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Original Article

Effects of Acupuncture at GB30, GB34, and BL40 on Functional Recovery after Sciatic Crushed Nerve Injury in Rats

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Background: Peripheral nerve injuries are a commonly-encountered clinical problem and often result in a chronic pain and severe functional deficits.
Objectives: The aim of this study was to evaluate the effects of acupuncture on the descending pain and the recovery of the locomotor function that follows sciatic crushed nerve injury in rats.
Method: In order to assess the effects of acupuncture on the descending pain and functional recovery, we investigated the walking track analysis, brain-derived neurotrophic factor (BDNF) and its receptor tyrosine receptor kinase B (TrkB) expression in the sciatic nerve, and on the expressions of c-Fos and nitric oxide synthase in the paraventricular nucleus (PVN) of the hypothalamus and in the ventrolateral periaqueductal gray (vlPAG) region resulting from sciatic crushed nerve injury in rats.
Results: Acupuncture treatment at Huantiao (GB30), Yanglingquan (GB34), and Weizhong (BL40) facilitated functional recovery. C-Fos and nitric oxide synthase expressions in the brain and BDNF and TrkB expressions in the sciatic nerve were decreased by acupuncture treatment. The most potent effects of acupuncture were observed at the GB30 acupoint.
Conclusion: It is possible that acupuncture can be used for pain control and functional recovery from sciatic nerve injury.

Key Words : Sciatic crushed nerve injury, Acupuncture, Functional recovery, Huantiao, Yanglingquan, Weizhong

Introduction

Acupuncture has been widely used for pain control. However, the mechanism of pain inhibition by acupuncture is still unclear and evaluation of the physiological effects of acupuncture is limited[1,2]. Many studies have indicated that objective physiological measurements are associated with the determination of the effects of acupuncture. Physiological changes of the acupuncture can be detected in the central nervous system[3,4]. Clinically, acupuncture and moxibustion have been used for relieving peripheral nerve disorder related symptoms such as pain, dysesthesia, numbness and weakness[5].

Peripheral nerves are often damaged by crush, compression, stretching, contusion, ischemia, and various diseases[6]. In the sciatic crushed nerve injury, the affected limb displays characteristics of painful neuropathy such as hyperalgesia, pain-related gait, and swelling[6]. These features are considered the abnormal responses to peripheral stimuli, reflecting the changes of nociceptive neural transmission in the central nervous system.

C-Fos expression can serve as a marker of neuronal

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excitation and injury[7]. It was reported that expression of c-Fos was induced by various stressful stimuli, such as immunological challenges[8,9], hemorrhage[8,10], noise[8,11], immobilization[12], pedal shock stimulus[13,14], and pain[15,16]. According to previous reports, up-regulation in the ventrolateral periaqueductal gray (vlPAG), nucleus raphe magnus (NRM), and dorsal raphe nucleus (DR) suggested activation of neurons[15,16].

The free radical nitric oxide (NO) is endogenously synthesized from L-arginine by nitric oxide synthase (NOS). Three isoforms of NOS have been characterized: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). Nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) is a histochemical marker specific for NOS in the central nervous system (CNS). Neurons containing NADPH-d were reported to be relatively resistant to various toxic insults and neurodegenerative disorders[17]. NOS has been implicated in the pathogenesis of many CNS diseases.

Neurotrophins are a family of proteins regulating the survival, differentiation, and maintaining of function of different neuron populations. The most prominent members of the mammalian neurotrophin family are the nerve growth factor (NGF) and the brain-derived neurotrophic factor (BDNF). BDNF is the most abundant neurotrophin in the CNS, and it is closely involved in neuronal cell survival and maintenance as well as in neural transmission[18].

Acupuncture is an important field of oriental medicine, and most diseases have been treated with it for several thousand years. Especially, *Huantiao* (GB30), *Yanglingquan* (GB34), *Weizhong* (BL40) acupoints are known to have good effects on lower back pain, sciatica[19,20]. These acupoints were also selected because experimental animals are small but we can locate these particular acupoints easily. Therefore in the present study, we investigated the effects of acupuncture on the functional recovery, expressions of c-Fos and NOS in the brain, and those of BDNF and TrkB in the sciatic nerve following sciatic crushed nerve injury in rats.

