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Pulsed Ultrasound and Pulsed Electromagnetic Field in the Treatment of Muscle Contusion in Rats

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Muscle contusion usually results from a direct blunt impact and is frequently associated with contact sports. Muscle contusion results from microscopic muscle fiber and capillary disruption causing a microhemorrhage dissecting torn fibers and remaining viable muscle fibers. Recent studies concluded that some physical methods, including pulsed ultrasound (PU) and pulsed electromagnetic field (PEMF) treatment, accelerate and facilitate wound healing, improve scar quality and have beneficial effects on muscle and tendon healing. However, there are few studies on the effects of the early use of physical methods, such as PU and PEMF, on the expression of neurotrophic factors. The objective of this study was to investigate the effects of the early application of PU and PEMF, measured through the expression of BDNF in the muscles (gastrocnemius) and spinal cords of rats after skeletal muscle contusion. In the spinal cords and muscles, there was a significant increase of BDNF expression in the PEMF and PU groups, a greater increase was found in the PEMF group than in the PU group. In conclusion, PEMF is a useful therapeutic method that improves muscle healing after muscle contusion.

Keywords : pulse ultrasound, pulse electromagnetic fields, muscle injury, BDNF

1. Introduction

Muscle contusion usually results from a blunt direct impact and is frequently associated with contact sports. Muscle contusion results from microscopic muscle fiber and capillary disruption which can cause microhemorrhages dissecting torn fibers and remaining viable muscle fibers [1].

Neurotrophins are well known for their roles in regulating neuronal survival, growth, plasticity and death [2]. Neurotrophins play a critical role in the synaptic plasticity and development of the nervous system in adults [3]. They protect neurons from degeneration and promote regeneration of injured nerves, they also enhance differentiation of neural stem cells by activating tyrosine kinase receptors (trk) and the down-stream signal pathways [2].

In addition, skeletal muscles express several neurotrophin receptors, providing the basis for neurotrophin signaling within the muscle compartment [4]. Muscle injuries results in the up-regulation of brain-derived neurotrophic factor (BDNF) expression, at the same time we see satellite cell activation and proliferation, suggesting that BDNF may play a role in mediating the satellite cell response to injury [5].

Recent studies concluded that some physical methods including therapeutic ultrasound and pulsed electromagnetic field (PEMF) treatments accelerate and facilitate wound healing, improve scar quality and have beneficial effects on muscle and tendon healing [6, 7].

Therapeutic ultrasound generates considerable heat in living tissues and can homogenize tissues. However low-intensity pulsed ultrasound (PU), which is nonthermogenic and nondestructive, is widely used to accelerate tissue healing [8].

The application of PEMF treatment to the area of injury produces changes in the cell environment and restores the integrity and function of tissues within the organism [9].

There are few studies on the effects of the early use of physical methods, such as PU and PEMF, on the expressions of neurotrophic factors. Therefore, the objective of this study is to investigate the effects of the early application of PU and PEMF, measured through the expression of BDNF in the muscles and spinal cords of rats after skeletal muscle contusion.

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2. Materials and Methods

2.1. Animals

30 male Sprague-Dawley rats weighing between 250 g and 300 g were used and maintained in a 12-hour on/12-hour off light/dark cycle with *ad libitum* access to food and water. All the experiments were conducted in accordance with the protocols established by the University of Daegu Animal Experiment Committee, based on the NIH Guidelines for the Care and Use of Laboratory Animals (NIH publication, 1996). The animals were randomly divided into 3 groups: control (n = 10), PU (n = 10) and PEMF (n = 10) groups.

2.2. Experimental procedure

Animals were anesthetized with intraperitoneal injection and muscle contusion was induced on the right gastrocnemius. The PU and PEMF groups were exposed to each modality for 3 days after injury.

A Diapulse machine (Diapulse Corp., USA) was used to deliver PEMF to the injury site. The PEMF was delivered at a frequency of 27.12 MHz with an intensity of 5 gauss and a pulse output of 450 W for 15 minutes per day.

Treatment with PU (Pulson 200, gymnaUniphy, Belgium) was 6 minutes in duration, at a frequency of 1.0 MHz, an intensity of 0.8 W/cm^2, an effective radiating area (ERA) of 1 cm^2, using a 50% duty cycle [10].

The experimental animals were killed 3 days after injury for the BDNF expression studies.

2.3. Sampling and immunohistochemistry

The animals were sacrificed via anesthesia with a mixture of 2 ml/kg 50% Zoletil and 50% Xylazine hydrochloride followed by perfusion through the heart with 200 ml of 0.9% NaCl solution followed by 4% paraformaldehyde solution. The muscles and spinal cords were removed, maintained in post-fixative overnight and subsequently sectioned to a thickness of 30 μm for immunohistochemistry.

