Treadmill exercise improves short-term memory by suppressing ischemia-induced apoptosis of neuronal cells in gerbils

Young-Je Sim\textsuperscript{a}, Sung-Soo Kim\textsuperscript{a}, Jee-Youn Kim\textsuperscript{a}, Mal-Soon Shin\textsuperscript{b}, Chang-Ju Kim\textsuperscript{b},\textsuperscript{*}

\textsuperscript{a} Research Institute of Sports Science, Korea University, #15-Ka Anam-dong, Songbuk-gu, Seoul 136-701, Republic of Korea
\textsuperscript{b} Department of Physiology, College of Medicine, Kyung Hee University, #1 Hoigi-dong, Dongdaemoon-gu, Seoul 130-701, Republic of Korea

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Abstract

In the present study, the effect of treadmill exercise on short-term memory, apoptotic neuronal cell death, and cell proliferation in the hippocampal dentate gyrus following transient global ischemia in gerbils was investigated. Step-down inhibitory avoidance task, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay, and immunohistochemistry for caspase-3 and 5-bromo-2′-deoxyuridine (BrdU) were used. Ischemia was induced by the occlusion of both common carotid arteries (CCA) of gerbils for 5 min. Gerbils in exercise groups were forced to run on a treadmill for 30 min once a day for 10 consecutive days. Such treadmill exercise improved short-term memory by suppressing the ischemia-induced apoptotic neuronal cell death in the dentate gyrus. In addition, treadmill running suppressed the ischemia-induced cell proliferation in the dentate gyrus. The present results suggest that treadmill exercise overcomes the ischemia-induced apoptotic neuronal cell death and thus facilitates the recovery following ischemic cerebral injury.

Keywords: Transient global ischemia; Apoptotic neuronal cell death; Cell proliferation; Short-term memory; Hippocampal dentate gyrus

Cerebral ischemia is induced by reduced cerebral blood flow due to the transient or permanent occlusion of cerebral arteries\cite{4}. Transient cerebral ischemia for 5 or 10 min leads to neuronal cell damage and eventually causes neuronal cell death\cite{1,20}. Tissue damage following cerebral ischemia is caused by the interaction of complex pathophysiological processes such as excitotoxicity, depolarization, inflammation, and apoptosis\cite{4}.

Apoptosis, also known as programmed cell death, plays a crucial role in the development and the maintenance of homeostasis in all multicellular organisms\cite{25,29}. Thompson reported that apoptosis is a form of cell death that constitutes part of a common mechanism in cell replacement, tissue remodeling, and removal of damaged cells\cite{25}. Inappropriate or excessive apoptosis, however, has been implicated in many diseases including cancer, autoimmune diseases, neurodegenerative disorders, acquired immunodeficiency syndrome (AIDS), and stroke\cite{25}. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) is an assay that detects the characteristic of apoptotic cell death DNA fragmentation\cite{10}. In ischemic animal models, TUNEL-positive cells represent apoptotic cell death\cite{20}. Another important characteristic of apoptosis is the activation of caspase-3. Caspase-3 is the most widely studied member of the caspase family and one of key executors of apoptosis\cite{3}. In ischemic animal models, the activation of caspase-3 is implicated in apoptotic neuronal cell death\cite{1}.

The neurogenesis in the hippocampus plays a central role in learning and memory process. The neurogenesis continues throughout life in the adult mammalian brain including humans\cite{6}. Cell proliferation in the hippocampal dentate gyrus is increased by learning, serotonin, N-methyl-D-aspartate (NMDA) receptor antagonists, and exposure to an enriched environment\cite{9}. Increased cell proliferation in the hippocampal dentate gyrus has also been observed in some pathological states including seizure, mechanical dentate gyrus lesions, and ischemic insult\cite{21}. Such up-regulation of cell

\*Corresponding author. Tel.: +82 2 961 0407; fax: +82 2 964 2195.
E-mail address: changju@khu.ac.kr (C.-J. Kim).

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proliferation during ischemic insult is considered as the compensatory adaptive response to the increased apoptotic neuronal cell death following ischemic injury [21].