Materials and Methods

1. Experimental animals

Male Sprague-Dawley rats weighing 220 ± 10 g (7 weeks of age) were used. The experimental procedures were performed in accordance with the animal care guidelines of National Institutes of Health (NIH) and the Korean Academy of Medical Sciences. The animals were housed at a controlled temperature (20 ± 2℃) and maintained under light-dark cycles, each consisting of 12 h of light and 12 h of darkness (lights on from 07:00 to 19:00 h), with food and water made available *ad libitum*. The rats were randomly divided into six groups (n=8 in each group): (A) the sham operation group, (B) the sciatic crushed nerve injury group, (C) the sciatic crushed nerve injury and *Huantiao* (GB30)-acupunctured group, (D) the sciatic crushed nerve injury and *Yanglingquan* (GB34)-acupunctured group, (E) the sciatic crushed nerve injury and *Weizhong* (BL40)-acupunctured group, and (F) the sciatic crushed nerve injury and non-acupoint-acupunctured group.

2. Surgical procedure

To induce crush injury on the sciatic nerve in rats, the previously described surgical procedure was performed[21]. In brief, the right sciatic nerve was exposed by incision on the gluteal muscle under Zoletil 50® anesthesia (50 mg/kg; Virbac Laboratories, Carros, France). The sciatic nerve was carefully exposed and crushed for 30 sec using a surgical clip (Pressure: 125 g; Fine Science Tools Inc., San Francisco, USA). The crushed location is between the sciatic notch and the point of trifurcation. Afterwards, the surgical wound was sutured and recovered. In the sham operation rats, the sciatic nerve was exposed, but the nerve was not crushed.

3. Acupuncture treatment

Starting on the day the surgery was given, daily acupuncture treatment was performed between 9:00 and 10:00 AM for 10 consecutive days. The rats in the acupuncture groups were lightly immobilized, and acupuncture needles were inserted to a depth of 3 mm at their respective acupoints bilaterally, turned at a rate of two spins per second for 30 sec[22,23]. These experiments were performed by skilled specialists using stainless steel acupuncture needles (Dongbang Co. 15 mm length×0.16 mm diameter). Acupoint *Huantiao* (GB30) located at the lateral 1/3 and medial 2/3 of the distance between the sacral hiatus and the greater trochanter of the femur. *Yanglingquan* (GB34) located in the depression anterior and inferior to the fibula capitulum. *Weizhong* (BL40), located in the center of the popliteal space. The non-acupoint was located in the flank.

4. Walking track analysis

For the assessment of motor nerve recovery, walking track analysis was carried out as described in previous reports with minor modifications[24](Fig. 1). The rats were allowed conditioning trials in an 8 × 66 cm walking track with a piece of white paper at the bottom of the track. The hind feet were dipped in red ink, leaving prints on the white paper. The print length (PL), toe spread (TS), and intermediary toe spread (IT) were thus obtained. In general, the maximal value was adopted for each measurement, and the data were recorded with the prefix E for the operated side and N for the normal, non-operated side. The sciatic function index (SFI), an indicator of the degree of nerve dysfunction, varies from 0 to -100, with 0 corresponding to normal function and -100 to complete dysfunction. It was calculated by the following formula in Fig. 1[24].

5. Tissue preparation

The animals were sacrificed on the 10th day following the sham operation, the sciatic crushed nerve injury group, the sciatic crushed nerve injury and acupunctured group. The animals were anesthetized using Zoletil 50®(10 mg/kg, i.p.; Virbac Laboratories, Carros, France), transcardially perfused with 50 mM phosphate-buffered saline(PBS), and fixed with a freshly prepared solution consisting of 4% paraform-aldehyde in 100 mM phosphate buffer (PB, pH 7.4). The brains were dissected and postfixed in the same fixative overnight and transferred into a 30% sucrose

$$SFI = -38.3\left(\frac{EPL-NPL}{NPL}\right) + 109.5\left(\frac{ETS-NTS}{NTS}\right) + 13.3\left(\frac{EIT-NIT}{NIT}\right)$$

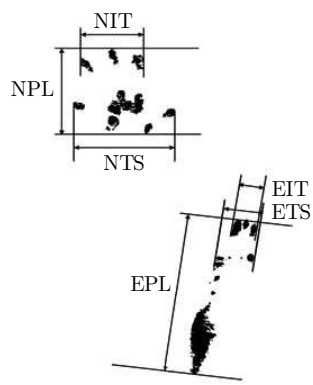


Fig. 1. Walking track analysis.

