Effects of Bee Venom and Sweet Bee Venom Acupuncture on Functional Recovery and c-Fos Expression in the Brain after Sciatic Crushed Nerve Injury in Rats

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**Background:** Peripheral nerve injuries are commonly encountered clinical problems and often result in severe functional deficit. Bee venom acupuncture has traditionally been used to treat several inflammatory diseases and chronic pain conditions.

**Objectives:** The aims of this study were to compare the effects of bee venom (general bee venom, BV) and sweet bee venom (allergen-removed bee venom, SBV) acupuncture on the recovery rate of locomotor function, the expression of brain-derived neurotrophic factor (BDNF) in the sciatic nerve, and the expression of c-Fos in the brain following sciatic crushed nerve injury in rats, and to evaluate differences due to administration areas.

**Method:** Walking track analysis, Western blot for BDNF and tyrosine receptor kinase B (TrkB), and immunohistochemistry for c-Fos were performed. In this study, comparative analyses of the effects of BV and SBV acupuncture in relation to administration sites, contralateral side or ipsilateral side, were conducted.

**Results:** In the present result, sciatic function index (SFI) in walking track analysis significantly decreased following sciatic crushed nerve injury. The expressions of BDNF and TrkB in the sciatic nerve increased after induction of sciatic crushed nerve injury. C-Fos expression in the ventrolateral periaqueductal gray (vlPAG) and paraventricular nucleus (PVN) also increased. BV and SBV acupuncture treatment improved the SFI in walking track analysis. Treatment with SBV at 1mg/kg showed more potent enhancing effect on SFI compared to BV. Treatment with 1mg/kg BV or 1mg/kg SBV acupuncture suppressed the BDNF and TrkB expression in the sciatic nerve. BV and SBV acupuncture treatment also suppressed c-Fos expression in the PVN and vlPAG regions. Treatment with SBV at 1mg/kg showed more potent suppressing effect on c-Fos expression compared to BV when injected into the contralateral side of the injured nerve. Generally we could not find significant difference in the effects between contralateral side and ipsilateral side of the injured nerve.

**Conclusion:** We have shown that BV and SBV acupuncture treatment can be used as the effective therapeutic modality to ameliorate the symptoms of sciatic crushed nerve injury.

**Key Words:** Sciatic nerve lesion, Bee venom, Walking, Brain-derived neurotrophic factor, TrkB receptor, C-Fos genes.

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**Introduction**

Peripheral nerve injuries are commonly encountered in clinical practice. In the sciatic crushed nerve injury (SCI), the affected limb displays characteristics of painful neuropathy such as hyperalgesia, pain-related gait, and swelling. Generally surgical repair is required for treating transection or crushed nerve...
injuries, while drug therapy or physical rehabilitation is a typical choice for treating injuries of moderate severity. Previous studies showing that acupuncture, electroacupuncture, pharmacopuncture (aquacupuncture, herbal acupuncture), complex herbs medication, single herb, specific component of herb and herbal bath may play a significant role in pain decrease or nerve regeneration after peripheral nerve injuries have been accumulated in Oriental medicine.

With its effects of anti-inflammatory, anti-nociceptive, immuno-regulation, etc., bee venom (BV) acupuncture is considered to be effective for autoimmune disease, chronic inflammation of various musculoskeletal diseases and various pain syndromes; however, BV acupuncture can result in severe side effects, such as hypersensitivity and anaphylaxis.

As a solution to this problem, sweet bee venom (SBV) is developed by removing allergens and shows significantly lower allergic responses both locally and throughout the body, upper LD50 of ICR mice, and 34.9% more containing of melittin, the main component (approximately 40-50% of dry weight) of BV, at same concentration than BV. This indicates the possibility of wider and easier therapeutic approach to the symptoms applicable to BV acupuncture with SBV.

There have been some previous studies on the clinical effects of SBV acupuncture: allergy response, knee joint osteoarthritis, whiplash injury, low back pain, obesity, cancer-related pain, molluscum contagiosum, stiff neck, spinal cord injury. However, little is known about the difference of effects between BV and SBV acupuncture against painful neuropathy induced by SCI.

C-Fos is an immediate early gene whose expression is sometimes used as a marker for stimulus-induced changes in the metabolic activity of neurons and frontal cortex, thalamus, and PAG, key structures for the coordination of pain perception. Delivery of neurotrophic factors to the injured spinal cord has been shown to stimulate neuronal survival and regeneration. TrkB mediates the multiple effects of neurotrophic factors, which includes neuronal differentiation and survival.