It has been reported that physical exercise exerts neuroprotective effect on hippocampal injury that induces memory impairment. Physical exercise has been known to facilitate recovery from injury and improves cognitive function [8,16]. In addition, physical exercise increases the expression of neurotrophic factors such as insulin-like growth factor-I (IGF-I) and basic fibroblast growth factor (bFGF). These neurotrophic factors are implicated in neuronal survival, differentiation, the alteration in synaptic plasticity, and memory function [27].

In the present study, the effect of treadmill exercise on short-term memory, apoptotic neuronal cell death, and cell proliferation in the hippocampal dentate gyrus following transient global ischemia in gerbils was investigated. The effect was assessed in step-down inhibitory avoidance task, TUNEL assay, and immunohistochemistry for caspase-3 and 5-bromo-2′-deoxyuridine (BrdU).

Adult male Mongolian gerbils (11–13 weeks of age) were used in this experiment. The experimental procedures were performed in accordance with the animal care guidelines of the National Institute of Health (NIH) and the Korean Academy of Medical Sciences. Gerbils were housed under the controlled temperature (20 ± 2 °C) and lighting (07:00–19:00 h) conditions. Food and water were made available ad libitum. Gerbils were randomly assigned into four groups (n = 10 in each group): the sham-operation (control) group, the sham-operation and exercise group, the ischemia-induction group, and the ischemia-induction and exercise group.

To induce transient global ischemia in gerbils, the surgical procedure were performed as previously described [15,19]. In brief, gerbils were anesthetized with 3% isoflurane in 20% O2–77% N2. Both common carotid arteries (CCAs) were exposed by bilateral neck incision. CCAs were occluded with 0.02% 3,3′-diaminobenzidine (DAB). Mayer’s hematoxylin (DAKO, Glostrup, Denmark) was used for counter-staining and the sections were finally mounted onto Permount®.

For the visualization of the DNA fragmentation, TUNEL staining was performed using In Situ Cell Death Detection Kit® (Roche, Mannheim, Germany) according to the manufacturer’s protocol [15,19]. Briefly, sections were fixed in ethanol-acetic acid (2:1) and rinsed. Then the sections were incubated with 100 µg/ml proteinase K, rinsed, incubated in 3% H2O2, permeabilized with 0.5% Triton X-100, rinsed again, and incubated in the TUNEL reaction mixture. The sections were rinsed and visualized using Converter-POD with 0.02% 3,3′-diaminobenzidine (DAB). Mayer’s hematoxylin (DAKO, Glostrup, Denmark) was used for counter-staining and the sections were finally mounted onto gelatin-coated slides. The slides were air dried overnight at room temperature, and coverslips were mounted using Permount®.

For the visualization of the caspase-3 expression, caspase-3 immunohistochemistry was performed as previously described [15,19]. Brain sections were incubated overnight with mouse anti-caspase-3 antibody (1:500; Santa Cruz Biotechnology, Santa Cruz, CA) and then for another 1 h with the biotinylated mouse secondary antibody. Bound secondary antibody was then amplified with Vector Elite ABC kit® (Vector Laboratories, Burlingame, CA). The antibody–biotin–avidin–peroxidase complex was visualized using 0.02% DAB. The sections were finally mounted onto gelatin-coated slides. The slides were air dried overnight at room temperature, and coverslips were mounted using Permount®.

For the detection of newly generated cells in the dentate gyrus, BrdU-specific immunohistochemistry was performed as previously described [19]. Brain sections were permeabilized by incubation in 0.5% Triton X-100 in PBS for 20 min,
The number of TUNEL-positive and caspase-3-positive cells in the dentate gyrus was counted hemilaterally in every eighth section throughout the entire extent of the dentate gyrus at 400× magnification. The number of BrdU-positive cells in the subgranular layer of the hippocampal dentate gyrus was counted in a similar fashion. The area of the granular layer of dentate gyrus was traced using Image-Pro® Plus image analyzer (Media Cybernetics Inc., Silver Spring, MD) at 40× magnification. The number of TUNEL-positive, caspase-3-positive, and BrdU-positive cells was expressed as the mean number of cells per millimetre square of the cross sectional area of the granular layer of the dentate gyrus. The data are expressed as the mean ± standard error mean (S.E.M.). For the comparison between groups, one-way ANOVA and Duncan’s post hoc test were performed. \( P < 0.05 \) was considered statistically significant.

