Exercise pre-conditioning strengthens brain microvascular integrity in a rat stroke model

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Increasing evidence indicates that physical activity reduces brain damage after stroke. The purpose of this study was to determine whether exercise-induced neuroprotection is associated with improved brain integrity in stroke.

Adult male Sprague–Dawley rats (3 months old, n=38) exercised on a treadmill, which required repetitive locomotor movement, for 30 minutes each day for 3 weeks. Then, using an intraluminal filament, stroke was induced by either 2 hours middle cerebral artery (MCA) occlusion followed by 24 or 48 hours of reperfusion. Brain damage was determined by evaluating brain infarction and brain edema, as well as ultrastructural alteration in endothelial–matrix–astrocyte interfaces.

Pre-ischemic motor exercise significantly (p<0.01) reduced infarct volume in the frontoparietal cortex and the dorsolateral striatum by 79%. By comparing the percentage difference in brain volume between the right (stroke site) and left hemispheres, we demonstrated a significant (p<0.01) reduction in brain edema associated with reduced infarct volume in a 3 week exercise group (Group 1, n=10) and a 3 week exercise plus 3 week rest group (Group 2, n=10). Edema in cortex and striatum was 19±4% without exercise pre-conditioning (n=10), in contrast to 5±3% (Group 1) or 6±4% (Group 2). The thickness of the basal lamina was enhanced by exercise. In ischemic rats without pre-exercise, alterations in microvessel ultrastructure with decreased luminal area, parenchymal edema and swollen astrocyte end-feet, as well as an abnormally thin basal lamina were observed. In contrast, exercise pre-conditioning significantly reduced the ischemic alterations, decreasing brain edema and increasing basal lamina thickness.

This study suggests that exercise pre-conditioning reduces brain injury by decreasing cerebral permeability and enhancing brain integrity after stroke. This exercise-induced endogenous neuroprotection could be an effective strategy to ameliorate ischemic brain injury from stroke.

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INTRODUCTION

Stroke is the third leading cause of death in the United States, with many survivors being left with physical and mental disabilities. There are currently limited treatment options for most types of stroke. There is increasing evidence that physical activity is associated with a decreased stroke incidence in men and women1–7, as well as with a substantial reduction in the risk of coronary heart disease7. Studies also indicate an inverse association linking physical activity with other risk factors such as body weight, blood pressure, serum cholesterol and glucose intolerance3–4.

An expanding literature substantiates the beneficial effects of exercise on stroke-induced brain injury8–18. A marked improvement in survival and a corresponding decrease in neuronal damage were observed in gerbils engaged in spontaneous locomotor activity for 2 weeks before a transient forebrain ischemia8. A reduced brain infarction was also found after a 60 minutes middle cerebral artery (MCA) occlusion in rats with 2 or 4 week pre-training on a treadmill10, as well as in mice with 3 week treadmill training15. Reduced infarction was reported to correlate with reduced brain edema10.

Recent research has focused on the functional interactions among capillaries, glia and neurons of the brain, which form the ‘neurovascular unit’ and were thought to play a vital role in maintaining brain microvascular integrity and reducing cerebral injury in stroke (see del Zoppo and Hallenbeck, 2000; del Zoppo and Mabuchi, 2003 and Lo et al., 2003 for reviewing). In this conceptual construct, the neurovascular unit consists of the endothelial cell of microvessels, the intervening basal lamina/matrix, the encircling astrocytic end feet and the adjacent neuron. In order to elucidate the integrity of the neurovascular unit in response to ischemic insults after
exercise pre-conditioning, we determined brain edema, as well as ultrastructural alterations of endothelial cells, intervening basal lamina–matrix and astrocyte that encircle the matrix circumferentially.

**MATERIALS AND METHODS**

**Subjects**

Adult female Sprague–Dawley rats (260–300 g, 3 months old, Charles River) were used and housed in the same animal care facility during a 12 hour light/dark cycle throughout the protocol. Animal care and surgical procedures were carried out in accordance with guidelines approved by the National Institutes of Health (NIH) and the Wayne State University Animal Investigation Committee. Three animal groups were studied for analysis using conventional light microscopy: (1) animals exercised for 3 weeks (n=10); (2) animals exercised for 3 weeks and housed for an additional 3 weeks after exercise (n=10); (3) non-exercised animals housed for 3 weeks (n=10). All these animals were subjected to transient focal ischemia (2 hour MCA occlusion followed by 2 days of reperfusion). Four additional animal groups were used for the study using electron microscopy: (1) treadmill-exercised animals with (n=2) or without (n=2) transient (2 hour MCA occlusion followed by 24 hours of reperfusion); and (2) non-exercised animals subjected (n=2) or not subjected to transient MCA occlusion (n=2).

**Motor exercise**

Animals were randomly assigned to one of two conditions: treadmill exercise or non-exercised control. An adapted human treadmill was used. During exercise, rats were restricted to a wood running track (15 × 60 cm) on a moving belt. This activity required repetitive locomotor movement. All animals were run at a speed of 15 m/min for 30 minutes each day. The distance of run was therefore 450 m/d for 3 weeks. Non-trained controls, as well as trained animals, were housed in small groups (n=3) in standard cages for equal time (3 weeks). In order to identify possible exercise-induced stress by treadmill running, body weight was monitored every 3 days. The animals that were not willing to run were excluded from further study.

