DOI 10.1007/s11062-020-09840-x
Neurophysiology, Vol. 51, No. 6, November, 2019

Physical Training Moderates Blood-Brain-Barrier Disruption and Improves Cognitive Dysfunction Related to Transient Brain Ischemia in Rats

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Received May 29, 2019

Cerebral ischemia induces structural and functional damage in the brain, which leads to cell death and cognitive dysfunction. According to the evidences, physical exercise training exerts a neuroprotective effect and may decrease ischemia-induced injuries in this case. We evaluated the protective effects of physical training on blood-brain-barrier (BBB) disruption, neuronal death, and cognitive dysfunction induced by cerebral ischemia in male rats. Thirty-six adult male rats were selected randomly and allocated into three groups, ischemia (I), ischemia+exercise (I+E), and sham (Sh). Brain ischemia was induced via occlusion of the common carotid arteries for 20 min. Before occlusion, animals of the I+E group ran on a treadmill 5 days a week for 4 weeks. Spatial memory performances of rats were evaluated by the Morris water maze test. Apoptotic cell death in the dentate gyrus (DG) of the hippocampus was detected by a TUNEL assay, while the level of disruption of the BBB was measured by an Evans blue assay. Cerebral ischemia caused spatial memory impairment; exercise training improved the index of memory impairment following ischemia significantly ($P < 0.05$). Also, exercise training significantly reduced the BBB permeability in I+E rats compared with the I group ($P < 0.05$). In addition, the number of TUNEL-positive cells was significantly greater in I rats, while exercise training significantly reduced apoptotic cell death ($P < 0.05$). Our results indicate that physical training exerts noticeable neuroprotective effects against brain ischemic injury, in particular by preserving the BBB integrity.

Keywords: cerebral ischemia, physical exercise, blood-brain-barrier (BBB), cell death, cognitive dysfunction.

INTRODUCTION

Reduction or cessation of blood flow to a part of the brain due to blockage of a cerebral artery leads to brain ischemia [1]. Cerebral ischemia is a standout cause of disability and death in the world, with over 80% of all cases activated by ischemic occasions [2]. Cerebral ischemia-reperfusion (IR) injury causes permanent degeneration in the CNS, which is a primary cause of death in neurological diseases [3]. During brain ischemia, cerebral blood flow, important metabolite levels, and that of oxygen are reduced; returned blood flow during reperfusion leads to intense cellular oxygenation and augmented generation of reactive oxygen species (ROSs). Then, the respective effects on cell signaling can lead to dramatic tissue damage [4]. Recent studies demonstrated that certain pathological features are related to the IR effects, including the creation of ROSs, energy failure, neuronal apoptosis, an inflammatory response, and, finally, to neurological dysfunction [5].

The hippocampus is vitally important for memory functions [6, 7]. This cerebral structure is more sensitive to ischemic insults than other areas of the brain. Ischemia readily leads to strong physical and functional damages in the hippocampus [8].

The blood-brain barrier (BBB) is a specialized endothelial structure in the CNS. The BBB is composed of specialized cerebral endothelial cells, which form a tight seal due to the presence of tight junctions, which restricts the entry of plasma components and blood cells into the brain [9]. Several neuropathological conditions,

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such as stroke, multiple sclerosis, parkinsonism, Alzheimer's disease, and epilepsy, can significantly alter the integrity of the BBB; as a result, permeability in the CNS can be significantly injured [10]. The BBB disruption is a major consequence of cerebral ischemia/reperfusion (CIR), and it leads to the impairment of neuronal and synaptic functions [11] and induces intense neuronal cell death [12, 13]. Therefore, reducing the BBB disruption can be considered as a potentially effective therapeutic intervention after CIR.

There is evidence that physical exercise may exert neuroprotective effects against ischemic injury at the cardiovascular, cerebral, and functional levels [14, 15]. However, the neuroprotective mechanisms of physical training against ischemia are still not completely clear.

We evaluated the effect of pre-ischemic physical exercise on BBB disruption, neuronal death, and cognitive dysfunction in male rats following transient cerebral ischemia.

METHODS

Animals and Experimental Groups. Thirty-six male Wistar rats (250–300 g) were acquired and housed in standard cages under controlled conditions (humidity 45–50%, 22–24°C, and a 12-h light/dark cycle) with free access to water and food. Animals were randomly divided into three groups, sham (Sh, served as controls), ischemia (group I), and ischemia+exercise (group I+E). There were 12 rats in each group; 6 rats were used for the assessment of the BBB permeability, and 6 rats were used for TUNEL staining. The common carotid arteries (CCAs) were occluded in animals of the I and I+E groups. The animals in the I+E group ran on a treadmill 4 weeks before the induction of ischemia. The sham animals were exposed to the same surgeries except occlusion of the CCAs.