In the present study, to compare the effects due to administration areas we conducted two experiments. The first experiment was to evaluate the effects of BV and SBV acupuncture at the contralateral side to the injured sciatic nerve on SCI; the second, at the ipsilateral side. For this study, walking track analysis, expressions of c-Fos in the PVN and vPAG regions, and expressions of BDNF and TrkB in the damaged sciatic nerve following SCI in rats were assessed.

Materials and Methods

1. Experimental animals and bee venoms

Adult male Sprague-Dawley rats, weighing 220 ± 10g (7 weeks old), were obtained from a commercial breeder (Orient Co., Seoul, Korea) for the experiment. The experimental procedures were performed in accordance with the animal care guidelines of the National Institute of Health (NIH) and the Korean Academy of Medical Sciences. Each animal was housed under controlled temperature (23 ± 2°C) and lighting (08:00 h - 20:00 h) conditions with food and water made available ad libitum before and after surgery.

Bee venoms were obtained from Korean pharmacopuncture institute (Seoul, Korea) for the experiment: Sunsu 2, general bee venom (BV); sweet bee venom, allergen-removed bee venom (SBV). BV and SBV were diluted into 0.01mg/kg, 0.1mg/kg and 1mg/kg. Each BV 0.005ml was injected into the Huantiáo (GB30) acupoint with disposable insulin syringe.

2. Experiment A: BV and SBV acupuncture treatment at contralateral side of injured sciatic nerve

In this study, the effects of BV and SBV acupuncture at the contralateral side of the injured sciatic nerve
on SCI were comparatively analyzed. The rats were randomly divided into eight groups (n=10 in each group): the sham-operation group, the SCI-induced group, the SCI-induced and 0.01mg/kg BV acupuncture-treated group, the SCI-induced and 0.1mg/kg BV acupuncture-treated group, the SCI-induced and 1mg/kg BV acupuncture-treated group, the SCI-induced and 0.01mg/kg SBV acupuncture-treated group, the SCI-induced and 0.1mg/kg SBV acupuncture-treated group, and the SCI-induced and 1mg/kg SBV acupuncture-treated group. Each BV 0.005ml was injected into the Huantiao (GB30) acupoint on the contralateral side of the damaged sciatic nerve. The rats in the sham-operation group and in the SCI-induced group received an equal amount of saline for the same duration. All rats received each treatment once a day for 12 consecutive days, starting three days after surgery.

3. Experiment B: BV and SBV acupuncture treatment at ipsilateral side of injured sciatic nerve

In this study, the effects of BV and SBV acupuncture at the ipsilateral side of the injured sciatic nerve on SCI were comparatively analyzed. The rats were randomly divided into four groups (n=10 in each group): the sham-operation group, the SCI-induced group, the SCI-induced and 1mg/kg BV acupuncture-treated group, and the SCI-induced and 1mg/kg SBV acupuncture-treated group. Each BV 0.005ml was injected into the Huantiao (GB30) acupoint ipsilateral side of the damaged sciatic nerve. The rats in the sham-operation group and in the SCI-induced group received an equal amount of saline for the same duration. All rats received each treatment once a day for 12 consecutive days, starting three days after surgery.

4. Induction of SCI

To induce crush injury on the sciatic nerve in rats, the previously described surgical procedure was performed\(^\text{36}\). In brief, the right sciatic nerve was exposed by incision on the gluteal muscle under anesthesia with Zoletil 50\(^\text{36}\) (50mg/kg; Virbac Laboratories, Carros, France). The sciatic nerve was carefully exposed and crushed for 30 sec using a surgical clip (Pressure: 125g; Fine Science Tools Inc., San Francisco, USA). The crushed location was 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.

5. Walking track analysis

For the assessment of motor nerve recovery, walking track analysis was carried out as described in previous reports\(^\text{37}\) with minor modifications (Fig. 1). The rats were tested in a walking track analysis 3, 6, 9 and 12 days after bee venom treatment. The rats were allowed conditioning trials in an 8 x 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), the toe spread (TS), and the 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 previously reported formula\(^\text{37}\).