**Induction of stroke with an intraluminal filament**

After 3 weeks, stroke was induced in exercised and non-exercised animals. Animals were anesthetized and maintained with 1–3% halothane in 70% N₂O and 30% O₂ with a facemask. Rectal temperature was maintained at 37°C with a circulating heating pad. MCA occlusion was induced with an intraluminal filament model as described by Zea Longa et al. and Belayev et al. Reliability and effectiveness of this model to induce stroke was enhanced using poly-L-lysine-coated intraluminal sutures, which yields consistently larger infarcts and greatly reduces interanimal variability. Briefly, the poly-L-lysine coated filament (4-0 nylon suture with blunted tip) was inserted into the right external carotid artery and lodged in the narrow proximal anterior cerebral artery, blocking the MCA at its origin. Animals were allowed to survive either for 24 or 48 hours after a 2 hour MCA occlusion followed by reperfusion that was established by withdrawal of the filament.

**Determination of infarct volume in Nissl stained sections**

Two days after surgery, all animals were deeply anesthetized with Nembutal (120 mg/kg, i.p.) and killed by cardiac perfusion of saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. Coronal brain frozen sections were cut on a microtome from 2.0 to 4.0 mm to bregma and stained with Nissl to determine infarct volume. All histological analyses were performed in a blinded fashion.

The infarct region, defined as the area with reduced staining or containing dark pyknotic necrotic cell bodies, was determined from serially cut sections through the MCA territory in the frontoparietal sensorimotor cortex and dorsolateral portion of the neostriatum. In order to minimize the error introduced by edema, an indirect method for calculating infarct volume was used. The non-infarcted region in the ipsilateral hemisphere was subtracted from that in the contralateral hemisphere. The infarct volume was presented as a percentage of the volume of the contralateral hemisphere. Statistical differences in infarct volume from two ischemia groups with or without exercise pre-conditioning were analysed by the Student’s t-test with a significance level at p<0.05.

**Brain edema measurement**

For histological analysis of brain edema, a series of coronal sections was obtained from right and left brain hemispheres containing frontoparietal cortex and dorsolateral striatum in ischemic rats after 48 hours of reperfusion. The degree of brain edema was expressed as the percentage difference in brain volume between the right and left hemisphere (ipsilateral or contralateral to stroke site). The effect of brain edema on infarct volume in rats has been previously determined in focal cerebral ischemia models. The increase in tissue volume corresponds well to water content determined by the conventional wet/dry technique. Total right or left brain volume was calculated respectively by summation of the entire brain slice, with each sequential area multiplied by the distance between sections from each hemisphere. This method has been successfully used to determine brain edema in ischemic rats. The differences in effect of ischemia and exercise pre-conditioning on the development of edema were statistically analysed by the Student’s t-test with a significance level at p<0.05.

**Tissue preparation for electromagnetic (EM) analysis**

After 24 hours of reperfusion, animals were processed for EM analysis using the techniques described by us previously. Briefly, each animal was perfused...
transcardially with a brief rinse of oxygenated saline followed by 2% paraformaldehyde/1.5% glutaraldehyde in 0.1 M PB (pH 7.4) at 4°C. Animals were then perfused with the same fixative containing 4% sucrose. The brains were removed and postfixed in 4% sucrose/fixative at 4°C for 1 hour, and placed in 4% sucrose in 0.1 M PB (pH 7.4) at 4°C overnight. Brain tissue containing the frontoparietal cortex or dorsolateral striatum from both hemispheres was treated with 1% osmium tetroxide in 0.1 M PB at 4°C and dehydrated in graded ethanols, cleared with propylene oxide, and then infiltrated with Epon resin overnight. After polymerizing at 60°C for 2 days, tissue was prepared for ultrathin sections. Section grids were viewed on a JEOL JEM 100 electron microscope at 100 kV. Morphological alterations of microvessels involving endothelial cells, basal lamina and perivascular end feet of astrocytes, as well as the spatial relationships of these elements were observed and compared in ischemic or non-ischemic tissue with or without exercise pre-conditioning.

RESULTS

Brain infarction

Infarct volume in frontoparietal cortex and dorsolateral striatum was presented as a percentage of the contralateral hemisphere in MCA occluded animals with or without prior exercise. Pre-exercised ischemic rats with reperfusion demonstrated an 11 ± 7% infarct volume as compared with a 52 ± 3% volume in ischemic rats without exercise (Figure 1A). This 79% reduction was highly significant (p<0.001). In addition, obvious differences in the extent of the lesion between exercised and non-exercised groups could be visually detected. A reduced lesion was restricted to the ischemic core in the striatum of pre-exercised ischemic rats, in contrast to the extensively infarcted area, which included both the dorsolateral portion of the neostriatum and frontoparietal cortex in rats without pre-ischemic exercise.

