Randomized Experimental Trial

Pulsed Radiofrequency Reduced Neuropathic Pain Behavior in Rats Associated with Upregulation of GDNF Expression

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Background: Pulsed radiofrequency (PRF) is a novel nondestructive interventional technique for the treatment of neuropathic pain (NP). However, this intervention is still lack of relevant regulation and the mechanism of action is insofar not clear. Historically, most studies have reported that PRF can relieve reduce hyperalgesia in multiple NP animal models by acting on the dorsal root ganglion. However, a few recent studies have shown that PRF can effectively treat hyperalgesia in pain models by a direct application on injured peripheral nerves.

Objectives: To observe changes in pain behavior and the pathology of the sciatic nerve (SN) after applying PRF at the ligation site in a chronic constriction injury (CCI) rat model and to investigate the effect of PRF on the expression of glia cell line-derived neurotrophic factor (GDNF) in nervous tissue.

Study Design: A randomized, experimental trial.

Setting: Experimental Animal Center, Beijing Tiantan Hospital, Capital Medical University.

Methods: Thirty-six adult Sprague-Dawley rats were randomly divided into 3 groups: Sham-Sham (SS), CCI-Sham (CS), and CCI-PRF (CP). The right SNs of the rats in the CS and CP groups were ligated to create a CCI model. For the SS group, the right SN was separated without ligation. On the 14th fourteenth day after surgery, PRF treatment was applied at the ligation site of the SN for the rats in the CP group using a 45 V output voltage at 42°C for 3 minutes. The electrode was placed in rats in the SS and CS groups without electricity applied. The hindpaw withdrawal threshold (HWT) and thermal withdrawal latency (TWL) were measured at various time points before and after the treatments in each group. Optical microscopic scores and electron microscopic observation were given to the right SN ligation sites of the rats in each group 14 days after the treatment . Meanwhile, the GDNF expression levels in the ligation site of the SN and in the L4-L6 spinal cord segments were determined for each group by enzyme-linked immunosorbent assay (ELISA).

Results: Fourteen days after PRF treatment, the HWT and TWL values in the CP group were significantly increased compared to those of the CS group ($P < 0.01$). Under the optical microscope, the axonal number, axonal diameter, and myelin sheath thickness in the CP group were significantly increased compared to those of the CS group 14 days after PRF treatment ($P < 0.01$). Under the electron microscope, the degeneration at the SN ligation site was significantly improved in the CP group compared to the CS group. The GDNF expression levels at the ligation site of the SN and the L4-L6 spinal segments in the CP and CS groups were increased compared to those of the SS group ($P < 0.01$). In addition, the GDNF expression in the CP group was significantly higher than that in the CS group ($P < 0.01$).

Limitations: GDNF expression was only measured at day 14 after the treatment rather than at various time points during the experiment.

Conclusions: The findings suggest that the application of PRF at the impaired SN relieved reduced the CCI-induced NP by through regulating the upregulation of the GDNF expression in the nervous tissues.

Key words: Pulsed radiofrequency, chronic constriction injury, sciatic nerve, spinal cord, hind paw withdrawal threshold, thermal withdrawal latency, optical microscopic, electron microscope, glia cell line-derived neurotrophic factor, enzyme-linked immunosorbent assay.

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Nowadays, neuropathic pain (NP) still lacks ideal treatment. As conservative medication treatment has not been effective, invasive treatments may apply to these patients. Conventional continuous radiofrequency technology showed actual effectiveness but inevitably caused impairment of nervous tissues (1). To reduce the side effects of treatment-related nerve impairment, Sluijter et al (2) first reported pulsed radiofrequency (PRF) as a nondestructive radiofrequency technology. The pulsed frequency emitted by the radiofrequency generator is 2 Hz, with an output voltage of 45 V and a 500 KHz high-frequency alternating current. The radiofrequency current lasts for 20 ms for each emission to form a relatively high voltage around the nervous tissues and is then stopped for 480 ms, allowing the heat generated by the radiofrequency current to diffuse in order to maintain a temperature of no more than 42°C and to avoid lesions in local tissues (2).

Clinically, PRF has been applied to the dorsal root ganglion (DRG) (34), brachial plexus (5), and suprascapular nerve (6) to reduce the pain of patients without nerve injury-related side effects. However, these reports are mostly descriptive studies or case reports that lack randomized control trial or treatment regulation. To date, most previous studies have reported that PRF could effectively relieve pain when applied to various NP animal models, for which the action targets were mostly located at the DRG (7). Recently only one study (8) reported that the application of PRF at the near end of the impaired sciatic nerve (SN) could significantly reverse the pain in a spared nerve injury (SNI) rat model. Another study performed by us recently reported that PRF treatment at the SN ligation site could relieve NP in a chronic constriction injury (CCI) model (9). Therefore, further studies to confirm the effectiveness and safety of PRF treatment directly on injured peripheral nerves are justified.