Exercise Training Protocol. Rats in the E group were trained to ran on a treadmill 5 days a week for 4 weeks [16–18]. At first, the animals were acclimatized to run on a treadmill for 10-15 min at speeds of 5-7 m/min with a 0% slope for two days before formal training sessions. Initially, weak electrical shocks (1.0 mA) were needed to force animals to run forward on the treadmill. Subsequently, the animals ran with no stimulating electrical shock. After an adaptive running session, the animals were subjected to formal training. Rats of the I+E group were trained to keep running on the treadmill during all 4 weeks. The formal exercise training was started for 35 min, with a speed of 18 m/min and a 0 deg slope in the first week (5 days per week). The intensity and duration of the training and treadmill slope were increased gradually, so that the rats kept running for 40 min at 18 m/min with a 5-deg slope, 45 min at 18 m/min with a 10-deg slope, and 50 min at 18 m/min with a 15-deg slope individually in the second, third and fourth weeks of the training period. Sedentary rats were placed daily on a stationary treadmill and were given electrical shocks in a way identical with that used for the I+E group.

Induction of Transient Global Cerebral Ischemia. For induction of transient cerebral ischemia [19, 20], animals were anesthetized with xylazine+ketamine (40 mg/kg, i.p.); then both CCAs were exposed and liberated from the carotid sheets, and the vagus nerves were cautiously isolated. Then the CCAs were occluded using microclips for 20 min. Afterwards, the arteries were released and inspected for reperfusion. Restoration of blood flow in the CCAs was affirmed by observation. The rectal temperature of animals was maintained throughout surgery at 36.5°C by using a heating system. After surgery, rats were returned to their home cage with free access to water and food for 4 days.

Behavioral Testing. The Morris water maze is the most common test used to assess cognitive functions related to memory. The apparatus comprised of a circular steel tank filled with water (22 ± 1°C) to a depth of 25 cm. The tank was divided into four quadrants, and an escape platform was located in the southeast quadrant at a fixed position submerged 2 cm beneath the water surface. In the spatial acquisition phase, the animals learned to find a submerged platform utilizing extra-maze cues. Each animal participated in 16 trials organized into daily blocks of 4 trials for 4 days. The animals were allowed to swim for a maximum of 60 sec or until the platform was found. If the platform was not found during this time, the rat was guided to the platform and allowed to stay there for 30 sec [21]. After testing, animals were sacrificed, and their brains examined for hippocampal injury assessment.

Assessment of the BBB Permeability. The BBB permeability was evaluated with the Evans blue (EB) extravasation method [22]. The EB infusion (1 ml/kg of 2% EB solution in saline, i.v. injection) was begun on the 5th day after surgery and proceeded

for 5 min. After 3 h, under deep anesthesia, the chest was opened [23]. Then the whole body was perfused with about 250 ml of warm saline solution infused into the left ventricle to wash the EB dye out of the circulation. After beheading of the rat, the brain was removed. Afterwards, the brain was weighed and homogenized in 2.5 ml of phosphate buffered saline. The homogenized solution was blended with 2.5 ml of trichloroacetic acid and centrifuged at 3,500 rpm for 30 min. The gathered supernatant was used to determine the EB absorbance at 610 nm by a spectrophotometer (Shimadzu, Japan). The EB concentrations were determined against a standard curve, and the obtained results were expressed as micrograms per 1.0 g of brain tissue [24].

TUNEL Staining. Rats were anesthetized; then, transcardiac perfusion was performed with saline (0.9%) followed by paraformaldehyde (4%) in 0.1 M phosphate buffer (pH 7.4). The brains were then extracted, postfixed in the similar fixative overnight, and embedded in paraffin. Subsequently, paraffin-embedded 7-μm-thick coronal sections were cut for immunohistochemical stains, between 3.3 and 4.2 mm posterior to the bregma level according to the Paxinos atlas [25]. The DNA fragmentation and apoptotic cell death in the DG area of the hippocampus were detected using a TUNEL assay [26]. First, paraffin blocks were cut in 7-μm-thick coronal sections. TUNEL staining was performed using an *In Situ* Cell Death Detection Kit (Roche, Germany). This kit for detection and quantification of apoptosis at the single-cell level is based on labeling of DNA strand breaks and was used according to the manufacturer's protocol. In summary, the sections (3 sections per animal) were deparaffinized in xylene, rehydrated by a consecutive series of alcohol, washed in PBS, and permeabilized by proteinase K (10 mM) at room temperature for 30 min. Afterwards, the sections were rinsed and incubated with 3% H_2O_2 in methanol for 10 min in the dark to block endogenous peroxidase. Then the sections were incubated in the TUNEL reaction mixture at 37°C in a humidified atmosphere for 60 min. After washing the sections with PBS, those were visualized using a converter-POD for 30 min at 37°C in humidified atmosphere in the dark and then washed with PBS. The DAB substrate (0.05% 3,3-diaminobenzidine, 50-100 μl) as a chromogen was added for 10 min; the slices were rinsed with PBS, mounted by coverslip, and analyzed by a light microscope. The TUNEL-positive cells were quantified using light microscopy at a ×400 magnification. These cells were counted within 0.160-mm^2 test zones of the DG area of the right hippocampus. All counting procedures were performed blindly.

Statistical Analysis. The one-way analysis of variance (ANOVA) was utilized to compare differences between the groups. Whenever a significant difference was uncovered, the Scheffe's or Dunnett's T3 *post-hoc* test was used to determine where the difference occured. At the point where the homogeneity of variance was indicated, the above-mentioned tests were used. The significance level was set at $P \leq 0.05$. All data were analyzed using SPSS software (Version 16.00).