After sciatic crushed nerve injury in rats, paired parameters of the print length (PL), toe spread (TS), and intermediary toe spread (IT) were calculated. (E) Experimental side, (N) normal side, (EPL) experimental print length, (NPL) normal print length, (ETS) experimental toe spread, (NTS) normal toe spread, (EIT) experimental intermediary toe spread, (NIT) normal intermediary toe spread, (SFI) sciatic functional index.

solution for cryoprotection. Coronal sections of 40 μm thickness were made with a freezing microtome (Leica, Nussloch, Germany).

6. C-Fos immunohistochemistry

For immunolabeling of c-Fos in the PVN, vlPAG, c-Fos immunohistochemistry was performed by the previously described method[25]. Free-floating tissue sections were incubated overnight with rabbit anti-c-Fos antibody (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and the sections were then incubated for 1 h with biotinylated anti-rabbit secondary antibody (1:200; Vector Laboratories, Burlingame, CA, USA). The sections were subsequently incubated with avidin-biotin-peroxidase complex (Vector Laboratories) for 1 h at room temperature. Immunoreactivity was visualized by incubating the sections in a solution consisting of 0.05% 3,3-diaminobenzidine (DAB) and 0.01% H_2O_2 in 50 mM Tris-buffer (pH 7.6) for approximately 3 min. The sections were then washed three times with PBS and mounted onto gelatin-coated slides. The slides were air-dried overnight at room temperature, and cover slips were mounted using Permount®.

7. Western blot analysis

Tissue samples harvested from the sciatic nerve were lysed in the protein lysis buffer containing 50 mM Tris-HCI (pH 7.5), 150 mM NaCl, 0.5% deoxycholic acid, 1% nonidet-P40 (NP40), 0.1% sodium dodecyl sulfate (SDS), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 100 μg/ml leupeptin. Protein concentration was measured using a Bio-Rad colorimetric protein assay kit (Bio-Rad). Protein of 50 μg was separated on SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane (Schleicher & Schuell GmbH, Dassel, Germany). Mouse anti-actin antibody (1:1000; Santa Cruz Biotech, Santa Cruz, CA, USA) and rabbit anti-BDNF antibody (1:1000; Santa Cruz Biotech) were used as primary antibodies. Horseradish peroxidase-conjugated anti-mouse antibody (1:1000; Santa Cruz Biotech) for Actin, and anti-rabbit antibody (1:2000; Santa Cruz Biotech) for BDNF were used as secondary antibodies. Band detection was performed using as enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biotech GmbH, Freiburg, Germany). The final amount of western blot product for BDNF expression was calculated densitometrically using Imaging-Pro®Plus (Media Cybernetics Inc., Silver Spring, MD, USA).

8. Data analyses

The data are expressed as the mean ± SD. For comparisons among the groups, one-way ANOVA and Duncan's post-hoc test were performed with $p < 0.05$ as an indication of statistical significance.

Results

1. Effect of acupuncture on the SFI

We measured SFI using a walking track analysis to assess functional recovery after sciatic crushed nerve injury in rats. The mean SFI in each group was calculated on the 3rd, 6th, and 9th day after causing sciatic crushed nerve injury.

The mean value of SFI in the A group was -5.34±9.14 on the 3rd day, -6.40±10.41 on the 6th day, and -6.74±3.76 on the 9th day from the commencement of the experiment. The mean value of SFI in the B group was -104.91±4.83 on the 3rd day, -77.88±1.86 on the 6th day, and -83.54±2.64 on the 9th day. The mean value of SFI in the C group was -104.91±4.83 on the 3rd, -70.61±5.97 on the 6th, and -51.72±6.32 on the 9th day of the experiment. The mean value of SFI in the D group was -104.91±4.83 on the 3rd, -74.47±4.16 on the 6th, and -74.07±2.74 on the 9th day. The mean value of SFI in the E group was -104.91±4.83 on the 3rd, -71.08±4.46 on the 6th, and -71.08±3.05 on the 9th

