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Pulsed Electromagnetic Fields to Influence Pain Relief Following Knee Osteoarthritis in Rats

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This study examined the effects of Pulsed Electromagnetic Fields (PEMF) therapy on the expression of c-fos protein in rats with knee osteoarthritis (OA). The aim of this study was to compare the improvement of pain relief in two experimental groups: PEMF and treadmill exercise. Thirty-two male Sprague-Dawley rats were randomly divided into four groups with eight rats in each: CON1 (including rats sacrificed 1 day after OA induction), CON7 (including rat sacrificed 7 days after OA induction), PEMF (including rats with applied PEMF for 7 days after OA induction), TE (including rats with applied treadmill exercise for 7 days after OA induction). A statistically significant reduction on expression of c-fos was observed in TE and PEMF groups compared to that in CON7 group. However, there was no significant difference in expression of c-fos between the TE group and PEMF group. It is suggested that PEMF could have positive effect on pain relief for OA confirming reduction on expression of c-fos in the spinal cord.

Keywords : pulsed electromagnetic fields, pain relief, osteoarthritis

1. Introduction

Osteoarthritis of the knee is one of the most common musculoskeletal disorders in the elderly. It is caused by continuous articular cartilage degeneration resulting in movement disorders and pain due to periarticular inflammation [1]. In addition, as osteoarthritis of the knee progresses, the rigidity of the joints and the intra-articular structural deformity causes an increase in the pain and limit the physical activity.

In order to treat symptoms of osteoarthritis such as pain and restricted movements, medications, surgery, and physical therapy are used clinically. The most common way to control pain is by use of medication, which is effective in relieving inflammation and pain; however, long-term use of medication can cause side effects such as dysfunction of several organs [2]. As regards surgery, it is difficult to consider the shape and size of other joints using a uniform surgical instrument; moreover, the cost to the patient is reportedly high with surgery [3]. In order to treat osteoarthritis, there are non-medical and non-surgical methods such as exercise therapy and electrotherapy.

Pulsed electromagnetic field (PEMF) is an electromedical stimulation therapy using high frequency and low frequency alternating currents of 15, 75, 150 Hz, and 20-200 kHz [4]. PEMF therapy has been reported to reduce the permeability of capillaries, thereby inducing wound healing, decreasing edema, increasing the range of motion, and promoting peripheral blood circulation. In clinical practice, it is one of the most effective physical therapies widely used to improve the motor function in osteoarthritis and reduce pain [5, 6]. However, recent systematic reviews of the clinical efficacy of clinical studies have shown that the effects of osteoarthritis pain reduce are inconsistent [7, 8]. This is because the distinction between electromagnetic field stimulation treatment using high frequency alternating current and electromagnetic field stimulation treatment using the low frequency alternating current is inconsistent. Moreover, in most clinical studies, the efficacy was inconsistent as it assessed the degree of subjective pain in humans [9].

There is a method of measuring the changes in expression of c-fos, a neurological pain factor, for the objective evaluation of pain. C-fos is expressed in the neurons of the posterior horn of the spinal cord after pain stimulation. In osteoarthritis, continuous stress on the joint and cartilage

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damage has been reported to increase the expression of c-fos by acting sensitively on the posterior horn of the spinal cord, a pain-related pathway [10].

These studies suggest that pulsed electric field stimulation therapy is effective in reducing pain in osteoarthritis patients. In addition, PEMF therapy is more effective in reducing pain in patients with knee osteoarthritis than general aerobic exercise, and provides a basis for objective physical therapy. However, studies evaluating the effect of PEMF therapy on changes in expression of c-fos are lacking. The aim of this study was to investigate the effect of general aerobic exercise and PEMF therapy on the expression of c-fos protein in rats with knee osteoarthritis.

