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Research Article

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Effect of Acute Phase Pain Control Using TENS on Pain Relief in Knee Osteoarthritis in a Rat Model

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| Abstract |

PURPOSE: This study examined the influence of treadmill exercise with initial pain control using transcutaneous electrical nerve stimulation (TENS) on induced pain of knee osteoarthritis in rats.

METHODS: Thirty adult male Sprague - Dawley rats were divided randomly into the TENS Group (TG, n = 10), Treadmill Exercise Group (TEG, n = 10), and Treadmill with TENS Group (TTG, n = 10). In the TG, TENS was performed for 20 min per day for two weeks with a TENS program at the knee joint. The TEG performed treadmill exercise 15 m/min for 20 min per day for two weeks. The TTG performed initial pain control by TENS program during the 1st ~ 3rd days, and treadmill exercise was performed using the TEG methods from the 4th day. The lumbar spine was extracted and processed using western blot analysis to evaluate pain (c-fos expression).

RESULTS: The results showed that c-fos expression was decreased significantly in all groups after each intervention ($p < .05$). In particular, TTG produced the most significant decrease compared to the other groups.

CONCLUSION: These results suggest that treadmill exercise with initial pain control using TENS is a suitable method for relieving pain in knee osteoarthritis.

Key Words: Knee osteoarthritis, Pain management, TENS, Treadmill exercise

Ⅰ. Introduction

Recent guidelines published by the Osteoarthritis Research Society International have suggested that exercise benefits osteoarthritis (OA) patients [1,2]. Exercise is the most widely recommended conservative therapeutic approach to improve the joint function in arthritis [3]. Although surgery and medical treatments, such as painkillers, are used widely for pain reduction in osteoarthritis, patients must take steps to prevent pain by controlling their pain-related risk factors. In particular, exercise can help prevent and treat the disease without the adverse effects of medication. Because exercise effectively improves the joint function by reinforcing the soft tissue in the knee joint and improving the regeneration power of the joint cartilage, exercise can be extremely beneficial in the

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treatment of OA [4-6].

Treadmill exercise has long been studied as a conventional treatment for OA. In general, treadmill exercise is commonly applied to patients with knee problems because of its beneficial effects on neuroprotection. Despite this, several studies have suggested that treadmill exercise may induce stress [7,8]. Such joint-specific strength and general aerobic conditioning are generally recommended for OA treatment [9].

Physical therapy modalities, including transcutaneous electrical nerve stimulation (TENS) and exercise, are well-known treatment methods for patients with knee OA. For example, Pietrosimone et al. [10] showed that the combination of TENS and exercise is more effective for the short-term treatment of knee OA than exercise alone. In addition, Chen et al. [11] reported that TENS plus exercise is more effective than an intra-knee injection of hyaluronic acid. There is sufficient evidence to support the effectiveness of TENS and exercise in the symptomatic relief of patients with knee joint OA [12,13].

This study examined the influence of treadmill exercise with initial pain control (TENS) on induced osteoarthritis in rats. This study provides information on the effectiveness of treadmill exercise and initial pain control by TENS that can be advised to patients in clinical practice.

Ⅱ. Methods

Thirty adult male Sprague-Dawley rats were randomly divided into three groups of 10 subjects each: the TENS Group (TG), Treadmill Exercise Group (TEG), Treadmill with TENS Group (TTG). All groups performed the intervention each day for two weeks.

1. Experimental Subjects

Thirty adult male Sprague-Dawley rats (8-10 weeks of age, weighing 250-300 g) were housed at 22°C under a 12:12-hour daylight cycle and were given access to food and water ad libitum. The subjects were divided randomly into the following three groups.