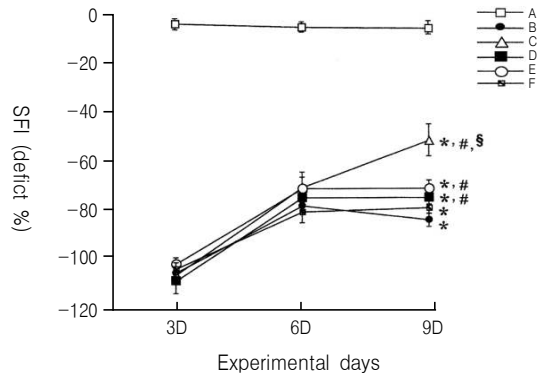


Fig. 2. Effect of acupuncture on the sciatic functional index (SFI).

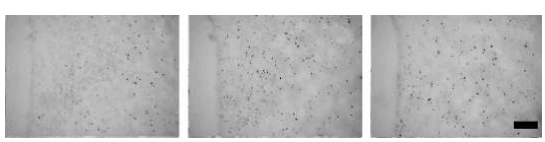
The values are represented as the mean ± SD. (□) sham operation group, (●) sciatic crushed nerve injury group, (△) sciatic crushed nerve injury and GB30-acupunctured group, (■) sciatic crushed nerve injury and GB34-acupunctured group, (○) sciatic crushed nerve injury and BL40-acupunctured group, (□) sciatic crushed nerve injury and non-acupoint-acupunctured group. * $P < 0.05$ compared to the 3rd day. # $P < 0.05$ compared to the sciatic crushed nerve injury group. § $P < 0.05$ compared to the sciatic crushed nerve injury and BL40-acupunctured group.

day. The mean value of SFI in the F group was -104.91±4.83 on the 3rd day, -80.17±4.52 on the 6th day, and -78.54±4.41 on the 9th day after commencement of the experiment (Fig. 2).

From the above results, the sciatic crushed nerve injury decreased the SFI value while acupuncture treatment had a tendency to increase recovery from sciatic crushed nerve injury in the treatment groups. On the 9th day from the commencement of the experiment, GB30-acupuncture showed significant increase of SFI.

2. Effect of acupuncture on c-Fos expression in the PVN

The number of c-Fos-positive cells in the PVN was 28.92±2.09 in the A group, 112.91±11.60 in the



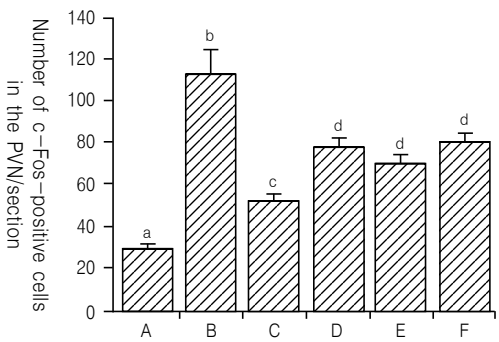


Fig. 3. Effect of acupuncture on c-Fos expression in the paraventricular nucleus (PVN).

Upper: Photographs of the c-Fos-positive cells. The scale bar represents 100 μm. (A) Sham operation group, (B) sciatic crushed nerve injury group, (C) sciatic crushed nerve injury and GB30-acupunctured group.
Lower: Mean number of c-Fos-positive cells in each group. (A) Sham operation group, (B) sciatic crushed nerve injury group, (C) sciatic crushed nerve injury and GB30-acupunctured group, (D) sciatic crushed nerve injury and GB34-acupunctured group, (E) sciatic crushed nerve injury and BL 40-acupunctured group, (F) sciatic crushed nerve injury and non-acupoint-acupunctured group. The values are represented as the mean ± SD.

B group, 52.54±2.90 in the C group, 78.50±3.72 in the D group, 70.67±3.39 in the E group, and 81.09 ±3.85 in the F group(Fig. 3).

The present results showed that sciatic crushed nerve injury increased c-Fos expression in the PVN and that acupuncture suppressed the sciatic crushed nerve injury-induced increase in the c-Fos expression in the PVN. Acupuncture at GB30 most potently suppressed c-Fos expression in the PVN.