Currently, the mechanism of PRF treatment of NP has not been revealed. In our preliminary study (9), PRF was shown to promote the reparation and regeneration of impaired peripheral nerves. Glia cell line-derived neurotrophic factor (GDNF) is an important neurotrophic factor that affects neuronal survival, growth, and directed differentiation in the central and peripheral nerve system (10-12). Additionally, researchers have confirmed that GDNF plays a critical role in regulating pain signal transmission, especially at the NP stage (13-15). However, there have been no reports concerning whether the mechanism of PRF-reduced NP involves the change of GDNF's concentration in nervous tissues. In this study, based on the hypothesis that PRF reduces the NP behavior in CCI through the upregulation of GDNF expression in nervous tissues, we investigated the GDNF levels in the ligation site of the SN and L4-L6 spinal segments after PRF treatment of the impaired SN.

Methods

Animals

Studies were performed with 36 adult male Sprague-Dawley rats (220 – 250 g, provided by Vital River Laboratories, Beijing). Animals were housed at 22 – 24°C on a 12 hour light/dark cycle, with food and water available ad libitum. Experimental procedures were approved by the Beijing Neurosurgical Institute Experimental Animal Welfare Ethics Committee.

Neuropathic Pain Model

The CCI procedure was established as described by Bennett and Xie (16). The right SN was exposed after anesthesia and 4 ligatures (4-0 chromic catgut) were placed superior to the 3 branches of the SN with a distance of one millimeter between each ligature. The ligatures were loosely tied until a short flick of the ipsilateral hind limb was observed.

Treatment Groups and Design

A total of 36 animals were randomly divided into 3 groups: (1) Sham-Sham (SS), (2) CCI-Sham (CS), and (3) CCI-PRF (CP). Each group's processing method is shown in Table 1, and the experiment procedures are shown in Table 2.

Table 1. *Each Group's group's Processing processing Methodmethod.*

Group	Number	CCI Procedures	PRF Treatment
Sham-Sham (SS)	12	Just expose the SN, without ligation	Just expose the SN to PRF electrode, with no treatment
CCI-Sham (CS)	12	Expose the SN, with ligation	Just expose the SN to PRF electrode, with no treatment
CCI-PRF (CP)	12	Expose the SN, with ligation	Exposure the SN to PRF electrode, with PRF treatment

CCI: chronic constriction injury; PRF: pulsed radiofrequency; SN: sciatic nerve.

Table 2. *Experimental procedures*.

Time (Day)	CCI Procedure/Sham Operating	PRF Treatment/ Sham Treatment	Behavioral Testing #	Tissue Collection
0	+		+	
2, 4, 6, 8, 10, 12 Days after SN Ligation			+	
14 after SN Ligation		+	+	
2, 4, 6, 8, 10, 12 Days after PRF Treatment			+	
14 Days after PRF Treatment			+	+

#: Behavioral test at day 0,14 after SN ligation, 14 after PRF treatment were prior to CCI procedures/sham operating, PRF treatment/sham treatment and tissue collection. CCI: chronic constriction injury; PRF: pulsed radiofrequency; SN: sciatic nerve.

Behavior Testing

Hindpaw Withdrawal Threshold (HWT) Test

Mechanical allodynia was tested using a graded series of Von Frey hairs (VFH) as described by Zhang et al (17). The pressure was started with 1 g. If no nociception was observed, the pressure was increased by one order of magnitude. The simulations were given 5 times at each pressure, and stopped for 30 seconds whenever a persistent slightly bent probe was observed for 3 – 5 seconds. The threshold of a hindpaw was determined when nociception was observed upon 3 out of 5 simulations.

Thermal Withdrawal Latency (TWL) Test

The rats were placed in separated transparent cages with glass plates on the floor for a 30-minute preadaptation. The heat center of the mobile infrared radiant heat stimulus generator (7371, Ugo Basile, Comerio, Italy) was pointed to the right hindpaw of the rats, and persistent radiation was applied. A digital timer was used to measure the latent time between the start of heating and the time at which reflective retraction of the rats was observed due to heat exposure. To prevent tissue damage, the measurement was performed every 2 minutes for a total of 3 times, and the average value over the 3 measurements was recorded.

Application of PRF or Sham Intervention

The PRF treatment trocar was vertically placed at the middle of the SN ligation site fixed by the three-dimensional frame. After the stylet was removed the radiofrequency electrode (PMK-21-50, Baylis Medical Inc., Montreal, Canada) was then inserted. The parameters of the PRF treatment device (PMG-230, Baylis Medical Inc., Montreal, Canada) were set as follows: pulse frequency 2 Hz, temperature 42°C, output voltage 45 V and duration 180 seconds. After the treatment, the wound was stitched closed. The rats were returned to their cages for routine breeding. For the Sham treatment, the SN was exposed, and the electrode was placed on it for 180 seconds without electricity.