RESULTS

Exercise Improves Ischemia-Induced Memory Deficits. Data analysis of the results of the Morris water maze test showed that the mean distance moved by a rat to reach the platform in the I group (534.33 ± 49.77 cm, mean ± s.e.m.) was significantly greater than that in the control Sh group (297.33 ± 42.53 cm, $P < 0.001$). In group I+E, the distance traversed to reach the platform (460.33 ± 43.36 cm) was significantly shorter compared to the respective value in the I group ($P < 0.05$; Fig. 1). Also, the mean time spent to reach the platform in the I group (36.5 ± 6.34 sec) was two times longer than in the Sh group (18.5 ± 3.61 sec, $P < 0.001$).

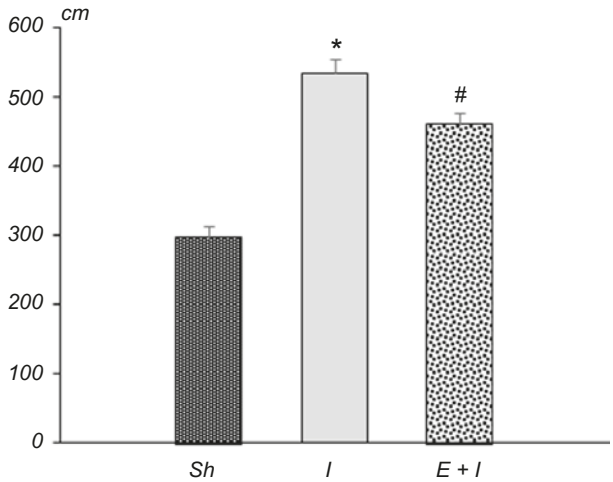


Fig. 1. Mean distances, cm, traveled to reach the platform in the Morris water maze test by animals of the studied groups (sham, Sh, ischemized, I, and exercise+ischemia, E+I). * Significant difference in comparison with the Sh group ($P < 0.001$); # that in comparison with the I ($P < 0.05$) and Sh ($P < 0.001$) groups. Means ± s.e.m. are shown.

The exercise (group I+E) significantly decreased the time to reach the platform (27.17 ± 6.91 sec) compared to the analogous value in the I group ($P < 0.05$; Fig. 2).

Exercise Reduces Ischemia-Induced Increase in the BBB Permeability. The effects of exercise on BBB disruption after ischemia are illustrated by Fig. 1. The average EB concentration in the brains of the I group (14.33 ± 3.83 μg/g tissue) was dramatically higher than that in the Sh group (2.00 ± ± 0.89 μg/g tissue, $P < 0.001$). Exercise was associated with a significantly lower EB concentration in the ischemic brains (8.33 ± 2.25 μg/g tissue) compared to the respective index in the I group ($P < 0.01$; Fig. 3).

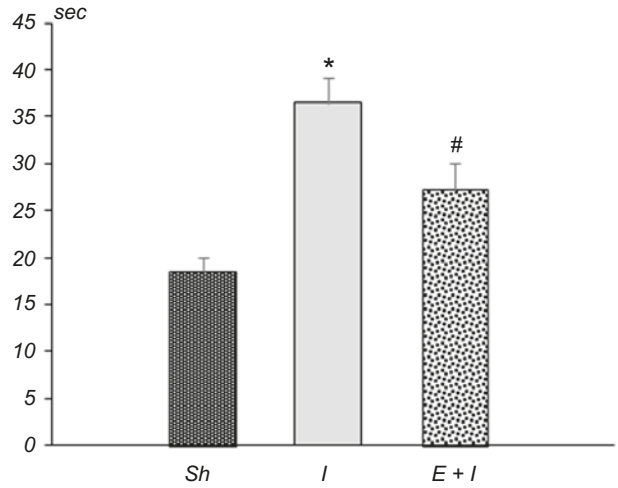


Fig. 2. Mean time, sec, to reach the platform in the Morris water maze test in the studied groups. * Significant difference in comparison with the Sh group ($P < 0.001$); # that in comparison with the I group ($P < 0.05$). Other designations are similar to those in Fig. 1

