Effects of Physical Activity and Ginkgo Biloba on Cognitive Function and Oxidative Stress Modulation in Ischemic Rats

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Abstract

Keywords

- ▶ physical activity
- ▶ Ginkgo biloba
- ▶ transient cerebral ischemia
- ▶ spatial memory
- ▶ oxidative stress
- ▶ hippocampus
- ▶ rat

Either exercise or Ginkgo biloba is reported to improve cognitive functioning. The aim of this study is to compare the protective effects of forced exercise and Ginkgo biloba on oxidative stress as well as memory impairments induced by transient cerebral ischemia. Adult male Wistar rats were treated with treadmill running or Ginkgo biloba extract for 2 weeks before cerebral ischemia. Memory was assessed using a Morris water maze (MWM) task. At the end of the behavioral testing, oxidative stress biomarkers were evaluated in the hippocampus tissue. As expected, the cerebral ischemia induced memory impairment in the MWM task, and oxidative stress in the hippocampus. These effects were significantly prevented by treadmill running. Indeed, it ameliorated oxidative stress and memory deficits induced by ischemia. In contrast, Ginkgo biloba was not as effective as exercise in preventing ischemia-induced memory impairments. The results confirmed the neuroprotective effects of treadmill running on hippocampus-dependent memory.

Cerebral ischemia is induced by the reduced cerebral blood flow due to the transient or permanent occlusion of the cerebral arteries. It is well known that the brain is vulnerable to ischemia. Indeed, transient cerebral ischemia for 5 or 10 minutes can lead to irreversible neuronal damage.^{1,2} Notably, general ischemic leads to extensive neuronal damage in the hippocampus and cerebral cortex and thus results in behavioral disorders, particularly impairment in learning and memory.³ Although the precise mechanisms underlying ischemia-induced hippocampal injury are still unclear, some evidence suggests that oxidative stress mediates may play an important role in neuronal cell death following cerebral ischemia.⁴ Ischemia causes formation of free radicals including reactive oxygen species (ROS) that can disrupt cell function by reacting with cellular macromolecules such as lipids, proteins, and nucleic acids.⁵ In the past few decades, many efforts have been made to find pharmacological and nonpharmacological treatments to prevent or even reverse the deleterious effects of ischemia on cognitive functions, especially memory.

In this regard, exercise and Ginkgo biloba are known to improve memory. Ginkgo biloba is one of the most widely used herbal remedies in the world to treat the symptoms of early-stage Alzheimer's disease, vascular dementia, peripheral claudication, and tinnitus of vascular origin.⁶ Despite multiple indications for ginkgo, it is commonly used as a memory enhancer. The results of studies on the efficacy of Ginkgo biloba on memory performance are conflicting. Although its beneficial effects have been reported in some studies,^{7,8} but others have found no effect.^{9–12} On the other hand, physical exercise as a nonpharmacological behavioral intervention has been shown to support brain health and function.¹³ It has been shown that physical exercise increases synaptic plasticity and stimulates neurogenesis¹⁴ in the areas involved in memory and learning processes, particularly in the hippocampus.¹⁵ Beneficial effects of voluntary exercise are well understood on hippocampus-dependent memory, while the effects of forced exercise are still controversial. Forced treadmill running is a type of training that obligates the animal to run according to the experimenters' demands

(duration and intensity), which could chronically activate the stress response.¹⁶ The physiological changes induced by the stress may lead to deleterious effects on memory.¹⁷ However, some investigations have reported that forced running could significantly improve memory.^{18,19}

Regarding the fact that each year millions of dollars are spent on ginkgo supplements²⁰ and ginkgo as a herbal remedy, it may have side effects,^{21,22} and exercise could be a low-cost, safe therapeutic option. Accordingly, this study was designed to verify and compare the neuroprotective effects of preischemic forced exercise training and Ginkgo biloba treatment on oxidative stress as well as memory impairments induced by transient cerebral ischemia in adult rats.

Materials and Method

Animals

Male Wistar rats (220–240 g) were used in this study and housed in pairs at the temperature of $21.0 \pm 2^\circ\text{C}$ and on a 12-hour reverse light/dark cycle (lights on 7:00 AM). Food and water were provided ad libitum, and body weights were recorded daily throughout the treatment and withdrawal periods. All procedures were approved by regional ethics committee of Tabriz University of Medical Sciences. Animals were randomly divided into four groups ($n = 10$ in each group) as follows¹: sham-operated group (SH)²; ischemic group (IS)³; preischemic exercise group (E + IS)⁴; preischemic Ginkgo biloba group (G + IS).

