The effects of exercise on the GAP-43 expression in the spinal cord of arthritis-induced rats

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Abstract. [Purpose] The purpose of the study was to investigate the effects of exercise on the recovery of spinal cord nerve cells damaged due to pain signals which are a major symptom of osteoarthritis. [Subjects and Methods] Adult male Sprague-Dawley rats (n=40) were used and induction of osteoarthritis by monosodium iodoacetate. Injected rats were randomly divided into 4 groups: Sham control group without MIA injection (SG), control group with injected MIA (CG), OA without exercise (NEG), OA with exercise (EG). Sham control group was injected normal cell line instead of MIA. The exercise group was submitted to 4-week training program on a treadmill for 5 days/week, 30 min/day, 16 m/min velocity, then spinal cord were removed and measured the GAP-43 expression by immunohistochemistry analysis. [Results] In this study, a results of measuring the expression of GAP-43. GAP-43 was observed in all groups, showed that the significant difference in each group. [Conclusion] It could be seen that exercise increased the GAP-43 expression in the spinal cord to promote the recovery of spinal cord nerve cells damaged due to chronic osteoarthritis.

Key words: Osteoarthritis, Treadmill exercise, GAP-43

INTRODUCTION

Knee osteoarthritis is known to cause not only restrictions in joint functions, but also major problems, such as the wear of articular cartilage and pain due to inflammatory damage to the surrounding tissues1, 2). If the pain due to osteoarthritis persists chronically, cytokines will increase in the spinal cord due to inflammatory stimulation coming from peripheral regions, and the increase in cytokines will induce inflammatory responses to cause damage to spinal cord nerve cells. If pain signals continuously extend from the peripheral joint regions to the spinal cord, spinal cord nerve cell damage will increase to develop chronic pain along with neuropathic pain, such as hyperalgesia3). In this case, to regenerate the synapses of damaged spinal cord nerve cells, growth-associated protein-43 (GAP-43), which is a substance related to nerve regeneration, increases in the posterior horn of the spinal cord4).

Among the many methods of treating osteoarthritis patients, exercise not only reinforces the regenerative capacity of articular cartilage and improves the collagen synthesis network, but it also strengthens the muscles and ligaments around the knee joints5). In addition, exercise is known to not only suppress the production of pre-inflammatory cytokines, such as IL-6, IL-8, and TNF-α, and increase the secretion of IL-10, which has anti-inflammatory effects to reduce inflammatory responses in the joints and the spinal cord, but it also increases the expression of neurotrophic factors, increased neurotrophic factor expression could promote the GAP-43 expression, thereby enhancing the nerve regeneration capacity3, 6, 7). As such, the purpose of exercise treatment for osteoarthritis patients is to not only enhance joint functions, but also relieve pain, thereby enhancing quality of life. Therefore, the present study was conducted to examine the effects of exercise on the recovery of spinal cord nerve cells damaged due to pain signals, which are a major symptom of osteoarthritis.
SUBJECTS AND METHODS

Experimental procedures were performed in accordance with the protocols established by the Institution of Animal Care and Use Committee (IACUC) at the Daegu University, based on the NIH Guidelines for the Care and Use of Laboratory Animals (NIH, 1996). Adult male Sprague-Dawley rats (8–10 weeks of age, weight 250–300 g, n=40) were used and housed at a controlled temperature at 25 ± 2°C with 12 h light/dark cycle and free access to food and water.

Induction of osteoarthritis by monosodium iodoacetate (MIA, Sigma, St Louis, MO, America) was performed briefly, 3 mg MIA in 50ul saline was injected through the patella tendon into the right knee joint48. Sham control group was injected normal cell line instead of MIA. Injected rats were randomly divided into 4 groups: Sham control group without MIA injection (SG), control group with injected MIA (CG), OA without exercise (NEG), OA with exercise (EG).

Three weeks after OA induction, exercise group was habituated on motor-driven treadmill at a speed of 10 m/min for 2 days to reduce their stress. The exercise group was submitted to 4-week training program on a treadmill for 5 days/week, 30 min/day, 16 m/min velocity which corresponded to approximately 60–70% VO2 max.