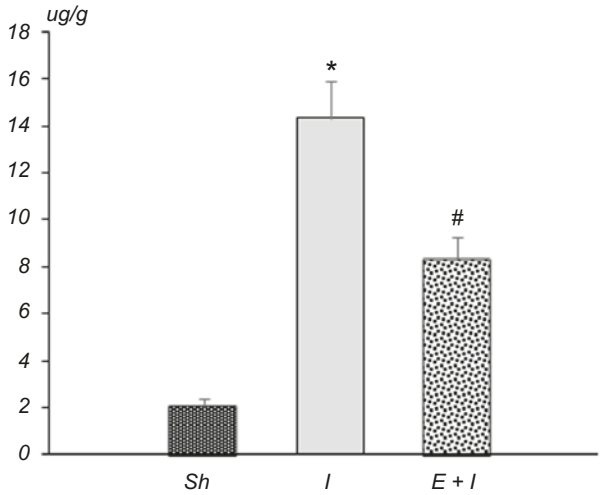


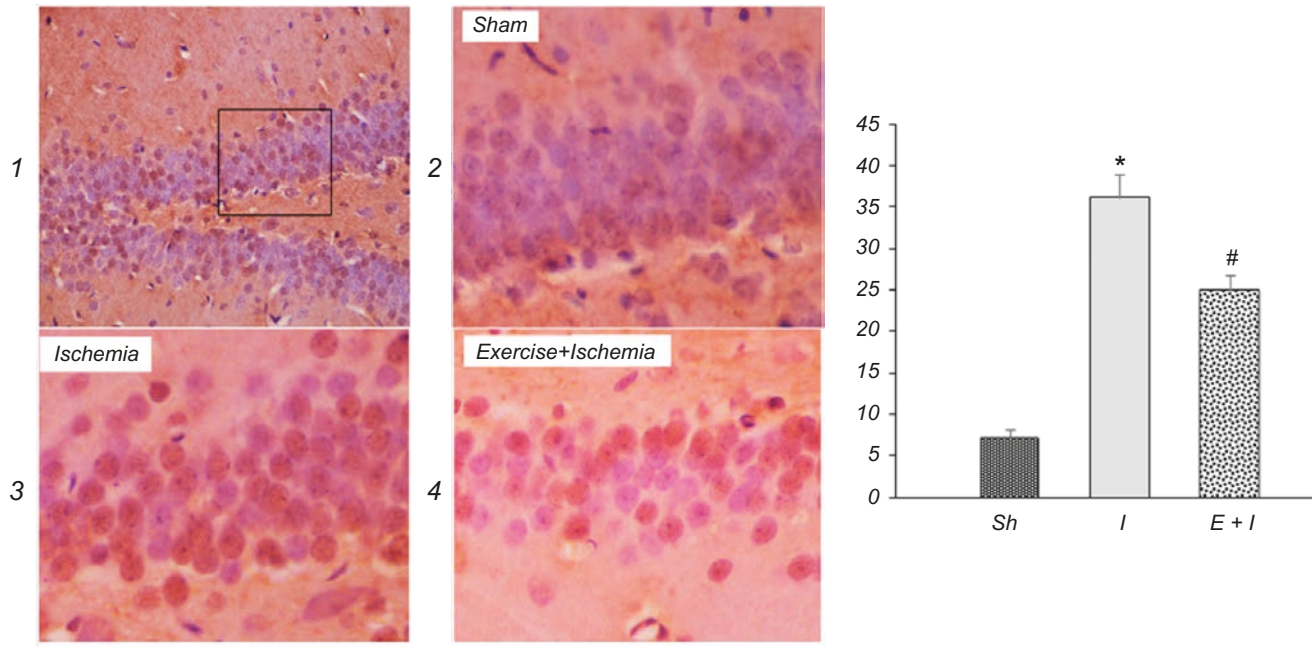
Fig. 3. Mean Evans blue concentration (μg/g tissue) in the brains of animals of the studied groups. * Significant difference in comparison with the Sh group ($P < 0.001$); # that in comparison with the I ($P < 0.01$) and Sh groups ($P < 0.01$). Other designations are similar to those in Fig. 1.

Exercise Reduces Ischemia-Induced Apoptotic Cell Death. The results of TUNEL staining demonstrated that the average number (density) of TUNEL-positive cells in the DG area of the hippocampus in group-I animals (36.17 ± 6.85) was significantly greater compared with that in the Sh group (7.17 ± 2.56, $P < 0.001$). Also, the number of TUNEL-positive cells in exercised rats (25 ± 4.47) was significantly smaller compared to the ischemic group ($P < 0.01$; Fig. 4).

DISCUSSION

Trying to evaluate possible neuroprotective effects of physical exercise against negative effects of ischemia, we investigated the effects of exercise on BBB disruption, neuronal death, and cognitive dysfunction in male rats subjected to transient cerebral ischemia. The results of assessment of the BBB permeability showed that such ischemia resulted in significantly increased BBB permeability. Also, cerebral ischemia induced intensification of apoptotic neuronal death in the hippocampal DG area and resulted in considerable memory dysfunction. Moreover, our study demonstrated that pre-ischemic physical exercise is capable of significantly reducing the ischemia-increased BBB permeability. Also, such pre-ischemic exercise significantly suppressed the ischemia-induced intensification of apoptotic cell death among hippocampal DG neurons. In addition, a repressive effect of pre-ischemic exercise on apoptotic cell death was associated with moderation of ischemia-induced cognitive dysfunction.

Our results showed that the increase of BBB disruption and worsening of the cognitive function were consistent with the cerebral ischemia, which implied that there was a tight relationship between the BBB permability and cognitive dysfunction in ischemized animals. Also, our findings prove the hypothesis that preserving the BBB integrity following an ischemic brain injury with physical exercise training decreases the level of apoptotic cell death and ameliorates functional outcomes. There are a number of reports supporting a neuroprotective role for exercise after ischemic insults [27].These results suggest that exercise training may protect



F i g. 4. TUNEL staining in the dental gyrus (DG) of the hippocampus. A) Photomicrographs of TUNEL stained units 1) illustration of the test area, 2–4) examples of staining in the examined groups (magnifications in 2–4 ×400). B) mean number of TUNEL-positive cells in the DG test-zones. * Significant difference in comparison with the Sh group ($P < 0.001$); # that in comparison with the I ($P < 0.01$) and Sh groups ($P < 0.001$).

the BBB function and structure after brain ischemic injury and, eventually, may improve the outcome. Recent studies have shown that exercise training promotes neurological recovery and functional restoration after ischemic stroke [28].

