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Investigating the changes of hippocampal m-RNA gene expression of neurotrophin-3 and its receptor (Tropomyosin Receptor Kinase C) in the recovery of movements of rats with spinal cord injury during two types of endurance exercises

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Abstract

Background: This study aimed to investigate the effect of four weeks of selected endurance training on neurotrophin-3 (NT-3) and tropomyosin receptor kinase C (TrkC) gene expression in hippocampal areas of rats with spinal cord injury (SCI).

Methods: In this experimental study, the rats were separated into six equal groups. First, the animals were put under general anesthesia and had their SCI. Then, for four weeks, they were subjected to two kinds of endurance training programs. However, the control injury group received no intervention or training. Following the completion of the training regimes, molecular tests were done using the qRT-PCR technique to evaluate changes in the gene expression of NT-3 and TrkC from the animals' hippocampus.

Results: The expression of NT-3 and TrkC genes were significantly reduced in the SCI model compared to the healthy control group, but it was increased in the SCI + exercise 1 and SCI + exercise 2 groups compared to the SCI group. NT-3 levels did not vary significantly between the SCI + exercise 1 and SCI + exercise 2 groups, although alterations in TrkC levels altered.

Conclusion: In addition to enhancing locomotion in animals with SCI, the endurance training regimens in this research were effective on the expression of NT-3 and TrkC genes and may play a role in axonal development and neuronal survival in SCI recovery.

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Introduction

Spinal cord injury is characterized by a complicated combination of pathological events that develop after the initial damage (1). Secondary damage occurs from minutes to weeks of the injury. It involves cell death owing to inflammation, glutamate release, excitatory amino acid release, glial wound development, and eventually neuronal cell apoptosis. Aside from these situations, chemicals and other substances are produced at the location of the injury, inhibiting axon development (2).

As a result, there is a need for innovative treatment techniques that may limit secondary damage and apoptosis while increasing axon development. Despite the efforts of researchers and considerable breakthroughs in post-lesion treatment and surgery, as well as the introduction of cell therapy approaches using stem cells in this patient population, no viable and ultimate cure for spinal cord injuries has been established. In their normal condition, neurons may engage the passive repair process that they have after producing a lesion by stimulating the production of a set of repair-related genes carried out by axons (3). Growth factors are responsible for triggering the activation of repair (4).

Nortrophin-3 is a neuro factor in the NGF (nerve growth factor) family of nerve cells. It is a protein growth factor that is active in some nerve cells of the peripheral and central nervous system (5). This factor promotes the survival and differentiation of existing neurons while also encouraging the development and differentiation of new neurons and synapses (6). Tropomyosin receptor kinase C seems to be a physiological receptor since it is the most dependent on neurotrophin-3 (NT3). This receptor mediates cellular activities by adding phosphate molecules to specific tyrosines in the cell, activating the cellular signal (7). Due to damage to the central nervous system, such as spinal cord injury, the amount of these factors changes (8). If neurotrophins are transferred to the spinal cord, they can improve sensory and motor function and also prevent the destruction of neurons. Exercise can change some neurotransmitters and the expression of neurotrophins (9).

Compounds produced during the secondary phase of injury may play an important role in spinal cord repair (10) despite the fact that the precise biological mechanism involved in spinal cord repair is still mainly unknown. Therefore, it appears necessary to comprehend and acknowledge the positive effects of regular physical activity and sports training in the secondary phase of spinal cord injury. (11). There is no consensus on what type of exercise and how much per day can have a better effect on improving spinal cord injury conditions. The purpose of this study is to investigate the effect of two types of aerobic exercise programs (one session per day compared with two sessions a day) on NT-3 and its receptor in rats with spinal cord injury.

Methods

This experimental study was conducted on Wistar rats in the animal laboratory of North Khorasan University of Medical Sciences. Adult and young male rats weighing 225 to 275 grams and aged 10 to 12 weeks were included in the study. Animals were randomly divided into six equal groups (healthy control group, healthy group with the first exercise protocol, healthy group with the second exercise protocol, control injury group, spinal cord injury group with the first exercise protocol, and spinal cord injury group with the second exercise protocol). Based on similar studies, seven rats were tested in each group (12).

