Behavioural Brain Research 300 (2016) 56–64

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Running exercise enhances motor functional recovery with inhibition of dendritic regression in the motor cortex after collagenase-induced intracerebral hemorrhage in rats

Yasuyuki Takamatsu[a,b], Keigo Tamakoshi[a,c], Yuya Waseda[a], Kazuto Ishida[a,*]

[a] Department of Physical Therapy, Program in Physical and Occupational Therapy, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan
[b] Department of Rehabilitation, National Hospital Organization Higashi Nagoya National Hospital, Nagoya, Aichi, Japan
[c] Department of Physical Therapy, Niigata University of Health and Welfare, Niigata, Japan

HIGHLIGHTS

- Motor functional recovery after ICH was enhanced by treadmill running exercise.
- Dendritic regression after ICH was inhibited by treadmill running exercise.
- TrkB expression after ICH was upregulated by treadmill running exercise.
- Nogo-A and ROCK 2 expression after ICH were not upregulated by treadmill running exercise.

ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form
12 November 2015
Accepted 3 December 2015
Available online 7 December 2015

Keywords:
Intracerebral hemorrhage
Running exercise
Motor functional recovery
Dendrite
Tropomyosin-related kinase B

ABSTRACT

Rehabilitative approaches benefit motor functional recovery after stroke and relate to neuronal plasticity. We investigated the effects of a treadmill running exercise on the motor functional recovery and neuronal plasticity after collagenase-induced striatal intracerebral hemorrhage (ICH) in rats. Male Wistar rats were injected with type IV collagenase into the left striatum to induce ICH. Sham-operated animals were injected with saline instead of collagenase. The animals were randomly assigned to the sham control (SC), the sham exercise (SE), the ICH control (IC), or the ICH exercise (IE) group. The exercise groups were forced to run on a treadmill at a speed of 9 m/min for 30 min/day between days 4 and 14 after surgery. Behavioral tests were performed using a motor deficit score, a beam-walking test and a cylinder test. At fifteen days after surgery, the animals were sacrificed, and their brains were removed. The motor function of the IE group significantly improved compared with the motor function of the IC group. No significant differences in cortical thickness were found between the groups. The IC group had fewer branches and shorter dendrite lengths compared with the sham groups. However, dendritic branches and lengths were not significantly different between the IE and the other groups. Tropomyosin-related kinase B (TrkB) expression levels increased in the IE compared with IC group, but no significant differences in other protein (brain-derived neurotrophic factor, BDNF; Nogo-A; Rho-A/Rho-associated protein kinase 2, ROCK2) expression levels were found between the groups. These results suggest that improved motor function after a treadmill running exercise after ICH may be related to the prevention of dendritic regression due to TrkB upregulation.

1. Introduction

Intracerebral hemorrhage (ICH) is a type of cerebrovascular accident that causes major neurological impairments and impacts the activities of daily living (ADL) and the quality of life (QOL) of patients with stroke [1]. Rehabilitative approaches improve motor functional recovery, ADL and QOL after stroke [2].

Neuronal activity-dependent plasticity in the peri-lesion area or the contralateral hemisphere contributes to motor functional recovery after stroke in human and animal studies and includes

Abbreviations: ICH, intracerebral hemorrhage; ADL, activities of daily living; QOL, Quality of life; SC, sham + control; SE, sham + exercise; IC, ICH + control; IT, ICH + exercise.

* Correspondence to: 1-1-20 Daikominami, Higashi-ku, Nagoya 461-8673, Japan. Fax: +81 52 719 1343.
E-mail address: ishida@met.nagoya-u.ac.jp (K. Ishida).

http://dx.doi.org/10.1016/j.bbr.2015.12.003
0166-4328/

altered plasticity of dendrites or synapses in neuronal cells [3,4]. Some exercises improve motor function and enhance neuronal plasticity changes in the brains of ICH animal models [5–9]. Our previous study showed that a treadmill running exercise improved motor functional recovery and altered dendritic morphology in the striatum after ICH in rats [6]. In the previous report, rehabilitative training improved recovery with enhanced dendritic complexity in the striatum and cortex after ICH in rats [5]. Furthermore, a previous study showed inhibition of dendritic regression, which meant a decrease in spine density due to melatonin-enhanced functional recovery following transient focal cerebral ischemia in rats [10]. These data demonstrate that dendritic plasticity contributes to functional recovery after ICH. However, the mechanisms by which dendritic plasticity is induced through rehabilitative exercises are not clear.

Brain-derived neurotrophic factor (BDNF), which is one of the growth-promoting factors for neurons, contributes to functional recovery after focal ischemia in rats [11]. Additionally, BDNF overexpression prevents dendritic atrophy in vitro [12]. Conversely, Nogo-A is one of the growth-inhibiting factors and acts on the plasticity of axons and dendrites as a negative regulator via RhoA/Rho-associated protein kinase (ROCK) signaling in the central nervous system (CNS) [13,14]. Some reports have suggested that anti-Nogo-A therapy results in functional recovery and neuronal plasticity after stroke in rats [15,16]. Both growth-promoting factors and growth-inhibiting factors have been related to motor functional recovery and neuronal plasticity after injury of the CNS [17]. Zhang et al. reported that exercise affects both types of factors in rats after ischemic stroke [18]. However, few studies have focused on both growth-promoting factors and growth-inhibiting factors to investigate the effects of exercise on motor function and dendritic plasticity after ICH in rats.