The BBB disruption universally culminates in neuronal neuroinflammation, dysfunction, and neurodegeneration. Cerebral ischemia is associated in most cases with BBB disruption and edema formation [29]. The BBB is a highly specialized brain endothelial structure in the CNS; it is mainly composed of microvascular endothelial cells [30]. The unique morphology and function of the BBB are determined by multiple factors. Therefore, breakdown of any of the constituents may contribute to the BBB disruption [11]. During brain ischemia-reperfusion, lack of the BBB integrity is a critical occurrence that contributes to the beginning of edema formation, activation of the inflammatory cascade, and ultimately, development of cognitive dysfunction [31].

Within recent years, there is increasing attention in alternative non-pharmacological treatments for cerebral ischemic stroke. Physical exercise training is an exciting prospect for clinical therapy of ischemic stroke. Early exercise after or before cerebral ischemia may improve the neurological status, conserve the integrity of the BBB, and decrease the cerebral infarct volume. These findings show that exercise training may significantly protect the BBB structure and function after ischemia-induced brain injuries [14].

Exercise training is an essential behavioral intervention that possesses numerous useful effects. Epidemiological studies indicated that physical activity leads, in principle, to positive systemic adaptations [32]. The mechanisms underlying the neuroprotective effects of exercise training after cerebral ischemia may involve several factors. It is well proven that oxidative stress, inflammatory events, excitotoxicity, and activity of proteinases are the main consequences of BBB disruption and causes of neuronal death after cerebral ischemia [33]. In addition to the role of exercise in amelioration of the risk factors, physical exercise clearly provides substantial endogenous neuroprotection against ischemia-related brain damage. These effects were shown to be mediated by various neuroprotective mechanisms, which increase neuronal survival after cerebral ischemia.

As was shown, BBB integrity plays a vital role in preserving cerebral vascular permeability. When the BBB is injured by ischemia (mostly due to changes in structural proteins, namely type IV collagen,

laminin, and fibronectin), the ability to selectively differentiate the products of the cerebrovascular system is damaged, and this can cause vasogenic edema and alteration of the properties of neuronal environment [34, 35]. It was shown that physical training enhances the integrity and thickness of the basal lamina in the BBB [36]. It appears that one of the important factors in this case is type IV collagen. Exercise training increases expression of the above protein, and this, in particular, reduces neuronal death after cerebral ischemia [37]. Therefore, increases in BBB resistivity can potentially reduce ischemia-induced cerebral damage [38]. This effect of exercise training can be considered an important mechanism for the corresponding neuroprotective effect on BBB disruption induced by ischemia.

Matrix metalloproteinases (MMPs) are up-regulated under conditions of cerebral ischemia, and this is closely associated with BBB disruption. It was also reported that physical training decreases the expression of the matrix metalloproteinase-9 (MMP-9), and this ameliorates the integrity of the BBB and decreases the amount of brain injury during ischemia [39]. MMP-9 is an enzyme produced by microglia, astrocytes, and endothelial cells. This enzyme provides degradation of the extracellular matrix and basal lamina proteins [40]. The MMP-9 activity is up-regulated after the beginning of cerebral ischemia and is closely linked with inflammation, leukocyte infiltration, and tissue damage. Moreover, increased expression of MMP-9 after cerebral ischemia leads to the development of brain edema by alterations in the BBB permeability [28]. As was shown, pre-ischemic exercise ameliorates BBB disruption and enhances the basal lamina integrity in ischemia by reducing the expression of MMP-9 and activating its endogenous inhibitor (tissue inhibitor of metalloproteinase-1, TIMP-1) [41]. Therefore, physical training, via reducing the MMP level and increasing the expression of integrins and collagen type IV, increases the BBB integrity and strengthens the neurovascular units; finally, this enhances neuronal protection capabilities [41].

In addition, it was shown that physical exercise re-establishes the expression of tight-junction proteins, such as occludin and claudin-4; the latter are the molecules acting as anchors in the endothelial cells to develop the BBB in the CNS and inhibiting the PECAM-1 expression. Therefore, it is suggested that physical exercise training maintains the BBB integrity via protecting tight junctions [42].

It was also established that some growth factors, such as insulin-like growth factor 1 (IGF-1), brain-derived neurotrophic factor (BDNF), and vascular endothelial growth factor (VEGF), might have the potential to cross the BBB in the CNS [43]. Also, it was proved that BDNF contributes to the improvement of memory performances after brain ischemia. It is now clear that exercise training can increase the levels of BDNF and IGF-1, increase tolerance of the brain to insult, and improve cognitive functions. Thus, exercise could considerably promote the brain functional plasticity following cerebral ischemia [44, 45].

