Exercise preconditioning reduces ischemia reperfusion-induced focal cerebral infarct volume through Up-regulating the expression of HIF-1α

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Abstract: To study the effect and mechanism of exercise preconditioning on focal cerebral ischemia reperfusion induced cerebral infarction via rat model; Sixty Sprague Dawley rats were divided into three groups at random: ischemia reperfusion group (IR, n=24), sham group (sham, n=12) and exercise preconditioning group (EP, n=24). Group EP carried out moderate exercise preconditioning for 4 weeks (swimming with non-weight bearing, 60 minutes/day, 6 days/week), Rats in Group EP and IR were established cerebral ischemia reperfusion injury model by Zea Longa's thread method. The cerebral infarct volume in rat of different group was evaluated after 2%TTC staining, expression of HIF-1α in rats' brain was detected by real-time RT-PCR, immunohistochmeistry method and western blot. No cerebral infarction and significant expression of HIF-1α in Group sham. Compared with Group IR, there was smaller infarct volume and stronger HIF-1α expression in Group EP ($P<0.05$). Moderate exercise preconditioning reduces ischemia reperfusion induced focal cerebral infarct volume through up-regulating the expression of HIF-1α.

Keywords exercise preconditioning, cerebral infarction, ischemia reperfusion, HIF-1α

INTRODUCTION

Cerebrovascular diseases (CVDs) commonly pose risks to human health and life; furthermore, CVDs are ranked as the second most common cause of adult disabilities and deaths in China (MOH, 2006) and in other countries (Bonita *et al*., 2004). The MOH (2005) state that among CVDs, cerebral infarction is the most common form induced by cerebral vascular obstructive ischemia, resulting in an incidence rate of 1.10/0.10 million that accounts for approximately 60% to 80% of the total incidence of CVDs. As such, studies in preventive and clinical medicine have focused on mechanisms that can effectively prevent the onset of cerebral infarction and reduce the size of affected body parts after blood vessels have been blocked.

Ischemic pre-conditioning (IPC) is a phenomenon that delays or relieves subsequent tissue ischemia-reperfusion injuries by stimulating the endogenous protective mechanism of the body. This mechanism is mobilized by recurrent transient ischemia. IPC is also a self-protective process that commonly occurs in animals. Neuronal ischemic pre-conditioning (NIPC) is a specific neuroprotective mechanism of the body triggered when one or more stimuli cause milder than permanent damage to the brain; as a result, the ability of the nervous system to resist lethal injury is enhanced, thereby preventing permanent ischemic damage (Nandagopal *et al*., 2001). Some researchers have shown that NIPC provides protection against severe cerebral ischemic injuries that occur subsequently. Studies have been conducted to investigate the protective effect and mechanism of exercise pre-conditioning as a special form of hypoxic-ischemic preconditioning on the central nervous system. Our preliminary studies showed that exercise pre-conditioning can relieve the apoptosis of cells in the cerebral cortex and the hippocampus of rats, eliciting protective effects against brain cell apoptosis induced by exhaustive exercise (Wang *et al*., 2012; Wang *et al*., 2009 However, studies have yet to determine whether or not it elicits protective effects. The related mechanism of a more severe form of ischemic cerebral infarction also remains unknown.

Hypoxia-inducible factor-1α (HIF-1α), which functions as a regulatory mechanism stabilizing intracellular environments as the body responds to ischemia and hypoxia, is a newly discovered transcription factor that can induce vascular endothelial growth factor (VEGF), erythropoietin (EPO), inducible nitric oxide synthase (iNOS), and other target genes after transcription. This study was designed to observe the effects of exercise pre-conditioning on the volume of cerebral infarction induced by focal cerebral ischemia reperfusion in rats using a rat model of middle cerebral artery occlusion (MCAO). This study also aimed to explore the related molecular mechanism.