Spinal cord were removed, postfixed for 30 min, then rinsed and stored in 0.2 M phosphate buffer (PB) overnight. Spinal cord were sectioned with a microtome in serial section of 30 um, collected on slides and sections were performed immunohistochemistry. Briefly, Sections were rinsed 5 min and incubated for 1hr RT in TBS (Tris-buffered saline) containing 5% donkey serum (Sigma Aldrich, Wien, Austria), 1% BSA (bovine serum albumin, Sigma-Aldrich). The sections were incubated for immunohistochemistry with the primary antibody (rabbit polyclonal GAP-43, 1:500, Chemion international, USA) overnight at 4°C. Subsequently, the sections were rinsed 5 min, after which the ABC complex (Vector Laboratories) was applied to each section for 1hr at room temperature, antibody binding was visualized using a commercially available DAB substrate solution (Vector Laboratories). For quantitative analysis of positive immunoreactivity was measured using computer-assisted image analysis and is reported here as the proportional area of tissue occupied by immunohistochemically stained. Data was calculated by pixel and were analyzed using SPSS for Windows version 18.0. and expressed as mean ± standard deviation (SD). Comparisons between groups were performed via the Bonferroni-Dunn test. P values less than 0.05 at the 95% confidence level were considered significant.

RESULTS

In this study, a results of measuring the expression of GAP-43, GAP-43 was observed in all groups, showed that the significant difference in each group (Table 1). Compared to the control group, significantly higher GAP-43 expression were found in NEG and EG groups. In particular, it was confirmed that the highest expression of GAP-43 in the group which performed treadmill exercise.

DISCUSSION

If osteoarthritis persists chronically, spinal cord nerve cells will be damaged. If the pain signals generated by the knee joint are continuously delivered to the spinal cord, cytokines, which are an inflammation-inducing substance, will increase in the spinal cord to cause inflammatory responses in the spinal cord so that nerve cells are damaged and the sensory neurons in the spinal dorsal horn become sensitive, resulting in the occurrence of neuropathic pain, such as hyperalgesia49).

When the central nervous system’s nerve cells have been damaged, activities for the protection and regeneration of the damaged region increase. Astrocytes, which are neuroglia cells, proliferate, neurotrophic factors, such as NGF, brain-derived neurotrophic factor (BDNF), and neurotrophic-3 (NT-3), increase, and these neurotrophic factors lead to increases in the expression of GAP-43, which acts directly in the secretion of neurotransmitters and axonal regeneration10,11).

In the present study, to examine whether exercise affects the expression of GAP-43, which plays important roles in nerve cell regeneration and recovery in osteoarthritic white rats’ spinal cords, rats were induced to perform treadmill exercises, and the state of the expression of GAP-43 in the posterior horn of the spinal cord was examined through immunohistochemical analysis.

Table 1. The comparison of expression of NGF in spinal cord between four groups unit; pixel

|                      | Expressions of GAP-43 (Mean ± SD) |
|----------------------|-----------------------------------|
|                      | SG (n=10) | CG (n=10) | NEG (n=10) | EG (n=10) |
| (n=40)               | 8,306.6 ± 541.7 | 11,558.2 ± 881.6† | 18,213.5 ± 603.3‡ | 22,986.4 ± 1239.6‡ |

SG: sham group; CG: control group; NEG: no exercise group; EG: exercise group; Mean ± SD: mean ± standard deviation
†Significant difference from SG. p<0.05
‡Significant difference from CG. p<0.05
#Significant difference from NEG. p<0.05
staining. Upon examining the state of the expression of GAP-43, it could be seen that GAP-43 increased in the CG compared to the SG, and this can be judged as indicating spinal cord nerve cell damage due to osteoarthritis, as presented in a study conducted by Orita et al.

In addition, Orita et al. observed the GAP-43 expression in the spinal cord, which did not clearly increase at the early stage of the onset of osteoarthritis, but it remarkably increased from 14 days after the onset, and the lesion progressed to 28 days after the onset when the experiment terminated. This is consistent with the results of the present study, indicating that the GAP-43 expression significantly increased in the NEG compared to in the CG. Therefore, GAP-43 seems to have increased over time through natural healing to heal the damaged nerve cells. The significantly higher expression could be seen in the EG, which performed exercise, compared to the NEG. Based on the results of an analysis of previous studies, this result can be judged as indicating that the damaged nerve cells were actively being recovered as GAP-43 increased thanks to exercise.

When the above-mentioned results were viewed comprehensively, it could be seen that exercise increased the GAP-43 expression in the spinal cord to promote the regeneration of spinal cord nerve cells damaged due to chronic osteoarthritis. Also, the increase in the regeneration of nerve cells damaged due to inflammatory responses is thought to have positive effects on treatment for pain relief.

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