6. Tissue preparation

The rats were sacrificed immediately after the last walking track analysis (15 days after induction of SCI). The animals were anesthetized using Zoletil 50\(^\text{58}\)(10 mg/kg, i.p.; Virbac Laboratories), transcardially perfused with 50 mM phosphate-buffered saline (PBS), and fixed with a freshly prepared solution consisting of 4% paraformaldehyde in 100mM phosphate buffer.
(PB, pH 7.4). Brains were dissected, post-fixed in the same fixative overnight, and transferred to 30% sucrose for cryoprotection. 40 thick coronal sections were made using a freezing microtome (Leica, Nussloch, Germany). Ten slice sections on average in the PVN and vlPAG regions were collected from each rat. The sections from 1.9mm to 2.1mm posterior from the bregma were used for PVN and the sections from 7.0mm to 7.2mm posterior from the bregma were used for vlPAG, and immunohistochemistry was conducted.

7. BDNF and TrkB western blot analysis

The crushed sciatic nerve tissues were collected, and then were immediately frozen at -80°C. The crushed sciatic nerve tissues were homogenized on ice, and lysed in a lysis buffer containing 50mM Tris-HCl (pH 7.5), 150mM NaCl, 0.5% deoxycholic acid, 1% Nonidet P40, 0.1% SDS, 1mM PMSF, and 100mg/ml leupeptin. Protein content was measured using a Bio-Rad colorimetric protein assay kit (Bio-Rad). Protein of 30μg was separated on SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane. Mouse actin antibody (1:500; Santa Cruz Biotech, CA, USA) and rabbit BDNF antibody and TrkB antibody (1:1000; Santa Cruz Biotech) were used as primary antibodies. Horseradish peroxidase-conjugated anti-rabbit antibody for BDNF and TrkB (1:2000; Vector Laboratories, Burlingame, CA, USA.) was used as secondary antibodies. The experiment was performed in normal lab conditions and at room temperature except for the membrane transfer. Membrane transfer was performed at 4°C with the cold pack and pre-chilled buffer. Band detection was performed using the enhanced chemiluminescence (ECL) detection kit (Santa Cruz Biotech).

8. c-Fos immunohistochemistry

For immunolabeling of c-Fos in the PVN, vlPAG, and c-Fos immunohistochemistry was performed by the previously described method⁵⁴. Free-floating tissue sections were incubated overnight with rabbit anti-c-Fos antibody (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and 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$_2$O$_2$ in 50mM 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®.

9. Data analyses

The area of PVN and vlPAG regions from each slice was measured using Image-Pro®Plus computer-assisted image analysis system (Media Cybernetics Inc., Silver Spring, MD, USA) attached to a light microscope (Olympus, Tokyo, Japan). The numbers of c-Fos-positive cells in the PVN and vlPAG regions were counted hemilaterally through a light microscope (Olympus, Tokyo, Japan).

To compare relative expression of BDNF and TrkB proteins, detected bands were calculated densitometrically using Molecular AnalystTM version 1.4.1 (Bio-Rad, Hercules, CA, USA).

Statistical analysis was performed using one-way ANOVA followed by Duncan’s post-hoc test, and the results are expressed as the mean ± standard error of the mean (S.E.M.). Significance was set as P < 0.05.

**Results**

1. Experiment A: treatment at contralateral side of injured sciatic nerve

1) Effect of BV and SBV acupuncture on SFI

We measured SFI using a walking track analysis to assess functional recovery after the sciatic crushed nerve was injured in rats (Fig. 2). The mean SFI in each group was calculated on the 3rd, 6th, 9th, and
12th days after SCI. The mean value of SFI was -5.30 ± 1.66 on the 3rd day, -9.62 ± 2.11 on the 6th day, -1.34 ± 0.00 on the 9th day, and -9.67 ± 2.21 on the 12th day in the sham-operation group, -104.90 ± 3.34 on the 3rd day, -89.64 ± 3.69 on the 6th day, -85.71 ± 2.39 on the 9th day, and -70.20 ± 2.11 on the 12th day in the SCI-induced group, -103.90 ± 4.11 on the 3rd day, -81.83 ± 2.96 on the 6th day, -88.38 ± 1.99 on the 9th day, and -53.83 ± 1.03 on the 12th day in the SCI-induced and 0.01mg/kg BV acupuncture-treated group, -101.90 ± 2.36 on the 3rd day, -85.40 ± 2.11 on the 6th day, -84.66 ± 1.98 on the 9th day, and -48.05 ± 1.12 on the 12th day in the SCI-induced and 0.1mg/kg BV acupuncture-treated group, -102.90 ± 3.11 on the 3rd day, -79.78 ± 1.99 on the 6th day, -78.04 ± 2.11 on the 9th day, and -45.63 ± 2.33 on the 12th day in the SCI-induced and 1mg/kg BV acupuncture-treated group, -105.90 ± 4.11 on the 3rd day, -81.83 ± 2.31 on the 6th day, -84.55 ± 1.97 on the 9th day, and -53.85 ± 2.13 on the 12th day in the SCI-induced and 0.01mg/kg SBV acupuncture-treated group, -104.60 ± 2.93 on the 3rd day, -82.60 ± 1.91 on the 6th day, -91.48 ± 2.33 on the 9th day, and -51.49 ± 1.46 on the 12th day in the SCI-induced and 0.1mg/kg SBV acupuncture-treated group, and -107.20 ± 2.89 on the 3rd day, -79.37 ± 2.46 on the 6th day, -85.78 ± 2.49 on the 9th day, and -31.45 ± 1.27 on the 12th day in the SCI-induced and 1mg/kg SBV acupuncture-treated group at the commencement of the experiment.