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MATERIALS AND METHODS

Animals and their corresponding groups

Rats were randomly divided into the ischemia-reperfusion group (IR group, *n*=24), exercise pre-conditioning group (EP group, *n*=24) and sham group (*n*=12). The middle cerebral artery (MCA) ischemia-reperfusion model was constructed using the improved method described by Longa (LONGA, 1989). The suture was removed after 2 h of ischemia to restore blood flow and induce reperfusion in the IR group. Sham operation was performed instead of ischemia-reperfusion in the sham group. In the EP group, the rats were subjected to load-free adaptive swimming exercise (10min/d) for the first 3 d and then to moderate load-free swimming exercise from 4d (60min/d, 6 d/w) for four weeks. Within 24h after the last exercise, ischemia reperfusion was performed with the method used in the IR group.

Experimental modeling

A rat model of focal cerebral ischemia reperfusion was established. The middle cerebral artery was sutured twice according to a previously described method (LONGA *et al*., 1989). with slight modifications. In brief, the rats were intraperitoneally anesthetized with 10% chloral hydrate (0.3 ml/kg). A straight incision was then made in the midline of the neck. These rats were subsequently subjected to surgery to form MCAO on the right side. The common carotid artery (CCA), external carotid artery (ECA) and intracervical artery (ICA) were exposed. Approximately 0.26cm diameter single-wire nylon fishing cord was obtained from one end of a paraffin-embedded with a total length of 0.5cm. The cord was marked at 2cm. The far end of the ECA was then ligated. Blood flow from the CCA and ICA was temporarily blocked by pulling the cord. A 0.3mm incision was made 5 mm from the near end of ligature. The cord was then inserted from the right side of the CCA incision to the ICA. The suture length from the CCA furcation was maintained at approximately 18 mm to 20 mm (depending on the body weight of each rat). The MCA was blocked on the right side. The skin incision was closed and the suture was fixed. After 2h of ischemia, the suture was carefully removed, and reperfusion was induced. The body temperature was maintained at 37±0.5°C during ischemia and in 2h after reperfusion. Once awake, the rats were then observed in terms of posture and behavior. The MCAO model was considered successful if the judgment criteria were observed, particularly limb paralysis and astasia as rats recover from surgical anesthesia as well as circular movements to the side in a tail suspension test.

In the sham group, the steps were the same as those performed in the IR group except no nylon fishing cord was inserted.

Preparation of materials

All of the rats were sacrificed by withdrawing blood from the femoral artery 24 h after MCAO operation was performed. The skin was then dissected to expose their heads and necks. The cervical cords were also cut from the corresponding cervical vertebrae. Hind neck muscles were separated and removed. The skulls were carefully removed using curved forceps to avoid scratching or damaging the brain tissues by the dura mater. The tissues of the skull base were slowly separated from the medulla, and the brain was extracted. The brain tissues from each group of rats were randomly divided into three groups. In one of these groups, the infarct volumes were measured by tetrazolium chloride (TTC) staining technique. In the second group, pathological examination was performed. In the third group, molecular biology experiments were conducted. The first, second, and third groups were cryopreserved at -20°C, fixed with 4% neutral paraformaldehyde and preserved in liquid nitrogen, respectively.

TTC staining

The brain tissues were frozen to -20°C for 20min. Each brain tissue was removed and then cut into five slices at spacing of 2mm. The first cut was performed at the midpoint between the forebrain pole and the optic chiasm. The second cut was placed at the optic chiasm. The third cut was conducted at the site of the funnel handle. The fourth cut was placed between the funnel handle and the caudal pole of the posterior lobe. The sliced tissues were placed in 2% TTC solution, covered with silver paper, and placed in an incubator at 37°C for 25min. The sliced brain tissues were overturned once at an interval of 5min to allow uniform staining. The tissues were fixed with 10% formaldehyde solution and left to stand overnight. A pathological image analyzer was used to measure the cerebral infarct size and the trapezoid rule was used to measure the cerebral infarct volume according to Yin. (Yin *et al*., 2003).

