Research article

The ameliorative effects of exercise on cognitive impairment and white matter injury from blood-brain barrier disruption induced by chronic cerebral hypoperfusion in adolescent rats

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HIGHLIGHTS

- Treadmill exercise alleviated the cognitive impairment induced by CCH.
- Treadmill exercise prevents myelin degradation and damage to microvessels in the motor cortex and hippocampus after CCH.
- Treadmill exercise may provide protective effects on BBB disruption from overexpression of MMP-9 induced by CCH.
- Exercise may improve ischemic neurological disorders by reducing white matter injury and BBB disruption from overexpression of MMP-9 in the brain.

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ABSTRACT

Vascular dementia is the progressive change in blood vessels that leads to neuronal injuries in vulnerable areas induced by chronic cerebral hypoperfusion (CCH). CCH induces disruption of blood-brain barrier (BBB), and this BBB disruption can initiate the cognitive impairment and white matter injury. In the present study, we evaluated the effect of treadmill exercise on the cognitive impairment, white matter injury, and BBB disruption induced by CCH. Vascular dementia was induced by permanent bilateral common carotid arteries occlusion (BCCA0) in rats. The rats in the exercise group were made to run on a treadmill for 30 min once a day for 14 weeks, starting 4 weeks after birth. Our results revealed that treadmill exercise group alleviated the cognitive impairment and myelin degradation induced by CCH. The disruption of BBB after CCH indicates degradation of occludin, zonula occluden-1 (ZO-1), and up-regulation of matrix metalloproteases (MMPs). Treadmill exercise may provide protective effects on BBB disruption from degradation of occludin, ZO-1, and overexpression of MMP-9 after CCH. These findings suggest that treadmill exercise ameliorates cognitive impairment and white matter injury from BBB disruption induced by CCH in rats. The present study will be valuable for means of prophylactic and therapeutic intervention for patients with CCH.

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1. Introduction

Cerebral ischemia is one of the most common neurological disorders, and it is related to vascular dementia and Alzheimer’s disease [1,2]. Vascular dementia is a progressive cognitive impairment that leads to neuronal injuries in vulnerable areas induced by chronic cerebral hypoperfusion (CCH) [3]. Permanent bilateral common carotid arteries occlusion (BCCA0) in rats has been used as the most popular animal model of chronic cerebral hypoperfusion ischemia [4,5]. Previous studies have shown that CCH induced by BCCA0 in rodents has been proposed as an experimental model of neuronal degeneration and white matter damage, especially the hippocampus and corpus callosum [6,7]. It has been reported that BCCA0 induced white matter injury in the hippocampus and corpus callosum, as well as learning and memory impairment [8–10]. There is wide agreement that learning and memory are dependent on the integrity of the white matter.

The neuropathological changes in these white matter lesions are associated with the blood-brain barrier (BBB) in vascular dementia [11]. The neurovascular unit of the BBB is composed
of the microvessels, pods of astrocytes, neurons, and pericytes. The integrity of brain microvessels integrity requires maintenance of the endothelial permeability barrier. The cerebral endothelium forms the largest barrier in the brain, and the BBB is a highly selective permeability barrier, primarily formed by brain endothelial cells, which are connected by tight junctions such as occludin and zonula occcluden-1 (ZO-1) [12]. When the BBB is damaged by various injuries, including ischemic stroke, BBB disruption generates the entrance of neurotoxic substances in the brain that lead to abnormal synaptic and neuronal functions [13]. Degradation of tight junction proteins of the BBB has been related to an increase in matrix metalloproteinases (MMPs) activity [14,15].

MMPs are calcium-dependent zinc-containing endopeptidase, and are capable of degrading all kinds of extracellular matrix proteins [16]. These have been considered as key molecules involved in the disruption of BBB after ischemia [17]. MMP-9 deficient knock-out mice are more protected against cerebral ischemia than wild-type mice [18]. Therefore, blocking MMP activation can be considered as a potential therapeutic intervention after CCH.

Regular physical activity is generally accepted as a means of promoting general health and well-being. Exercise is generally known to increase neuronic plasticity, alter the expression of various genes [19], and improve memory function [20] by inhibiting apoptotic neuronal cell death in the hippocampus [21]. Exercise may ameliorate neurological impairments in Alzheimer’s and Parkinson’s disease [22,23]. Many previous studies have shown that exercise reduces brain damage by decreasing cerebral permeability, MMP-9 expression, and brain integrity after ischemia [24,25].