First, the rats were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). After specifying the cutting site (vertebrae T9 to T11), the skin was cut 2.5 cm towards the head and tail of the animal and along the spine. After cutting the superficial and deep fascia and removing the muscles adjacent to the spinous appendage of the vertebrae, a dental microphoresis (Bohr device) was used to cut the lamina. After laminectomy, the vertebrae were fixed by a stereotaxic device, and a blow was applied to the spinal cord from a height of 25 mm using a 10-gram weight or a hollow cylinder (13). Then, the muscles and fascia were immediately sutured using 4-0 absorbable threads. Healthy groups only underwent laminectomy surgery, and no spinal cord injury was inflicted on them. It should be noted that the nutritional conditions were the same as healthy rats, and animals with spinal cord injury had no restrictions on access to standard food and water.

After ensuring that a spinal cord lesion was obtained and two weeks after producing the lesion in the rodents, the following aerobic exercise protocols from prior studies were implemented (14). The first training regimen consisted of running on a treadmill at a constant pace of 9 meters per minute for four weeks and six-morning sessions per week. First week: 10 minutes, second week: 15 minutes, third week: 20 minutes, and fourth week: 30 minutes. The second endurance exercise protocol was 12 sessions per week (6 morning sessions and six evening sessions) for four weeks. All training sessions were at a speed of 8 meters per minute. The training time in the first week was 5 minutes; in the second week, 5-10 minutes; in the third week, 10-15 minutes and in the fourth week, it was 15 minutes.

The BBB score motor test was carried out to assess the motor improvement of the lower limbs of rodent spinal cord injury models. The BBB test was conducted 24 hours after surgery, and animals were excluded from the study if their score was less than 3. Two weeks after the spinal cord injury, the motor recovery evaluation based on this test was initiated (15).

Forty-eight hours after the last training session and in a 12-hour fasting state, rats were first anesthetized with ketamine-xylazine injection; then, the rats underwent surgery to collect tissue samples. In this way, after breaking the skull,

the brain was completely removed without damage. Then, the two hemispheres were separated without destruction with the help of a 24-inch scalpel blade. Finally, the hippocampus was separated from the cerebral hemisphere and transferred to a 1.5 ml microtube containing RNA-Later buffer. At the end, the samples were transferred to a -20 freezer for storage. The remains of the animal were destroyed in the septic tank. Molecular tests were performed using the qRT-PCR method. Based on the RNA extraction kit (Addbio, made in Korea), RNA extraction and cDNA synthesis were performed and stored in a -20 freezer. The primer was designed as an exon junction-exon by PRIMER3 software and IDT DNA online software and was ordered by Gene Fanavaran company based in Isfahan for synthesis. Finally, the obtained data were calculated using the formula 2-ΔΔct, and the expression level of the target genes was normalized with the result of the reference gene β-Actin. According to the instructions mentioned in the previous section, RNA was extracted. After checking its concentration and purity (based on NanoDrop and 260 nm and 280 nm ratio of absorbance), an extracted RNA sample was transferred to a 1.5% agarose gel. Observing the bands related to 18s rRNA (1.9Kb) and 28s rRNA (5Kb) confirmed the accuracy of RNA extraction.

The inferential statistics of one-way analysis of variance (ANOVA) were utilized to compare the variance changes between groups, taking into account the normality of the data as determined by the Shapiro-Wilk test, the validity of the research and data analysis hypotheses, and the significance of the mean difference. In addition, the post hoc Tukey test was utilized to compare the groups. The SPSS 20.0 and GraphPad Prism programs (GraphPad Prism. ver 8.4.3.686) were used for data analysis and graphing, respectively.