Immunohistochemical staining

The paraformaldehyde-fixed specimen was embedded in paraffin and cut into 5 mm slices (three slices per brain specimen). The deparaffinized section was hydrated, dehydrated using graded ethanol, and treated with 3% H_2O_2 and normal serum. Anti-mouse HIF-1α (a monoclonal antibody; Santa Cruz Corp., USA, working concentration of 1:1000) was added and the specimen was stored at 4°C overnight. A few drops of goat anti-mouse IgG antibody (Cell Signal Corp., USA, working concentration of 1:1000) and serum protein (working concentration of 1:200) were also added. The specimen was incubated at 37°C for 60 min, developed with 3,3'-diaminobenzidine, and counter stained with hematoxylin. Afterward, the specimen was dehydrated using graded ethanol, vitrified with dimethylbenzene, and sealed with neutral balsam. The primary antibodies were replaced with phosphate-buffered saline and used as a blank control sample. The images of the specimen were obtained using a computer image collection and analysis

system (Version 3.0; Nikon & SPO, USA). Image Pro-Plus was used to analyze protein expression quantitatively. The images of each slice were randomly analyzed in 10 high-power fields, and the selected parts of each slice from different group are similar.

Examination by fluorescence quantitative RT-PCR

Total RNA was eluted by Trizol (working concentration of 100mg/ml) from the brain tissue cryopreserved by liquid nitrogen. The content and purity of RNA were determined using a UV spectrophotometer. TaKaRa Prime Script™ reagent kit (TaKaRa Biotechnology, Dalian., Ltd.) was used to obtain cDNA after reverse transcription. TaKaRa SYBR® Premix Dimer Eraser™ (TaKaRa Biotechnology) was used for real-time PCR. The primer sequences were listed as follows: HIF-1α (amplification fragment length 234bp) upstream: 5′-CGGCGGCGAGAACGAGAAGAAAAAG-3′, downstream: 5′-TTCTCACACGTAAATAACTGAT GGT-3′; β-actin (amplification fragment length 99 bp) upstream: 5′-CGT AAA GAC CTC TAT GCC AAC A-3′, downstream: 5′-TAG GAG CCA GGG CAG TAA TC-3′. At the end of the reaction, the melting curve of the PCR product was analyzed to determine the specificity of the reaction, and data processing was performed according to the Ct value of each specimen.

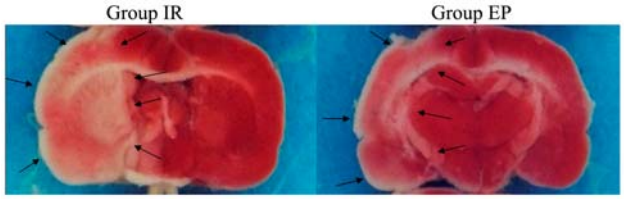

Fig. 1: TTC staining results of rats' brain in each group after MCAO

Western blot assay

The brain issue was removed from liquid nitrogen and placed in a pre-cooled mortar to grind. After the ground tissue was rinsed, 500μl/100 mg protein lysate (BioTeke Corporation) was added and mixed with the tissue in the mortar. The suspension was then poured into an Eppendorf tube. The following protease inhibitors were added before the protein lysate was used: 1% PMSF ([174 mg/10 ml isopropanol); 1/500 leupeptin (1 mg/ml); and 1/1000 pepstatin A (5 mg/ml). Afterward, the suspension was cooled in an ice bath for 20 min to ensure sufficient lysis and centrifuged for 20 min at 14,000 r/min and 4 °C. The supernatant or the total protein in rat brain tissue was collected and determined using a BCA protein assay kit (Wuhan Boster Biological Engineering Co., Ltd.). Approximately 20 μg of proteins from each specimen was added to a loading buffer and heated on a hot plate for 10 min. The proteins were transferred to polyvinylidene fluoride membrane after SDS-PAGE electrophoresis was conducted. Signals were detected by western blot after the X-ray film was properly exposed. The antibodies used in western blot were the same as those in the immunohistochemistry assay. Quantity One image analysis software was used to analyze electrophoresis results quantitatively.

STATISTICAL ANALYSIS

SPSS 17.0 was used for statistical analysis. Experimental results were expressed as mean ± standard deviation. The differences between the groups were analyzed using *t*-test by pairwise comparison. $P<0.05$ indicates statistical differences.

