Pulsed radiofrequency inhibits expression of $P2X_3$ receptors and alleviates neuropathic pain induced by chronic constriction injury in rats

Miao Fu[1], Lan Meng[2], Hao Ren[3], Fang Luo[2]

[1]Department of Anesthesiology, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University Cancer Hospital and Institute, Beijing 100142, China;
[2]Department of Pain Management, Beijing Tiantan Hospital, Capital Medical University, Beijing 100050, China;
[3]Department of Anesthesiology and Pain Management, Beijing Tiantan Hospital, Capital Medical University, Beijing 100050, China.

Abstract

Background: Pulsed radiofrequency (PRF) is a minimally invasive interventional technique that provides a novel and effective treatment strategy for neuropathic pain (NP). PRF is advantageous because it does not damage nerves and avoids sensory loss after treatment. At present, animal studies have demonstrated that PRF is safe and effective for relieving the NP associated with sciatic nerve damage in rats with chronic constriction injury (CCI). However, the mechanism through which this effect occurs is unknown. An increasing body of evidence shows that the expression of the P2X ligand-gated ion channel 3 ($P2X_3$) receptor is closely related to NP; this study was to investigate whether the expression of this receptor is involved in NP relief due to PRF.

Methods: A total of 36 healthy adult male Sprague-Dawley (SD) rats were randomly divided into three groups: Sham group, CCI group, and PRF group. The right sciatic nerve was ligated in CCI group and PRF group to establish a CCI model; the right sciatic nerve was separated but not ligated in Sham group. On day 14 after the operation, PRF was administered to the ligated sciatic nerve in PRF group (42°C, 45 V, 2 min). A non-live electrode was placed at the exposed sciatic nerve for the rats in Sham and CCI groups. The hindpaw withdrawal threshold (HWT) and thermal withdrawal latency (TWL) were measured at the right hindpaw at different time points before and after PRF or sham therapy. On day 28 after treatment, the dorsal root ganglion (DRG) and spinal dorsal horn of the right L4–6 were harvested from each group to determine the mRNA and protein levels of the $P2X_3$ receptor.

Results: On day 28 after PRF treatment, the HWT (8.33 ± 0.67 g *vs.* 3.62 ± 0.48 g) and TWL (25.42 ± 1.90 s *vs.* 15.10 ± 1.71 s) were significantly higher in PRF group as compared to CCI group ($P < 0.05$). The mRNA expression of the $P2X_3$ receptor in the DRG in PRF group was 23.7% lower than that in CCI group ($P < 0.05$), in the spinal dorsal horns in PRF group was 22.7% lower than that in CCI group ($P < 0.05$). The protein expression of the $P2X_3$ receptor in the DRG in PRF group was 27.8% lower than that in CCI group ($P < 0.05$), in the spinal dorsal horns in PRF group was 35.6% lower than that in CCI group ($P < 0.05$).

Conclusion: PRF possibly reduces NP in CCI rats by inhibiting the expression of the $P2X_3$ receptor in the L4–6 DRG and spinal dorsal horns.

Keywords: Pulsed radiofrequency; $P2X_3$ receptor; Neuropathic pain; Chronic constriction injury

Introduction

In 2008, the Neuropathic Pain Special Interest Group (NeuPSIG) of the International Association for the Study of Pain (IASP) defined neuropathic pain (NP) as a symptom which arises "as a direct consequence of a lesion or disease affecting the somatosensory system".[1] NP is characterized by spontaneous pain, hyperalgesia, and allodynia. Moreover, it presents a challenging public health problem. Many researchers have studied the neuroanatomical and neurophysiological mechanisms of the development and maintenance of NP, but specific details remain unknown. As a result, no targeted treatment is available for NP and no breakthrough therapies have been developed for NP to date. Therefore, it is of great importance and urgent necessity to seek novel treatments for NP.

In 1997, Sluijter *et al*[2] first proposed the use of pulsed radiofrequency (PRF), a technique that does not damage nerves. PRF was developed based on continuous

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Miao Fu and Lan Meng contributed equally to this work.

Correspondence to: Prof. Fang Luo, Department of Pain Management, Beijing Tiantan Hospital, Capital Medical University, Beijing 100050, China
E-Mail: luofangwt@yahoo.com

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radiofrequency (CRF). With an emission frequency of 2 Hz, an output of 45 V, and a 500-kHz high-frequency alternating current during each session, the RF current lasts 20 ms with an intervening 480 ms interval. These settings allow the dispersion of heat generated by the RF current through the nerve tissue, thereby preventing the temperature at the electrode tip from exceeding 42°C and avoiding the associated degeneration of local tissues.[2,3] Given its clinical efficacy and avoidance of associated permanent nerve injury, so far PRF has been widely applied to treat NP.[4-6] Meanwhile, it can be used in theory on various targets of the nociception pathway. Studies have demonstrated that PRF can be administered to dorsal root ganglions (DRG) to effectively treat peripheral NP.[7,8] Despite these advantages, performing DRG radiofrequency on the percutaneous puncture path is complex and risks significant tissue damage. In contrast, it is easier to administer radiofrequency to the peripheral nerves. Furthermore, animal studies have demonstrated that PRF is safe and effective at reducing the NP associated with sciatic nerve damage in rats.[9-11] However, the mechanism underlying this observation remains unclear.