2. Materials and Methods

2.1. Animals

32 male Sprague-Dawley rats (8 weeks old, weighing between 250-300 g) were used and were housed with 12-hour light/dark cycle, with ad libitum access to food and water. Rats were randomly divided into four groups with 8 rats in each. CON1 (including rats sacrificed 1 day after OA induction), CON7 (including rat sacrificed 7 days after OA induction), TE (including rats with applied treadmill exercise for 7days after OA induction), PEMF (including rats with applied PEMF for 7 days after OA induction). All the experimental protocols received approval from the University of Daegu Animal Experiment Committee, based on the NIH Guidelines for the Care and Use of Laboratory Animals (NIH publication, 1996).

2.2. Experimental procedure

Induction of osteoarthritis by monosodium iodoacetate (MIA, sigma, St Louis, MO, America) was performed. Briefly, MIA (4.8 mg/60 ul) was injected intraarticularly through the infrapatellar ligament of the right knee each rats as previously described [11]. Starting 10 days after MIA injection, rats were randomly assigned to the CON1, CON7, TE, PEMF. TE group was submitted to 7 days training program on a treadmill for 30 min per day with 16 m/min velocity which corresponded to approximately 60-70 % VO_2 max. On the other hand, PEMF group was used a Diapulse machin (Diapulse Corp., USA) at a frequency of 27.12 MHz with 5 gauss intensity for 30 min per day on right knee joint.

2.3. Sampling and western blot analysis

After the animals were anesthetized and sacrificed, the spinal cord of each group were collected and washed twice in PBS, and then homogenized and lysed RIPA Lysis and Extraction Buffer (Thermo Scientific) for 1 hour on ice. The lysates were centrifuged for 10 min at 15,000 rpm at 4 °C, and the protein concentration was determined. Equal amounts of protein (40 μg) were resolved via 12 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. The blots were washed with TBS-T (10 mM Tris·HCl [pH 7.6], 150 mM NaCl, 0.05 % Tween 20), blocked with 5 % skim milk for 1 hour, then incubated with the anti c-fos (1:1000) antibody at the dilutions recommended by the suppliers. The membranes were washed, and the primary antibodies were detected using horseradish peroxidase-conjugated goat anti-mouse IgG (1:3000). The bands were then visualized via enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The thickness of the bands was photographically measured by Scion image.

2.4. Statistical analysis

The results were expressed as the means ± standard deviation (SD). All experiments were analyzed via One-way analysis of variance (one-way ANOVA), and Fisher's LSD test was used for post hoc evaluations. The statistical significance level was set at $p < 0.05$.

3. Results and Discussion

We investigated the influence of PEMF on the in vivo reduction of pain in osteoarthritis by decreasing the rate of expression of c-fos. The values on expression of c-fos were represented in Table 1. A statistically significant reduced expression of c-fos was seen in the TE and PEMF groups after 7 days compared to the CON7 group

Table 1. Comparison of change expression of c-fos between each group (n = 32).

	CG (n = 16)		EG (n = 16)	
	CON1	CON7	PEMF	TE
Expression of c-fos	8131.4 ± 872.5	17121.4 ± 2014.4 [a]	2980.8 ± 511.2 [a b]	3762.8 ± 402.6 [a b]

CG; control group, EG; experimental group
Values are expressed as mean ± SD
[a]significant difference from CON1, $p < 0.05$
[b]significant difference from CON7, $p < 0.05$

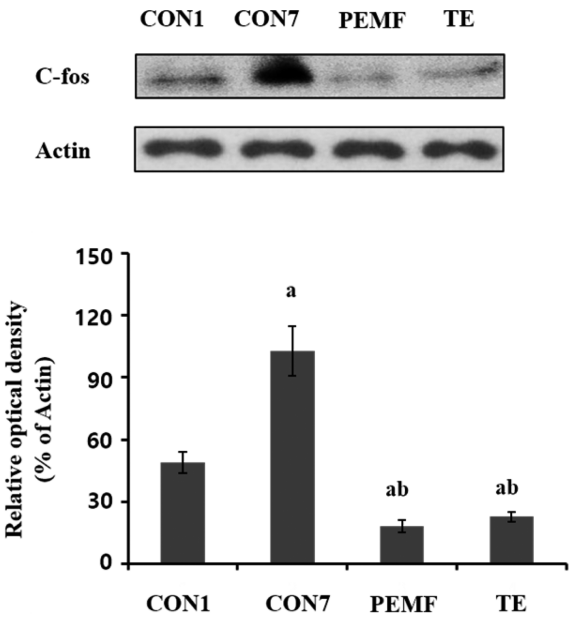

Fig. 1. Western blot analysis for expression of c-fos in rat spinal cord in each group. The values represent the means ± SD. $^{a}p < 0.05$ versus CON1, $^{b}p < 0.05$ versus CON7.