Optical and Electron Microscopic Analysis

On the fourteenth day after the treatment, the ligation site of the SN of the rats in the CP and CS groups and corresponding right SN in the SS group were retrieved. The samples were fixed in a mixture of 2% glutaraldehyde and 2% formaldehyde, embedded in resin, made into semi-thin slides, stained with azure methylene blue, positioned, made into ultra-thin slides, and dyed with osmic acid. Thin sections were observed under a transmission electron microscope (H-7650 HITACHI, Hitachi, Japan). Semithin sections were observed under an optical microscope (LEICA DM6000B, Leica, Germany). For morphometric analysis, 3 sections from each rat were randomly selected, and 10 randomly selected areas (using a counting frame of 2,500 μm2) per section were observed under 40 X magnification to perform the statistical analysis. Axon counts, axonal diameter, and myelin sheath thickness were calculated by IMAGE-PRO PLUS program (Media Cybernetics, Inc., 4340 East-West Hwy, Suite 400, Bethesda, USA) according to the method of Bagriyanik et al(18).

Enzyme-Linked Immunosorbent Assay (ELISA)

The L4-L6 spinal segments from each group (n = 6), and the ligation site of the SN in the CP and CS groups and the corresponding right SN in the SS group were retrieved 14 days after the treatment. The ligature was separated on an ice plate. The samples were cleaned with physiological saline, dried with filter paper, weighed, homogenized, and centrifuged at 2,500 rpm for 1 minute. The supernatant was collected and stored in a -70°C freezer for future tests. The supernatant was strictly processed according to the instructions on the ELISA kit (Elabscience Biotechnology Co, Ltd, Wuhan, China), and the optical density (OD) values at 450 nm were determined with a microplate reader (BIO-RAD

680, BIO-RAD, USA). The GDNF concentrations in the samples were identified by the OD values based on the standard curve. The expression levels of GDNF in the SN and the L4-L6 spinal segments were calculated accordingly.

Statistical Analysis

A statistical analysis was performed for the experiment data using SPSS 18.0. The measurement data were expressed as the means ± S.D. Pain behavior differences of 3 groups were evaluated by repeated measures analysis of variance (ANOVA). Optical microscope and GDNF expression differences between groups were evaluated by one-way ANOVA, and S-N-K test was used in post hoc analysis. A $P < 0.05$ was regarded as a significant difference.

Results

Behavioral Testing

Effect of a CCI Lesion

On day 14 after the ligation of the SN, both the HWT and TWL values in the CS and CP groups decreased significantly compared to those of the SS group ($P < 0.01$) (Figs. 1 and 2).

Effect of the PRF Treatment

On the sixth day after the PRF treatment, the HWT scores of the treated hindpaw in the CP group was significantly higher than that of CS group ($P < 0.05$). From the eighth day after the treatment until the end of the experiment, the HWT scores in the CP group were significantly higher than that of the CS group ($P < 0.01$) (Fig. 3).

In the CP and CS groups, the TWL measurements of the hindpaw at the ligation site showed no significant differences during the first 4 days after the PRF/Sham treatment ($P > 0.05$). Beginning at the sixth day after the treatment, the TWL of the treated hindpaw in the CP group was significantly higher than that of the CS group ($P < 0.01$) (Fig. 4).

Histological Evaluation

Optical Microscope Analysis

Fourteen days after the Sham treatment, the axonal number, axonal diameter, and myelin sheath thick-

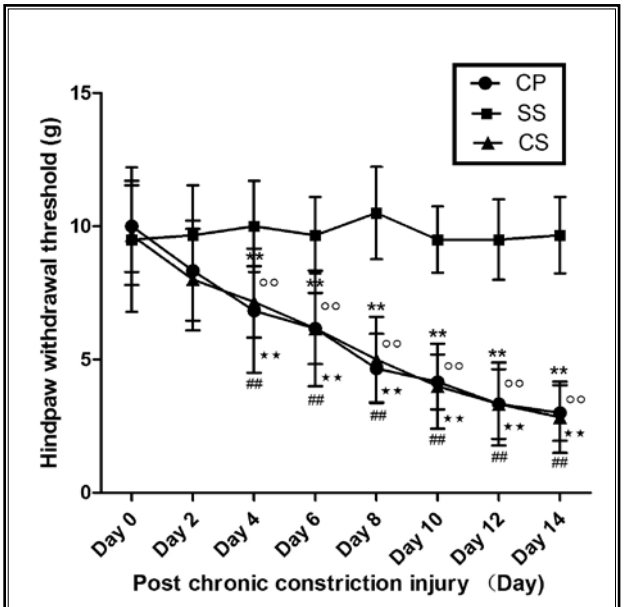


Fig 1. *HWT variations within 15 days after the ligation of the right sciatic nerve.*
**: CP group compared to before ligation, $P < 0.01$; ##: CS group compared to before ligation, $P < 0.01$; ##: comparison between the CP group and SS group, $P < 0.01$; ll: comparison between the CS group and SS group, $P < 0.01$.