RESULTS

TTC staining

After TTC staining was performed, normal brain tissue exhibited a brick-red appearance, whereas the ischemic infarction area appeared pale, showing clear boundaries with normal tissues. The boundaries indicated the patent infarction area. In the IR group, a large infarct volume was observed in the brain tissue. In the EP group, cerebral infarct volume was significantly reduced compared with that in the IR group ($P<0.05$). No infarct was observed in the sham group (table 1; fig. 1).

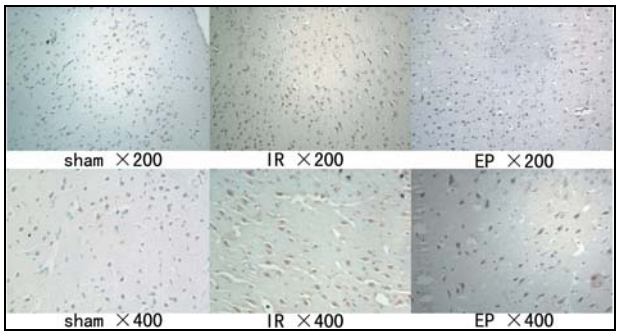

Fig. 2: Immunohistochemical results of HIF-1α in brain tissue of different group.

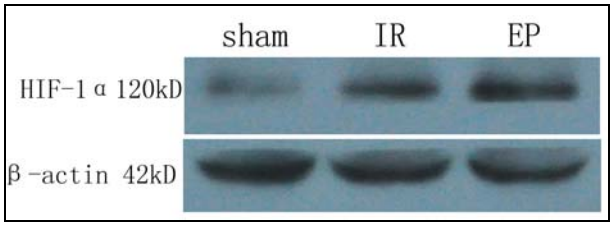

Fig. 3: Detection of HIF-1α protein in different group by western blot assay.

Immunohistochemical of HIF-1α

Low HIF-1α expression was observed in the sham group; however, images could not be analyzed because of low expression levels. Many HIF-1α-positive cells were observed in both IR and EP groups. The cells showed yellowish brown nuclei under a light microscope. A partially positive expression was observed in the cytoplasm. HIF-1α was expressed in most areas of the

brain tissue. The image analysis results revealed that the HIF-1α expression in the EP group was significantly stronger than that in the IR group (P<0.01, table 2; fig. 2).

Table 1: Comparison of cerebral infarct volume in rat of different group after MCAO ($\overline{X}$ ±S)

Group	N	Total infarct volume (mm^3)	Infarct volume ratio (%)
sham	4	0	0
IR	8	168.37±45.14	33.65±8.12
EP	8	122.98±38.51#	21.76±9.02#

Table 2: The change of HIF-1α positive cell in different groups ($\overline{X}$ ±S)

Group	N	HIF-1α
sham	4	0
IR	8	15.52±3.08
EP	8	27.40±2.84*

Note: #: P<0.01, Comparing group EP to group IR

Table 3: The expression of HIF-1α via RT-PCR detection in different groups ($\overline{X}$ ±S)

Group	N	HIF-1α($2^{-\Delta\Delta CT}$)	The relative expression rate
sham	4	0.13±0.02	1
IR	8	0.49±0.08#	3.77
EP	8	0.72±0.04	5.54

Note: #: P<0.05. Comparing to Group EP and Group sham.

Table 4: Comparison of Western blot strips gray value in different groups ($\overline{X}$ ±S)

Group	N	HIF-1α	β-actin
sham	4	37.17±8.58	189.78±63.62
IR	8	114.85±42.31*	209.46±42.94
EP	8	192.76±55.71	169.39±51.34

Note: #: P<0.01. Comparing to Group EP and Group sham.