These results show that the SFI was decreased by induction of SCI, and BV and SBV acupuncture treatment at the contralateral side of the injured nerve enhanced the SFI. A significant difference in effects between BV and SBV acupuncture was not observed, although 1mg/kg SBV acupuncture showed

Fig. 3. Effect of BV and SBV acupuncture at contralateral side of injured nerve on the expression of BDNF and TrkB.

Actin was used as an internal control (46 kDa).

A: Sham-operation group
B: SCI-induced group
C: SCI-induced and 0.01mg/kg BV acupuncture-treated group
D: SCI-induced and 0.1mg/kg BV acupuncture-treated group
E: SCI-induced and 1mg/kg BV acupuncture-treated group
F: SCI-induced and 0.01mg/kg SBV acupuncture-treated group
G: SCI-induced and 0.1mg/kg SBV acupuncture-treated group
H: SCI-induced and 1mg/kg SBV acupuncture-treated group
more potent increasing effect on SFI. These results indicate that BV and SBV acupuncture treatment promotes functional recovery following SCI.

2) Effect of BV and SBV acupuncture on BDNF and TrkB expressions

When the level of mature BDNF (14 kDa) in the sham-operation group was set as 1.00, the level of mature BDNF was 3.33 ± 0.32, 3.37 ± 0.56, 2.75 ± 0.33, 2.11 ± 0.24, 3.22 ± 0.53, 3.03 ± 0.45, and 2.02 ± 0.31 in the SCI-induced group, in the SCI-induced and 0.01mg/kg BV acupuncture-treated group, in the SCI-induced and 0.1mg/kg BV acupuncture-treated group, in the SCI-induced and 1mg/kg BV acupuncture-treated group, and in the SCI-induced and 1mg/kg SBV acupuncture-treated group.

When the level of TrkB (95-145 kDa) in the sham control group was set as 1.00, the level of TrkB was 2.31 ± 0.17, 2.52 ± 0.21, 2.40 ± 0.20, 1.61 ± 0.19, 2.51 ± 0.32, 1.93 ± 0.32, and 1.23 ± 0.13 in the SCI-induced group, in the SCI-induced and 0.01mg/kg BV acupuncture-treated group, in the SCI-induced and 0.1mg/kg BV acupuncture-treated group, in the SCI-induced and 1mg/kg BV acupuncture-treated group, and in the SCI-induced and 1mg/kg SBV acupuncture-treated group.

3) Effect of BV and SBV acupuncture on c-Fos expression in the PVN

Photomicrographs of c-Fos-positive cells in the PVN region are presented in Fig. 4. The number of c-Fos-positive cells was 30.66 ± 4.31/section in the sham-operation group, 104.62 ± 11.43/section in the SCI-induced group, 85.16 ± 9.12/section in the SCI-induced and 0.01mg/kg BV acupuncture-treated group, 82.22 ± 11.33/section in the SCI-induced and 0.1mg/kg BV acupuncture-treated group, 72.42 ± 8.69/section in the SCI-induced and 0.01mg/kg SBV acupuncture-treated group, 77.25 ± 10.96/section in the SCI-induced and 0.1mg/kg SBV acupuncture-treated group, and 53.20 ± 6.66/section in the SCI-induced and 1mg/kg SBV acupuncture-treated group.

These results show that c-Fos expression in the PVN region was increased by induction of SCI, and BV and SBV acupuncture treatment at the contralateral side of the injured nerve significantly suppressed the c-Fos expression. A significant difference of effects between BV and SBV acupuncture was not observed, although 1mg/kg SBV acupuncture showed more potent suppressing effect on c-Fos.

