Treadmill exercise increases cell proliferation in hippocampal dentate gyrus in alcohol-intoxicated rats


Aim: Effect of treadmill exercise on hippocampal cell proliferation under normal conditions has been well documented; however, this effect under alcohol intoxication conditions is not clarified, yet. In the present study, the effect of treadmill exercise on cell proliferation in the dentate gyrus in alcohol-intoxicated rats was investigated.

Methods: Experimental design: comparative investigation on number of 5-bromo-2'-deoxyuridine (BrdU)-positive cells in the dentate gyrus 8 days after commencement. Setting: animal laboratory. Participants: male Sprague-Dawley rats of 5 weeks in age weighing 150±10 g. Intervention: animals were divided into 4 groups: the control-rest group, the control-exercise group, the alcohol-treated-rest group, and the alcohol-treated-exercise group. Animals of the alcohol-treated groups were injected intraperitoneally with alcohol (2 g/kg) once a day for 3 days. All animals were injected BrdU (50 mg/kg) intraperitoneally, and rats of exercise groups were made to run on treadmill for 30 min each day for 5 days following alcohol administration.

Measures: mean number of BrdU-positive cells in dentate gyrus was observed via immunohistochemistry.

Results: Treadmill exercise significantly increased the number of BrdU-positive cells in the dentate gyrus. Also, treatment with alcohol for 3 days inhibited cell proliferation and treadmill exercise alleviated alcohol-induced inhibition of new cell formation.

Conclusion: These results suggest the possibility that treadmill exercise may help in improvement following alcohol-induced brain damage.

Key Words: Exercise test - Alcoholism - Cell proliferation - Dentate gyrus - Bromodeoxyuridine - Immunohistochemistry.

Alcohol is one of the most extensively used and abused drugs in the world. Exposure to alcohol during neural development is known to lead to substantial neuronal loss in multiple brain regions.1 Animal models have revealed the life-long sequelae of prenatal alcohol exposure including poor somatic growth, major organ malformation, craniofacial anomalies, and associated central nervous system (CNS) dysfunctions.2 CNS dysfunction is expressed as reduced capacity for basic adaptive functions, including impaired neural plasticity, poor learning, and abnormal response to challenging situations.3 Alcohol was reported to enhance granule cell death by increasing the occurrence of apoptosis.4, 5

Most neurons are integrated into the CNS during discrete developmental periods.6 Recent studies have shown that the hippocampus of adult mammal retains the ability to produce and incorporate new granule neurons.7 It is
well known that the hippocampus plays a pivotal role in learning and memory. Previous studies have shown that cell proliferation is increased by serotonin, enriched environments, estrogen, NMDA receptor antagonists, ischemia, seizure, and physical exercise, whereas adrenal steroids, stress and aging decrease neurogenesis.

van Praag et al. reported that wheel running enhances neurogenesis in the dentate gyrus of the hippocampus, and Trejo et al. reported that treadmill exercise increases the number of 5-bromo-2'-deoxyuridine (BrdU)-positive cells in the hippocampal dentate gyrus and that this increase in cell formation is associated with the release of insulin-like growth factor 1 (IGF-1). The effect of exercise on hippocampal cell proliferation has been well documented; however, little has been published on the effect of treadmill exercise on cell proliferation under alcohol intoxication conditions. In the present study, the effect of treadmill exercise on cell proliferation in the hippocampal dentate gyrus in acutely alcohol-intoxicated rats was investigated via immunohistochemistry.

Materials and methods

Animals and treatment

Adult male Sprague-Dawley rats weighing 150±10 g (5 weeks in age) were obtained from a commercial breeder (Daehan Biolink Co., Chungbuk, Korea). 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. Each animal was housed under controlled temperature (20±2°C) and lighting (07:00 hr-19:00 hr) conditions with food and water made available ad libitum. The animals were equally divided into four groups: the control-rest group (n=5), the control-exercise group (n=5), the alcohol-treated-rest group (n=5), and the alcohol-treated-exercise group (n=5). Animals of the alcohol-treated groups were injected intraperitoneally with alcohol (2 g/kg) once per day for 3 consecutive days, while those of the control groups were injected with an equivalent amount of 0.9% NaCl once a day for the same duration of time. On the 3rd day of the experiment, blood was collected via cardiac puncture 1 hr after the last injection of alcohol, and the blood alcohol concentration was measured using a Sigma Diagnostics Kit (St. Louis, MO, USA) according to the manufacturer’s protocol.

Treadmill exercise

Animals of the exercise groups, those of the control-exercise and the alcohol-treated exercise groups, were made to run on treadmill for 30 min each day for 5 consecutive days starting on the 3rd day of the experiment, while those of the other groups, the control-rest and the alcohol-treated rest groups, were left on treadmill without running for 30 min. The exercise load consisted of running at a speed of 7 m/min for 10 min, at 10 m/min for another 10 min, and at 13 m/min for the last 10 min, at 0 degree of inclination. BrdU (50 mg/kg in saline; Sigma, St. Louis, MO, USA) was injected intraperitoneally into all animals for the 5 days following alcohol administration.