3. Effect of acupuncture on c-Fos expression in the vlPAG

The number of c-Fos-positive cells in the vlPAG was 7.92±0.68 in the A group, 48.42±2.33 in the B group, 20.75±1.53 in the C group, 33.67±2.18 in the D group, 34.25±1.60 in the E group, and 37.08±2.25 in the F group (Fig. 4).

The results show that sciatic crushed nerve injury increased c-Fos expression in the vlPAG and that acupuncture suppressed c-Fos expression in the vlPAG. Acupuncture at GB30 most potently suppressed c-Fos expression in the vlPAG.

4. NOS expression in the PVN

The number of NADPH-d-positive cells in the PVN was 76.18± 11.79 in the A group, 177.00±9.61 in the B group, 108.36±4.20 in the C group, 141.20±12.45 in the D group, 136.64 ±7.53 in the E group, and 139.82±6.13 in the F group (Fig. 5).

The results show that sciatic crushed nerve injury increased NOS expression in the PVN and that acupuncture suppressed NOS expression in the PVN. Acupuncture at GB30 most potently suppressed NOS expression in the PVN.

5. NOS expression in the vlPAG

The number of NADPH-d-positive cells in the vlPAG was 24.27± 3.57 in the A group, 65.09±2.73 in the B group, 36.83±2.69 in the C group, 51.33± 3.86 in the D group, 42.73±3.78 in the E group, and 52.67±4.32 in the F group (Fig. 6).

The present results show that sciatic crushed nerve injury increased NOS expression in the vlPAG and that acupuncture suppressed NOS expression in the vlPAG. Acupuncture at GB30 and BL 40 potently suppressed NOS expression in the vlPAG.

6. Effect of acupuncture on BDNF expression

Photomicrographs of BDNF expression in the sciatic nerve are shown in Fig. 7. The expression of

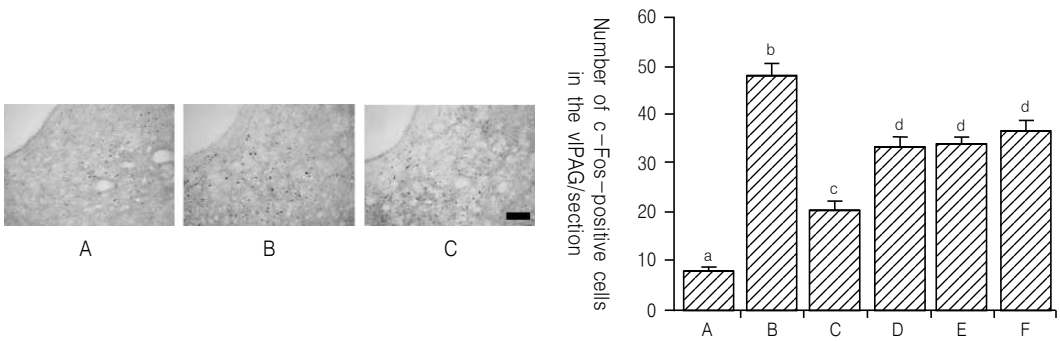


Fig. 4. Effect of acupuncture on c-Fos expression in the ventrolateral periaqueductal gray (vlPAG).

Upper: Photographs of the c-Fos-positive cells. The scale bar represents 100 μm. (A) Sham operation group, (B) sciatic crushed nerve injury group, (C) sciatic crushed nerve injury and GB30-acupunctured group.
Lower: Mean number of c-Fos-positive cells in each group. (A) Sham operation group, (B) sciatic crushed nerve injury group, (C) sciatic crushed nerve injury and GB30-acupunctured group, (D) sciatic crushed nerve injury and GB34-acupunctured group, (E) sciatic crushed nerve injury and BL40-acupunctured group, (F) sciatic crushed nerve injury and non-acupoint-acupunctured group. The values are represented as the mean ± SD.

