Pre-ischemic and Post-ischemic Swim Training on Neurological Outcomes in Brain Ischemia Rats

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Abstract

Swimming is frequently preferred as an exercise model for small laboratory animals and as a health promotion exercise for ordinary people. The purposes of the present study are to examine the pre-ischemic and post-ischemic training effects of swimming exercise on infarction in rats. For the pre-ischemic training effect, rats were randomly assigned to either of the 2 groups (n=9 for each group): control and pre-ischemic swim training for 2 weeks. For the post-ischemic training effect, rats were divided into 2 groups (n=9 for each group): control and post-ischemic swim training for 2 weeks. Cerebral infarction was induced by middle cerebral artery occlusion (MCAO) for 60 min, followed by reperfusion. After the predetermined period (24 hours or 2 weeks), rats were killed and brain slices were then stained to assess lesion size. Neurological examination was performed before killing. Pre-ischemic swim training for 2 weeks can reduce the infarction size and neurological deficits caused by MCAO ($P < 0.05$). However, the post-ischemic swim training for 2 weeks did not result in a better recovery compared with the spontaneous recovery. The present study provides evidence that pre-ischemia swim training increases ischemia tolerance in an animal model of cerebral ischemia.

Key Words: swimming, brain ischemia, protective effect, recovery effect, male rats

Introduction

Stroke is a major contributor to human disability in many countries (3). Early mobilization was reported to reduce secondary complications and mortality and promotes long-term functional outcome (8). However, the optimum program of rehabilitation after a stroke is not identified (15). Also, it is not known if or how exercise can protect the brain from damage or can influence the recovery process after stroke.

Moderate physical activity can significantly reduce the risk of stroke and heart attacks in men both with and without pre-existing ischemic heart disease (13). However, more vigorous activities do not confer any further protection (13). Our previous study has demonstrated the effect of pre-ischemic locomotor training in reducing both infarction size and edema (12). The effect of exercise on recovery after stroke has not yet been systematically studied, to the present authors' opinion, even though stroke is a major cause of death with increasing incidences of cerebral infarction as of today. Swimming is frequently preferred as an exercise model for small laboratory animals and also as a health promote exercise for ordinary people (4), however, such exercise effect on ischemic brain damage has not been studied.

The middle cerebral artery occlusion (MCAO) model is considered to be reliable and reproducible of cerebral ischemia (5). The pathohistological consequences of the MCAO model have been studied extensively. Damage following MCAO in rats is demonstrated in striatum and overlying cortex, similar to that observed in human thrombotic/embolic occlusion of the middle cerebral artery (MCA) (9,12). The purposes of the present study are to examine the pre-ischemic and post-ischemic training effects of

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swimming exercise in MCAO rats.

Materials and Methods

Pre-ischemic Training Effects

Young male rats (3-4 months of age, n=18) of the Sprague-Dawley strain were used. The rats were housed in a temperature-controlled room (22 ± 10°C) with daily artificial illumination for 12 hours (06.00AM - 06.00PM). All animals were given food and water ad libitum. Rats were randomly assigned to one of the two groups: the control (rest for 2 weeks) and the swimming training (swam 30 min per day, 5 days a week for 2 weeks) group. All rats underwent the stroke model - middle cerebral artery occlusion (MCAO) procedures after the predetermined 2 weeks. Rats were then killed 24-hour post MCAO procedure to quantify the infarct volume.

Post-ischemic Training Effect

Eighteen young male Sprague-Dawley rats were housed in groups of 2 and maintained under a 12 hour light/dark cycle with food and water available ad libitum. All rats underwent the stroke model - middle cerebral artery occlusion (MCAO) procedures and then were randomly assigned to one of the 2 groups. Rats in control group (n=9) remained relatively inactive for 2 weeks. Rats in the swimming training group (n=9) were scheduled to swim 30 min per day, 5 days a week for 2 weeks. Rats were then killed after the predetermined 2-week period to quantify the infarct volume.

Exercise Protocol - Swimming Training

Animals were trained to swim for 30 min per day, 5 days a week. Rats swam in groups of two in plastic tanks measuring 27 inches deep by 21 inches. Water temperature was maintained at approximately 34°C throughout the 30 min swim period (4). Following the swim session each animal was partially dried with a towel and returned to the home cage.

MCAO Procedures

Middle cerebral artery occlusion procedure leading to focal ischemia was conducted under chloral hydrate anesthesia (with single 0.5 g/kg i.p. bolus in 1 ml of saline provided anesthesia lasting at least 2 hours). Rectal temperature was monitored throughout the surgical procedures and maintained at normothermic (37.0 ± 0.5°C) by a heating blanket controlled by an electronic temperature controller (HB 101/2, Debiomed).