4) Effect of BV and SBV acupuncture on c-Fos expression in the vlPAG

Photomicrographs of c-Fos-positive cells in the vlPAG region are presented in Fig. 5. The number of c-Fos-positive cells was 19.50 ± 3.19/section in the sham-operation group, 80.66 ± 8.97/section in the SCI-induced group, 51.60 ± 7.11/section in the SCI-induced and 0.01mg/kg BV acupuncture-treated group, 46.40 ± 5.98/section in the SCI-induced and 0.1mg/kg BV acupuncture-treated group, 40.91 ± 8.01/section in the SCI-induced and 1mg/kg BV acupuncture-treated group, 41.44 ± 4.99/section in the SCI-induced and 0.01mg/kg SBV acupuncture-treated group, and 40.39 ± 3.79/section in the SCI-induced and 0.1mg/kg SBV acupuncture-treated group.
These results show that c-Fos expression in the vIPAG region was increased by induction of SCI, and BV and SBV acupuncture treatment at the contralateral side of the injured nerve significantly suppressed the c-Fos expression. The significant difference of effects between BV and SBV acupuncture
Effects of Bee Venom and Sweet Bee Venom Acupuncture on Functional Recovery and c-Fos Expression in the Brain after Sciatic Crushed Nerve Injury in Rats

was not observed, however, 1mg/kg SBV acupuncture showed more potent suppressing effect on c-Fos.

2. Experiment B: treatment at ipsilateral side of injured sciatic nerve

1) Effect of BV and SBV acupuncture on SFI

We measured SFI using a walking track analysis to assess functional recovery after sciatic crushed nerve injury in rats (Fig. 6). The mean SFI in each group was calculated on the 3rd, 6th, 9th, and 12th days after SCI. The mean value of SFI was -6.85 ± 0.00 on the 3rd day, -3.46 ± 2.66 on the 6th day, -8.51 ± 1.78 on the 9th day, and -6.99 ± 2.39 on the 12th day in the sham-operation group, -91.49 ± 3.97 on the 3rd day, -85.55 ± 2.36 on the 6th day, -75.71 ± 2.47 on the 9th day, and -71.31 ± 3.13 on the 12th day in the SCI-induced group, -88.04 ± 2.34 on the 3rd day, -73.80 ± 2.86 on the 6th day, -69.62 ± 1.99 on the 9th day, and -66.15 ± 1.08 on the 12th day in the SCI-induced and 1mg/kg BV acupuncture-treated group, and -91.59 ± 2.59 on the 3rd day, -72.50 ± 2.17 on the 6th day, -66.23 ± 1.95 on the 9th day, and -50.30 ± 1.37 on the 12th day in the SCI-induced and 1mg/kg SBV acupuncture-treated group at the commencement of the experiment.

These results show that the SFI was decreased by induction of SCI, and BV and SBV acupuncture treatment at ipsilateral side of injured nerve enhanced the SFI. Especially, 1mg/kg SBV acupuncture showed more potent increasing effect on SFI compared to the 1mg/kg BV acupuncture. These results indicated that BV and SBV acupuncture treatment at the ipsilateral side of the injured nerve promoted functional recovery following SCI.

2) Effect of BV and SBV acupuncture on BDNF and TrkB expressions

When the level of mature BDNF (14 kDa) in the sham control group was set as 1.00, the level of mature BDNF was 3.92 ± 0.16, 2.61 ± 0.18, and 2.30 ± 0.03 31 respectively in the SCI-induced group, the SCI-induced and 1mg/kg BV acupuncture-treated group, and BV acupuncture-

Fig. 6. Effect of BV and SBV acupuncture at ipsilateral side of injured nerve on sciatic functional index (SFI) following sciatic crushed nerve injury.

(A) Sham-operation group
(B) SCI-induced group
(C) SCI-induced and 1mg/kg BV acupuncture-treated group
(D) SCI-induced and 1mg/kg SBV acupuncture-treated group.

* represents P < 0.05 compared to the each group at 3 days after SCI induction.
# represents P < 0.05 compared to the SCI-induced group at 12 days after SCI induction.
treated group, and the SCI-induced and 1mg/kg SBV acupuncture-treated group.