Tissue preparation

On the 7th day of the experiment, animals were sacrificed 2 hr after injection of BrdU in the control groups; while in the exercise groups, treadmill exercise was performed 1 hr after BrdU injection, and then 30 min after, were sacrificed. Animals were fully anesthetized using Zoletil (10 mg/kg, i.m.; Vibac, Carros, France), transcardially perfused with 0.05 M phosphate buffer saline (PBS) and fixed with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB) at pH 7.4. Brains were removed, postfixed in the same fixative overnight, and transferred into 30% sucrose solution for cryoprotection. Coronal sections of 40 μm thickness were made using a freezing microtome (Leica, Nussloch, Germany). Five sections on average were selected in each brain from the region spanning from Bregma -3.30 mm to -4.16 mm.

Immunohistochemistry

For detection of cell birth in the dentate gyrus, BrdU-specific immunohistochemistry was performed according to a previously described method. In brief, sections were first pretreated by immersing in 50% formamide-2X standard saline citrate (SSC) at 65°C for 2 hr, denaturating in 2 N HCl at 37°C for 30 min, and rinsing twice in 0.1 M sodium borate (pH 8.5). After the pretreatment, the sections were incubated overnight at room temperature with a BrdU-specific mouse monoclonal antibody (1:600) (Boehringer Mannheim, Mannheim, Germany). The sections were then washed three times with PBS and incubated for 1 hr with a biotinylated mouse secondary antibody (1:100), (Vector Laboratories, Burlingame,
CA, USA). The sections were incubated for another 1 hr with an avidin-biotin-horseradish peroxidase complex (1:100), (Vector Laboratories, Burlingame, CA, USA). For staining, the sections were reacted with 0.02% 3,3'-diaminobenzidine containing nickel chloride (40 mg/ml), (Nickel-DAB) and 0.03% hydrogen peroxide in 50 mM Tris-HCl (pH 7.6) for 5 min, and were mounted onto gelatinized glass slides.

Data analyses

The mean number of BrdU-positive cells in each group was obtained according to a previously described stereological method. The area of the dentate gyrus region was measured hemilaterally in each of the selected sections after immunostaining with mouse anti-neuronal nuclei (NeuN) antibody using an image analyzer (Multiscan, Fullerton, CA, USA). The results are expressed as number per mm² of the area of the granular layer of the dentate gyrus. Statistical differences were determined using one-way ANOVA followed by Scheffe’s post-hoc analyses, and results were expressed as mean ± standard error mean (SEM). Differences were considered significant for p<0.05.

Results

The blood alcohol concentration was 155.95±11.44 mg/dl 1 hr after the last alcohol injection in the alcohol-treated groups, while it was 0 or negligible in animals of the control groups. Typical BrdU-positive cells in each group are represented in Figure 1.

In the control-rest group, the mean number of BrdU-
positive cells was 256±13/mm², and this figure was increased to 411±12/mm² in the control-exercise group. In this result, treadmill exercise significantly increased the number of BrdU-positive cells in the dentate gyrus. The present result is consistent to previous reports stating that voluntary wheel exercise in mice and treadmill exercise in rats increase BrdU-positive cells in the dentate gyrus of the hippocampus.13-15

The mean number of BrdU-positive cells was 142±14/mm² in the alcohol-treated rest group, and 225±18/mm² in the alcohol-treated exercise group. In the present study, it was shown that treatment with alcohol for 3 days inhibits cell proliferation in the dentate gyrus and that treadmill exercise alleviates alcohol-induced inhibition of new cell formation (Figure 2).

Discussion

In the present results, treadmill exercise increased new cell formation in the dentate gyrus not only under normal conditions but also under alcohol-intoxicated conditions. It was also revealed that alcohol treatment decreases cell proliferation in the dentate gyrus significantly. Alcohol induces apoptosis by inhibiting trophic factors, and causes impairments in learning and memory.2, 16

Physical activity has been reported to induce the expression of trophic factors in brain regions related to higher cognitive function.17 Gomez-Pinilla et al.18 suggested that learning and physical activity may contribute to enhancing the expression of trophic factors. Newly formed granule cells have been shown to be capable of extending axons from new synapses on CA3 neuronal targets and thus promote recovery of hippocampal function.19 Enhanced cell proliferation has been associated with improved spatial memory performance and upregulation of synaptic plasticity and learning.7, 15

Exercise facilitates recovery from brain injury such as stroke and enhances improvement from sequelae.14, 15, 20 In the present study, it was demonstrated that treadmill exercise is effective in increasing cell proliferation in alcohol-intoxicated rats.

Conclusions

In the present study, treatment with alcohol for 3 days inhibited cell proliferation, and treadmill exercise increased new cell formation in the dentate gyrus not only under normal conditions but also under alcohol-intoxicated conditions. These results suggest the possibility that treadmill exercise may help in improvement following alcohol-induced CNS damage. However, further studies are needed to clarify details of the mechanism of alcohol-induced impairment and functional recovery by treadmill exercise.

References


