The Effects of Pulsed Elctromagnetic Fields on Expression of Neurotrophic Factors after Spinal Cord Hemisection in Rats

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The purpose of this study was to identify the effect of pulsed electromagnetic fields on the expression of neurotrophic factors after spinal cord injury. Sprague-Dawley male rats were given a spinal cord hemisection and randomly divided into 2 groups, the control and experimental groups. The experimental group was administered a fifteen minutes session of pulsed electromagnetic field once a day, five days a week. In order to observe the effect of these pulsed electromagnetic fields, this study observed the BDNF expression in the rat's lumbar spinal cord and the H&E staining in the gastrocnemius at 3, 7, 14, 21 days group after spinal cord hemisection. The results of this showed that the immunoreactivity of the BDNF in the rat's spinal cord gradually increased in each group. At 21 days, there is a significant difference between the control and experimental groups. The morphological shape of the gastrocnemius was gradually changed from 3days to 21days, and the gastrocnemius at 21 days was significantly degraded. However, the experimental group showed a slightly more organized gastrocnemius than the control group at 21days. The Results of this study suggest that pulsed electromagnetic field application decreases the degeneration of a rat's gastrocnemius morphology, and increases the immunoreactivity of the BDNF in the rat's spinal cord after spinal cord hemisection.

Keywords : pulse electromagnetic fields, BDNF, spinal cord hemisection

1. Introduction

Spinal cord injury can cause motor abnormality or sensory disturbance depending on the injury site, it leads to various limitations in daily life due to complications and the limiting of daily motions. In particular, it accompanies permanent disability by causing not only the loss of motor and sensory functions below the injured site but also by amyotrophy [1].

In Korea, the largest cause of spinal cord injury is traffic accident (42.7%), followed by falling accidents (19.4%), which is similar to the international injury pattern (Bong-Ok Kim, 1984). About 60% of those who suffer serious motor injuries such as spinal cord injury need a certain degree of assistance for basic daily life [2,3].

These disabilities affect not only the private life of victims, but also their relationships with their families and colleagues, causing problems in functioning socially. Thus, spinal cord injuries that cause various disabilities need a rehabilitation process and the goal of rehabilitation is to return them to a social life that is as close to normal as possible.

Pulsed Electromagnetic Fields (PEMF) are being used for pain control and treatment of degenerative diseases, particularly for accelerating the repair of fractured or damaged tissues [4]. Many studies on PEMF have reported that it is effective in the stimulation of descending inhibition paths resulting in an increase of central beta-endorphin production, hyperpolarization at motor nerve terminals leading to muscular relaxation [5, 6], and the stimulation of chondrogenesis [5].

There are few studies on the effects of stimulants such as PEMF on the expression of neurotrophic factors. Thus, this study was conducted to investigate the effects of PEMF on the expression of neutrotrophic factors in rats after spinal cord hemisection. This study will partially reveal the mechanism by which PEMF acts in the recovery of motor functions in patients with spinal cord injury.

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2. Study Method

2.1. Laboratory Animals

Spraque-Dawley masculine rats aged 8-10 weeks with a weight of 250-300 g which had lived in a standard cage and had no neurological problems were used. After selecting the laboratory animals that had no neuromotor problems, laminectomy and hemisection were performed. The animals that scored 0-2 in the modified Tarlov test which is a neuromotor behavior test at 4 days after hemisection were selected and used for the experiment.

2.2. Experimental Method

The laboratory animals that were judged to be appropriate for this experiment were randomly divided into a control group (n=12) and an experimental group (n=12), and test were performed on each group at 3 days (n=3), 7 days (n=3), 14 days (n=3), and 21 days (n=3).

The control group consisted of laboratory animals that did not receive any treatment after inducement of spinal cord injury and the experimental group consisted of laboratory animals to which PEMF was applied after the inducement of spinal cord injury.