One of the conceivable mechanisms for the neuroprotective capability of physical training could be its ability to limit ROS formation [46]. ROS-induced oxidative stress plays a key role in the ischemic cascade [47]. Earlier studies showed that physical training improves antioxidant activity and also enhances expression of the Cu/Zn superoxide dismutase (SOD1) protein [46]. Treadmill training might also protect against ischemia-induced cognitive impairments by decreasing the lipoperoxidation through an increase in the antioxidant capacity in the hippocampus [48].

Heat-shock protein (HSP-70) helps optimum folding of proteins during normal and stress-inducing conditions (including ischemia) [49]. Overexpression of HSP-70 was recently shown to be neuroprotective in the ischemized brain [50]. There is evidence that HSP-70 is involved in the inhibition of MMP expression. It was indicated that pre-ischemic exercise can diminish the brain infarction volume by up-regulation of HSP-70 [49].

Tumor necrosis factor (TNF)-α is a disadvantageous pro-inflammatory cytokine, which is up-regulated after ischemic injury. Regardless of its inflammatory and harmful effects, there is evidence that TNF-α is a useful factor in neuroprotection and tissue repair. Moreover, this protein can serve as an inductor of endogenous neuroprotection after exercise training [41]. It was demonstrated that exercise produces a chronic low-rate increase in the TNF-α level, eventually increasing neuronal tolerance and protection in the setting of ischemia injury [51]. In addition to the above-mentioned, a recent study [52] showed that physical training decreases the levels of IL-6 and reduces the IL-6/IL-10 and IL-1β/IL-1ra ratios. Accordingly, it can be assumed that physical activity reduces the intensity of inflammation and, as a consequence, can help to maintain BBB integrity; thus, this noticeably influences cognitive function [53].

The results of our study suggest that physical exercise may protect the BBB structure and function and suppress apoptotic cell death in the hippocampus. Ultimately, this improves cognitive dysfunction following ischemic brain injury. Thus, these results show that exercise training may exert a protective effect against cerebral ischemia. These neuroprotective effects of exercise provide a prophylactic and therapeutic approach and can be used as an efficient procedure in reducing the complications of brain ischemia.

Acknowledgment. The authors give special thanks to the Ilam University for valuable support.

All assessments were performed in accordance the Helsinki Declaration. In this study, all issues relating to the welfare of animals have been considered.

The authors of this work, N. Shamsaei, H. Abdi, and F. Moradi, confirm the absence of any conflicts regarding commercial or financial relations, relations with organizations or persons that could in any way be connected with the research, as well as co-author relationships.

