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Original Article

Effect of exercise on the expression of nerve growth factor in the spinal cord of rats with induced osteoarthritis

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Abstract. [Purpose] We examined the impact of exercise on the expression pattern of nerve growth factor in the spinal cord of rats with induced osteoarthritis of the knee joint. [Subjects and Methods] To produce monosodium iodoacetate-induced arthritis, rats were administered 3 mg/50 µL monosodium iodoacetate through the interarticular space of the right knee. The animals were randomly divided into four groups: rats sacrificed 3 weeks after 0.9% saline solution injection (shame group, n = 10), rats sacrificed 3 weeks after monosodium iodoacetate injection (control group, n = 10), rats with 4 weeks rest from 3 weeks after monosodium iodoacetate injection (no exercise group, n = 10), and rats with 4 weeks treadmill training from 3 weeks after monosodium iodoacetate injection (exercise group, n = 10). Serial coronal sections of the lumbar spine were cut and processed for immunohistochemistry. [Results] The expression of nerve growth factor was significantly increased in the EG compared with the SG, CG, and NEG. [Conclusion] Increased nerve growth factor expression in the spinal cord due to exercise-induced stimulation can be effective in treating chronic pain. Such treatment will contribute not only to improving the joint function of patients with chronic pain but also their quality of life.
Key words: Osteoarthritis, Treadmill exercise, Nerve growth factor

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INTRODUCTION

Osteoarthritis-associated knee pain accompanied by restricted joint function occurs as the joint cartilage is exfoliated from the joint after degenerative changes and the production of osteophytes, which result in mechanical stimulation of nerves in the subchondral bone during weight loading[1, 2)]. It is also characterized by an increase in inflammatory cytokines in the soft tissue and cartilage surrounding the joint[3, 4)]. The latter causes a local inflammatory reaction, thereby increasing the expression of diverse pain signaling molecules such as bradykinin, serotonin, and histamine[5)]. These pain signals subsequently activate the peripheral nociceptors surrounding the joint, causing pain[6)].

If the pain signal continuously flows into the spinal cord due to the activation of peripheral invasive sensory receptors, a local inflammatory reaction occurs in the dorsal horn part of the spinal cord. At the same time, the activation of glia cells, such as astrocytes and microglia, leads to the secretion of large amounts of cytokines, and this further accelerates spinal cord inflammation[7)]. This inflammatory reaction damages the nerve cells of the spine, increasing their sensitivity and stimulating the secretion of inflammation-inducing peptides. As a result, neuropathic pain, such as hyperalgesia, occurs[8, 9)].

Nerve growth factor (NGF) is a neurotrophic factor that controls the differentiation and survival of sensory nerves and sympathetic nerves, growth of the neuraxis, and transfer of neural signals in general[10)]. In the early phase of arthritis, NGF plays a role in providing protection against inflammation-related neuronal damage caused by proinflammatory cytokines and neuronal regeneration[11)]. In an albino rat model of induced osteoarthritis of the ankle joint, Pezet et al. reported that the expression of the NGF receptors tyrosine kinase A and p75 (or NGFRp75), which are closely related to neuronal plasticity, increased in laminae 3–5 of the dorsal horn at the L3–L4 level of the spinal cord[12)]. NGF plays a crucial role in the growth, proliferation, and differentiation of nerve cells, and an increase of this indicates the existence of nerve damage[13)].