($p < 0.05$). There was, however, no significant difference in expression of c-fos between the TE and PEMF groups ($p < 0.05$) (Fig. 1).

Exercise is the most commonly recommended intervention in osteoarthritis because surgeries such as total knee replacement may not be optimal in younger populations with osteoarthritis due to its expensive cost and side effects [3, 12]. A recent meta-analysis indicated that running has protective effects against knee joint surgery in OA; moreover, exercise also unmasked the protective and pain-alleviating effects [13, 14]. Similarly, we observed that the TE group demonstrated less expression of c-fos than the CON7 group. Our observation that 7 days of treadmill exercise-induced pain relief may be due to suppression of inflammatory factors including brain-derived neurotrophic factor (BDNF), glial activation, macrophage infiltration, and cytokines within the spinal cord of neuropathic pain. These observations are similar to that of previous studies in that exercise might be helpful in diminishing inflammatory factors within the dorsal root ganglion and the brain [15, 16].

PEMF has been used as a therapeutic method for promoting tissue healing; however, some studies have found that PEMF has no clinical benefit for the healing of long-bone improving pain control, or joint stiffness in knee OA [8, 17]. The outcomes of PEMF has been known to be inconsistent; however, some studies have demonstrated that PEMF influenced the different responses in different tissues, especially in those involved on the recovery of arthritis and pain control due to knee osteoarthritis [18, 19].

C-fos is a small nuclear protein and it is used a marker for the activation of nociceptive neurons in the dorsal horn of the spinal cord. A study that investigated the expression of c-fos during treadmill exercise showed that the intensity and duration of treadmill exercise adjusted the expression of c-fos [20]. In addition, a number of studies confirmed a wide number of peripheral stimuli such as stress or inflammation that trigger the upregulation of the immediate early gene c-fos and its protein product c-fos in the dorsal horn neurons [10, 21]. Similar to these studies, our results also showed that CON7 groups upregulated the expression of c-fos compared to the CON1 group. Also, this study found that a significantly lower expression of c-fos in the spinal cord was observed in the PEMF groups than in the CON7 groups. Consequently, our findings suggest that the application of PEMF may induce an analgesic effect on osteoarthritis in the acute phase.

The release of endogenous opioids in the central nervous system has been suggested as a general mechanism of pain relief; moreover, it may improve pain through inflammatory responses by the glia or peripheral immune cells [22]. This is consistent with the results of previous studies that reported that exercise stimulates the release of endogenous opioid peptides and increases nociceptive thresholds in animal models; endogenous opioids could mediate potential exercise-associated anti-inflammatory effects that diminish joint pain. In addition, there is evidence that PEMF actually changes brain wave activity and the symptom-altering effects of the electromagnetic waves are the results of direct effects on the central nervous function [23, 24]. Hence, our results may reflect that both the TE and PEMF groups were down-regulated expression of c-fos when compared with CON7 group. Although there was no significant difference between TE and PEMF groups, it means that PEMF is as effective to reduce pain as treadmill exercise. Therefore, our findings suggest that PEMF is effective for pain relief in osteoarthritis models.

In conclusion, in order to determine the effectiveness of PEMF, this study evaluated whether PEMF can improve pain relief when compared to exercise. In TE and PEMF group, protective effects and diminished pain were observed in rats with OA. Taken together, this study indicates potential effectiveness of PEMF treatment in the pain relief of OA. Consequently, this study suggested that PEMF could have positive influences on pain relief of OA through reducing expression of c-fos in the spinal

cord.

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