When the level of TrkB (95-145 kDa) in the sham control group was set as 1.00, the level of TrkB was 4.34 ± 0.02, 2.12 ± 0.00, and 1.85 ± 0.04 in the SCI-induced group, the SCI-induced and 1mg/kg BV acupuncture-treated group, and the SCI-induced and 1mg/kg SBV acupuncture-treated group, respectively. These results show that BDNF and TrkB expressions in the damaged sciatic nerve were increased by induction of SCI. BV and SBV acupuncture treatment at the ipsilateral side of the injured nerve decreased the BDNF and TrkB expressions. There was no significant difference between BV and SBV acupuncture in relation with BDNF and TrkB expressions.

3) Effect of BV and SBV acupuncture on c-Fos expression in the PVN

Photomicrographs of c-Fos-positive cells in the PVN region are presented in Fig. 8. The number of c-Fos-positive cells was 28.50 ± 2.31/section in the sham-operation group, 95.31 ± 10.33/section in the SCI-induced group, 67.98 ± 6.98/section in the SCI-induced and 1mg/kg BV acupuncture-treated group, and 54.33 ± 4.97/section in the SCI-induced and 1mg/kg SBV acupuncture-treated group.

These results show that c-Fos expression in the PVN region was increased by induction of SCI, and BV and SBV acupuncture treatment at the ipsilateral side of the injured nerve significantly suppressed the c-Fos expression. A significant difference of effects between BV and SBV acupuncture was not observed.

4) Effect of BV and SBV acupuncture on c-Fos expression in the vILPAG

Photomicrographs of c-Fos-positive cells in the vILPAG region are presented in Fig. 5. The number of c-Fos-positive cells was 14.81 ± 2.17/section in the sham-operation group, 51.11 ± 6.23/section in the SCI-induced group, 27.90 ± 3.49/section in the...
SCI-induced and 1mg/kg BV acupuncture-treated group, 25.45 ± 2.99/section in the SCI-induced and 1mg/kg SBV acupuncture-treated group.

These results show that c-Fos expression in the vIPAG region was increased by induction of SCI, and BV and SBV acupuncture treatment at the ipsilateral side of the injured nerve significantly suppressed the c-Fos expression. No significant difference of effects between BV and SBV acupuncture was observed.

Fig. 8. Effect of BV and SBV acupuncture at ipsilateral side of injured nerve on the number of c-Fos-positive cells in the paraventricular nucleus.

Upper: Photomicrographs of c-Fos expression in the paraventricular nucleus.
(1) Sham-operation group, (2) SCI-induced group, (3) SCI-induced and 1mg/kg BV acupuncture-treated group, (4) SCI-induced and 1mg/kg SBV acupuncture-treated group.
Sections were stained for c-Fos like immunoreactivity (brown). Scale bar represents 250μm.
Lower: (A) Sham-operation group, (B) SCI-induced group, (C) SCI-induced and 1mg/kg BV acupuncture-treated group, (D) SCI-induced and 1mg/kg SBV acupuncture-treated group.

Fig. 9. Effect of BV and SBV acupuncture at ipsilateral side of injured nerve on the number of c-Fos-positive cells in the ventrolateral periaqueductal gray.

Upper: Photomicrographs of c-Fos expression in the ventrolateral periaqueductal gray.
(1) Sham-operation group, (2) SCI-induced group, (3) SCI-induced and 1mg/kg BV acupuncture-treated group, (4) SCI-induced and 1mg/kg SBV acupuncture-treated group.
Sections were stained for c-Fos like immunoreactivity (brown). Scale bar represents 250μm.
Lower: (A) Sham-operation group, (B) SCI-induced group, (C) SCI-induced and 1mg/kg BV acupuncture-treated group, (D) SCI-induced and 1mg/kg SBV acupuncture-treated group.
Peripheral nerve injuries are commonly encountered in clinical practice due to accidental trauma, deliberate surgery, or acute compression, and for treating transection or crush nerve injuries of moderate severity, drug therapy or physical rehabilitation are typical choices. Therefore, searching for effective treatment methods, including the ones of natural origin, has attracted considerable research interest.