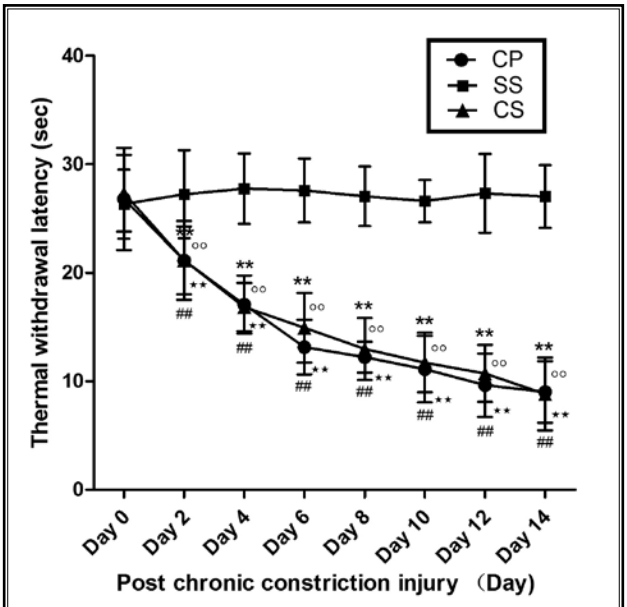


Fig 2. *TWL variations within 15 days after the ligation of the right sciatic nerve.*
** CP group compared to before ligation, $P < 0.01$; ##: CS group compared to before ligation; ##: comparison between the CP group and SS group, $P < 0.01$; ll: comparison between the CS group and SS group, $P < 0.01$.

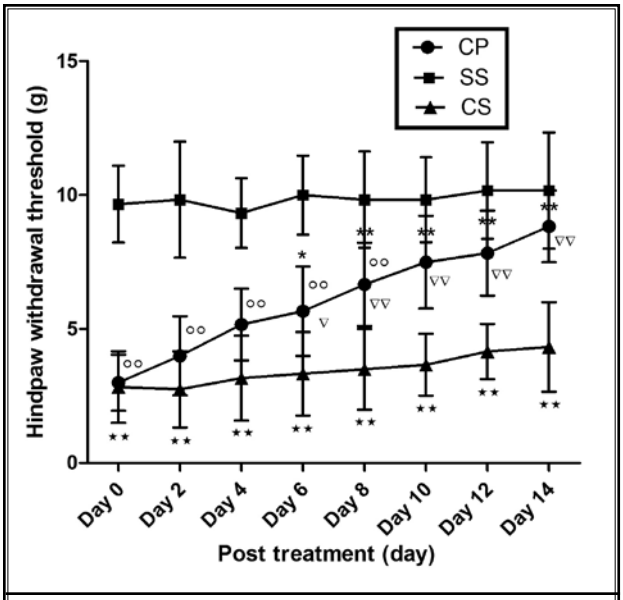


Fig 3. *HWT variations within 15 days after the PRF/Sham treatment in the CP, CS and SS groups.*
*: CP group compared to before PRF treatment, $P < 0.05$;
**: CP group compared to before PRF treatment, $P < 0.01$;
oo: comparison between the CP group and SS group, $P < 0.01$;
□□: comparison between the CS group and SS group, $P < 0.01$;
▷: comparison between the CP group and CS group, $P < 0.05$,
▷ ▷: comparison between the CP group and CS group, $P < 0.01$.

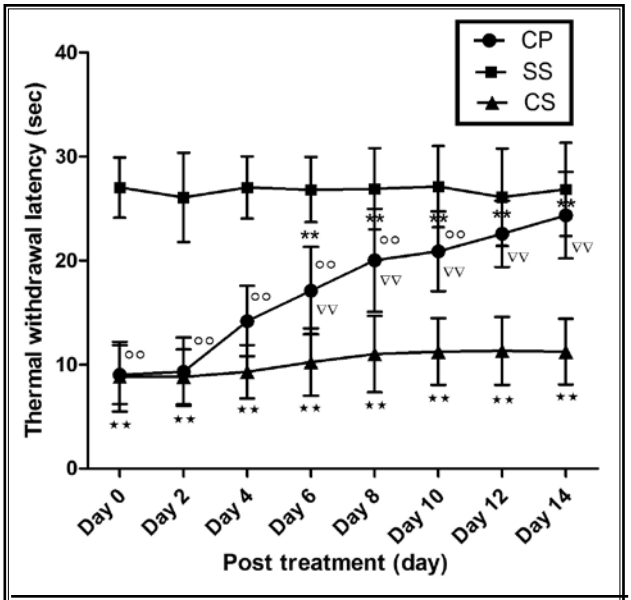


Fig. 4. *TWL variations within 15 days after the PRF/Sham treatment in the CP group, CS group and SS group.*
**: CP group compared to before PRF treatment, $P < 0.01$;
oo: comparison between the CP group and SS group, $P < 0.01$;
□□: comparison between the CS group and SS group, $P < 0.01$;
▷: comparison between the CP group and CS group, $P < 0.05$;
▷ ▷: comparison between the CP group and CS group, $P < 0.01$.