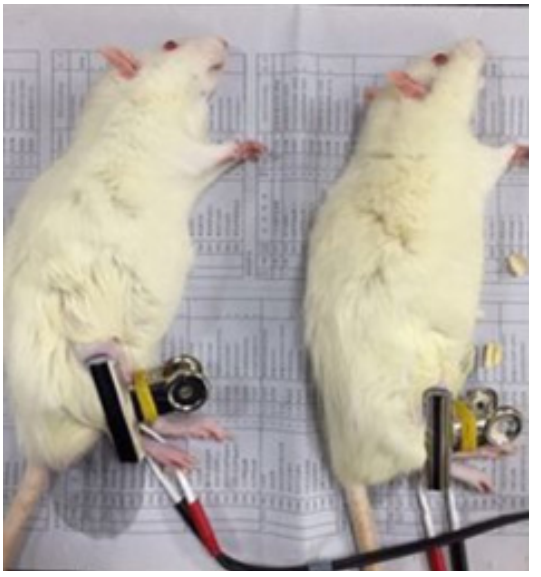

Fig. 1. TENS application.

2. Experimental Design

1) TENS Group (TG)

Five days after a Monosodium Iodoacetate (MIA) injection, TENS (BM-420, Hanil, Korea) was applied, and the rats were anesthetized with tribromoethanol (Fig. 1). After anesthesia, electrodes with a modified double clip were attached to the knee joint, and current flow was performed for 20 min per day for two weeks using a program controlled within a constant mode, an asymmetrical biphasic waveform, frequency range of 0-100 Hz and a pulse with a range of 20-700 μs.

2) Treadmill Exercise Group (TEG)

Five days after the MIA injection, a motor-driven treadmill (JD-A-09 type, JEUNGDO Bio & Plant Co., Ltd., Korea) was used for the treadmill exercise (Fig. 2). Before the treadmill exercise, the rats experienced one day of

Fig. 2. motor-driven treadmill (JD-A-09 type, JEUNGDO Bio & Plant Co., Ltd., Korea).

adaptive running exercise at 6-9 m/min for five min. The running speed and duration were 15 m/min for 20 min per day for two weeks, according to a previously published protocol [1].

3) Treadmill with TENS Group (TTG)

Five days after the MIA injection, the TTG underwent initial pain control using the TENS program for one to three days, such as TG. The treadmill exercise was then performed using the previously TEG methods.

3. Experimental Procedure

1) Induction of Osteoarthritis in the Rat

Osteoarthritis was induced via an intra-articular injection of 50 μl of 3 mg MIA (Sigma Aldrich Chimie, Lyon, France) through the patellar ligament of the right knee. Up to five days after the MIA injection, substantial inflammation of the synovial joints was observed in this model. The general health of the animals was monitored throughout this research.

2) Western Blot Analysis

Chronic pain begins, in many cases, as nerve injury pain or persistent psychogenic pain. In the case of OA, the neuropathic pain is caused by the continuous water-soluble infringement loss of articular cartilage. To evaluate the analgesic effect and the pain standard, observing the production of the c-fos protein expression of the early genes immunohistochemically is popular. The dorsal horn of the spinal cord contains active synaptic sites via C fibers, with the A fiber mainly delivering the feelings of pain via this route [14].

Western blot analysis was performed as previously reported [15]. Briefly, equal amounts of the total protein (20 μg) in pooled samples were loaded into each 12% SDS-PAGE well. After electrophoresis, the proteins were transferred to a nitrocellulose membrane (Whatman, Germany), which was then overnight incubated with c-fos (Chemicon, 1:500 dilution) at 4° C. The blots were then incubated with anti-rat horseradish peroxidase (HRP) (Enzo, 1:2000 dilution) for one hour at room temperature.

The ECL PLUS Western blot analysis system (Amersham BioSciences UK Ltd., UK) was used to detect the immunoreactive proteins. The thickness of the bands was measured photographically using Scion Image software (Scion, Frederick, MD), in which a distance of 250 mm was equivalent to 733 pixels.

3) Statistical Analysis

Significant variation analysis was used to calculate the one-way ANOVA, and evaluate the statistical significance of the differences among the three groups. A Tukey test was used for the post hoc evaluations. The differences between before and after in c-fos were compared using a paired t-test. All the analyses were done using Origin 18.0 software, and p-values $< .05$ were considered significant.

Ⅲ. Results

In the intragroup comparison of each group, a significant decrease in c-fos expression was observed after the

Table 1. Comparison of c-fos between the Three Groups before and after the Experiment (unit: %).