There are studies showing evidence that electroacupuncture, pharmacopuncture (aqua-acupuncture, herbal acupuncture), complex herb medication, specific components of herbs and herbal baths may play a significant role in pain decrease after peripheral nerve injuries; acupuncture, electroacupuncture, pharmacopuncture, complex herb medication, single herbs on nerve regeneration; single herbs on osteoporosis prevention; warm needle moxibustion on local inflammatory factors inhibition; and their complex application, more effective.*

Bee venom (BV) acupuncture, the therapeutic application of diluted BV, exerts not only pharmacological actions from the bioactive compounds isolated from BV but also a mechanical function from acupuncture stimulation. According to the previous studies, the effects of BV acupuncture are anti-inflammatory, anti-nociceptive, immuno-regulation, blood circulation-stimulation, anti-bacterial, radioactivity-protection, allergic reactions, etc. Bee venom contains several bioamines such as apamin, histamine, procaine, serotonin, and norepinephrine, which facilitate nerve transmission and healing in a variety of nerve disorders. Kim et al. reported that BV acupuncture produces a very potent and long-lasting anti-nociceptive effect in both acute and chronic rodent pain models. Roh et al. reported BV acupuncture might be an effective alternative therapy for patients with painful peripheral neuropathy, especially for those who are poorly responsive to opioid analgesics.

But BV acupuncture can cause severe side effects, for example hypersensitivity and anaphylaxis, injury to central nerve system, cardiovascular system, peripheral blood system, and renal dysfunction. For this reason, sweet bee venom (SBV) was developed by removing allergens (molecular weight over 10,000) such as phospholipase A2, hyaluronidase, etc. from the BV through gel filtration chromatography and propionic acid/urea polyacrylamide gel electrophoresis.

There are some studies on the comparison between BV and SBV acupuncture: BV, outstanding antibacterial activity against gram positive S. aureus vs. SBV, outstanding antibacterial activity against gram positive S. aureus and gram negative E. coli; BV, superior antioxidant and lipid peroxidation effects; BV, not showing lipolysis effect vs. SBV, increased lipolysis in low dosage and decreased lipolysis in high dosage; SBV acupuncture, more activating effect of the autonomic nervous system within normal range; BV and SBV acupuncture, similar pain control and function promotion on low back pain with radiating pain; BV acupuncture, superior pain relief on the knee joint osteoarthritis; SBV acupuncture (each 0.1mg/ml), superior pain relief on the knee joint osteoarthritis; BV acupuncture, superior pain relief and function promotion on chronic lower back pain; and BV and SBV acupuncture, similar treatment effects on stiff neck.

However, little is known about the difference of effects between BV and SBV acupuncture against painful neuropathy induced by SCI.

In BV acupuncture, the method of injecting is important for improving its effectiveness. Several studies suggest that the effect of BV was intensified by acupuncture stimulation, which may help achieve the therapeutic goal. Moreover, comparative studies of the acupoint versus non-acupoint stimulation on an adjuvant induced neuropathy pain animal model with BV acupuncture were carried out to examine...
Effects of Bee Venom and Sweet Bee Venom Acupuncture on Functional Recovery and c-Fos Expression in the Brain after Sciatic Crushed Nerve Injury in Rats

In this study, we used the acupoint, named Huantiao (GB30), useful for pain relieving on the disease of hip joints and legs. In the previous studies on neuropathic pain, pharmacopuncture applied at GB30 also showed the effect of pain decrease.

Generally the sciatic functional index (SFI) is a quite useful tool for the evaluation of functional recovery of the sciatic nerve of rats in a number of experimental injuries and treatments. It can be used repeatedly to measure functional recovery over time in the same animal. Thus walking track analysis is a widely accepted technique for functional evaluation after sciatic nerve repair in rats. In the present result, the walking track analysis revealed that induction of SCI decreased SFI score. This indicates SCI-induced functional damage in the walking track analysis.

Our present study showed that BV and SBV acupuncture treatment significantly enhanced the SFI in the SCI rats. Treatment with SBV acupuncture at 1mg/kg showed more potent enhancing effect compared to the BV acupuncture. There was no significant difference on the effects between the contralateral and ipsilateral sides of the injured nerve.

Generally after peripheral nerve transaction or injury, a series of events occurs during regeneration that involves the nerve cell body, the axonal fiber, the target muscle, and the surrounding chemical and cellular environment. Distal nerve endings undergo Wallerian degeneration, and skeletal muscle begins to atrophy because of lack of electrical or chemical stimulation and a decrease in myotrophic factors. Nerve cell bodies enter a restorative phase that is critical for successful regeneration to occur. Thus, trophic factors appear to play an important role in supporting the neuronal cell body and the axons during this period of regeneration.