The purinergic receptor (P2X) ligand-gated ion channel 3 ($P2X_3$) receptor, a member of the P2X family, is highly selectively expressed in the body and the peripheral and central axons of the primary afferent sensory neurons associated with nociceptive transmission; the central axon terminal travels in the spinal cord and stops at the spinal dorsal horn. During peripheral nerve injury, the nerve releases ATP, which activates the $P2X_3$ receptor on the postsynaptic membrane, leading to membrane depolarization and subsequent rapid synaptic transmission. In addition, the released ATP activates the $P2X_3$ receptor on the presynaptic membrane, resulting in a pain signal that passes through DRG neurons and enters the spinal cord via C and Aδ nerve fibers and finally arrives at the pain center in the brain.[12] Peripheral nerve injury facilitates the release of endogenous ATP to reinforce the activation of $P2X_3$ receptors to amplify the nociceptive signaling. Therefore, activation of the neuronal $P2X_3$ receptor is engaged in the initiation and development of chronic neuropathic pain.[13] Inhibition of $P2X_3$ in the brainstem or the spinal dorsal horn reduces hypersensitivity following nerve injury, suggesting that $P2X_3$ plays a role in pathological pain.[14] Though evidence exists supporting the implication of the $P2X_3$ receptor in regulating NP, no studies have validated whether PRF reduces NP by modulating the expression of the $P2X_3$ receptor yet. This study investigated pain behaviors and the expression of the $P2X_3$ receptor in the L4–6 DRG and spinal dorsal horns after administering PRF to ligated sciatic nerves in chronic constriction injury (CCI) rats, in an attempt to answer whether PRF reduces NP by modulating the expression of the $P2X_3$ receptor.

Methods

Ethical approval

All animal procedures were approved by the Beijing Neurosurgical Institute Experimental Animal Welfare Ethics Committee. A total of 36 clean, healthy adult male Sprague-Dawley (SD) rats (Vital River Laboratories, China) weighing 220 to 250 g were housed at 22 to 24°C environment, with and relative humidity of 40% to 60%, 12-h alternative light-dark cycle, and free access to food and water.

Sciatic nerve CCI model

The rat CCI model was established with reference to the method described by Bennett *et al*[15] After anesthesia (10% chloral hydrate, 400 mg/kg, intraperitoneal injection), an incision was made along the lateral midline of the right lower extremity. The sciatic nerve trunk was exposed after blunt dissection along the lateral biceps femoris, and a 4–0 chromic thread was applied to loop and ligate the proximal bifurcation of the sciatic nerve four times with a 1 mm spacing (the intensity was monitored by the slight twitch of the calf muscle). After this procedure, the rats were returned to their original housing environment.

Treatment groups and design

A total of 36 rats were randomly divided into three groups: Sham group, CCI group, and PRF group. The treatment for each group is shown in Table 1.

Hindpaw withdrawal threshold (HWT) test

The rats were placed in separate transparent cages with a metal mesh bottom and acclimated for 2 h. During the experiment, different pressures of Von Frey hair (VFH; Stoelting, Kiel, WI, USA) were applied to vertically stimulate the sole of the rats' right hindpaw. The pressure started at 1 g and was increased to the next level if paw withdraw was not elicited. Pressure was applied until the VFH slightly bent for 3 to 5 s, and this procedure was repeated for five times at a 30 s interval. The HWT was determined when paw withdraw was elicited for at least three out of the five times.

Table 1: Group assignment and treatment.

Groups	CCI procedure	PRF treatment
Sham	The sciatic nerve was exposed with no ligation	A non-live electrode was placed at the ligated site
CCI	The sciatic nerve was exposed and ligated	A non-live electrode was placed at the ligated site
PRF	The sciatic nerve was exposed and ligated	An electrode was placed at the ligated site for PRF treatment

CCI: Chronic constriction injury; PRF: Pulsed radiofrequency.

Thermal withdrawal latency (TWL) test

The rats were placed in separate transparent cages with a glass plate at the bottom and acclimated for 30 min. A portable infrared radiation thermal stimulator (7371, Ugo Basile, Comerio, Italy) was adopted to direct the center of heat to the right hindpaw of the rats for continuous radiation, and a digital timer was utilized to determine latency, which was defined as the time from the start of heat exposure to paw withdrawal due to heat. To prevent tissue damage, the TWL was measured three times in every 2 min, and the mean value was employed for analysis.

Application of PRF or sham intervention

For PRF group, the ligated sciatic nerve was exposed and treated with PRF. A PRF trocar (PMF-21-50-2, Baylis, Canada) was vertically placed at the center of the ligated sciatic nerve and secured with a space frame. Next, the core was removed, and an RF electrode was inserted (PMK-21-50, Baylis, Canada). An RF instrument (PMG-230, Baylis Medical Inc., Montreal, Canada) was adopted for PRF therapy at 2 Hz, 42°C, and 45 V for 120 s. At the end of the treatment, the incision was sutured, and the rats were returned to the original housing environment. For CCI group and Sham group, the sciatic nerves were exposed, and a non-line electrode was placed at the sciatic nerve for 120 s.