The latency in the sham-operation group was 175 s, ranged from 160 to 180 s, and in the sham-operation and exercise group was 180 s, ranged from 175 to 180 s. The difference between the sham-operation group and the sham-operation and exercise group was not statistically significant. The latency in the ischemia-induction group was 46 s, ranged from 30 to 66 s, and in the ischemia-induction and exercise group was 117 s, ranged from 70 to 167 s (Fig. 1). The latency in ischemic gerbils was shorter than control gerbils. Treadmill exercise increased the latency in ischemic gerbils significantly. The present results show that treadmill exercise improved the ischemia-induced short-term memory impairment.

The number of TUNEL-positive cells in the dentate gyrus of the sham-operation group was 34.98 mm\(^{-2} \pm 2.73\) mm\(^{-2}\) and in the sham-operation and exercise group was 34.10 mm\(^{-2} \pm 5.01\) mm\(^{-2}\). The difference of the number of TUNEL-positive cells between the sham-operation group and the sham-operation and exercise group was not statistically significant. In the ischemia-induction group, the number of TUNEL-positive cells in the dentate gyrus was increased to 67.99 mm\(^{-2} \pm 5.31\) mm\(^{-2}\). In the ischemia-induction and exercise group, on the other hand, the number of TUNEL-positive cells in the dentate gyrus was reduced to 39.95 mm\(^{-2} \pm 3.92\) mm\(^{-2}\) (Fig. 2). The present results show that ischemic insult-induced apoptotic neuronal cell death in the dentate gyrus and treadmill exercise significantly suppressed the ischemia-induced apoptosis of neuronal cells.

The number of caspase-3-positive cells in the dentate gyrus of the sham-operation group was 16.41 mm\(^{-2} \pm 2.78\) mm\(^{-2}\) and in the sham-operation and exercise group was 29.84 mm\(^{-2} \pm 6.24\) mm\(^{-2}\). The difference of the number of caspase-3-positive cells between the sham-operation group and the sham-operation and exercise group was not statistically significant. In the ischemia-induction group, the number of caspase-3-positive cells in the dentate gyrus was increased to 91.21 mm\(^{-2} \pm 23.92\) mm\(^{-2}\). In the ischemia-induction and exercise group, on the other hand, the number of caspase-3-positive cells in the dentate gyrus was reduced to 35.94 mm\(^{-2} \pm 7.23\) mm\(^{-2}\) (Fig. 3). The present results show that ischemic insult induced the caspase-3 expression in the dentate gyrus and treadmill exercise significantly suppressed the ischemia-induced caspase-3 expression.

The number of BrdU-positive cells in the dentate gyrus of the sham-operation group was 215.72 mm\(^{-2} \pm 11.63\) mm\(^{-2}\) and in the sham-operation and exercise group was 232.54 mm\(^{-2} \pm 11.33\) mm\(^{-2}\). Treadmill exercise increased cell proliferation in control gerbils. In the ischemia-induction group, the number of BrdU-positive cells in the dentate gyrus was increased to 469.82 mm\(^{-2} \pm 37.63\) mm\(^{-2}\) (Fig. 4). The present results show that cell proliferation in the dentate gyrus was increased in response to transient global ischemia and treadmill exercise.
exercise suppressed the ischemia-induced cell proliferation in the dentate gyrus.

Cerebral ischemia induces the deprivation of oxygen and glucose resulting in tissue infarction and neuronal cell death [1,4,20]. Apoptosis is implicated in ischemia and neurodegenerative diseases including Parkinson’s disease and Alzheimer’s disease [25]. The morphological characteristics of the apoptotic cell death are cell shrinkage, chromatin condensation, membrane blebbing, and DNA fragmentation [20]. In addition, the up-regulation and activation of caspase-3 enzyme at the early stage of apoptosis following ischemia has been reported [1]. In the present study, the number of TUNEL-positive and caspase-3-positive cells in the dentate gyrus was significantly increased following transient global ischemia. This indicates that ischemia induces apoptotic neuronal cell death in the dentate gyrus.