Results

Out of 42 rats used in this study, 33 survived until the end of the study and were studied. The evaluation of the motor test 24 hours after surgery and the creation of a spinal cord lesion showed that the score was less than 3 for the animals. The average motor test scores of the two groups were evaluated in the weeks after the injury. According to the obtained results, the average motor test scores in the group receiving the second exercise in the second week after the injury and the first exercise group in the third week after the spinal cord injury had a significant increase compared to the motor test scores of the injury group ($p<0.05$). Also, the scores of the healthy group were significantly different in all weeks compared to other groups ($p<0.001$).

The expression of the NT3 gene (Figure 1) in the spinal cord injury model had a significant decrease compared to the control group ($P \leqslant 0.0001$). Also, NT3 had a significant increase in expression in the group of spinal cord injury + exercise 1 and the group of spinal cord injury + exercise 2 compared to the group of spinal cord injury. Still, this increase in expression was not significantly different between the two groups of spinal cord injury + exercise. On the other hand, in exercise groups 1 (exercise 1 without spinal cord injury) and 2 (exercise 2 without spinal cord injury) compared to the control group, an increase in NT3 gene expression was observed. However, the expression of NT3 in the 1st exercise group was not significantly different from the 2nd exercise group. TrkC (Figure 2) in the spinal cord injury model had a significant decrease in expression compared to the control group ($P \leqslant 0.0001$), and it also increased significantly in the spinal cord injury + exercise 1 group and the spinal cord injury + exercise 2 group compared to the spinal cord injury group. No significant increase in expression was observed between the spinal cord injury + exercise 2 group compared to the spinal cord injury + exercise 1 group. On the other hand, in exercise 1 (exercise 1 without spinal cord injury) and exercise 2 (exercise 2 without spinal cord injury) compared to the control group, a significant increase in expression was observed. Exercise group 2 showed a significant increase compared to exercise group 1.

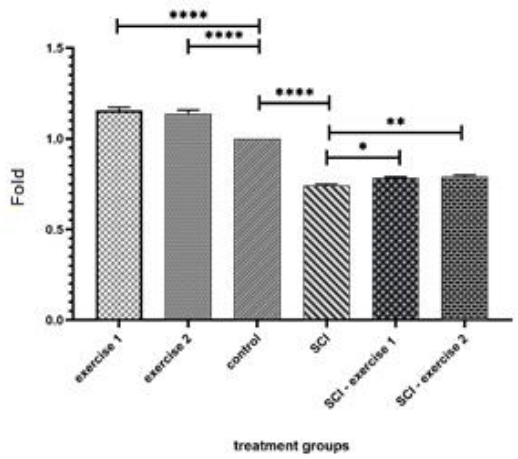

Figure 1. Neutrophin 3 gene expression results in the studied groups from animal hippocampal tissue. *p<0.05, **p<0.01, ***p<0.001, data are presented as mean ± standard deviation.

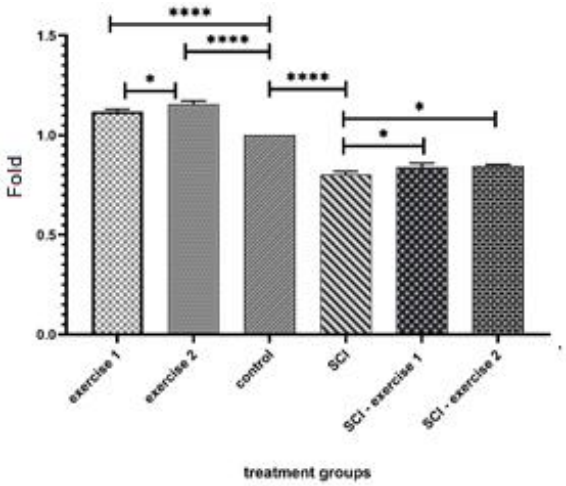

Figure 2. The results of TrkC gene expression in the studied groups from animal hippocampus tissue. *p<0.05, **p<0.01, ***p<0.001, data are presented as mean ± standard deviation.

Discussion

This study aimed to investigate the effect of two types of endurance training (4 weeks) on NT3 and tropomyosin receptor kinase C gene expression in hippocampal rats with spinal cord injury. The results of the present study showed that the expression of NT3 and TrkC in the hippocampal tissue showed a significant increase in both exercises on the treadmill (one session per day and two sessions per day) compared to the control group and the spinal cord injury group.

