have indicated there are beneficial effects of preconditioning exercise on cerebral ischemia, including enhanced survival rates, alleviation of oxidative damage, improvement of cerebral blood flow, and maintenance of neurovascular integrity [6–9]. Through these mechanisms, precon-

Abbreviations: MK, midkine; MCAO, middle cerebral artery occlusion; CCA, common carotid artery; ECA, external carotid artery; ICA, internal carotid artery; TTC, 2,3,5-triphenyltetrazorlium chloride; NT, nitrotyrosine.

* Corresponding author at: Course of Physical Therapy, School of Health Sciences, Faculty of Medicine, Kagoshima University, 8-35-1, Sakuragaoka, Kagoshima 890-8544, Japan. Fax: +81 99 275 6801.
E-mail address: sakaki@health.nop.kagoshima-u.ac.jp (H. Sakakima).

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ditioning exercise may reduce the neural deficits associated with the development of brain infarction [5,6,10].

Neurotrophic factors play a role in neuronal survival, proliferation, maturation and outgrowth in the developing brain and are also neuroprotective after insults in the mature brain. Brain-derived neurotrophic factor (BDNF), a neurotrophin family member, facilitates neuronal survival and development, but also modulate synaptic plasticity [11]. Running exercise increased BDNF and nerve growth factor (NGF) mRNA levels in several brain areas of intact adult rats [12]. Pre- and post-ischemic physical activity reduced neurological deficits and infarct volume due to cellular BDNF expression in the brain regions supplied by experimental brain ischemia [3,6].

Midkine (MK) is a heparin-binding growth factor with a molecular weight of 13 kDa [13]. MK is an endogenous neurite outgrowth factor involved in central nervous system development [14]. MK has been implicated in neuronal tissues repair, as it is expressed during the early stages of cerebral infraction [15], spinal cord injury [16], and peripheral nerve injury [17]. Mice lacking the MK gene exhibited delayed degeneration and regeneration after peripheral nerve and muscle injuries [18,19]. Low intensity exercise after stroke increased cellular expression of MK and NGF in areas surrounding the lesion, and the increased expression levels of MK and NGF were associated with a reduction of infarct volume and decreased apoptotic cell death after brain ischemia [2]. Although MK may have neuroprotective and regenerative effects, it is unknown whether MK expression levels are increased by pre-conditioning exercise prior to ischemia and whether MK overexpression is associated with neuroprotection after stroke.

Apoptosis and oxidative damage play critical roles after ischemic reperfusion. Physical exercise provides neuroprotection by inhibiting apoptotic activity [2,20]. Long-term exercise can enhance antioxidant activity in the brain [21]. Regular exercise training can reduce the peroxidation levels of lipids and proteins in the rat brain [22]. Peroxynitrite ($ONOO^-$), a powerful oxidant and cytotoxic agent, is generated from the reaction of nitric oxide (NO) with superoxide (O_2^-) [23]. Nitrotyrosine in the cerebrospinal fluid is an *in vivo* marker for peroxynitrite-induced neurotoxicity after oxidative nitric oxide damage [24,25]. However, it remains unknown whether preconditioning exercise prior to ischemia can reduce peroxynitrite-induced neurotoxicity in the brain. Therefore, we investigated whether preconditioning exercise reduced apoptotic activity and levels of peroxynitrite, as measured by the expression of nitrotyrosine (NT), after ischemia.

In this study, we investigated the effects of preconditioning exercise on brain damage and sensorimotor function improvement, by determining the expression levels of MK and BDNF, astrocyte proliferation, angiogenesis, apoptotic activity, and oxidative damage in the ischemic rat brain.

2. Materials and methods

2.1. Animals

Thirty-four male Sprague-Dawley rats (Kyudo Company, Saga, Japan) (280.1 ± 44.9 g; mean ± standard deviation) were used in this study. The rats were pair-housed under temperature-controlled conditions (22 ± 1 °C) on a 12 h light/dark cycle, with food and water available *ad libitum*. The experimental protocol was approved by the Ethics Board of the Institute of Laboratory Animal Sciences of Kagoshima University.

2.2. Exercise training protocol

All rats performed 3 days of motor-driven treadmill running (MK-680, MUROMACHI KIKAI CO., LTD, Japan) at a speed 15–25 m/min for 10 min per day (familiarization). After familiarization, the rats were randomly assigned to 4 groups: exercise and ischemia (Ex group, $n = 11$), no exercise and ischemia (No-Ex group, $n = 11$), exercise and no ischemia (Ex-only group, $n = 6$), and no exercise or ischemia as a control (Control group, $n = 6$). Before the middle cerebral artery occlusion (MCAO) procedure, the rats in the Ex and Ex-only groups exercised at a speed of 25 m/min for 30 min for 5 days a weeks for 3 weeks. The rats in the No-Ex and Control groups were allowed to move freely in their cage, and no additional treadmill running was employed. The exercise was performed at room temperature and during the daytime. In order to monitor the stress induced by treadmill running, body weight was periodically measured periodically. Additionally, the stress hormone, serum cortisol, was evaluated in the Ex-only and Control groups after 3 weeks by the Clinical Pathology Laboratory inc (Kagoshima, Shiroyama, Japan).

2.3. Middle cerebral artery occlusion model

After 3 weeks of the running program, the rats were anaesthetized using an injection of 4% chloral hydrate (10 mL/kg) administered intraperitoneally. Subjects' rectal temperatures were monitored throughout the surgical procedures and maintained at 37 °C using a heating blanket (KN-474, NATUME, Tokyo, Japan). Stroke was induced by a 60 min left middle cerebral artery occlusion (MCAO) using an intraluminal filament, as previously described [3]. Briefly, a midline incision was made, and the left common carotid artery (CCA), the external carotid artery (ECA), and the internal carotid artery (ICA) were exposed. The distal ECA branch was completely coagulated. The CCA and ECA were then tied with a white thread. A 4–0 monofilament nylon suture (Cat. No. 3035, Doccol Co., Albuquerque, NM) coated with poly-L-lysine (a tip diameter with coating of 0.3–0.33 mm) was inserted into the left CCA *via* an arteriotomy. It was then passed up the lumen of the ICA into the intracranial circulation and lodged into the narrow proximal anterior cerebral artery, blocking the MCA at its origin. After 60 min of MCA occlusion, reperfusion was established by withdrawal of the filament. After 48 h, the rats were sacrificed with an overdose of 4% chloral hydrate. The brain, including the ischemic region, was analyzed histologically and immunohistochemically. Brain tissues was also used for 2,3,5-triphenyltetrazorlium chloride (TTC) studies.

2.4. Evaluation of ischemic infarct

Rats were deeply anaesthetized *via* an injection of 4% chloral hydrate (10 ml/kg, intraperitoneally), and transcardially perfused with physiological saline before being decapitated. The brain was carefully removed and cut into six 2-mm-thick coronal sections from the frontal tip using a brain slicer. The slices were then immersed in a 1% solution of TTC in phosphate buffered saline (PBS, pH 7.4) at 37 °C for 10 min. After staining, the sections were scanned to determine the ischemic infarct volume. The infarctions were measured using Scion Image software BETA 4.0.3 (Scion Corp., Frederick, MD). The total infarct area (mm^3) was multiplied by the thickness of the brain sections to obtain the infarct volume.

2.5. Evaluation of neurological scores, the sticky tape-removal test, motor behavior, and locomotor function

Animals in each group were evaluated for neurological scores, sensorimotor dysfunction, and motor function using the sticky tape-removal test, beam walking and rotarod tasks test 48 h after

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