Running wheel exercise enhances the neurogenesis in the dentate gyrus of adult mice [28]. Trejo et al. showed that physical exercise increases cell proliferation and the uptake of the neurotrophic factor IGF-I from the bloodstream into specific brain areas including the hippocampus in rats [27]. In the present study, treadmill exercise increased the generation of new cells in the dentate gyrus in sham-operation gerbils. In regard to apoptotic neuronal cell death in sham-operation gerbils, the difference between exercise group and non-exercise group was not statistically significant. Mild-intensity treadmill exercise was reported to increase cell proliferation without inducing apoptosis in the dentate gyrus under normal conditions [19].

Physical exercise facilitates recovery of ischemia-induced neurological impairment and reduces the expression of
Fig. 4. The effect of treadmill exercise on cell proliferation in the dentate gyrus after transient global ischemia. (Upper): Photomicrographs of 5-bromo-2′-deoxyuridine (BrdU)-positive cells. A scale bar represents 100 μm. (Lower): The mean number of BrdU-positive. The data are represented as the mean ± S.E.M. *P < 0.05 compared to the sham-operation group. #P < 0.05 compared to the ischemia-induction group. (A) Sham-operation group; (B) sham-operation and exercise group; (C) ischemia-induction group; and (D) ischemia-induction and exercise group.

mRNA of apoptosis-associated genes including Bcl-xL and Dp5 [16,26]. Guezennec et al. reported that treadmill running down-regulates the expression of glutamate receptors, which are implicated in excitotoxicity [12]. Carro et al. showed that treadmill running at low intensity prevents the neuronal cell loss as well as the impairment induced by excitotoxic damage or energy imbalance [2]. The present results also showed that treadmill exercise significantly suppresses the ischemia-induced increase in DNA fragmentation, the caspase-3 expression, and cell proliferation in the dentate gyrus. The suppression of cell proliferation by treadmill exercise may be attributed to the reduction of apoptotic cell death by treadmill exercise [19]. It has been suggested that the hippocampal neurogenesis may be affected by memory formation [11]. Ischemic injury to the hippocampus is known to induce memory impairment [24]. Physical exercise alters neurotransmitter levels and neuronal metabolism in the rat hippocampus. It has been reported that activation of cholinergic fibers increases acetylcholine release and produces vasodilation in the rat hippocampus, resulting in increased cerebral blood flow in the hippocampus during exercise [5,23]. Nakajima et al. demonstrated that the increase in cerebral blood flow in the hippocampus during walking is at least partly responsible for the improvement in spatial learning ability associated with hippocampal activation or hippocampal neurogenesis [23]. Increase in blood flow in the hippocampus due to physical exercise also results in the provision of sufficient oxygen and glucose to the hippocampus; this appears to be advantageous for the neurons of the hippocampus in the sense that protection against delayed neuronal cell death induced by insufficiency of blood supply is afforded [13,17]. It was reported that running wheel enhances the hippocampal-related spatial learning in rats and aerobic exercise improves cognitive function such as learning and memory in the elderly [7,28]. It has been shown that physical exercise prevents cognitive decline during aging and facilitates functional recovery after central nervous system injury [18,22].

We evaluated the effect of treadmill exercise on the ischemia-induced short-term memory impairment using latency of step-down inhibitory avoidance task. The difference in the latency between exercise group and non-exercise group in gerbils without ischemia was not statistically significant. The latency shortened by transient global ischemia, on the other hand, was significantly improved by treadmill exercise. The present study shows that treadmill exercise improves short-term memory by suppressing the ischemia-induced apoptotic neuronal cell death in the dentate gyrus. The present results thus provide direct evidence that the improvement in short-term memory associated with treadmill exercise is due to the reduction of apoptotic neuronal cell death by treadmill exercise. This suggests that treadmill exercise may alleviate central nervous system complications and short-term memory deficits induced by cerebral ischemia.

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References