In brief, the sections were washed (3 × 10 min) in a 0.01 M phosphate-buffered saline solution (PBS; pH 7.2) and incubated for 12 h at room temperature with mouse monoclonal anti-BDNF (Chemicon, USA). The antibody was diluted to 1:200 with a solution of Triton X-100 and normal donkey serum. Following incubation in the primary antibody, the sections were rinsed (3 × 10 min) in PBS, incubated for 90 min at room temperature with anti-mouse IgG (Vector Laboratories Inc, USA), and diluted to 1:25 with a solution of Triton X-100 and normal donkey serum. Following incubation in the secondary antibody, the sections were rinsed (3 × 10 min) in PBS, and subsequently incubated for 1 h at room temperature with a Vectastain Elite ABC-kit (Vector Laboratories, Inc., USA). The sections were rinsed in PBS and incubated for 10 min in 0.04 mg of 3,3'-diaminobenzidine (DAB, in 200 ml distilled water). The sections were then incubated for 1 min in DAB solution with 35% H_2O_2. The DAB sections were rinsed in PBS (3 × 10 min) to halt the chromagen reaction, wet-mounted onto gelatin/chromium-coated slides and permitted to air-dry overnight. The sections were subsequently dehydrated using a series of alcohols, soaked in xylene and cover-slipped with Clarion (Biomedia, USA).

2.4. Statistical analysis

The results were expressed as means ± standard error (S.E.). All experiments were analyzed via the analysis of variance, some experiments were analyzed via comparisons of the treatment mean with the controls via the Bonferroni-Dunn test. The difference was regarded as statistically significant when $p < 0.05$.

3. Results and Discussion

In this study, we examined the use of PEMF and PU as physical methods to improve tissue healing. We induced a muscle contusion and observed the expression of BDNF in the spinal cords and muscles of rats after 3 days.

PEMF and PU treatments produce athermal effects that promote tissue healing and reduce pain and inflammation [11, 12]. PU therapy is works by a transient cavitation effect that leads to changes in volume and pressure caused by bubbles forming in the liquid medium, when these bubbles hit one another, they release energy that may break chemical bonds, thereby producing reactive free radicals and provoking chemical changes in cells. A change in pressure induced by the bubbles may modify the permeability of the cellular membrane to calcium and sodium ions, increasing protein synthesis. In addition, organelles may be altered due to irradiation forces [12]. However, there are few studies of the relationship between organic tissues and PEMF. Moreover, research evidence to support the effects of the early use of PEMF and PU is also lacking.

Our results demonstrate that the PEMF and PU significantly increase BDNF expression. In the spinal cords, there was a significant increase in BDNF expression for the PEMF and PU groups, a significantly greater increase was found in the PEMF group compared to the PU group (Figs. 1, 2). In the muscles, the PEMF and PU groups showed significant increases in BDNF expression compared to the control group. However, there were not signi-

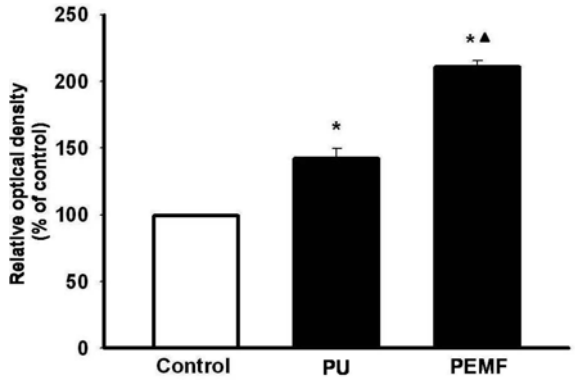

Fig. 1. The effect of pulsed ultrasound and pulsed electromagnetic field on expression of BDNF in rat spinal cords after muscle contusion. Muscle (gastrocnemius) contusions in adult Sprague-Dawley rats were induced. Rats were allowed to survive for 24 hours after muscle injury. The spinal cord sections from the control, PU and PEMF groups were stained for BDNF. The amounts of BDNF were detected via immunohistochemistry, as described in the Materials and Methods section. Each example shown is representative of three experiments. The values shown represent the mean ± SE of the results relative to the control group 3 days after injury. *$p < .05$ vs. control. $p < .05$ vs. PU.