Examination of HIF-1α by fluorescence quantitative RT-PCR

The HIF-1α/β-actin amplification melting curves of the specimen in each group reached the peak values, indicating that the primer exhibited good specificity without producing a primer dimer or inducing non-specific amplification. At the end of the reaction, the Ct value of each reaction was obtained from the RT-PCR instrument. The ΔΔCt value was calculated with β-actin gene as the internal control by using the following formula: ΔΔCt = (the mean Ct value of the target gene in the specimen of the group to be measured the mean Ct value of the housekeeping gene in the specimen of the group to be measured) – (the mean Ct value of a target gene in a specimen of the control group the mean Ct value of the housekeeping gene in the specimen of the control group). The corresponding expression level of HIF-1α gene in the specimen of each group was equal to $2^{-\Delta\Delta CT}$. The HIF-1α gene expression levels in IR and EP groups were significantly higher than those in the sham group (P<0.05). The HIF-1α gene expression level of the EP group was significantly higher than that of the IR group (P<0.05, table 3).

HIF-1α protein by western blot assay

The expression levels of HIF-1α protein in the rat brain tissue of IR and EP groups were significantly up regulated compared with those in the sham group (fig. 3). The level of up regulation was higher in the EP group than in the IR group. The gray-scale value of each strip measured using the image analysis software is shown in table 4.

DISCUSSION

Cerebral infarction is a condition of severe CVD from hypoxic-ischemic brain cells induced by cerebral vascular occlusion. Brain is the organ which most sensitive to injury and constant blood flow can continuously deliver oxygen and glucose to neurons. This blood flow is very important for the normal function of brain cells. If the blood flowing to the brain is interrupted even for a short duration, cell trauma or cell death occurs. Damage to the lesion site becomes permanent when neuronal death occurs.

Mammalian brains are highly sensitive to the loss of energy supply (such as in brain ischemia and hypoxia). However, studies have shown that mammalian brains can adapt to this adverse condition and achieve metabolic stability, resisting ischemia. This metabolic state is referred to as NIPC, a transient non-fatal ischemic event (ischemic pre-conditioning) followed by a period of reperfusion. Thus, these events increase the ischemic tolerance of the brain. The causes of ischemia and hypoxia in the body or organ can be divided into three categories: (1) reduction in ambient oxygen content, resulting in difficulty to obtain sufficient oxygen in normal physiological processes (e.g., in specific hypoxic and airtight environment in China's Qinghai-Tibet Plateau); (2) insufficient oxygen supply to the body, stimulating pathophysiological processes, including hypoxia in the heart, brain, and respiratory system (e.g., blockage in blood vessels); and (3) consumption of higher oxygen level than the body can physiologically metabolize, resulting in a relatively insufficient oxygen supply (e.g., during strenuous exercise). Ischemic tolerance induced by non-fatal vascular occlusion in the brain produces strong neuroprotective effects and related mechanisms in cases of severe cerebral ischemia and hypoxia (EBRAHIMI *et al*., 2012; Fang *et al*, 2013;Luca *et al*., 2013; NARAYANAN *et al*., 2013). NIPC also elicits protective effects on the brain. However, further