Table 3. *Optical microscopic analysis on the 14 days after the PRF/Sham treatment for each group (mean ± S.D.).*

Group	Number	Axonal Number	Axonal Diameter (μm)	Myelin Sheath Thickness (μm)
Sham-Sham (SS)	6	44.89 ± 2.97	4.57 ± 0.26	0.34 ± 0.03
CCI-Sham (CS)	6	23.78 ± 2.39#	3.33 ± 0.25#	0.27 ± 0.02#
CCI-PRF (CP)	6	34.17 ± 1.95*,#	4.34 ± 0.28*	0.32 ± 0.03*

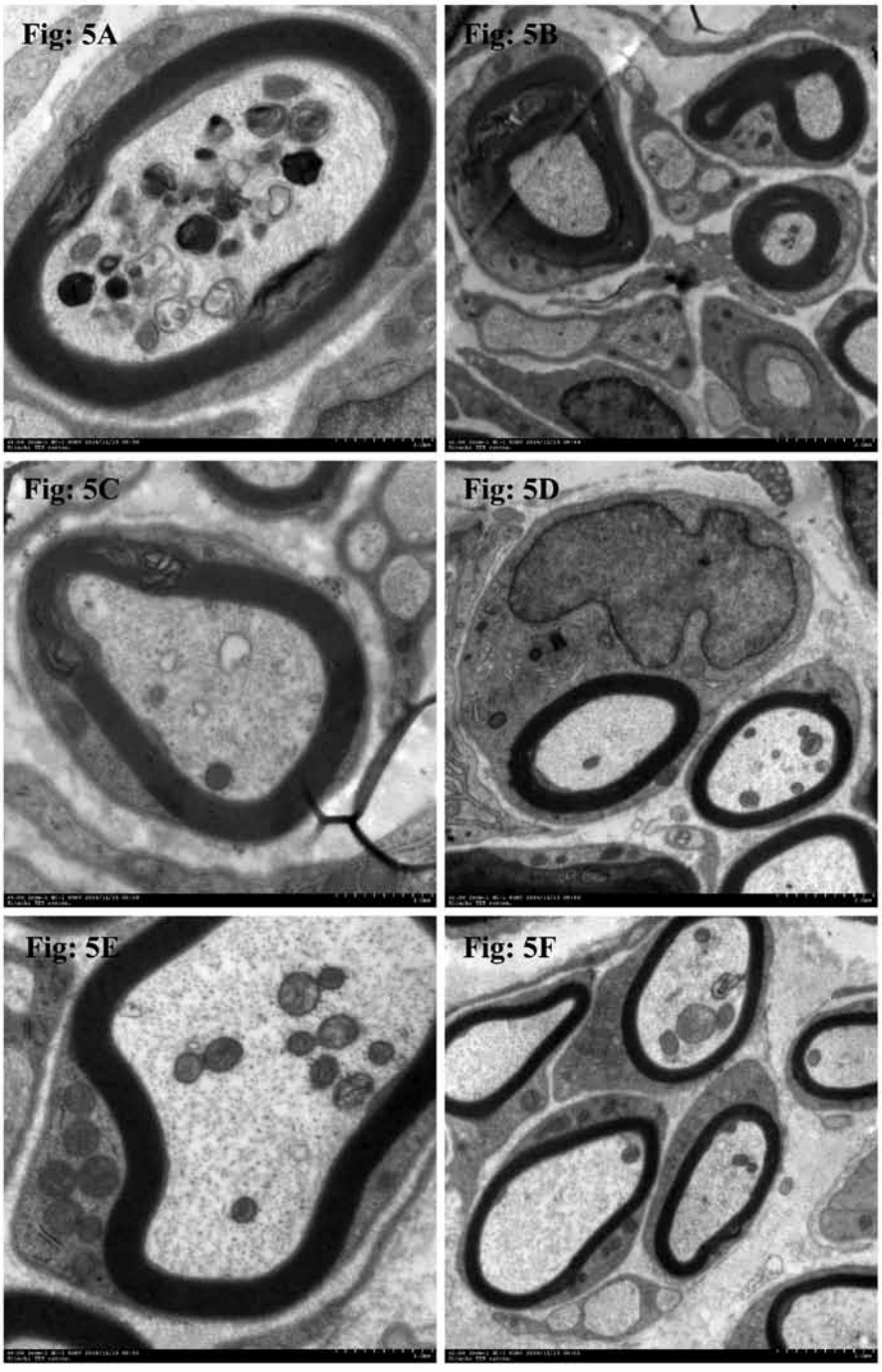
*: compared to the CS group, $P < 0.01$; #: compared to the SS group, $P < 0.01$.

ness in the CS group showed significant decreases compared to those of the SS group ($P < 0.01$).The axonal diameter and myelin sheath thickness in the CP group exhibited no significant differences compared to those of the SS group ($P > 0.05$) after the PRF treatment, but the axonal number was significantly lower than that of the SS group ($P < 0.01$). Compared to the CS group, the axonal number, axonal diameter, and myelin sheath thickness in the CP group all presented significant increases ($P < 0.01$) (Table 3).