For PEMF, we used a Diapulse treatment machine (Diapulse Corp., America) at a frequency of 27.12 MHz, a pulse rate of 80-600 times per second, a pulse width of 65 μs, an output range per pulse of 293-975 W, aduty cycle of 0.5-3.9%, and with quadrangle pulses. For this experiment, a pulse rate of 300 times per second, a pulse width of 65 μs, and a pulse output of 450 W were applied for 15 minutes per day.

2.3. Histological Examination

After the experiment, the animals in each group were sacrificed by heart perfusion (0.9% NaCl). After the fixing of tissues (4% paraformaldehyde, pH 7.4), the gastrocnemiuses of the spinal cord and right lower limb were extracted.

For the spinal cord tissues, after posterior fixing for 12 hours (4% paraformaldehyde, pH 7.4), the tissues were quickly frozen to −30 °C. The tissues on the coronal plane were cut down to the thickness of 30 μm using a microtome (Leica SM 2000R) and treated with 0.01 mol phosphate buffer (PB). Then a immunohistochemical procedure was carried out.

For the gastrocnemius, posterior fixing was performed for 12 hours, and the general tissue making process of dehydration, clearing, and paraffin embedding were performed for the production of histologic sections. The histologic sections were cut to a 10 μm thickness using a microtome and slides were made with them, these were dried in a dryer (C-SLS, Changshin Science Co., Korea) at 40 °C for one day before Hematoxylin-Eosin staining.

2.4. Data Processing

For morphological and immunohistochemical observation of the tissues, an optical microscope ScanScope CS (Aperio Technologies, USA) was connected to a PC and image analysis was performed using Aperio ImageScope v9.1.19.1571 (Aperio Technologies, USA). For morphological observations, the radiologic changes of the tissues were analyzed after 3, 7, 14, and 21 days. To calculate the BDNF immunoreactive nerve cells, 20 sheets of sections were selected from the spinal cord of each animal and the number of cells on the optical microscope image were counted using Photoshop CS3 Extended for windows. Statistical analysis was performed using SPSS Win 12.0. For comparison by period, an independent sample t-test was used, and for comparison of the immunohistochemical expression patterns, one-way ANOVA was performed. When a significant difference was found by one-way ANOVA, Duncan's multiple range test was performed to confirm the differences between the groups. The statistical significance level for this study was set to .05.

3. Results

3.1. Immunohistochemical Analysis of BDNF Expression

To observe the effects of the application of PEMF in the control group and experimental group on the expression of BDNF in ratswith experimentally induced unilateral spinal cord injury, the laboratory animals that showed a score of 0-2 in the modified Tarlov test at 4 days after operation were selected. Then, an immunohistochemical method was applied to the radiocarpal ampullary region using the spinal cords of rats which were separated on day 3, 7, 14, and 21.

3.1.1. Comparison by application period

The experimental group showed an increasing trend measured by the modified Tarlov test: 3358.90 ± 871.80 on day 3, 4817.85 ± 1458.61 on day 7, 6437.25 ± 1589.91 on day 14, and 7159.05 ± 2633.28 on day 21. One way layout dispersion analysis was conducted to verify the statistical significance of the difference between the groups by application period, the difference was found to be significant with a P value of .000 (F = 18.716, p < .05). For verification, Duncan's multiple range test was conducted, and significant differences were found between the 3 day group and the 7 day, 14 day, and 21 day groups, and also between the 7 day group and the 14 day group.

Table 1. The change of BDNF expression

unit: pixel

Group	3 days	7 days	14 days	21 days
Experimental	3358.90 ± 871.80	4817.85 ± 1458.61*	6437.25 ± 1589.91*	7159.05 ± 2633.28*
Control	3468.30 ± 1331.72	3785.90 ± 1017.79	4387.50 ± 1176.22*	4846.30 ± 1646.45*

Experimental group : Pulsed electromagnetic fields application group at 3, 7, 14 and 21 days after spinal cord injury.
Control group: Breeding group at 3, 7, 14 and 21 days after spinal cord injury.
All values are expressed as a mean ± standard error. Values with different superscripts in the same column are statistically significant ($p < .05$) by Duncan's multiple range test.