Drug Administration

Extract of Ginkgo biloba leaf was purchased from the Ahura Darou drug company (Shiraz, Iran) and consisted of 24% ginkgo flavoglucosides and 6% terpenoid lactones. A solution was prepared by mixing the Ginkgo biloba extract in 0.5% methylcellulose and was administered intragastrically via gavage at the dose of 100 mg/kg daily, in a volume of 1 mL/kg for 2 weeks before cerebral ischemia. All animals, which did not receive ginkgo were given orally appropriate volumes of the vehicle (0.5% solution of methylcellulose).

Exercise

Rats were familiarized with the treadmill apparatus to minimize novelty stress and were randomly assigned to different experimental groups (sedentary and exercised groups). The moderate-intensity forced-exercise training program was used, which consisted of running sessions on an adapted motorized rodent treadmill at 60% of each rat's maximal oxygen uptake.²³ Animals in the exercise groups were trained on a treadmill (0-degree inclination) for 60 minute/day, at a treadmill speed of 17 miles/minute, and 5 days/week for 2 weeks before ischemia, as previously described in our previous study.²⁴ Animals that were not able to perform the exercise were excluded from the sample. At the same time of the day, the sedentary rats were placed on a stationary treadmill, with the shock grid turned off, for the duration of the treadmill training session.

Transient Cerebral Ischemia

Rats were subjected to transient cerebral ischemia induced by bilateral common carotid occlusion. Animals were intraperi-

toneally anesthetized with ketamine–xylazine combination (50 and 6 mg/kg, respectively). A ventral midline incision was made, and the bilateral common carotid arteries were exposed and carefully separated from the carotid sheath and vagus nerve, and then occluded by artery clips. During the surgical procedure, the body temperature of the rat was kept stable at $36.5 \pm 0.5^\circ\text{C}$ using a heating pad. SH rats received the same operation without ligation.

Morris Water Maze

Twenty-four hours after the ischemia period, rats were submitted to behavioral testing for spatial memory in the Morris water maze (MWM) described previously.²⁴

Oxidative Stress Parameters

Tissue Preparation

The rats were sacrificed by cervical dislocation under ether anesthesia for 24 hours after the probe trial. The hippocampus was rapidly dissected on an ice-cold surface, and frozen in liquid nitrogen and subsequently stored at -80°C until biochemical analysis. The frozen hippocampal tissues were prepared according to the method of Fontella et al.²⁵

Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity was determined using a RANSOD kit (Randox Laboratories, Crumlin, United Kingdom) according to the method of Delmas-Beauvieux et al.²⁶

Glutathione Peroxidase Activity

Glutathione peroxidase (GPx) activity was determined according to the method of Paglia and Valentine²⁷ using a RANSEL kit (Randox Laboratories).

Catalase Activity

Catalase (CT) activity was determined spectrophotometrically according to the method of Aebi.²⁸

Malondialdehyde

Malondialdehyde (MDA) concentration as an indicator of lipid peroxidation was determined according to the method of Draper and Hadley.²⁹

Statistical Analysis

Data from the MWM task were analyzed using one-way analysis of variance (ANOVA), with or without repeated measures where appropriate. Data from the biochemical measures were analyzed by one-way ANOVA. All analyses were followed by the Tukey's test for multiple comparisons, whenever appropriate. Differences with $p < 0.05$ were considered statistically significant. Data were expressed as mean $\pm$ standard error of the mean.

Results

Morris Water Maze Test

A repeated measure one-way ANOVA revealed a significant main effect of block ($F(2,35) = 110.585$; $p < 0.0001$) on

Fig. 1 Effect of training on time latency to find the hidden platform. (A) The average escape latencies during training blocks on the hidden platform task. (B) The effect of ischemia, exercise, and ginkgo administration on escape latency to find the hidden platform. Cerebral ischemia significantly impaired spatial learning performance in the Morris water maze test. Both exercise and ginkgo improved spatial learning in ischemic rats. Data are mean $\pm$ SEM (standard error of the mean). (*) significantly different from sham-operated group ($p < 0.05$). (+) significantly different from ischemic group ($p < 0.05$).

escape latency as indicated by a significant reduction in the time to find the hidden platform over three training blocks ($\rightarrow$ Fig. 1A). In addition, data analysis showed a significant main effect of group ($F(3,36) = 13.339$; $p < 0.0001$). No interaction effect of block with group was found. As shown in $\rightarrow$ Fig. 1B, there were significant differences between IS and SH groups. Indeed, cerebral ischemia caused significant deficits in spatial learning. In addition, Posthoc analyses showed that E + IS and G + IS groups required significantly less time to locate the hidden platform than IS group ($p < 0.05$).

The analysis of swim speed revealed no main effects of block, group and their interaction (data not shown), indicating that neither block nor group had any significant effect on swimming speed.