studies should be conducted to determine the correlation between factors, including pre-conditioning methods, duration, hypoxic-ischemic level, and interval between pre-conditioning and severe ischemia. The protective effects of pre-conditioning should also be determined. The effects of exercise pre-conditioning as a unique form of cerebral hypoxia-ischemia pre-conditioning on vital organs, such as the heart and the brain, have been studied by sports medicine researchers. For instance, Xu (Xu *et al*., 2012) used rats subjected to intervention via medium-load treadmill exercise and found that exercise pre-conditioning can improve myocardial superoxide dismutase activity, thereby preventing subsequent myocardial damages caused by heavy exercise. Wang and Yuan (Wang *et al*., 2009) and Wang (Wang *et al*., 2012) conducted a swimming exercise pre- conditioning in rats and found that early exercise pre-conditioning can relieve the exhaustive exercise-induced apoptosis of nerve cells in the brain, thereby producing protective effects. Wei (Wei *et al*., 2012) also showed that exercise pre-conditioning can increase antioxidant capacity and free radical elimination in the brain. These studies have focused on the protective effects of exercise pre-conditioning on cardiac and cerebral ischemia induced by strenuous exercise. However, studies have yet to determine whether or not exercise pre-conditioning elicits a protective effect on severe cerebral vascular obstructive ischemia. Ding (Ding *et al*., 2006) found that early exercise pre-conditioning for three weeks can improve cerebral micro vascular integrity in rats after stroke. Curry (Curry *et al*., 2010) also reported that exercising on a treadmill for three weeks at a rate of 30 min/day increases matrix metalloproteinase-9 activity by up regulating the expression of tumor necrosis factor-α; as a result, inflammatory reactions in rats are inhibited after cerebral infarction occurs. Kang (Kang *et al*., 2011) revealed that exercise pre-conditioning for four weeks increases the expressions of VEGF and laminin, maintains micro vascular integrity after focal cerebral ischemia, and reduces the cerebral infarct volume. In the present study, the rats in the EP group with focal cerebral ischemia reperfusion after these rats were subjected to exercise for four weeks exhibited a lower cerebral infarct volume than the rats in the IR group that did not undergo exercise pre-conditioning as shown by TTC staining results. Moderate load swimming exercise for four weeks can also improve the resistance to severe ischemia and hypoxia to some extent, and this resistance protects the brain from obstructive cerebral infarction. The possible mechanism of this protective effect was also discussed.

Hypoxia-inducible factor (HIF) was first discovered in Hep3B cells by Semenza (Semenza *et al*., 1992) in 1992, and subsequent studies have explored the possible functions of HIF. In common brain ischemia models, including focal cerebral ischemia and neonatal cerebral ischemia models, HIF levels in brain tissues after ischemia and hypoxia are significantly up regulated, and the expressions of a series of downstream genes are regulated. HIF is a heterodimer consisting of α and β subunits. Three kinds of α subunits, namely, HIF-1α, HIF-2α, and HIF-3α, have been discovered; HIF-1α participates in protecting the body from ischemia and hypoxia at a greater extent that other subunits (Gidday, 2006). HIF-1α mainly maintains the stability of tissues and cells in a hypoxic-ischemic state by expressing inducible glycolytic gene, VEGF, iNOS, and EPO (Tao *et al*., 2013; MORTEN *et al*., 2013; KUMAR *et al*., 2012; BENTOVIM *et al*., 2012). HIF-1α is present in almost all normoxic cells, but its expression is low. The effects of hypoxia on HIF-1α protein expression are mainly categorized into two. (1) At the beginning of hypoxia, the oxygen-sensing mechanism in the body immediately regulates hypoxia by protein translation. Thus, HIF-1α mRNA can continuously synthesize HIF-1α protein, induce HIF-1α and HIF-1β to form heterodimers, and bind to hypoxia response elements (HREs) in the target gene DNA-regulating region, along with other transcription factors, such as p300/CRB. (2) With further reduction in the oxygen content, von Hippel-Lindau protein (pVHL) is separated from HIF-1α protein. pVHL is an important protein that regulates HIF-1α protein degradation. Such separation reduces HIF-1α protein degradation with a sudden increase in HIF-1α protein content in cells, and HIF-1α protein forms compounds with HIF-1β and p300/CRB. HIF-1α lso regulates the translation of these genes, along with the HREs of the HIF target gene.

WACKER (WACKER *et al*., 2012) showed that hypoxia pre-conditioning induces ischemic tolerance in mice by activating the HIF/SphK2/CCL2 signaling pathway. Sarkar (Sarkar *et al*., 2012) also found that the expression of HIF-1α gene in endothelial cells at the acute phase of ischemic pre-conditioning-induced myocardial protection substantially increases, and the activation of the gene is necessary to induce the protective effects of IPC. This result indicates that physical ischemic stimuli can improve HIF-1α expression level. Several studies have shown that exercise can also affect the expression of HIF-1α. For instance, Fan (Fan *et al*., 2012) found that simple exercise stimuli and simple hypoxia stimuli similarly increase HIF-1α expression in rat brain tissue; exercise combined with hypoxia further increases HIF-1α expression level. Xu (Xu *et al*., 2011) studied the effects of different modes of hypoxic endurance exercises on the expression of HIF-1α in rat liver tissues and found that simple hypoxia, simple exercise, and different modes of hypoxic exercises can significantly increase HIF-1α expression in liver tissue. This finding is consistent with that of Liu (Liu *et al*., 2010). Wang (Wang *et al*., 2009) also found that sports training, particularly HiHiLo, can upregulate the mRNA expressions of HIF-1α and VEGF in the cortex renis. However, other reports have suggested