Although the beneficial effects of exercise on chronic ischemia have been documented, it has not been well clarified whether treadmill exercise has some neuroprotective effect on BCCAO-induced cognitive impairment and white matter injury in rats. In the present study, we investigated the question of whether involuntary exercise ameliorates cognitive impairment and white matter injury from BBB disruption induced by CCH in rats.

2. Materials and methods

2.1. Experimental animals

Male Wistar rats (80 ± 10 g, 4 weeks old) were used in this experiment. The experimental procedures were performed in accordance with the animal care guidelines of the National Institutes of Health (NIH) and the Korean Academy of Medical Sciences. The rats were randomly divided into three groups (n = 10 in each group): The Sham group (Sham), the BCCAO group (BCCAO), and the BCCAO and treadmill exercise group (BCCAO+Ex).

2.2. Bilateral common carotid arteries occlusion (BCCAO)

Adult male Wistar rats (body weight 250–350 g, 12 weeks old) were anesthetized in 3% halothane in 70% N2O and balance of O2. The vessel occlusions were carefully performed to avoid damage of the surrounding tissue, particularly near the vagus nerve. For bilateral occlusion, a ventral midline incision was made to expose both carotid arteries. Each carotid artery was double-ligated with 3-0 silk (Ailee, Korea) just below the carotid bifurcation. Sham animals underwent the same operation procedure without vessel ligation.

2.3. Treadmill exercise protocol

The rats in the exercise group were made to run on a treadmill for 30 min once a day for 14 weeks starting 4 weeks after birth, according to the previously described method [26]. The treadmill exercise load consisted of running at 2 m/min for the first 5 min, at 3 m/min for the next 5 min, and then at 5 m/min for the last 20 min at 0° of inclination. The rats in the non-exercise groups were left in treadmill without running for the same period as the exercise group.

2.4. Radial 8-arm maze task

Spatial learning was tested using a radial 8-arm maze apparatus, as previously described [27]. Four weeks after BCCAO, the number of correct choices on the radial 8-arm maze task was determined to evaluate specific memory capability. A small receptacle filled with water (3 cm in diameter and 1 cm in depth) was located at the end of each arm. The rats were deprived of water for 48 h and then allowed to explore for water and to drink during a period of 8 min. Re-entry into the previously visited arm was counted as an error. In addition, the number of correct choices before the first error was counted.

2.5. Immunohistochemistry and immunofluorescence

Serial coronal sections of 40 μm thickness were obtained using a freezing microtome (Leica, Nussloch, Germany). For the visualization of myelin basic protein (MBP), rat endothelial cells antigen-1 (RECA-1) immunohistochemistry was performed. After being blocked with 10% normal hours and rabbit serum for 1 h, the sections were incubated overnight at 4°C with MBP antibody (1:200; Abcam, Cambridge, UK), and RECA1 antibody (1:1000; Abcam, Cambridge, UK). The sections were then incubated for 2 h with the biotinylated rat and mouse secondary antibody (1:200; Vector Laboratories). The bound secondary antibody was then amplified using a Vector Elite ABC kit® (Vector Laboratories). The antibody–biotin–avidin-peroxidase complex was visualized using 0.02% DAB.

For immunofluorescence, the sections were incubated overnight at 4°C with a mixture of two of the following primary antibodies: mouse monoclonal antibody to RECA1 (1:1000; Abcam, Cambridge, UK), and goat monoclonal antibody to occludin (1:500; LifeSpan BioSciences, Inc., Seattle, USA). The sections were then incubated with a mixture of Alexa Fluor 488-conjugated donkey anti-goat IgG and Alexa Fluor 594-conjugated goat anti-mouse IgG (1:1000; Molecular Probes, Eugene, OR) for 2 h at room temperature. Nuclei were visualized with 4', 6-diamidino-2-phenylindole (DAPI).