Although surgery and medical treatments such as painkillers are widely used for pain reduction in osteoarthritis, it is important that patients take steps to prevent pain by controlling pain-related risk factors. In particular, exercise can help prevent and treat the disease without the adverse effects of medicine. As exercise is effective in improving

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Table 1. Comparison of NGF expression in the spinal cord between the four groups (unit: pixels)

	Expression of NGF (mean ± SD)			
Group (N = 40)	SG (n = 10)	CG (n = 10)	NEG (n = 10)	EG (n = 10)
	11,152.1±671.7	14,315.3±805.8 †	16,069±488.5 †	20,257±747.5 †‡§

†Significant difference from SG, $p < 0.05$.
‡Significant difference from CG, $p < 0.05$.
§Significant difference from NEG, $p < 0.05$.
‖Significant difference from EG, $p < 0.05$.
Mean ± SD: mean ± standard deviation.
NGF: nerve growth factor, SG: shame group, CG: control group, NEG: no exercise group, EG: exercise group

joint function by reinforcing the soft tissue in the knee joint and improving the regeneration power of the joint cartilage, exercise can be expected to be extremely beneficial in osteoarthritis treatment[1, 14, 15].

In the present study, we examined the expression pattern of NGF in the spinal cord of a rat model of induced osteoarthritis of the knee joint to clarify the impact of exercise on NGF expression.

SUBJECTS AND METHODS

All experimental protocols were performed in accordance with the guidelines of Daegu University, based on the National Institutes of Health guidelines for the care and use of laboratory animals (1996). Forty 8–10-week-old male Sprague-Dawley rats, weighing 250–300 g, were housed at a temperature of 25 ± 2 °C and maintained in a 12-h light-dark cycle. The animals had free access to food and water.

For the induction of monosodium iodoacetate (MIA; Sigma, St Louis, MO, USA; cat no. I2512)-induced arthritis, rats were anesthetized with 2 mL/kg of a 50% Zoletil/50% xylazine hydrochloride mixture and given 3 mg/50 μL MIA through the interarticular space of the right knee[2, 16]. Three weeks after MIA injection, the rats were subjected to a treadmill exercise according to previously described methods[17]. Briefly, the treadmill velocity was set at 8 m/min per 20 minutes for 2 days and at 16 m/min per 30 minutes for 4 weeks.

The rats were randomly divided into four groups: rats sacrificed at 3 weeks after 0.9% saline solution injection (shame group, SG, n = 10), rats sacrificed at 3 weeks after MIA injection (control group, CG, n = 10), rats with 4 weeks rest from 3 weeks after MIA injection (no exercise group, NEG, n = 10), and rats with 4 weeks treadmill training from 3 weeks after MIA injection (exercise group, EG, n = 10).

At the end of the exercise period, the rats were sacrificed and their spinal cords were removed. The lumbar spinal cord region was selected for immunohistochemistry analysis performed according to a previously described method[18]. Briefly, rats were anesthetized with 2 mL/kg of a 50% Zoletil/50% xylazine hydrochloride mixture, then intracardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. Serial coronal sections (30 μm) of the lumbar spine (L4–S1) were cut by using a microtome (BRIGHT5040) on a cryostat, and processed for immunohistochemistry. Tissue sections were incubated at 4 °C overnight in a solution containing an anti-NGF antibody (diluted 1:500 in PBS) and added with rabbit polyclonal anti-NGF antisera (Vector, USA), which specifically recognizes rat NGF.

Multiple comparisons between groups were performed by using SPSS for Windows version 18.0. The results are expressed as means ± standard deviation (SD). Differences between groups were tested by using one-way ANOVA, followed by a post-hoc test (Duncan test) when a difference was detected. Values of $p < 0.05$ at the 95% confidence level were considered significant.

RESULTS

The results of the present study, which examined the impact of exercise on NGF expression, showed that NGF was expressed in all groups of rats. NGF showed a statistically significant increase in rats with osteoarthritis induction (CG) compared with those without osteoarthritis induction (SG) ($p < 0.05$). The expression of NGF was also significantly elevated in the NEG compared with that in the SG ($p < 0.05$); however, no statistically significant difference was observed when compared with that of the CG ($p > 0.05$). On the other hand, NGF expression increased significantly in the EG compared with that in the SG, CG, and NEG ($p < 0.05$) (Table 1).