Group	Pre-Day	Post-Day	p
TENS	7302.80 ± 152.40	5074.50 ± 199.50	.000**
Treadmill	7274.40 ± 133.94	4920.70 ± 122.07	.000**
TENS Treadmill	7333.40 ± 156.70	2769.00 ± 111.88	.000**

*p < .05, **p < .01
M ± SD: mean ± standard deviation

Table 2. Induced Arthritis, Treadmill, TENS, and Treadmill with TENS Groups According to c-fos: Comparisons Before and After the Experiment (unit: %).

Period	TENS	Treadmill	Treadmill with TENS	p	Post-hoc
Pre	7302.80 ± 152.40	7274.40 ± 133.94	7333.40 ± 156.70	.676	
Post	5074.50 ± 199.50	4920.70 ± 122.07	2769.00 ± 111.88	.000**	TENS > Treadmill TENS > Treadmill with TENS Treadmill > Treadmill with TENS

*p < .05, **p < .01
M ± SD: mean ± standard deviation

intervention (Table 1). A significant difference in c-fos expression was observed after the intervention in each group (Table 2).

Ⅳ. Discussion

Pain is a major symptom of OA and is largely the focus of the treatment for knee OA patients who desire a reduction in symptoms [16,17]. Pain management is critical in osteoarthritis because osteoarthritis causes pain, which weakens the muscle strength. The weakened muscle strength reduces the activity of daily life and the quality of life. Furthermore, muscle strength is also important because it is involved in cartilage regeneration. Therefore, in this study, TENS and treadmill exercises were used in OA rats. The c-fos protein was measured by Western blot analysis to evaluate each exercise and reduce knee OA pain and pain in the spine [18].

c-fos expression decreased in all groups after the intervention. In TG, c-fos expression decreased significantly from 7302.980 ± 152.40 to 5074.50 ± 199.50. These results concur with a previous study in which performed a three-week TENS intervention on patients with knee osteoarthritis [19]. This is in line with a previous study in which 36 patients with knee osteoarthritis were treated with TENS intervention for six weeks and significant pain reduction after intervention [20]. This result is because TENS selectively increases the input of the large fibers, thereby reducing the presynaptic inhibition of pain-transmitting cells (T-cells) and reducing the perception of conscious pain [21]. On the other hand, there was not much pain reduction compared to the other groups because it was similar to the pain delivered to the spinal cord during stimulation [17].

In TEG, c-fos expression was decreased significantly from 7274.40 ± 133.94 to 4920.70 ± 122.07. These results are in line with a previous study in that walking exercise reduces the risk of pain in patients with knee osteoarthritis [22]. Intense treadmill exercise has been shown to stimulate the expression of the nerve growth factor (NGF), which controls the growth, differentiation, and apoptosis of nerve cells [23]. In addition, it is in line with a previous study

reporting that treadmill exercise intervention reduced pain in rats with knee osteoarthritis [24]. These results suggest that the motion of the knee joint with a treadmill may increase the circulation in the joints and may have contributed to the regeneration of injury caused by osteoarthritis and the inflammation of the synovial membrane [25].

In TTG, c-fos expression decreased the most from 7333.40 ± 156.70 to 2769.00 ± 111.88. In osteoarthritis, intense pain causes a decrease in activity. If this condition persists, it will lead to a decrease in muscle strength. Continued lowering of muscle strength reduces the joint stability and reduces the ability of the cartilage to recover, further increasing pain [26,27]. Therefore, exercise should be combined with pain control to prevent the decrease in muscle strength. In the TEG, the effect was limited because of the limitation of exercise participation due to pain. In TTG, there was a significant effect because of active participation due to initial pain intervention. A limitation of this study was that the number of subjects was small, and no human subjects were included. Future studies with a large number of people will be needed.

Ⅴ. Conclusion

This study examined the effects of treadmill exercise, TENS, and treadmill exercise with initial pain control (TENS) on osteoarthritis pain in rats.

In this study, the three groups showed improved c-fos expression after the intervention. The TTG had a greater impact on pain relief than the other two groups. This result suggests that treadmill exercise with initial pain control is an efficient method to relieve pain in osteoarthritis.

Further investigation should be considered regarding the effects of treadmill exercise with initial pain control on knee joint cartilage regeneration.

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