In the adult brain, NT3 expression is largely restricted to the dentate gyrus of the hippocampus, a region that exhibits significant neurogenesis. NT3 facilitates hippocampal plasticity, learning, and memory by regulating neurogenesis (16). In a 2021 study, Ge Li et al. showed that in a rat model of spinal cord injury treated with a neurotrophin-3-releasing bio-scaffold, the host nerve fibers regenerated in the transplant and established synaptic connections with donor neurons, supporting spinal cord injury repair. Based on this, the recovery of locomotor performance in mice with spinal cord injury, which received NSC-derived neural network tissue grafts with modified TrkC, was significantly improved (17). The results of the present study also show improvement in animal movement and changes in the expression of NT3 and tropomyosin receptor kinase C. In a 2017 study, Hua Fang et al. showed that neurotrophic factors (NF) and Trk signaling mechanisms underlie the promotion of motor recovery after acute spinal cord injury. In the NF/Trk group, the expression of NF/Trk pathway components increased significantly (18). The results of the present study also showed changes in NT3 and TrkC gene expression, which can directly induce the neurotrophin signaling pathway and stimulate the growth and differentiation of nerve cells.

Several previous research studies have shown that high-level competitive weight lifters are capable of performing two training sessions on the same day with no injuries or decrements in performance (4-8,10,16). In fact, given the clients' recovery after TDT, an increase in performance was also observed (7,10).

In this study, an increase in motor efficiency was observed in rats with spinal cord injury by using exercise protocols. The results of movement evaluations also showed improvement in the movement of animals with spinal cord injury compared to the spinal cord injury group and healthy groups. A number of authors have suggested that engaging in two training sessions per day could potentially have a more significant biological impact than just one session per day (19,20). The results of this study showed that the movement score has a significant difference between performing the movement in two training sessions per day. In contrast, molecular tests did not show significant changes. Treadmill exercise is widely considered an effective strategy to restore motor function after spinal cord injury. However, the specific training intensity that optimizes recovery and the underlying mechanical basis of this recovery is unclear; therefore, in this study, we investigated the effect of two types of training with different intensities on the treadmill in rats with spinal cord injury through the molecular evaluation of hippocampal tissue. Accompanying the treadmill exercise protocol with receiving common drugs in the treatment of spinal cord injury, measuring the amount of spinal neurogenesis in the studied groups and measuring neutralizing RNAs and miRNAs of the PI3K/AKT pathway can also be investigated in future research.

Conclusion

The results of this study showed that the NT3 and tropomyosin receptor kinase C gene expression was significantly decreased in the spinal cord injury model compared to the control group, as well as the expression of these genes in the spinal cord injury group + exercise 1 and the spinal cord injury group + exercise

2 increased compared to the spinal cord injury group. NT3 did not show a significant difference between the two groups of spinal cord injury + exercise, but the changes in tropomyosin receptor kinase C between the two groups were significant. Therefore, the exercise protocols in this study, in addition to improving movement in animals with spinal cord injury, are effective on tropomyosin receptor kinase C and can be a factor for axonal growth and neuronal survival in the recovery of spinal cord injury.

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Ethical statement

All the steps of maintaining and performing the necessary experiments on animals were carried out in full compliance with the ethical principles of working with laboratory animals based on the Helsinki protocol. The tissues of this study were taken from another study on spinal cord rats by the same groups (The approval of the Ethics Committee of the Research and Technology Vice-Chancellor of North Khorasan University of Medical Sciences; IR.NKUMS.REC.1402.036).

Conflicts of interest

The authors declare that they have no conflict of interest.

Author contributions

Sadegh-Cheragh-Birjandi: concept development. Mohamad Amin Younessi Heravi: data analysis, manuscript writing. Sina Jalili Rasti: Data collection and manuscript writing. Ali Yaghubi: concept and manuscript writing.

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