DISCUSSION

Osteoarthritis pain can be linked to central nervous system diseases that can affect many factors, from peripheral nerve function to central nervous system mechanisms[19]. Recently, a number of researchers have argued that neurological-related factors should be included in considerations of osteoarthritis treatment strategies[20].

The primary cause of chronic pain in cases of peripheral tissue damage is increased irritability of peripheral nociceptors located near the damaged part. A secondary cause is increased irritability of nerve cells within the central nervous system[21]. This is because inflammatory pain in peripheral areas, stimulation of peripheral primary afferent sensory nerves, and inflammation-related neuropathic pain in the spinal dorsal horn are all interrelated[7, 9].

Neurotrophins (NTs) are essential for the growth, proliferation, differentiation, and survival of nerve cells, which include NGF, brain-derived neurotrophin factor, NT-3, and NT-4[13]. Among these, NGF is a metabolically active peptide that controls the survival and differentiation of sensory nerve cells and sympathetic nerves, the growth of axons, and the

transfer of neural signals. Combined with the low-affinity NGF receptor p75NGFR, NGF plays a central role in the recovery, regeneration, and growth of nerve cells damaged by inflammation induced by proinflammatory cytokines[11].

Pezet et al. examined the expression pattern of the NGF receptor in the spinal dorsal horn over time after inducing arthritis in albino rats. In a control group without arthritis induction, the NGF receptor was expressed mainly on the surface of laminae 1–2, with some expression near the edge of laminae 3–5. The expression pattern did not change over time. In the experimental group with arthritis induction, the expression increased near laminae 3–5 until the acute phase (i.e., 3 weeks after the injection with an arthritis-inducing drug). In particular, the expression of the NGF receptor dramatically increased in the deep part. In the postacute phase, starting from the 8th week, the expression pattern began to decrease in laminae 3–5, and it decreased to a level similar to that of the control group in the recovery phase of the 12th week[12].

In the present study, NGF significantly increased between laminae 3 and 5 in the CG, where osteoarthritis was induced 3 weeks after the MIA injection, compared with that of the normal group (SG). The expression of NGF increased in the NEG exposed to an additional 4 weeks of treatment compared with that of the CG, although the increase was not significant.

Taking into consideration the results of previous studies, the stimulation of NGF expression during the active period of arthritis in the CG and NEG was likely due to a natural healing response to inflammation-related nerve cell damage. During this response, molecules are induced to protect and regenerate nerve cells, and these eventually increase the levels of NGF[9–12].

In our study, we observed a relatively higher expression of NGF in the EG that underwent exercise training compared with the other groups. With osteoarthritis induction, the damage to the spinal cord neurons subsequently increased the expression of NGF. We surmise that exercise further stimulated the NGF expression, affecting the regeneration of nerve cells in the neuraxis.

Many previous studies have reported that exercise stimulated the regeneration of nerve cells by increasing the expression of NGF. According to Chae and Kim, treadmill exercise of moderate intensity stimulated the expression of NGF, which controls the growth, differentiation, and apoptosis of nerve cells[22]. They argued that exercise restricted apoptosis, thereby preventing nerve cell damage. In an experiment with albino rats with ischemic stroke induced by surgery, Chung et al. reported that 2 weeks of treadmill exercise was effective in increasing NGF expression in the brain compared with a control group that did not undergo exercise training. They also reported that the exercise function of the experimental group also improved[23]. Matsuda et al. found that NGF expression increased in an albino rat with ischemic stroke after 20 minutes of treadmill exercise for 28 days[24]. They also reported that the area of brain tissue destroyed by ischemia decreased and that behavioral function improved after the exercise.

The results of the present study suggest that exercise-induced stimulation of spinal cord nerve cells, damaged by continuous osteoarthritis-related pain due to increased NGF expression in the spinal cord, can be effective in treating chronic pain, which, thus far, has proved difficult to treat. Such treatment will contribute not only in improving the joint function of patients with chronic pain but also their quality of life.

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