REFERENCES

1. C. Iadecola, F. Zhang, S. Xu, et al., "Inducible nitric oxide synthase gene expression in brain following cerebral ischemia," *J. Cereb. Blood Flow Metab.*, **15**, No. 3, 378–384 (1995).
2. Z. Liu, X. Chen, Y. Gao, et al., "Involvement of GluR2 up-regulation in neuroprotection by electroacupuncture pretreatment via cannabinoid CB1 receptor in mice," *Sci. Rep.*, **5**, 9490 (2015).
3. D. Mozaffarian, E. J. Benjamin, A. S. Go, et al., "Heart disease and stroke statistics-2016 Update: A Report from the American Heart Association," *Circulation*, **133**, No. 4, e38–360 (2016).
4. B. K. Siesjö, "Pathophysiology and treatment of focal cerebral ischemia: Part I: Pathophysiology," *J. Neurosurg.*, **77**, No. 2, 169–184 (1992).
5. J. Bai and P. D. Lyden, "Revisiting cerebral postischemic reperfusion injury: New insights in understanding reperfusion failure, hemorrhage, and edema," *Int. J. Stroke*, **10**, No. 2, 143–152 (2015).
6. S. Erfani, M. Khaksari, S. Oryan, et al., "Visfatin reduces hippocampal CA1 cells death and improves learning and memory deficits after transient global ischemia/reperfusion," *Neuropeptides*, **49**, 63–68 (2015).
7. J. Robin, M. Hirshhorn, R. S. Rosenbaum, et al., "Functional connectivity of hippocampal and prefrontal networks during episodic and spatial memory based on real-world environments," *Hippocampus*, **25**, No. 1, 81–93 (2015).
8. B. C. White, J. M. Sullivan, D. J. DeGracia, et al., "Brain ischemia and reperfusion: molecular mechanisms of neuronal injury," *J. Neurol. Sci.*, **179,** Nos. 1/2, 1–33 (2000).
9. B. T. Hawkins and T. P. Davis, "The blood-brain barrier/neurovascular unit in health and disease," *Pharmacol. Rev.*, **57**, No. 2, 173–185 (2005).
10. B. V. Zlokovic, "The blood-brain barrier in health and chronic neurodegenerative disorders," *Neuron*, **57**, No. 2, 178–201 (2008).
11. N. J. Abbott, L. Rönnbäck, and E. Hansson, "Astrocyte-endothelial interactions at the blood–brain barrier," *Nat. Rev. Neurosci.*, **7**, No. 1, 41–53 (2006).
12. L. J. Noble, F. Donovan, T. Igarashi, et al., "Matrix metalloproteinases limit functional recovery after spinal cord injury by modulation of early vascular events," *J. Neurosci.*, **22**, No. 17, 7526–7535 (2002).
13. V. Gerzanich, S. K. Woo, R. Vennekens, et al., "*De novo* expression of Trpm4 initiates secondary hemorrhage in spinal cord injury," *Nat. Med.*, **15**, No. 2, 185–191(2009).
14. Y. Zhang, P. Zhang, X. Shen, et al., "Early exercise protects the blood-brain barrier from ischemic brain injury via the regulation of MMP-9 and occludin in rats," *Int. J. Mol. Sci.*, **14**, No. 6, 11096–11112 (2013).
15. A. Schmidt, J. Wellmann, M. Schilling, et al., "Meta-analysis of the efficacy of different training strategies in animal models of ischemic stroke," *Stroke*, **45**, No. 1, 239–247 (2014).
16. N. Shamsaei, M. Khaksari, S. Erfani, et al., "Exercise preconditioning exhibits neuroprotective effects on hippocampal CA1 neuronal damage after cerebral ischemia," *Neural. Regen. Res.*, **10**, No. 8, 1245–1250 (2015).
17. F. Zhang, J. Jia, Y. Wu, et al., "The effect of treadmill training pre-exercise on glutamate receptor expression in rats after cerebral ischemia," *Int. J. Mol. Sci.*, **11**, No. 7, 2658–2669 (2010).
18. F. Zhang, Y. Wu, and J. Jia, "Exercise preconditioning and brain ischemic tolerance," *Neuroscience*, **177**, 170–176 (2011).
19. N. Aboutaleb, N. Shamsaei, H. Rajabi, et al., "Protection of hippocampal CA1 neurons against ischemia/reperfusion injury by exercise preconditioning via modulation of Bax/Bcl-2 ratio and prevention of caspase-3 activation," *Basic Clin. Neurosci.*, **7**, No. 1, 21–29 (2016).
20. M. LotfiAski, M. E. Rezvani, M. Khaksari, et al., "Neuroprotective effect of berberine chloride on cognitive impairment and hippocampal damage in experimental model of vascular dementia," *Iran. J. Basic Med. Sci.*, **21**, No. 1, 53–58 (2018).
21. S. Erfani, N. Aboutaleb, S. Oryan, et al., "Visfatin inhibits apoptosis and necrosis of hippocampus CA3 cells following transient global ischemia/reperfusion in rats," *Int. J. Pept. Res. Ther.*, **21**, No. 2, 223–228 (2015).
22. M. Kaya, M. Küçük, R. B. Kalayci, et al., "Magnesium sulfate attenuates increased blood-brain barrier permeability during insulin-induced hypoglycemia in rats," *Can. J. Physiol. Pharmacol.*, **79**, No. 9, 793–798 (2001).