However, no significant difference was found between the 14 day and 21 day groups.

The control group showed an increasing trend after the modified Tarlov test: 3468.30 ± 1331.72 on day 3, 3785.90 ± 1017.79 on day 7, 4387.50 ± 1176.22 on day 14, and 4846.30 ± 1646.45 on day 21. The differences were significant with a p-value of .007 (F = 4.386, $p <$ .05). Duncan's multiple range test for verification showed significant differences between the 3 day group and the 14 day and 21 day groups, and between the 7 day group and the 21 day group. However, no significant differences were found between the 3 day group and the 7 day group, between the 7 day group and the 14 day group, and between the 14 day group and the 21 day group (Table 1).

3.1.2. Comparison by groups

The comparison between control group and experimental group by period did not find any significant difference on day 3 at a significance level of .941 (F = 0.61, $p >$ .05). However, significant differences were found on day 7, 14, and 21 at significance levels of .043 (F = 3.321, $p <$ .05), .000 (F = 9.123, $p <$ .05), and .009 (F = 5.177, $p <$ .05), respectively. The verification did not show significant differences in all groups on day 3, but significant differences were found on day 7, 14, and 21 (Fig. 1).
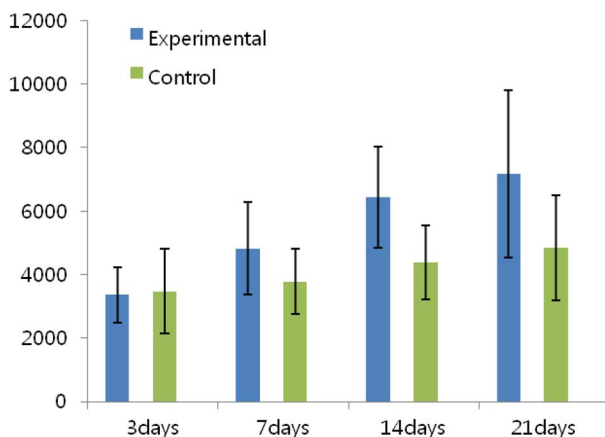
Fig. 1. (Color online) The change in BDNF expression.

3.2. Morphological comparison of tissues through Hematoxylin-Eosin staining of the gastrocnemius

To investigate the effects of the application of vibration and the action of the pulsed electromagnetic field after inducing unilateral spinal cord injury through surgical methods, members of the experimental group and the control group were sacrificed on day 3, 7, 14, and 21. Thenm their gastrocnemiuses were removed and stained with the Hematoxylin-Eosin staining and the following results were obtained. The morphology of the muscles was observed on day 3 after the application of the pulsed electromagnetic field to the animals with induced spinal cord injury, no significant difference was found between the control and experimental groups. The muscles showed a normal morphology. On day 7, there was no significant morphological difference between the two groups, but the muscular arrangement became distorted by atrophy and the gaps between muscle fibers and epimysiums gradually widened. Moreover, a similar degree of nuclei loss was observed. Observation of the morphology of muscles in the experimental group on day 14 found a slightly improved distortion of muscular arrangement and gaps between muscle fibers and epmysiums compared to day 7, but the loss of nuclei did not increase. In the case of the control group, however, the gaps between muscle fibers and perimysiums widened and the muscular arrangement was much more distorted and the loss of nuclei was greater compared to the experimental group. On day 21, the experimental group showed distortion of the muscular arrangement, a few more gaps between muscle fibers and epimysiums, and a slightly increased loss of nuclei. In the case of the control group, however, the muscular arrangement had completely broke down, the gaps between muscle fibers and epimysium had increased considerably compared to day 14. In particular, the connections of muscle tissues were broken and the loss of nuclei had greatly increased (Fig. 2, 3).