Our study further demonstrated a significant (p<0.01) reduction (Figure 1A) in infarct volume from rats subjected to transient MCA occlusion after 3 weeks of exercise followed by 3 weeks of rest. In addition, no obvious difference could be detected in the location of the lesion which included the dorsolateral neostriatum and frontoparietal cortex in rats without pre-ischemic exercise. Before exercised and non-exercised animals were subjected to MCA occlusion, their body weight was found to gradually increase during the procedure without significant differences among animals.

Brain edema

By comparing the percentage difference in brain volume between the right (stroke site) and left hemispheres, we demonstrated a significant (p<0.01) reduction in brain edema associated with reduced infarct volume in animals exercised for 3 weeks and 3 weeks
plus 3 weeks of rest (Figure 1B). Edema in cortex and striatum was 19 ± 4% without exercise preconditioning (n = 10), in contrast to 5 ± 3% (exercise for 3 weeks, n = 9) or 6 ± 4% (3 weeks of exercise plus 3 weeks of rest, n = 9).

**Ultrastructural vascular morphology**

Profiles of blood vessels in the striatum from normal control, exercise (3 weeks), ischemic (2 hour MCA occlusion) and exercised-ischemic are shown in Figure 2. The thickness of the basal lamina was enhanced by exercise. In ischemic rats without pre-exercise, alterations in blood vessel ultrastructure with decreased luminal area (curved lumen), parenchymal edema and swollen astrocyte end-feet, as well as an abnormally thin basal lamina were observed. Tight junction morphology, however, appeared normal, suggesting that endothelial-astrocyte-matrix interactions provide the central trigger for brain injury in stroke. In contrast, exercise pre-conditioning significantly reduced the ischemic alterations, decreasing brain edema and increasing basal lamina thickness.

**DISCUSSION**

This study demonstrates that physical activity can effectively reduce brain injury after focal ischemia. The reduced brain infarction is associated with decreased brain edema and the maintenance of microvascular ultrastructure. Because disruption of the blood–brain barrier (BBB) leads to the formation of vasogenic brain edema, the results suggest that exercise pre-conditioning induces neuroprotection by improving microvascular integrity (e.g. reinforcement of the basal lamina) and permeability of the BBB (brain edema) after stroke.

Recent studies have emphasized that brain microvessel integrity requires maintenance of the endothelial permeability barrier and basal lamina within neurovascular unit. The neurovascular unit consists of the endothelial cell of microvessels, the intervening basal lamina/matrix, the encircling astrocytic end feet and the adjacent neuron. Basal lamina derived from the extracellular matrix (ECM) not only provides structural support for cells but also acts as a physical barrier to or as a selective filter for soluble molecules. Generated by endothelial cells and astrocytes in concert during development, the basal lamina forms a biologically active connection between the two cell components.

The importance of the neurovascular unit in maintaining vascular integrity in stroke has been emphasized. Focal ischemia abruptly alters the stable relationships among endothelial cells, astrocytes and the intervening ECM. Soon after MCA occlusion, the primary vascular...
permeability barrier is lost concomitant with alterations in major vascular matrix constituents, such as laminin, collagen and fibronectin. Persistent obstruction induces structural changes related to increased permeability, edema formation, loss of ECM, and swelling and separation of astrocytes and/or endothelium from the matrix in ischemic regions.

Angiogenesis in the brain occurs normally during early development. Conversely, endothelial cell proliferation is low in the adult brain. Physical activity on a running wheel induces an increased blood vessel density in brain and daily forced exercise with a treadmill induces cortical and striatal angiogenesis. The exercise-induced angiogenesis was observed to associate with reduced brain damage. It is likely that the induced vascularization by exercise in adult rats resists ischemia/reperfusion insult by improving BBB function and enriching the functionally integrated neurovascular unit.

Similarly, astrogliosis was reported to play a role in inducing BBB properties. Studies on brain endothelial cell and astrocyte interactions have indicated that astrocyte end feet covers well over 90% of the cerebrovascular surface and restricts permeability of the vascular bed. Focal ischemia abruptly alters the stable relationships among endothelial cells, astrocytes and the intervening ECM. The endothelial–astrocyte–matrix interactions provide the central trigger for angiogenesis, in frontoparietal cortex and dorsolateral cortex, two regions involved in motor behavior. This study suggests that the therapeutic effects of exercise on BBB enhancement may be attributed to the endothelium, astrocytes and interfering basal lamina in newly formed vessels after stroke in exercise pre-conditioned rats.

Our study demonstrated that physical activity reduces brain damage in association with enhanced brain integrity after a transient focal ischemia. The beneficial effects of exercise on brain damage after stroke suggest that exercise is a neuroprotective factor independent from other stroke risk factors. Although further studies are needed to establish direct cause-and-effect evidence, the therapeutic effect of exercise could be attributable to exercise-induced angiogenesis and astrocytosis. Finally, exercise-induced endogenous neuroprotection may provide a powerful protective strategy to ameliorate ischemia/reperfusion brain injury from stroke.

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