The right middle cerebral artery (MCA) was exposed using microsurgical techniques (5,12). Briefly, a 2-mm burr hole was drilled at the junction of the zygomatic arch and the squamous bone, following a 2-cm vertical skin incision midway between the right eye and ear and splitting of the temporalis muscle. The right MCA trunk was ligated immediately above the rhinal fissure with 10-0 suture. Complete interruption of blood flow was confirmed by using an operating microscope. Both common carotid arteries (CCAs) were then occluded using nontraumatic aneurysm clips. After the predetermined duration of ischemia (60 minutes), the aneurysm clips and ligation were removed from both CCAs and MCA. Restoration of blood flow in all three arteries was observed directly under the microscope. Free access to food and water was allowed after recovery from anesthesia.

Quantitative Analysis of Infarct Volume

After a period of 24 hours or 2 weeks post ischemia procedure, the rats were killed under ketamine anesthesia by intracardiac perfusion with 200 ml of 0.9% NaCl. The brain was removed carefully and dissected into coronal 2-mm sections using a brain slicer. The fresh brain slices were immersed sequentially into a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) in normal saline at 37°C for 30 minutes, and then fixed in 10% phosphate-buffered formalin at 4°C. The cross-sectional area of infarction identified as the unstained area in the cerebral cortex of the right MCA territory of each brain slice was measured by an image analyzer, Image-Pro Plus Data Analysis Program (Media Cybernetics, Silver Spring, MD, USA). The damaged areas measured were mainly confined to cerebral cortex including its adjacent caudate nucleus, putamen, and hippocampus. Total measured infarct volume (MV) for each brain was calculated by summation of the infarct area of all brain slices (area of infarct in square millimeters times thickness, 2 mm) from the same hemisphere. Both right hemisphere volume (RV) and left hemisphere volume (LV) were also measured and calculated. To compensate for the effect of brain edema on MV in the ischemic hemisphere, the corrected infarct volume was calculated and used as the infarct volume in the present study. The corrected infarct volume equals to: LV – (RV – MV) (11).

Neurological Examination

Neurological examination was performed before killing. A neurologic grading system with a four-point scale (0-4) described by Menzies et al. (6) was

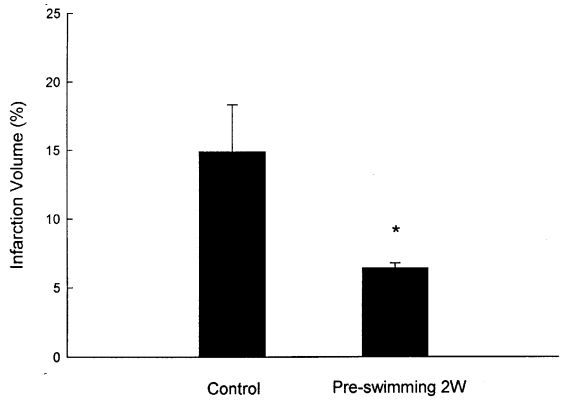

Fig. 1. Infarction volumes (presented as infarction volume / ipsilateral brain volume × 100%) at 24 hours post middle cerebral artery occlusion in rats of control, and pre-ischemic swimming training for 2 weeks (Pre-swimming 2W). * $P < 0.05$ vs. control group.

used: 0 = no apparent deficits; 1 = left forelimb flexion; 2 = decreased grip of the left forelimb while tail pulled; 3 = spontaneous movement in all directions; left circling only if pulled by tail; 4 = spontaneous left circling.

Statistical Analysis

Infarct volumes were expressed as means ± standard error of means. The training effect on infarct volume was tested using an independent *t* test. Neurological scores were expressed as median and range and the difference between two groups was analyzed with Mann-Whitney *U* test. A probability value of less than 0.05 was considered to be significant.

Results

Pre-ischemic Training Effect

There were significant differences between the 2 studied groups (n=9 for each group) for both infarct volume (t = -2.318, P = 0.045) (Fig. 1) and neurological score (z = -2.063, P = 0.034) (Table 1). The mean infarct volume measured 24 hours post ischemic procedure was 14.90 ± 3.63% of total volume of the ipsilateral hemisphere for rats in the control group, and 6.43 ± 0.39% for rats underwent a two-week swimming training before ischemic procedure (Fig. 1).

Post-ischemic Training Effect

The 2-week swimming training after MCAO did not result in a decrease in infarct volume compared with 2-week spontaneous recovery (t = 0.598, P = 0.560) (Fig 2). The mean infarct volume measured 2 weeks post ischemic procedure is 13.60 ± 1.39% of total volume of the ipsilateral hemisphere for rats in the control group (n=9) and 15.16 ± 2.21% for rats (n=9) underwent the two-week swimming training after ischemic procedure (Fig. 2). With regard to the neurologic function, no significant difference was found between the 2-week swimming training group and 2-week spontaneous recovery group (z = -1.770, P = 0.077) (Table 1).

Table 1. Neurological scores of rats in different groups

	Number	Neurological score
Pre-ischemic training effect		
Control	9	1 (0-2)
Pre-swimming for 2 weeks	9	0 (0-1)[a]
Post-ischemic training effect		
Control	9	1 (0-2)
Post-swimming for 2 weeks	9	2 (0-2)

Neurological scores are expressed as median (range).
[a] $P < 0.05$ versus control.