that the HIF-1α gene expression level exhibits slight changes in skeletal muscles after a six-week exercise is completed under normoxic conditions (Zhao *et al*., 2009). In the present study, the HIF-1α levels in mRNA and protein in the brain of rats in both EP and IR groups were higher than those in the sham group after focal cerebral ischemia-reperfusion for 2 h. This result indicated that acute ischemia and hypoxia may activate the HIF-1α gene expression in brain nerve cells, maintain the cells in a specific metabolic state, and resist severe stimulus induced by lack of energy. Immunohistochemical analysis, fluorescence quantitative RT-PCR, and western blot results after a four-week medium-load exercise was conducted show that HIF-1α expression was apparently higher in the EP group than in the IR group not subjected to pre-conditioning. This result was observed in the gene transcription level or in the protein expression level. Exercise, as a unique and mild cerebral hypoxic-ischemic stimulus, can promote HIF-1α expression, mobilize the self-protection mechanism of the body, and enable a certain degree of hypoxia tolerance in brain cells. Thus, the brain can resist severe hypoxic-ischemic stimuli.

Considering the interval between pre-conditioning and ischemic events, researchers found that NIPC may produce two major effects, namely, rapid tolerance and delayed tolerance (Lin *et al*., 2009; Zhang *et al*., 2008). Delayed tolerance was discovered earlier than rapid tolerance and considered as the most typical form of cerebral tolerance. Delayed tolerance requires the synthesis of a large amount of proteins and strict timing; under delayed tolerance, protective effects are manifested within 24 h to 48 h after pre-conditioning; the peak is reached after 3 d, and this peak gradually disappears after one week (Barone *et al*., 1998). Rapid tolerance does not require the synthesis of new proteins but can only elicit protective effects on nerve cells for a short time; rapid tolerance appears within several minutes after pre-conditioning and lasts less than 3 d (PEREZ-PINZON *et al*., 1997; PEREZ-PINZON *et al*., 1999). In the present study, focal cerebral ischemia/reperfusion was performed in 24h after the four-week exercise pre-conditioning was completed. The cerebral hypoxic-ischemic stimulus induced by medium-load in rats is small, but the changes in the expressions of some proteins in the body occur with relatively longer duration of action, particularly in more evident hypoxic-ischemic organ cells. These proteins may be involved in the protective effects of delayed tolerance. Exercise pre-conditioning elicits protective effects against focal ischemic cerebral infarction after 24 h. However, the present study did not investigate whether or not exercise pre-conditioning causes problems in rapid tolerance. Differences in terms of protective effects between the two kinds of tolerance were not discussed, but these topics will be considered in our future studies.

The mechanism of hypoxic-ischemic pre-conditioning is very complicated. Exercise pre-conditioning and its stimulating effect on the whole body may involve changes in numerous physiological processes, including angiogenesis, microvascular contraction, blood and iron metabolism, cell proliferation, and energy metabolism. Furthermore, the signal transduction system regulating these physiological processes is complicated and varied, but a common protein (HIF protein) is present in numerous upstream regulators. In the present study, HIF-1α was used as the target factor to explain the possible mechanism of exercise pre-conditioning. Medium-load exercise pre-conditioning for four weeks affected HIF-1α expression and significantly improved the mRNA and protein levels of this protein. However, the influencing factors downstream of this gene are varied and functionally complicated. Therefore, the signaling pathways that specifically induce protective effects should be studied further.

CONCLUSION

Four-week medium-load exercise pre-conditioning can reduce the infarct volume of cerebral infarction induced by focal ischemia. The protective effect is probably instigated by upregulating HIF-1α expression.

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