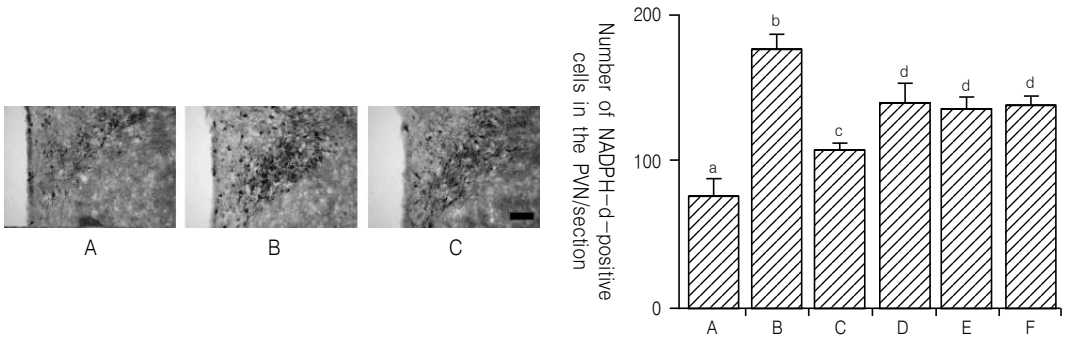


Fig. 5. Effect of acupuncture on NADPH-d expression in the paraventricular nucleus (PVN).

Upper: Photographs of the NADPH-d-positive cells. The scale bar represents 100 μm. (A) Sham operation group, (B) sciatic crushed nerve injury group, (C) sciatic crushed nerve injury and GB30-acupunctured group.
Lower: Mean number of NADPH-d-positive cells in each group. (A) Sham operation group, (B) sciatic crushed nerve injury group, (C) sciatic crushed nerve injury and GB30-acupunctured group, (D) sciatic crushed nerve injury and GB34-acupunctured group, (E) sciatic crushed nerve injury and BL40-acupunctured group, (F) sciatic crushed nerve injury and non-acupoint-acupunctured group. The values are represented as the mean ± SD.

BDNF in the sciatic nerve was 1.00±0.05 in the A group, 1.58±0.24 in the B group, 1.08±0.30 in the C group, 1.22±0.38 in the D group, 1.25±0.31 in the E group, and 1.55±0.29 in the F group (Fig. 7).

These results show that sciatic crushed nerve injury enhanced and that acupuncture decreased BDNF expression in the sciatic nerve. Acupuncture at GB 30, GB34, and BL40 decreased BDNF expression in the sciatic nerve.

7. Effect of acupuncture on TrkB protein expression

Photomicrographs of TrkB expression are shown in Fig. 7. The expression of TrkB in the sciatic nerve was 1.00±0.23 in the A group, 1.94±0.36 in the B group, 1.13±0.34 in the C group, 1.48±0.33 in

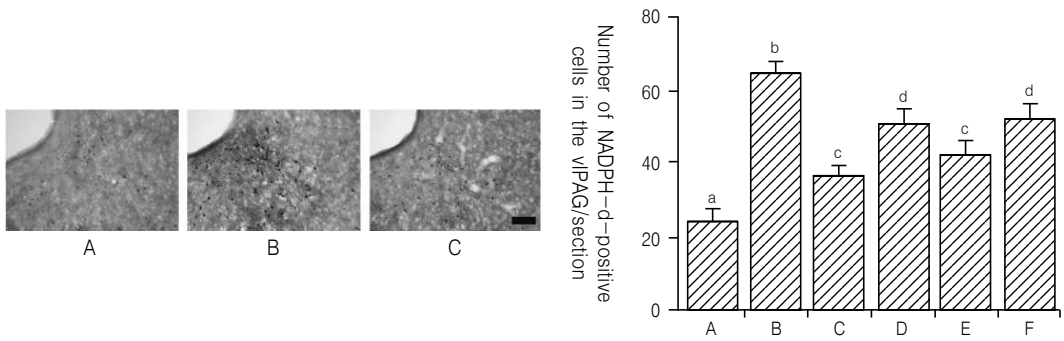


Fig. 6. Effect of acupuncture on NADPH-d expression in the ventrolateral periaqueductal gray (vlPAG).

Upper: Photographs of the NADPH-d-positive cells. The scale bar represents 100 μm. (A) Sham operation group, (B) sciatic crushed nerve injury group, (C) sciatic crushed nerve injury and GB30-acupunctured group.
Lower: Mean number of NADPH-d-positive cells in each group. (A) Sham operation group, (B) sciatic crushed nerve injury group, (C) sciatic crushed nerve injury and GB30-acupunctured group, (D) sciatic crushed nerve injury and GB34-acupunctured group, (E) sciatic crushed nerve injury and BL40-acupunctured group, (F) sciatic crushed nerve injury and non-acupoint-acupunctured group. The values are represented as the mean ± SD.