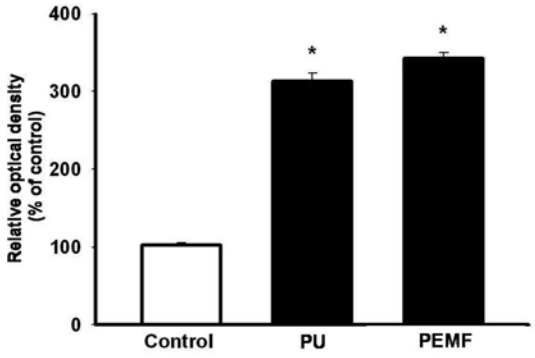

Fig. 3. The effect of pulsed ultrasound and pulsed electromagnetic field on expression of BDNF in rat muscles after muscle contusion. Muscle (gastrocnemius) contusions in adult Sprague-Dawley rats were induced. Rats were allowed to survive for 24 hours after muscle injury. The spinal cord sections from the control, PU and PEMF groups were stained for BDNF. The amounts of BDNF were detected via immunohistochemistry, as described in the Materials and Methods section. Each example shown is representative of three experiments. The values shown represent the mean ± SE of the results relative to the control group 3 days after injury. *$p < .05$ vs. control.

ficant differences between the two groups ($p > 0.05$), though the increase in BDNF expression for the PEMF group was slightly greater (Figs. 3, 4).

BDNF plays a critical role during neuronal development, including modulating cell survival, neuronal differentiation, dendritic outgrowth, axonal guidance, spine formation and synaptic plasticity [13]. Furthermore, BDNF is expressed in myogenic progenitors known as satellite cells and plays a role during muscle regeneration in vivo [14].

Kang *et al.* (2011) reported that PEMF application decreases the degeneration of a rat's gastrocnemius morphology, and increases the expression of BDNF in the rat's spinal cord after spinal cord hemisection [15]. In our previous study, we investigated differences in pain with varying PEMF application time, measured through the expression of c-fos in the spinal cords of rats after skeletal muscle crush injuries. The effects of early PEMF application on pain, after muscle injury, was reported [16].

In the primary phase of muscle healing, the rupture and necrosis of myofibers and hematoma formation occurs in the injured portion, and growth factors, such as chemokines and cytokines, are required by inflammatory cells for recovery [17].

Our study suggests that early application of PEMF and PU enhances BDNF expression in the spinal cord and muscle after muscle contusion. Moreover, there was significantly greater increase in BDNF expression for the PEMF group compared to the PU group. However, this result does not mean that PU has little value as a treatment for muscle injuries. There was a difference in the therapeutic doses of the PEMF and PU administered.

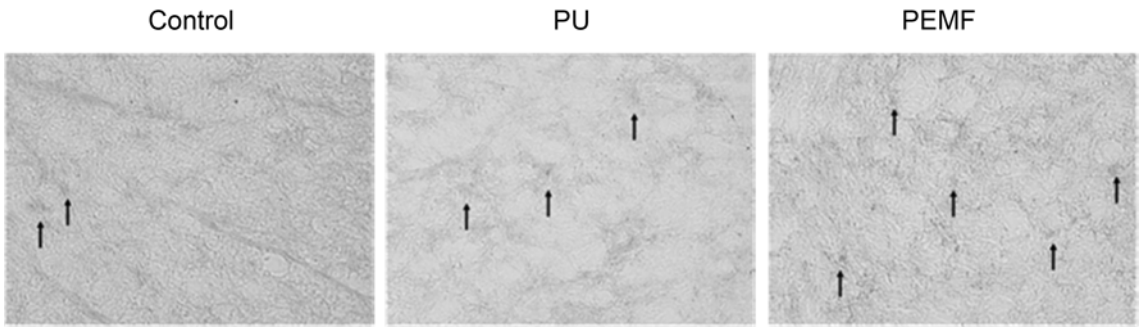

Fig. 2. Expression of BDNF in rat spinal cords.

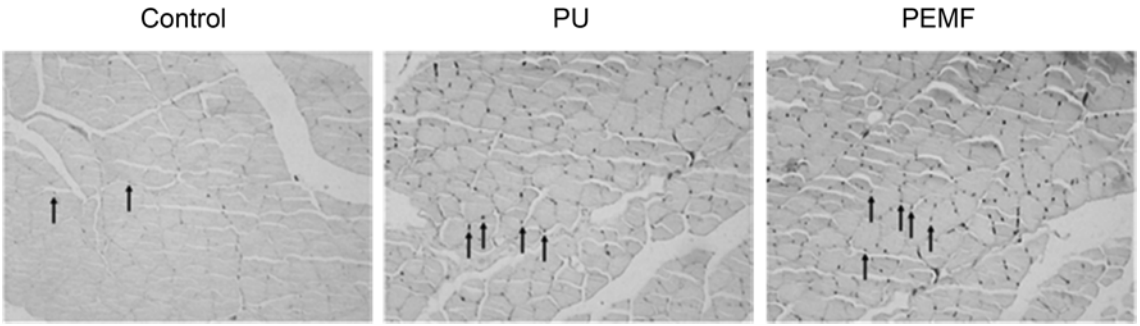

Fig. 4. Expression of BDNF in rat muscles.

Therefore, we can conclude that the this provides clear evidence that PEMF is a useful therapeutic method that improves muscle healing after muscle contusion.

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