23. J. Y. Lee, H. E. Lee, S. R. Kang, et al., "Fluoxetine inhibits transient global ischemia-induced hippocampal neuronal death and memory impairment by preventing blood-brain barrier disruption," *Neuropharmacology*, **79**, 161–171 (2014).
24. W. L. Yeh, D. Y. Lu, C. J. Lin, et al., "Inhibition of hypoxia-induced increase of blood-brain barrier permeability by YC-1 through the antagonism of HIF-1 accumulation and VEGF expression," *Mol. Pharmacol*., **72**, No. 2, 440–449 (2007).
25. G. Paxinos and C. Watson, *The Rat Brain In Stereotaxic Coordinates*, 7th Edition, Elsevier, Burlington, (2005).
26. F. Z. Mehrjerdi, N. Aboutaleb, H. Pazoki-Toroudi, et al., "The protective effect of remote renal preconditioning against hippocampal ischemia reperfusion injury: role of kATP channels," *J. Mol. Neurosci*., **57**, No. 4, 554–560 (2015).
27. N .Aboutaleb, N. Shamsaei, M. Khaksari, et al., "Pre-ischemic exercise reduces apoptosis in hippocampal CA3 cells after cerebral ischemia by modulation of the Bax/Bcl-2 proteins ratio and prevention of caspase-3 activation," *J. Physiol. Sci*., **65**, No. 5, 435–443 (2015).
28. B. Langhammer and B. Lindmark, "Functional exercise and physical fitness post stroke: The importance of exercise maintenance for motor control and physical fitness after stroke," *Stroke Res. Treat*., **2012**, 864835 (2012).
29. M. Asahi, X. Wang, T. Mori, et al., "Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood-brain barrier and white matter components after cerebral ischemia," *J. Neurosci*., **21**, No. 19, 7724–7732 (2001).
30. P. Ballabh, A. Braun, and M. Nedergaard, "The blood-brain barrier: an overview: structure, regulation, and clinical implications," *Neurobiol. Dis*., **16**, No. 1, 1–13 (2004).
31. M. Khan, T. S. Dhammu, H. Sakakima, et al., "The inhibitory effect of S-nitrosoglutathione on blood-brain barrier disruption and peroxynitrite formation in a rat model of experimental stroke," *J. Neurochem.* (Suppl.), **123**, No. 2, 86–97 (2012).
32. D. Szalewska, M. Radkowski, U. Demkow, et al., "Exercise strategies to counteract brain aging effects," *Adv. Exp. Med. Biol*., **1020**, 69–79 (2017).
33. M. A. Moskowitz, E. H. Lo, and C. Iadecola, "The science of stroke: mechanisms in search of treatments," *Neuron*, **67**, No. 2, 181–198 (2010).
34. G. J. delZoppo, "The neurovascular unit in the setting of stroke," *J. Int. Med*., **267**, No. 2, 156–171 (2010).
35. G. J. delZoppo and T. Mabuchi, "Cerebral microvessel responses to focal ischemia," *J. Cereb. Blood Flow Metab*., **23**, No. 8, 879–894 (2003).
36. R. Kochanski, D. Dornbos, and Y. Ding, "Neuroprotection and physical preconditioning: Exercise, hypothermia, and hyperthermia," Innate tolerance in the CNS, *Springer Series in Translational Stroke Research*, Springer, New York (2013), pp. 105–131.
37. W. Davis, S. Mahale, A. Carranza, et al., "Exercise preconditioning ameliorates blood–brain barrier dysfunction in stroke by enhancing basal lamina," *Neurol. Res*., **29**, No. 4, 382–387 (2007).
38. D. Dornbos III and Y. Ding, "Mechanisms of neuroprotection underlying physical exercise in ischemia-reperfusion injury," First ed. Intech. Open Access Publisher, Rijeka, Croatia, 299–326 (2012).
39. K. Chaudhry, R. Rogers, M. Guo, et al., "Matrix metalloproteinase-9 (MMP-9) expression and extracellular signal-regulated kinase 1 and 2 (ERK1/2) activation in exercise-reduced neuronal apoptosis after stroke," *Neurosci. Lett*., **474**, No. 2, 109–114 (2010).
40. E. H. Lo, T. Dalkara, and M. A. Moskowitz, "Mechanisms, challenges and opportunities in stroke," *Nat. Rev. Neurosci*., **4**, No. 5, 399–415 (2003).
41. M. Guo, B. Cox, S. Mahale, et al., "Pre-ischemic exercise reduces matrix metalloproteinase-9 expression and ameliorates blood–brain barrier dysfunction in stroke," *Neuroscience*, **51**, No. 2, 340–351 (2008).
42. P. S. Souza, E. D. Gonçalves, G. S. Pedroso, et al., "Physical exercise attenuates experimental autoimmune encephalomyelitis by inhibiting peripheral immune response and blood-brain barrier disruption," *Mol. Neurobiol*., **54**, No. 6, 4723–4737 (2017).
43. C. W. Cotman, N. C. Berchtold, and L. A. Christie, "Exercise builds brain health: key roles of growth factor cascades and inflammation," *Trends Neurosci*., **30**, No. 9, 464–472 (2007).
44. C. W. Cotman and N. C. Berchtold, "Exercise: a behavioral intervention to enhance brain health and plasticity," *Trends Neurosci*., **25**, No. 6, 295–301 (2002).
45. C. Phillips, M. A. Baktir, D. Das, et al., "The link between physical activity and cognitive dysfunction in Alzheimer disease," *Phys. Ther*., **95**, No. 7, 1046–1060 (2015).
46. L. Hoffman-Goetz and P. Spagnuolo, "Effect of repeated exercise stress on caspase 3, Bcl-2, HSP 70 and CuZn-SOD protein expression in mouse intestinal lymphocytes," *J. Neuroimmunol*., **187**, Nos. 1/2, 94–101 (2007).
47. C. Allen and U. Bayraktutan, "Oxidative stress and its role in the pathogenesis of ischaemic stroke," *Int. J. Stroke*, **4,** No. 6, 461–470 (2009).
48. F. Cechetti, P. V. Worm, V. R. Elsner, et al., "Forced treadmill exercise prevents oxidative stress and memory deficits following chronic cerebral hypoperfusion in the rat," *Neurobiol. Learn. Mem*., **97**, No. 1, 90–96 (2012).
49. B. Liebelt, P. Papapetrou, A.Ali, et al., "Exercise preconditioning reduces neuronal apoptosis in stroke by up-regulating heat shock protein-70 (heat shock protein-72) and extracellular-signal-regulated-kinase 1/2," *Neuroscience*, **166**, No. 4, 1091–1100 (2010).
50. R. G. Giffard and M. A. Yenari, "Many mechanisms for hsp70 protection from cerebral ischemia," *J. Neurosurg. Anesthesiol*., **16**, No. 1, 53–61 (2004).

51. R. Reyes, Y. Wu, Q. Lai, et al., "Early inflammatory response in rat brain after peripheral thermal injury," *Neurosci. Lett*., **407**, No. 1, 11–15 (2006).
52. M. A. Małkiewicz, A. Szarmach, A. Sabisz, et al., "Blood-brain barrier permeability and physical exercise," *J. Neuroinflammation*, **16**, No. 1, 15 (2019).
53. M. U. Chupel, L. G. Minuzzi, G. E. Furtado, et al., "Exercise and taurine in inflammation, cognition, and peripheral markers of blood-brain barrier integrity in older women," *Appl. Physiol. Nutr. Metab*., **43**, No. 7, 733–741 (2018).