2.6. Western blot analysis

Protein isolated from the motor cortex was prepared with a lysis buffer containing 50 mMTris-HCl (pH 7.5), 150 mM NaCl,
0.5% deoxycholic acid, 1% nonidet-P40, 0.1% sodium dodecyl sulfate (SDS), 1 mM phenylmethylsulfonyl fluoride, and 100 μM/ml leupeptin. Protein concentration was measured using a colorimetric protein assay kit (Bio-Rad, Hercules, CA, USA). Protein of 30 μg was separated on SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane (Schleicher & Schuell GmbH, Dassel, Germany). The membranes were blocked in 5% nonfat skim milk in TBST for 1 h at room temperature followed by incubation with antibodies against MMP-9 (1:1000; Bioworld Technology, St. Louis Park, MN, USA), occludin (1:500, LifeSpan BioSciences, Inc., Seattle, USA), and ZO-1 (1:50; LifeSpan BioSciences). The primary antibodies were detected with horseradish peroxidase-conjugated secondary antibody (1:2000; Santa Cruz Biotech). Band detection was performed using an enhanced ECL detection system (Amer sham Pharmacia Biotech GmbH, Freiburg, Germany). To compare the relative expressions of proteins, the detected bands were quantitated by densitometric scanning using Image-Pro® Plus software (Media Cybernetics Inc., Silver Spring, MD, USA).

2.7. Statistical analysis

The results are expressed as mean ± standard error of the mean (S.E.M). SPSS Statistics version 21 (IBM SPSS, Chicago, IL, USA) was used for statistical analysis. Statistical analysis was performed using one-way ANOVA followed by Tukey’s post-hoc test. Differences between groups were considered significant at p < 0.05.

3. Results

3.1. Treadmill exercise alleviates memory impairment after CCH

The number of correct choices on the radial 8-arm maze task is presented in Fig. 1. The BCCAO group was decreased the number of correct choices on the radial 8-arm maze task as compared with the sham group. In contrast, the BCCAO+Ex group was increased as compared with the BCCAO group. These results showed that treadmill exercise alleviated spatial memory impairment induced by CCH (p < 0.05).

3.2. Treadmill exercise prevents loss of MBP expression in the white matter after CCH

Photomicrograph of MBP expression in the motor cortex and corpus callosum is presented in Fig. 2. The BCCAO group was decreased the MBP expression in the motor cortex and corpus callosum as compared with the sham group. In contrast, the BCCAO + Ex group was increased as compared with the BCCAO group. These
results showed that treadmill exercise decreased loss of MBP expression in the white matter induced by CCH ($p < 0.05$).

3.3. Treadmill exercise prevents damage to microvessels in the motor cortex and hippocampal CA1 region after CCH

Photomicrograph of RECA1-positive microvessels in the motor cortex and hippocampal CA1 region is presented in Fig. 3. Although the number of RECA1-positive microvessels in both regions was increased in the BCCAO group, the length of RECA1-positive microvessels in both regions was decreased. These results showed that BCCAO tissue commonly demonstrated microvessels with short lengths (fragments) and sections with narrow and constricted diameters (Data not shown). Treadmill exercise alleviated CCH-induced microvessels damage in the motor cortex and hippocampal CA1 region as compared with the BCCAO group ($p < 0.05$).

3.4. Treadmill exercise prevents the loss of tight junction protein and activation of MMP-9 in the motor cortex after CCH

Photomicrograph of occludin immunofluorescence staining in the motor cortex is presented in Fig. 4A. Representative blots and quantitative data are shown in Fig. 4B, which indicate that the level of ZO-1 in the motor cortex was dramatically decreased after CCH. In contrast, The BCCAO + Ex group was decreased the expression of ZO-1 in the motor cortex as compared with the BCCAO group. Treadmill exercise significantly attenuated the loss of ZO-1 in the motor cortex as compared with the BCCAO group ($p < 0.05$). MMP-9 plays a crucial role in ischemic disruption of the BBB. The level of MMP-9 in the motor cortex was increased after CCH. In contrast, The BCCAO + Ex group was decreased the expression of MMP-9 in the motor cortex as compared with the BCCAO group. These results showed that treadmill exercise reduced the activation of MMP9 in the motor cortex, which was induced by CCH ($p < 0.05$).
4. Discussion