4. Discussion

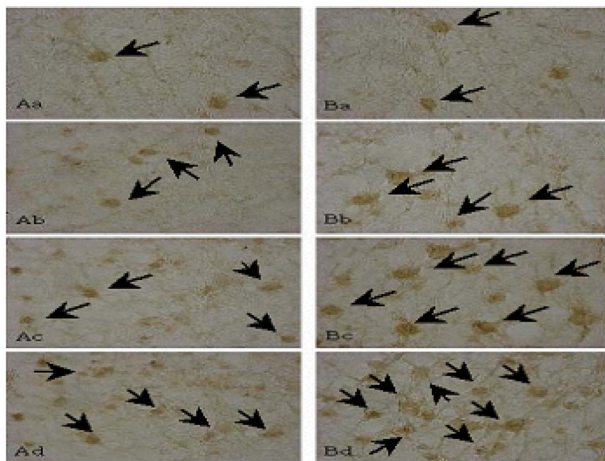The present study investigated the effects of PEMF

Fig. 2. (Color online) The immunohistochemical reactivity of BDNF on the spinal cord after pulsed electromagnetic field application in rats.
Aa: Immunostained photographs for BDNF on the 3rd day in the control group
Ab: Immunostained photographs for BDNF on the 7th day in the control group
Ac: Immunostained photographs for BDNF on the 14th day in the control group
Ad: Immunostained photographs for BDNF on the 21th day in the control group
Ba: Immunostained photographs for BDNF on the 3rd day in the experimental group
Bb: Immunostained photographs for BDNF on the 7th day in the experimental group
Bc: Immunostained photographs for BDNF on the 14th day in the experimental group
Bd: Immunostained photographs for BDNF on the 21th day in the experimental group
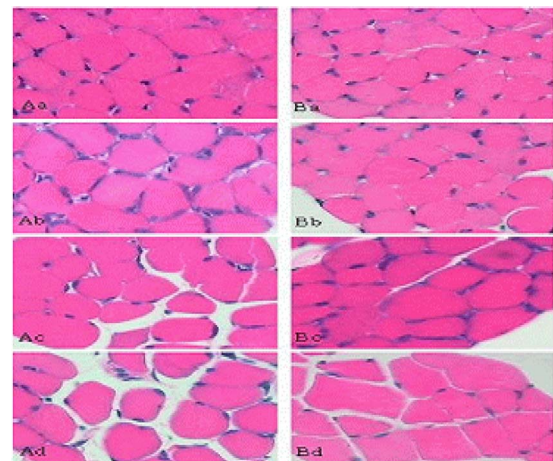

Fig. 3. (Color online) The histological changes of muscle fiber after pulsed electromagnetic field application in rats.
Aa: H&E stained photographs on the 3rd day in the control group
Ab: H&E stained photographs on the 7th day in the control group
Ac: H&E stained photographs on the 14th day in the control group
Ad: H&E stained photographs on the 21th day in the control group
Ba: H&E stained photographs on the 3rd day in the experimental group
Bb: H&E stained photographs on the 7th day in the experimental group
Bc: H&E stained photographs on the 14th day in the experimental group
Bd: H&E stained photographs on the 21th day in the experimental group

treatment on the recovery of patients with spinal cord injury by observing functional changes, morphological changes of lower limb muscles, and the expression of BDNF in the spinal cord in laboratory animals.

In studies related to the spinal cord injury inducement in rats, Erchbamer et al. (2006) found no significant differences in recovery by the application of an environmental program and a physical training program when incomplete injuries were induced by shock injury. Ho-Yeon Lee et al. (2003) studied the recovery of the somatic sensory evoked potential using the excision of the spinal cord in a spinal injury model. They also used the spinal cord injury model [12] with hemisection to analyze the gait recovery after spinal cord injury [9], the recovery of locomotion [10], the graphical analysis of motor recovery modes [9], and the expression of neurotrophic matters in the spinal cord motor nerves.

Yeong-Il Lee et al. (2007) reported a reduction in the expression of BDNF in the spinal cord of rats which dis-

agrees with the results of the control group in this study. However, Zvarova et al. (2007) reported an increase in the expression of BDNF after spinal cord injury which agrees with the findings of this study.