Electron Microscope Analysis

On the fourteenth day after the Sham treatment, the SN fiber myelin sheath at the ligation site was significantly thinner in the CS group. Partial demyelination of the nerve fibers and completely damaged myelin lamellar structures were observed. Axons in the myelin sheath were shown to have degenerated and dissolved. The microfilaments and microtubules in the axons dissolved and disappeared. Mitochondria were swollen and were rarely observed. In addition, a large amount of collagen fibers appeared around the severely damaged myelin sheath. Schwann cells were observed to form heterochromatin and were in the mitotic phase with various cytoplasmic organelles absent (Fig. 5A, 5B).

On the fourteenth day after the PRF treatment, the SN fibers showed loose arrangements in the CP group. Both myelinated and non-myelinated nerve fibers were almost in normal structures. The myelin sheath struc-



5A: The myelin sheath of the nerve fiber was significantly thinner, axons in the myelin sheath had degenerated and dissolved, and mitochondria were swollen and rarely observed (×4.0 K).
5B: Demyelination of nerve fibers was observed, myelin sheath was distorted, and the myelin lamellar structure was significantly damaged, Schwann cells had formed heterochromatin and were in the mitotic phase with various cytoplasmic organelles absent (×2.0 K).
5C: Myelin sheath showed mostly complete structure, with partial myelin sheath showing irregular shapes. The myelin lamellar structure appeared in loose fuzzy segments and with formation of myelin sheath balls (×4.0 K).
5D: The chromatin of Schwann cells was homogenous and loose, mitochondrial hyperplasia was observed in the axons, and swollen mitochondria, broken cristae, and vacuolization were observed in a small number of nerve fibers (×2.0 K).
5E: The ultra-microstructure of the myelinated nerve fibers showed a regular morphology, demyelination of nerve fibers was observed, swollen mitochondria and hyperplasia were rarely observed in the axons, and the microfilaments and microtubules in the axons were also clear and complete (×4.0 K).
5F: The chromatin of the Schwann cells was homogenous, the nerve fiber structure was dense, the ultra-microstructure of the myelin nerve fibers showed a regular morphology, and swollen mitochondria and hyperplasia were occasionally observed in the axons (×2.0 K).

Fig. 5. Morphometric analysis of SN 15 days after the PRF/Sham treatment in the CS (5A, 5B) group, CP (5C, 5D) group, and SS (5E, 5F) group.

tures were mostly complete, with only partial irregularly shaped myelin sheath. The myelin lamellar structure appeared in fuzzy segments, loose and with formation of myelin sheath balls. The chromatin of Schwann cells was homogenous and loose. In addition, a large amount of mitochondrial hyperplasia was observed in the axons. Swollen mitochondria, broken cristae, and vacuolization were observed in a small number of the nerve fibers (Fig. 5C, 5D).

In the SS group, the myelin lamellar structure of the SN was clear. The microfilaments and microtubules in the axons were also clear and complete. Schwann cells and other subcellular fractions in the axons were also observed (Fig. 5E, 5F).

ELISA

By the end of the experiment, the GDNF level at the ligation site of the SN in the CP group ranks highest among the 3 experimental groups. While the GDNF concentration at the SN at the ligated site in the CS group was significantly higher in comparison with that in the SS group ($P < 0.01$) (Fig. 6).

The GDNF level in the L4-L6 spinal segments in the CP groups takes the highest place of the 3 groups in experiments. The GDNF concentration in the L4-L6 spinal segments in the CS group was significantly higher than that of the SS group ($P < 0.01$) (Fig. 7).

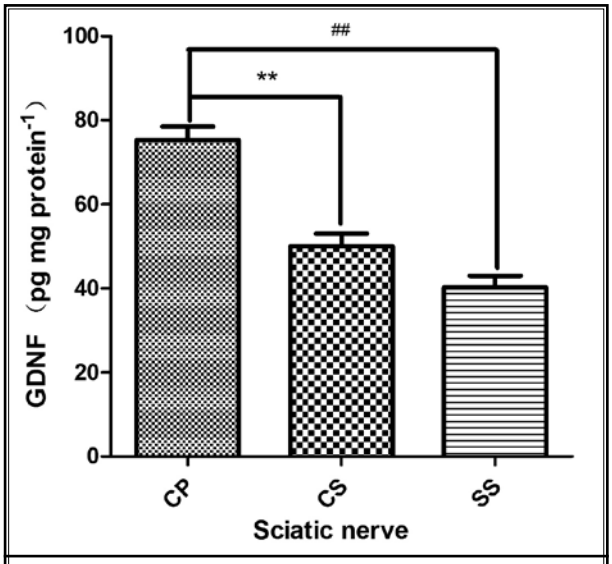


Fig. 6. *Variations of GDNF expression in the sciatic nerve tissue of rats in each group on the fourteenth day after the PRF treatment.*
**: Compared to the CS group, P < 0.01; ##: compared to the SS group, $P < 0.01$.