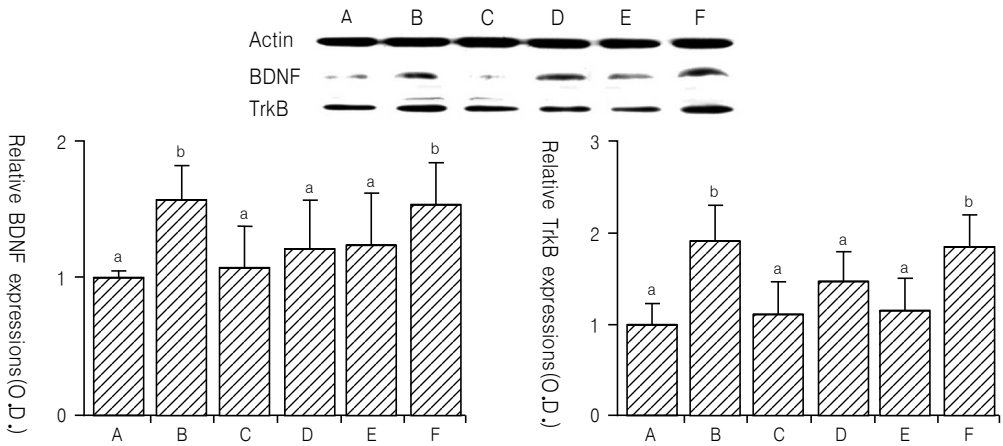


Fig. 7. Effects of acupuncture on BDNF and TrkB expression.

Upper: Representative expression of the protein level of BDNF, TrkB, and actin in the sciatic nerve.
Lower: Density of BDNF and TrkB protein expression in the sciatic nerve. (A) Sham operation group, (B) sciatic crushed nerve injury group, (C) sciatic crushed nerve injury and GB30-acupunctured group, (D) sciatic crushed nerve injury and GB34-acupunctured group, (E) sciatic crushed nerve injury and BL40-acupunctured group, (F) sciatic crushed nerve injury and non-acupoint-acupunctured group. Molecular weights of BDNF and TrkB = 15 kDa; Actin = 42 kDa.

the D group, 1.18±0.35 in the E group, and 1.89± 0.34 in the F group (Fig. 7).

The present results show that sciatic crushed nerve injury enhanced and that acupuncture inhibited TrkB expression in the sciatic nerve. Acupuncture at GB 30, GB34, and BL40 decreased TrkB expression in the sciatic nerve.

Discussion

Acupuncture is a meridian-based therapy, and needles are inserted into precisely defined, specific points on the body, each of which has distinct therapeutic actions[26]. When an acupoint is stimulated, treatment effects occur on the specific parts of the body along a particular meridian that contains this specific acupoint. Investigators have demonstrated that the nervous system and neurotransmitters respond to needling stimulation and electroacupuncture[27].

Damage to the nervous system caused by a transient sciatic crushed nerve injury often causes complicated changes in the CNS[28,29]. Many changes affecting the ascending facilitatory system and the descending inhibitory system occur within the CNS, resulting in the development of persistent pain. Treatment goals generally target alleviating pain and improving physical functions[30]. In the sciatic crushed nerve injury model serving as unilateral peripheral neuropathy, Vogelaar et al.[31] reported that when persistent pain still existed, the animals did not support their weight on the injured paw, due to compensation; sensory and motor reinnervation of the paw were fully established at 3 weeks after nerve injury. In the acute stage of sciatic crushed nerve injury, they exhibit flexion contracture of the toes and a curvature of the feet subjected to walk by their dorsum of the affected foot or to load their weight on the medial part of the affected foot. These observations might be due to compensatory immobilization to painful dysesthesia as well as neurological loss[31].

Therefore in the present study, we investigated the effects of acupuncture by measured SFI using a walking track analysis to assess functional recovery

after sciatic crushed nerve injury in rats. SFI value significantly decreased after the sciatic crushed nerve injury, while subsequent acupuncture treatment enhanced SFI value. On the 9th day after commencement of the experiment, acupuncture at GB 30 showed the most potent functional recovery effect, implying that GB30 is one of the most valuable acupoints curing for sciatic crushed nerve injury.