Xiao et al. (2007) reported that the expression of BDNF increased temporarily after transaction of the spinal cord. In a study on the expression of BDNF after hemisection of the lower thoracic cord, Ernfors et al. (1993) reported that neurotrophic factors in the radiocarpal motor nerves rapidly decreased after spinal cord injury and that BDNF expression was affected by the activity of the spinal cord circuit and the integration of the spinal cord pathway and upper pathway, and also that the neurotrophic factors affected the anatomical and functional reorganization of motor circuits and indicated the possible recovery of walking ability and holding function after spinal cord injury.

Compared to the control group who showed low expression speed depending on the period, the experimental

group showed higher expression speed over time. This result appears to be associated with the effect of the application of PEMF.

The observation of morphological changes through Hematoxylin-Eosin staining suggests that physiological stimulation by PEMF changes the functions of cells in the bones and nervous tissues, and this appears to affect the regeneration ability of these tissues [17] which seems to have an effect over time.

It was reported that the application of PEMF to normal limbs does not bring about noticeable increase in the phalangeal blood flow and does not affect the blood flow for treatment of bone malunion. It was also reported that the test results for the total blood flow of bones as well as skin and muscles with plethysmography including occlusion showed that the increase of blood flow in bones was around 10% of the total and this affected the blood flow to all tissues [18].

A study on the effects of PEMF on the regeneration of facial nerves using rats reported that the transaction of facial nerves affected the initial regeneration [17]. Critical factors in the PEMF inducing nerve regeneration are actual intensity and the electromagnetic field used. Experiments using electrostatic or electromagnetic fields did not show differences in nerve regeneration. For peripheral nerves, PEMF restricts fibroplasia in the injured region, activates the more effective enzymes for protein synthesis, and causes advantageous axoplasmic flow and electrochemical changes of nerves. It also improves the predation activity of Schwann cells and is involved in the control of the nerve growth factor of Schwann cells to a certain degree.

It was claimed that PEMF increases protein synthesis during nerve regeneration and the injury level of nerve cell bodies decreases during the transformation phase of the injured cells [19]. Loss of skeletal muscles after spinal cord injury rapidly decreases the total muscular surface by up to 25% within the first 6 weeks [20]. Such severe muscular atrophy is classified into the decrease of muscular protein synthesis and the acceleration of the decrease of muscular protein [21]. The denervated model of animals shows the loss of muscular protein within the first 24 hours after neurotomy. However, the cell-level mechanism of this early atrophy process as indicated by the decrease of proteins is not known.

Maria *et al.* (2007) reported that the synthesis of metallothionein, the increased production of each protein, localization of proteins in the early stages of spinal cord injury increases at the terminal region of cells and have the effect of protecting the skeletal muscles.

This study confirmed that PEMF treatment delayed amyotrophy after spinal cord injury, restored function, and increased the expression of nerve regeneration. However, we could not find the answer to the question of whether this effect brings about the complete recovery of functions along with the recovery of nerves.

Although PEMF after spinal cord injury has effects on functional recovery, nerve regeneration, and prevention of amyotrophy, our understanding of the effects of its application to patients in a clinical setting is still insufficient. Therefore, many studies on the functional recovery of patients in a clinical setting will be necessary in the future.

5. Conclusion

This study examined the expression of the neurotrophic factor BDNF which plays an important role in nerve regeneration on day 3, 7, 14, and 21 for a group of rats that had been grown in standard cages with no experimental intervention after the hemisection of the thoracic cord and a group of rats to which PEMF was applied after the hemisection of the thoracic cord. The observation of muscular tissues in the gastrocnemius showed that PEMF was effective for increasing the expression of BDNF which is known to play a key role in nerve recovery in rats after hemisection of the spinal cord. Furthermore, PEMF was found to be effective in the prevention of amyotrophy due to spinal cord injury.

In the future, more clinical studies with patients are necessary, and the results of this study are expected to be used as basic data for these studies.

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