BDNF is a member of neurotrophin family that promotes the survival of specific neurons in the central nerve system and peripheral nervous system during development. The levels of BDNF mRNA and protein were altered following peripheral nerve injury. In the previous study, BDNF is up-regulated in injured sciatic nerves 3 days after the nerve lesions and this up-regulated lasts for several weeks.

TrkB is the high affinity catalytic receptor for several neurotrophins, which are small protein growth factors that induce the survival and differentiation of distinct cell populations. The neurotrophins that activate TrkB are BDNF, NT-4 (neurotrophin-4), and NT-3 (neurotrophin-3).

In many studies, neurotrophic factors supplied endogenously or exogenously at the site of the injured lesion have been reported to enhance peripheral nerve regeneration. However, controversy showing that the infusion of neurotrophic factor delays the onset of regeneration and suppresses regenerative responses of peripheral nerve injury in rats, and that needle TENS can decrease the expression of nerve growth factor after peripheral nerve injury in rats has continued to date. Therefore it is still unclear whether excessive expressions of neurotrophic factors including BDNF promote or delay functional recovery from nerve injury.

Our results showed that BDNF and TrkB protein expression in the crushed sciatic nerve were significantly increased following SCI. These results suggest that induction of sciatic crushed injury caused more expressions of BDNF and its receptor TrkB to repair sciatic injury. Treatment with 1mg/kg BV acupuncture or 1mg/kg SBV acupuncture suppressed the BDNF and TrkB expression in the crushed sciatic nerve. The present results suggest that BV and SBV acupuncture controlled SCI-induced pain and injury, thereafter suppressed SCI-induced over-expression of BDNF and TrkB. There was no significant difference on the suppression of BDNF and TrkB between contralateral side and ipsilateral side of the injured nerve.

C-Fos is an immediate early gene whose expression is sometimes used as a marker for stimulus-induced
changes in the metabolic activity of neurons\textsuperscript{34}. It has been reported that expression of the c-Fos gene is induced by various stressful stimuli, such as immunological challenges\textsuperscript{66,67}, immobilization\textsuperscript{68}, pedal shock stimulus\textsuperscript{69,70}, and pain\textsuperscript{71,72}.

The levels of c-Fos in the frontal cortex, thalamus, and PAG, which are key structures for the coordination of pain perception, were significantly increased in the rats following sciatic nerve ligation\textsuperscript{73}. The inhibition of c-Fos expression in the spinal cord by BV acupuncture in the several nociceptive models was suggested as the possible mechanism of pain control\textsuperscript{43,74}. Wu et al. reported that subcutaneous injection of BV suppressed c-Fos expression in the spinal cord and brain of the spontaneous pain model\textsuperscript{75}.

In the present study, expressions of c-Fos in the vlPAG and PVN were increased following sciatic crushed nerve injury, which represents that severe peripheral pain induced by SCI caused neuronal activation in these brain areas. In contrast, BV and SBV acupuncture treatment significantly suppressed SCI-induced c-Fos expression in the vlPAG and PVN, which represents BV and SBV acupuncture alleviated severity of pain, and resulted in a decrease of neuronal activation in these brain areas. Treatment with SBV acupuncture at 1mg/kg showed more potent suppressing effect on c-Fos compared to the BV acupuncture. In the case of injection into the ipsilateral side of the injured nerve, there was no significant difference in the effects between SBV and BV acupuncture.

We suggest that BV and SBV acupuncture can be useful therapeutic intervention for pain control and functional recovery following peripheral nerve injury.

1. Sciatic function index (SFI) in walking track analyses significantly decreased following sciatic crushed nerve injury. The expressions of BDNF and TrkB in the sciatic nerve were increased by induction of sciatic crushed nerve injury. C-Fos expression in the ventrolateral periaqueductal gray (vlPAG) and paraventricular nucleus (PVN) were increased by sciatic crushed nerve injury.

2. BV and SBV acupuncture treatment improved the SFI in walking track analysis. Treatment with SBV at 1mg/kg showed more potent enhancing effect on SFI compared to the BV.

3. Treatment with 1mg/kg BV or 1mg/kg SBV acupuncture suppressed the BDNF and TrkB expression in the sciatic nerve.

4. BV and SBV acupuncture treatment also suppressed of c-Fos expression in the PVN and vlPAG regions. Treatment with SBV at 1mg/kg showed more potent suppressing effect on c-Fos expression compared to the BV in the case of injection onto the contralateral side of the injured nerve.

5. Generally we could not find significant differences in the effects between the contralateral and ipsilateral sides of the injured nerve.

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