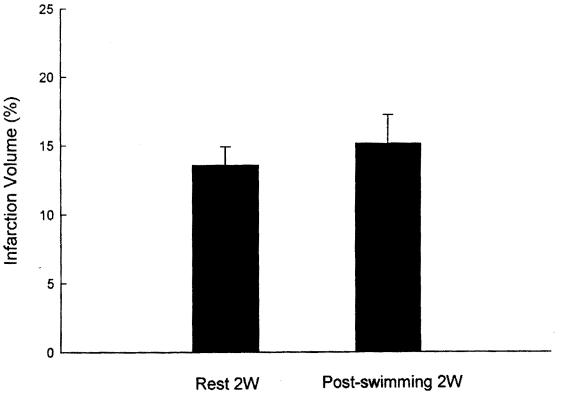

Fig. 2. Infarction volumes (presented as infarction volume / ipsilateral brain volume × 100%) at 24 hours post middle cerebral artery occlusion in rats of control, and post-ischemic swimming training for 2 weeks (Post-swimming 2W).

Discussion

The present study was designed to determine whether a swimming exercise program would result in a protective effect or a post-training recovery effect in MCAO rats regarding the ischemic damage.

Our results demonstrated that swim training for 2 weeks in advance could reduce the infarction volume secondary to ischemic damage in rats. Our previous study found that pre-ischemic treadmill training for at least 2 weeks could also reduce infarction size (12).

Such protective effect for cerebral ischemic damage can be induced by different types of exercise - swimming and treadmill training. The mechanisms for the training-induced ischemia tolerance is not immediately known at present time. However, angiogenesis and increasing endogenous opioids may be the partial mechanisms underlying the ischemia tolerance. Physical exercise requiring the continuing stimulation of cortical and subcortical centers for muscular control may affect the vascular blood supply of these cerebral regions (2). Although the significance of present observed protection is not clear, induction of angiogenesis process may support a reduction of infarction from ischemia. Also, an enhanced release of endogenous opioids has been found due to physical activity (1). The elevated opioid level may inhibit the excitotoxic stimulation of NMDA-receptor complexes by glutamate resulting from tissue injury (1).

Swimming is frequently preferred as an exercise model for small laboratory animals, and it has several advantages over other types of exercise. The intensity of labor during exercise is greater than running for equal periods, and aversive stimulation used to promote running is not used in swimming (4). On the other hand, swimming exercise certainly includes some emotional factors. This is especially true in inexperienced animals, and the endocrine response to this mixture of emotional and physical stress was found to be different from that to exercise alone (4,16). Swimming may cause an enhanced release of epinephrine from the adrenal medulla and a reduced output of norepnephrine from sympathetic nerve endings. In contrast, physical exercise with less emotional component resulted in increased norepinephrine to epinephrine ratio. Corticosterone is also found to be increased in proportion to the level of emotional component of swimming exercise in rats (4). Our rats could tolerate the swimming exercise for 30 min, however, it has been observed that this is an exhaustive exercise for rats with ischemic damage, indicated by observations of submerged periods of the animals for 20 - 30 seconds. This may be the reason for not observing a swimming training effect on recovery in our MCAO rats. The 2-week swim training after MCAO did not result in a better recovery compared with the 2-week spontaneous recovery regarding the infarct volume and neurological score. The results of our study should be accepted to reflect a mixture of emotional and physical components of the swimming exercise, which may not be suitable for rats immediately following the ischemic insult.

Tissue perfusion is well regulated by local mechanisms, mainly on the basis of the metabolic status of a given tissue reflected by the level of certain metabolites. If the tissues have a sufficient autoregulating capacity, any alteration can be tolerated and the impairment in tissue perfusion does not occur (10). However, if the autoregulatory reserve has already been used as a result of existing vascular problems, such as the ischemic lesion, the extra load induced by swimming may cause the impairment in perfusion. Such impairment in perfusion may result in different training effects between pre-ischemic training for 2 weeks and post-ischemic training for 2 weeks.

The reduction of infarct volume accompanied by the improvement of neurological function was noted in our pre-ischemic training rats. The correlation between infarct volume and neurological score after focal cerebral ischemia in rats has been demonstrated (7). Witte indicated that the perilesional dysfunction brain area contributes to neurological deficit, and recovery occurs when this perilesional dysfunction resolves (14). Our results support the inference addressed by Witte.

In conclusion, we found that swimming, as the treadmill training exert cerebral protection against ischemic damage with an extended therapeutic window. Its use provides beneficial effects on motor measures and it significantly reduces infarct volume. However, swim training does not have an effect on recovering from ischemic damage induced by MCAO as expected. Future studies should be directed at elucidating the mechanisms by which exercise affect the ischemic damage.

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