In this study, we investigated the effect of a treadmill running exercise on the motor functional recovery and dendritic plasticity in the motor cortex by focusing on both growth-promoting factors and growth-inhibiting factors after collagenase-induced striatal ICH in rats.

2. Materials and methods

2.1. Animals and experimental design

The experimental procedures were performed in accordance with the animal care guidelines of Nagoya University. Male Wistar rats weighing 250–300 g (8 weeks in age) were used in this experiment. The rats were housed in controlled temperature (25 °C) and lighting conditions (8:00–20:00) with food and water made available ad libitum throughout the experiments. The animals were randomly assigned to two groups: the sham group or the ICH group. Each group was divided into two subgroups: the exercise group and the control group. Therefore, this study followed four groups in total: the sham control (SC), the sham exercise (SE), the ICH control (IC), and the ICH exercise (IE) group. All experiments were performed following the design, as shown in Fig. 1. All efforts were made to minimize suffering and the number of animals used. This study was unblinded to experimental group during behavioral evaluation and during histological and biochemical analyses.

2.2. Induction of ICH

ICH was induced based on previous studies with minor modifications [19,20]. To induce hemorrhage, the animals were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and were placed in a stereotaxic frame. Through a hole drilled in the skull, a needle (φ = 400 μm) was implanted into the left striatum at the following coordinates: 3.0 mm lateral to the midline, 0.2 mm anterior to the coronal suture, and 6.0 mm deep from the surface of the brain. Then, 1.2 μl of saline containing 0.24 U collagenase (Type IV; Sigma–Aldrich, St. Louis, MO, USA) was infused over 6 min. The needle remained in place for an additional 7 min after the infusion and was subsequently withdrawn slowly. Sham-operated animals were infused with 1.2 μl saline instead of collagenase. The injection of collagenase damaged the animals' left striatum and paralyzed their right forelimbs and hindlimbs.

2.3. Treadmill exercise

Animals in the exercise groups (SE, IE) were forced to run on a motorized treadmill for 30 min at a speed of 9 m/min once a day for 11 consecutive days from day 4 to day 14 after ICH induction. A previous study showed that the same exercise protocol was effective for motor functional recovery following ICH in rats [6].

2.4. Behavioral tests

To evaluate motor function after ICH, the animals performed three behavioral tests: a motor deficit score, a beam-walking test and a cylinder test (SC: n = 11, SE: n = 11, IC: n = 14, IE: n = 14).

2.4.1. Motor deficit score

A motor deficit score was evaluated to assay gross motor dysfunction at 1, 3, 7, 10 and 15 days after surgery. The specific tests included the following: (1) observation of spontaneous ipsilateral circling, which was graded from 0 (no circling) to 3 (continuous circling); (2) contralateral hindlimb retraction, which measured the ability of the animal to replace the hindlimb after it was displaced laterally by 2 cm to 3 cm and was graded from 0 (immediate replacement) to 3 (replacement after minutes or no replacement); (3) beam walking ability, which was graded 0 for a rat that readily traversed a 2.4 cm wide, 80 cm long beam to 3 for a rat unable to stay on the beam for 10 s; and (4) bilateral forepaw grasp, which measured the ability of the rat to hold onto a 2-mm diameter steel rod and graded 0 for a rat with normal forepaw grasping behavior to 3 for a rat unable to grasp with the forepaws [21]. The scores from all 4 tests were added to give a motor deficit score (maximum possible score, 12). Animals that had a low motor deficit score were excluded from the experiment. The specific exclusion criterion was defined as a total score less than 7 out of 12.

2.4.2. Beam-walking test

The beam-walking test (1.0 cm diameter, 80 cm long beam) was performed to evaluate hindlimb dysfunction at 1, 3, 7, 10 and 15 days after surgery. We evaluated the function of the right paralyzed hindlimb. Performance was rated on a 7 point scale: (1) the rat is unable to place the affected hindlimb on the horizontal surface of the beam; (2) the rat places the limb on the beam and maintains balance but is unable to traverse the beam; (3) the rat traverses the beam dragging the affected hindlimb; (4) the rate traverses the beam and places the affected hindlimb on the horizontal surface of the beam once; (5) the rat crosses the beam and places the affected hindlimb on the horizontal surface of the beam to aid during less than half of the steps; (6) the rat uses the affected hindlimb to aid in more than half of the steps; and (7) the rat traverses the beam with no more than two foot slips [22].

2.4.3. Cylinder test

The cylinder test was performed to evaluate the asymmetry of forelimb usage one day prior to surgery and at 3, 7 and 15 days after surgery. We evaluated the right paralyzed forelimb function. The rats were placed in a Plexiglas cylinder (20 cm in diameter, 35 cm