Expressions of c-Fos and NOS are commonly used to represent activation of neurons in the brain by external inputs, of which frontal cortex, thalamus, and PAG are key structures for the coordination of pain perception[32]. Among previous reports the levels of c-Fos were significantly increased in the rats following sciatic nerve ligation[33]. Expression of c-Fos was induced by painful stimuli such as formalin, noxious visceral stimulation and tail pinch[34,35,36]. C-Fos was also observed in the lateral reticular nucleus, which receives spinal afferents[37] and in the ventrolateral medulla which receives afferents from the nucleus tractus solitarius (NTS) and sends projections to the PVN[38]. Some studies suggested that c-Fos may be useful as a sensitive marker of the neuronal activation and plasticity following sciatic nerve injury[39,40]. Narita et al.[41] reported that the neuropathic pain-like state causes a substantial change in the expression of c-Fos in the rat brain, and practically the levels of c-Fos in the frontal cortex, thalamus, and PAG were significantly increased in the rat following sciatic nerve ligation.

Peripheral nerve lesions are well known to induce a dramatic increase of NOS expression in the affected neuronal cell bodies. NOS inhibitors can maintain the homeostasis of oxidative stress-related biomarkers, in neuronal cell bodies, thus facilitated the axonal regeneration[42]. Balaratnasingam et al.[43] demonstrated that NOS was up-regulated in a time-dependent manner after pressure elevation. High mRNA expression of iNOS began to be shown in the sciatic nerve injury, and treatment with the extract of Gingko biloba promoted the regeneration of nervous tissues, probably by inhibiting the expression of iNOS[44].

In this study, expressions of c-Fos and NOS in the vlPAG and PVN were increased following sciatic crushed nerve injury, reflecting severe peripheral pain causing neuronal activation in these brain areas. Acupuncture treatment significantly suppressed these expressions in the vlPAG and PVN, suggesting acupuncture alleviated severity of pain, and resulted in a decrease of neuronal activation in these brain areas.

BDNF is a 12.4-kDa basic protein initially isolated from pigs' brains and widely expressed in the nervous system. BDNF also plays a role in activity-dependent neuronal plasticity[45]. The exogenous administration of BDNF has protective properties for injured neurons and stimulates axonal regeneration[46]. Based on these properties, these molecules may be used as therapeutic agents for treating degenerative diseases and traumatic injuries of both the central and peripheral nervous system[45,46]. In a dramatic advance, the three members of the tropomyosin-related kinase (Trk) receptor tyrosine kinase family were shown to be a second class of neurotrophin receptors[47]. The neurotrophins directly bind and dimerize to these receptors, which results in activation of the tyrosine kinases present in their cytoplasmic domains. BDNF and NT-4 are specific for TrkB. TrkB must be capable of activating intracellular signaling pathways important for neuronal survival and differentiation[47]. Neuronal deficits are also generated by the loss of BDNF or TrkB[48,49]. Therefore BDNF and TrkB play major roles in neuronal deficits. The present results show that expressions of BDNF and TrkB increased following sciatic nerve injury. These results suggest that induction of sciatic crushed injury caused more BDNF and its receptor TrkB expressions to repair sciatic injury.

Yang et al.[50] reported that Ohyaksungi-san (Wuyaoshunqi-san) and electrical acupuncture have good effects on nerve regeneration after crush injury

in rat sciatic nerve. Ahn et al.[51] reported that electroacupuncture and Cervi Pantortichum Cornu pharmacopuncture may play a significant role in pain decrease and nerve regeneration after crush injury of sciatic nerve in rats. However, while many studies of acupuncture have focused on pain control, little is known about the effect of acupuncture on the painful neuropathy induced by sciatic crushed nerve injury.

In this study, it was shown that acupuncture at GB30, GB34, and BL40 increased SFI following the sciatic crushed nerve injury in rats. Acupuncture at GB30 showed the most potent recovery effect. The recovery mechanisms of SFI by acupuncture treatment are supposed to decrease c-Fos and NOS expressions in the brain, and BDNF and TrkB expressions in the sciatic nerve. Based on the present result, it is possible that acupuncture can be used for pain control and functional recovery from sciatic nerve injury.

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