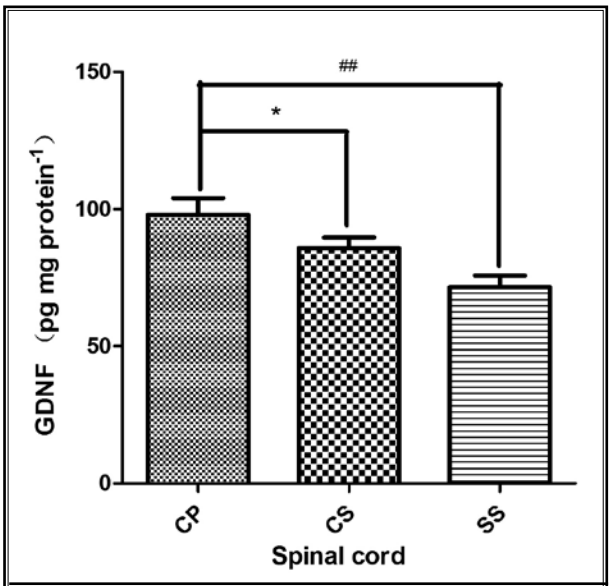


Fig. 7. *Variations of GDNF expression in rat L4-L6 spinal segments on the fourteenth day after the PRF treatment.*
*: Compared to the CS group, P < 0.01; ##: compared to the SS group, $P < 0.01$.

Discussion

In this study, the HWT and TWL in the CS and CP group were significantly lower than those before the ligation and those of the SS group. This indicated the successful establishment of the NP model in the CS and CP group. Right after the PRF treatment, the pain behavior of the rats did not improve significantly. On the sixth day after the treatment, the mechanical and thermal pain threshold of the hindpaw at the ligated site in the CP group showed significant improvement compared to that of the CS group. This improving trend persisted until the end of the experiment. Our study showed that the application of PRF on the peripheral nerve could reduce NP behavior in a CCI model. This result was consistent with the results from Vallejo et al (8) who first reported that PRF treatment on the injured peripheral nerve could relieve the NP in SNI model.

The symptoms of the CCI model have demonstrated extreme similarity to the clinical characteristics of human peripheral NP (19). The DRG is the first level of sensory neurons. The impairment of the DRG would only reduce the sensation without affecting motor

functions. Therefore, the DRG has been a common target for the treatment of chronic pain in clinical practice. Different from most studies in the past, {who?} have reported that PRF could effectively relieve NP in CCI models by action on the corresponding DRG, the results showed that PRF treatment the injured SN could also reduce the hyperalgesia in the CCI model. Compared to the puncture of the peripheral nerve, percutaneous puncture of the DRG involves complicated operations, great trauma, and risk, while the puncture of the nerve trunk is more convenient. As a technique that is theoretically nondestructive to the nerves, PRF can be applied to various targets along the nociceptive pathway, including the hybrid nerves, especially motor fibers. The results of the study provided a theoretical basis for the selection of targets of PRF during the clinical treatment of NP.

Vallejo et al (8) applied 42°C PRF directly at the impaired nervous tissue for 3 minutes. The ipsilateral hindpaw showed significantly increased HWT scores on the second day after the treatment compared to that of the control group. The parameters used in this study were the same as those of Vallejo et al (8), and significant pain reduction was observed from the sixth day after the treatment. This result suggested that the onset time of PRF treatment may not be completely the same due to the different NP models used in these experiments. The delayed onset time of PRF revealed in this experiment was in agreement with our clinical observations for the PRF treatment on patients with trigeminal neuralgia (20). This may be caused by the tissue damage during the puncture in PRF treatment and the gradual onset of the PRF neuromodulation effect. In addition, on the fourteenth day after the treatment, PRF could not completely relieve the mechanical and thermal hyperalgesia, which suggested that further observing of the long-term effects and technical modifications of PRF to enhance its effectiveness are merited.