In the present study, treadmill exercise improves cognitive impairment and white matter injury induced by CCH in rats. Consistent with our study, the majority of reports have demonstrated that exercise alleviated cognitive impairment in rats through the Morris water maze [28] and 8-arm maze test [29], in a manner similar to the effect of exercise on Alzheimer’s disease and CCH [30,31]. Learning and memory function is impaired in ischemic stroke [21] and white matter structure within the hippocampal formation has previously been shown to relate to associative cognitive function [32]. White matter injury is an important determinant of cognitive impairment after brain injury especially MBP in hippocampus and corpus callosum [33]. We have demonstrated the relationship between white matter abnormalities and cognitive dysfunction affected by BCCAO in adolescent rats. In a previous study, the degradation of myelin sheath after CCH causes damage of white matter [34]. MBP expression was decreased in response to BCCAO in the hippocampus and white matter [8,35]. Similarly, our data showed that expression of MBP in the motor cortex and corpus callosum was decreased by chronic hypoperfusion, but was recovered by treadmill exercise. Lee et al., [33] suggested that CCH induces white matter and hippocampal damage via inhibition of inflammation. Previous studies have shown that exercise ameliorates ischemia-induced inflammation, neuronal apoptosis, and microglia activation [21,22]. Our findings suggest that treadmill exercise may provide protective effects on cognitive impairment and myelin degradation induced by CCH.

CCH causes not only impairment of neurons, but also microvessels [11,36]. Interestingly, in our study, the morphological change of microvessels in the motor cortex and the hippocampus was found only in the BCCAO group. Physical activity enhances angiogenesis and neurotrophin overexpression [37] and the hippocampus shows angiogenic and neurogenic plasticity rapidly following physical activity [38]. In the present study, many broken fragments and constricted microvessels could not supply sufficient blood to the brain tissues. These results indicate that treadmill exercise prevents damage to microvessels in the motor cortex and hippocampal CA1 region after CCH.

Cerebral ischemia is closely associated with BBB disruption, edema formation, and hemorrhagic transformation. The BBB disruption is recognized as a key mechanism of white matter injury in various central nervous system diseases [39]. The BBB is a highly specialized brain endothelial structure in the central nervous system that mainly consists of microvascular endothelial cells [12]. The BBB is composed of pericytes, astrocytes, neurons, and extracellular-matrix, which have been redefined as the neurovascular unit. The individual components of the neurovascular unit work in concert to regulate microvascular permeability, ion gradients, nutrient uptake, toxin removal, and cerebral hemodynamics [40]. Previous studies have been demonstrated that the neurovascular unit components are damaged, when they are in Alzheimer’s, dementia, and ischemia and chronic hypoperfusion [41,42]. In these pathological processes, the loss of permeability and selectivity of BBB, the degradation of the extracellular matrix and basal lamina components, and an inflammatory response. The preservation of the tight junction is governed by essential transmembrane proteins.
including claudins, occludin, and junction adhesion molecules [43]. Tight junction proteins in brain microvascular endothelial cells are an important structural component of the BBB [44]. Occludin was the first integral membrane protein discovered within the tight junction of endothelial cells. ZO plays a vital role in maintaining the continuity and integrity of tight junction. Many studies have demonstrated that the levels of occludin and ZO-1 decreased after ischemia [45,46]. Similarly, our data showed that ZO-1 expression in the motor cortex was decreased by BCCAO, but were redeemed by treadmill exercise. Zhang et al. [47] suggested that early exercise could significantly suppress the loss of occludin in the brain. Their findings indicate that treadmill exercise may offer protective effects on BBB disruption by CCh.

MMPs are up-regulated in cerebral ischemia and are closely associated with BBB disruption. BBB disruption induced by cerebral ischemia is attenuated in MMP-9 knockout mice by reducing degradation of ZO-1 protein as compared to wild-type mice [39]. Elevation of MMP-9 has been associated with ischemia-induced brain injury [48]. Similarly, our data showed that MMP-9 expressions in the motor cortex were increased by BCCAO, but were suppressed by treadmill exercise. A previous study showed that the injury to MBP was attenuated in MMP-9-deficient animals [39]. Alleviation of MMP-9 was able to ameliorate BBB damage after cerebral ischemia. Guo et al. [25] suggested that pre-ischemic exercise could significantly suppress the activation of MMP-9 in the brain following BBB disruption. Based on these findings, our results indicate that treadmill exercise may provide protective effects on BBB disruption from overexpression of MMP-9 induced by CCh. In conclusion, treadmill exercise ameliorates cognitive impairment and white matter injury from BBB disruption induced by CCh in rats. Our results would support the finding that exercise may improve ischemic neurological disorders by reducing white matter injury and BBB disruption from overexpression of MMP-9 in the brain. Thus, these results suggest that treadmill exercise can be used as a means of prophylactic and therapeutic intervention for patients with chronic hypoperfusion disorders such as chronic heart failure, atherosclerosis and other circulatory disorders.

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References


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