On the probe trial, one-way ANOVA revealed a significant effect of group ($F(3,36) = 8.69$; $p < 0.001$) on the time spent in the target quadrant and the number of platform crossing ($F(3,36) = 7.558$; $p < 0.001$). Actually, IS rats showed a worse learned bias to navigate toward the goal quadrant, which previously contained the platform, and they spent less time in the quadrant than SH rats ($p < 0.0001$; $\rightarrow$ Fig. 2A). IS group also crossed the platform location fewer times than SH group ($p < 0.0001$; $\rightarrow$ Fig. 2B). In contrast, E + IS, but not G + IS, rats displayed an improved learned bias and spent more time in the quadrant and crossed ($p < 0.05$) the location of the platform significantly more times ($p < 0.05$) than did the IS rats. The performance of G + IS groups did not differ significantly from IS group.

Fig. 2 (A) The effect of ischemia, exercise, and ginkgo administration on percentage of distance traveled in the target quadrant during the probe test. Cerebral ischemia significantly decreased the traveled distance in the target quadrant. Preischemic exercise group (E + IS) group spent more time in the quadrant than ischemic (IS) rats. (B) The effect of ischemia, exercise, and ginkgo administration on the number of platform crossing. IS group also crossed the platform location fewer times than sham-operated (SH) group. E + IS group crossed the location of the platform significantly more times than did the IS rats. The performance of preischemic Ginkgo biloba group did not differ significantly from IS group. Data are mean $\pm$ SEM (standard error of the mean). (*) significantly different from SH, group ($p < 0.05$). (+) significantly different from IS group ($p < 0.05$).

Fig. 3 The effect of cerebral ischemia, exercise, and ginkgo on superoxide dismutase (SOD) activity. The level of SOD activity was significantly decreased in ischemic (IS) rats compared with sham-operated (SH) rats. Both exercise and ginkgo increased activities of SOD in the hippocampus of ischemic rats. Data are mean $\pm$ SEM (standard error of the mean). (*) significantly different from SH group ($p < 0.05$). (+) significantly different from IS group ($p < 0.05$).

Oxidative Stress Measurements

A one-way ANOVA revealed only a significant main effect of group ($F(3, 36) = 7.784$; $p < 0.0001$) on SOD activity. The SOD level was significantly lower in IS group compared with SH, E + IS, and E + IS groups ($\rightarrow$ Fig. 3).

A one-way ANOVA conducted on GPx activity showed significant effect of group ($F(3,36) = 2.950$; $p < 0.05$). The level of GPx activity was significantly lower in IS group compared with SH group ($\rightarrow$ Fig. 4). No meaningful differences were observed for GPx measurements between E + IS, G + IS, and IS groups.

Statistical analysis revealed a significant effect of group ($F(3,36) = 3.544$; $p < 0.05$) on CT activity. An obvious decrease in the level of CT activity was observed in IS group compared with SH group ($\rightarrow$ Fig. 5). Exercise increased activities of CT in

Fig. 4 The effect of cerebral ischemia, exercise, and ginkgo on glutathione peroxidase (GPx) activity. The GPx activity was significantly reduced in ischemic (IS) group compared with sham-operated (SH) group. No differences were observed between preischemic exercise group, preischemic Ginkgo biloba group, and IS group. (*) significantly different from SH group ($p < 0.05$). Data are mean $\pm$ standard error of the mean.

Fig. 5 The effect of cerebral ischemia, exercise and ginkgo on catalase (CT) activity. The CT activity was significantly reduced in ischemic (IS) group compared with sham-operated (SH) group. No meaningful differences were observed between preischemic exercise group, preischemic Ginkgo biloba group, and IS group. (*) significantly different from SH group ($p < 0.05$). Data are mean $\pm$ SEM (standard error of the mean).

the hippocampus of ischemic rats. No difference was observed between G + IS and IS groups.

Statistical analysis of MDA level with one-way ANOVA revealed significant main effects of group ($F(3,36) = 4.762$; $p < 0.01$). As can be seen in $\rightarrow$ Fig. 6, the MDA level was significantly higher in IS group compared with other groups. No differences were observed in the MDA level between E + IS, G + IS, and SH groups.