After the peripheral nerve was impaired, the number, diameter, and myelin sheath thickness of the nerve were routinely used to assess nerve regeneration (18). In this experiment, we discovered that at the fourteenth day after the Sham treatment, the rats in the CS group showed significant decreases in above indexes compared to the SS group, which was consistent with observations by other scholars (18,21). On the fourteenth day after the PRF treatment, the rats in the CP group showed no significant differences in the axonal diameter and myelin sheath thickness, except for the axonal number at the ligated SN in comparison with that of the SS group. Compared to the CS group, all the above indexes in the CP group showed significant increases. Therefore, we suggested that PRF could promote the reparation and regeneration of impaired nerves to a certain extent. However, 14 days after the PRF treatment, the impaired nerve fibers failed to recover to their normal number, which indicated the limitations of either the effectiveness of PRF or the short duration of the observation.

The safety of PRF treatment of the SN has attracted attention from clinical physicians. Choi et al (22) applied 42°C PRF directly on the normal SN of rats for 2 minutes. On the second day after the procedure, only a slight injury and edema in the myelinated axons were observed. The PRF treatment itself could induce minor, reversible pathological changes to nervous tissues, which may be another reason for its gradual onset. In this study, we discovered that the SN in CS group showed a large number of collagen fibers emerged around the severely injured myelin sheath. Schwann cells were observed to form heterochromatin and were in the mitotic phase, which suggested that the pathological changes in the injured SN in the CCI model were significant. These results were in agreement with the observations from Gabay et al (23) and Wagner et al (24). The electron microscopic results revealed that rats in the CP groups showed almost normal myelinated and non-myelinated nerve fiber structures at the ligated site of the SN after the PRF treatment. The injury was significantly reduced compared to that of the CS group, which suggested that the PRF treatment of the impaired peripheral nerve was safe and effective. In addition, we observed a considerable amount of mitochondrial hyperplasia in the myelin fibers of the ligated SN in the CP group after PRF treatment. Such mitochondrial hyperplasia was rarely observed in the CS and SS groups, which indicated that PRF could promote the hyperplasia of mitochondria in injured nerve fibers and that this effect benefited the reparation and regeneration of nervous tissues.

GDNF is widely distributed in the peripheral and central nervous systems and provides nutrition, protection, and post-injury reparation for multiple types of neurons (25-27). Several studies have shown that both direct intrathecal administration of exogenous GDNF (28,29) and upregulation of GDNF expression in vivo through transgenic technology (30) could significantly relieve NP. Consistent with previous studies, on the twenty-eighth day after the ligation of the SN, the GDNF expression at both the SN and spinal cord L4-L6 segments of the rats in the CS group with Sham treat-

ment significantly increased compared to those of the SS group. In this study, in the CS group, the pain behavior showed a slight improvement, but the pathological injury of the tissue remained severe; this result suggested that the upregulation of GDNF could not completely improve the prognosis of impaired nerves. Exogenous supplementation of GDNF or other methods to increase the level of it may be required to relieve NP.

The actual action mechanism of PRF treatment requires further studies to clarify. Vallejo et al (8) reported that after direct PRF treatment of an impaired SN in an SNI model, the expression of many pain-related genes along the nociceptive pathway would change to effectively relieve hyperalgesia. On the fourteenth day after PRF treatment, the GDNF expression levels at the SN at the ligation site and L4-L6 spinal cord segments in the CP groups were significantly higher than those in the CS group (increased by 50.5% and 14.1%, respectively); the increases were even more significant when compared to the SS group (increased by 87.2% and 38.8%, respectively). On the fourteenth day after PRF treatment, the hyperalgesia in the NP rats were reduced significantly. As observed with optical and electron microscopes, the degree of injury was significantly reduced in the nerve fibers after PRF treatment. Since Choi et al (22) has confirmed that PRF directly on the normal SN can only induce minor, reversible pathological changes to nervous tissues, we suggest that PRF may promote the reparation of injured peripheral nerves through the enhancement of GDNF expression to ameliorate the neuropathic pain state; however, up to now, it's not clear whether GDNF can provide nervous protection through the peripheral or central nervous system. On the basis of our results, we speculated that PRF could reduce NP behavior through the upregulation of GDNF expression both in the peripheral and central nervous system.

Conclusions

The direct application of PRF at the ligation site of the SN can reduce CCI-induced hyperalgesia and hyperthermalgesia and, to a certain extent, can repair the lesions in the injured SN after ligation. The mechanism of these events may be realized through the upregulation of GDNF expression in the nervous tissues.

Limitations

Long-term studies are required to clarify whether the rats can recover to the same condition as before the surgery after PRF treatment. The effectiveness of the PRF treatment to the SN and the DRG should be compared in the same NP model in order to find a more ideal target. The different therapeutic effects resulting from different PRF parameters also require further studies to confirm. The GDNF expression was only measured at the fourteenth day after the treatment, with a lack of sequential measurements. The correlation between the variation of GDNF expression and the pain behavior indexes is meaningful for how the GDNF might be an artifact of the experiment. The other potential mechanisms of PRF remain to be studied in the future.

Acknowledgment

Profs. Ji Nan and Luo Fang corresponded equally to this work in designing and supervising the project.

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