Discussion

The hypothesis of this study was based on the some reports in the literature that Ginkgo biloba treatment and exercise training could attenuate the effect of ischemia on memory performance function³⁰ and oxidative stress ROS production.^{31,32} In line with

Fig. 6 The effect of cerebral ischemia, exercise and ginkgo on the malondialdehyde (MDA) level. Cerebral ischemia increased the level of MDA in the hippocampus of ischemic (IS) rats compared with sham-operated (SH) rats. Both exercise and ginkgo decreased the concentration of MDA in the hippocampus of ischemic rats. Data are mean $\pm$ SEM (standard error of the mean). (*) significantly different from SH group ($p < 0.05$). (+) significantly different from IS group ($p < 0.05$).

previous studies, our data showed that transient cerebral ischemia was associated with learning and memory impairments.^{30,33,34} Cerebral ischemia also resulted in elevated MDA level and decreased activity of SOD, GPx, and CT enzymes in the hippocampus.^{35–38} Preischemic administration of both Ginkgo biloba extract and treadmill running significantly increased SOD activity and prevented the elevation of MDA level in ischemic rats. Therefore, this study confirms the findings of other researchers that exercise³⁹ and ginkgo⁴⁰ ameliorate oxidative stress.

The exact mechanisms underlying the effects of ischemia on memory function are unclear. Some evidence suggests that oxidative stress may play an essential role in the ischemia-induced cognitive function disturbance. As both exercise and ginkgo extract attenuated oxidative stress in hippocampus, it was expected that both of them would prevent memory dysfunction induced by ischemia, but the results did not support this expectation. Actually, ischemic rats subjected to treadmill training exhibited improved memory acquisition and retention in the MWM task, whereas ginkgo-treated ischemic rats showed significant improvement only in acquisition of spatial memory. Indeed, reduced oxidative stress was not accompanied by amelioration of ischemia-induced memory deficit in ginkgo-treated ischemic rats. While ginkgo supplements are commonly used as memory enhancers, but the results of studies on the efficacy of ginkgo on memory performance are not equal. In this regard, some studies have reported its beneficial effects^{7,8} and free radical scavenging by ginkgo has been suggested as a possible mechanism.^{31,41} However, others have found no such effect.^{9–12} Our findings do not confirm the studies reporting the effectiveness of Ginkgo biloba in treating memory impairments due to its antioxidant properties,^{42,43} but are in agreement with those reports showing that the retention deficit caused by ischemia was not statistically reduced by ginkgo extract.¹²

It is worth mentioning that as far as we know, no study has compared the antioxidant and memory protective activities of preischemic ginkgo extract treatment and exercise. In this study, we have done this comparison in rats subjected to cerebral ischemia. While both treadmill exercise and ginkgo extract reduced oxidative stress in the hippocampus, only exercise significantly reduced the effect of ischemia on memory performance. Consequently, it can be suggested that the extract of Ginkgo biloba does not provide significant neuroprotection against ischemia-induced memory impairments compared with forced exercise. In other words, exercise induced stronger neuroprotection in comparison with Ginkgo biloba. Also, it can be concluded that inhibition of oxidative stress cannot provide a full explanation for the observed improvement in memory in exercised ischemic rats. However, it may be part of the mechanisms responsible for the neuroprotective effects of exercise pretreatment.

The beneficial effects of exercise on the brain functions are well-documented.¹³ It has been shown that exercise prior to ischemia may attenuate, or even prevent, impairments in cognitive function deficits, particularly learning and memory.^{30,44,45} For instance, Carro et al showed that preischemic exercise prevents impairments in spatial memory performance.⁴⁶

In the same line of evidence, our results indicate that even 2-week treadmill running prior to transit cerebral ischemia prevent memory deficits in the MWM task. They also confirm the effects of exercise in increasing the antioxidant ability.³² Taken together, this study reinforces previous findings that preischemic treadmill exercise as preconditioning has neuroprotective benefits.⁴⁷ Although the precise mechanisms underlying the protective effects of preischemic exercise are not yet completely clear, but a variety of potential mechanisms have been suggested, including: (1) increasing the expression of neurotrophins such as brain-derived neurotrophic factor¹³ and nerve growth factor⁴⁸ that can increase resistance of neurons to ischemia damage,⁴⁹ (2) reducing glutamate release and overexpression of glutamate receptors⁵⁰ and promoting glutamate uptake,⁵¹ (3) increasing brain mitochondrial biogenesis,⁵² (4) improving neurovascular unit integrity through strengthening of blood–brain barrier,⁵³ (5) inducing mild oxidative stress that stimulates the expression of antioxidant defense enzymes⁵⁴ and improves antioxidant capacity,⁵⁵ and (6) reducing inflammatory responses after cerebral ischemia and reperfusion injury.⁵⁶

In conclusion, transit global cerebral ischemia has a deleterious effect on learning and memory functions. Exercise pretreatment for 2 weeks effectively provides protection against impairments of memory caused by ischemia. On the other hand, Ginkgo biloba extract pretreatment for 2 weeks did not effectively protect memory performance from ischemia-induced injury. Further studies should be performed to compare the effects of exercise and Ginkgo biloba extract treatment on memory.

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