RT-qPCR detection of the mRNA expression of the $P2X_3$ receptor

Behavior indicators were measured on day 28 after PRF treatment. The rats were sacrificed by breaking their necks, and the DRG and spinal dorsal horns of the right L4–6 were promptly removed and stored in liquid nitrogen. The tissue was equally divided into two samples, one of which was used for RT-qPCR. A TRIzol kit (Invitrogen Corporation, USA) was adopted to extract the total RNA from the L4–6 DRG and spinal dorsal horns of the ligated side. An Moloney Murine LeukemiaVirus (MMLV) first-strand synthesis kit (New England Biolabs, USA) was utilized to obtain the first strand cDNA from the extracted RNA via reverse transcription. The ΔΔCT method was employed for the relative quantification of the mRNA level and β-actin as the reference. The following sequences were adopted for the primers for the $P2X_3$ mRNA: NM_031075, primer F: 5'-CC AAGTCGGTGGTTTGAAGAG-3', primer R: 5'-TACT-GAGGACTCAATGGCGGTGT-3'. The sequences of the primers for β-actin mRNA were as follows: NM_031144, primer F: 5'-CCCATCTATGAGGGTTACG- ', primer R: 5'-TTTAATGTCACGCACGATT-3'. ABI StepOnePlus (ABI, USA) was applied to perform the qPCR, and Fast SYBR Green Master Mix (Thermo Fisher Scientific, USA) was used in the reaction system. The PCR conditions were 95°C for 20 s, followed by 95°C for 3 s and 60°C for 30 s for 40 cycles. The melting curve was plotted.

Western blotting analysis of the protein expression of $P2X_3$ receptor

The second DRG and spinal dorsal horn sample was grounded on ice in an Radio Immunoprecipitation Assay (RIPA) lysis buffer. After centrifugation, the supernatant was collected to determine the protein concentration via Bicinchoninic Acid (BCA) methods. The sample was loaded for electrophoresis at constant voltages of 70 V and 90 V for stacking and resolving gels, respectively and then transferred to a Polyvinylidene Fluoride (PVDF) membrane at a constant current of 200 mA for 70 min. The membrane was blocked in 5% Bull Serum Albumin (BSA) at room temperature for 2 h. After the TBST wash (5 min, three times), the membrane was incubated in a bag with Tris Buffered saline Tween (TBST) -diluted antibody (1:100, Santa Cruz Biotechnology, USA) at 4°C overnight. After the second TBST wash (5 min, three times), the membrane was incubated with the Horseradish Peroxidase (HRP)-labeled goat anti-rabbit secondary antibody (1:3000, Santa Cruz Biotechnology) at room temperature for 2 h. After the third TBST wash (10 min, three times), electrochemiluminescence (ECL) reagents were added to the membrane, and the DNR MicroChemi chemiluminescence gel imaging system was adopted for analysis. The relative gray value of the $P2X_3$ receptor band represented the relative expression level of the $P2X_3$ receptor.

Statistical analysis

SPSS 20.0 (Chicago, IL, USA) was utilized for statistical analysis. Measurement data was expressed as the mean ± standard error of the mean (SEM). The HWT and TWL were analyzed with a repeated-measures analysis of variance (ANOVA). The expression level of the $P2X_3$ receptor was also analyzed with a one-way ANOVA. A value of $P < 0.05$ was considered significant.

Results

Behavioral testing

No significant between-group difference was observed in the HWT before sciatic nerve ligation ($P > 0.05$). On day 7 after ligation, the HWT showed a significant decrease in CCI group and PRF group ($P < 0.01$). The HWT decreased to 2.71 ± 0.53 g and 2.69 ± 0.60 g in these groups, respectively, on day 14 after ligation. No significant difference was found between CCI group and PRF group ($P > 0.05$). The HWT in these two groups was significantly lower than that in Sham group (9.55 ± 0.64 g; $P < 0.01$). In addition, no significant between-group difference was observed in the TWL before sciatic nerve ligation ($P > 0.05$). On day 7 after ligation, the TWL showed a significant decrease in CCI group and PRF group ($P < 0.01$). The TWL decreased to 11.42 ± 1.45 s and 11.04 ± 1.54 s in these groups, respectively, on day 14 after ligation. No significant difference was found between the CCI group and PRF group ($P > 0.05$). The TWL in these two groups was significantly lower than that in Sham group (27.80 ± 1.51 s; $P < 0.01$) [Figure 1].

On day 7 after PRF treatment, the HWT was significantly higher in PRF group than that in CCI group ($P < 0.01$); from day 14 to day 28, the HWT continued to increase in PRF group and was significantly higher than that in CCI group ($P < 0.01$). During the first 14 days after PRF treatment, the HWT gradually increased in PRF group but

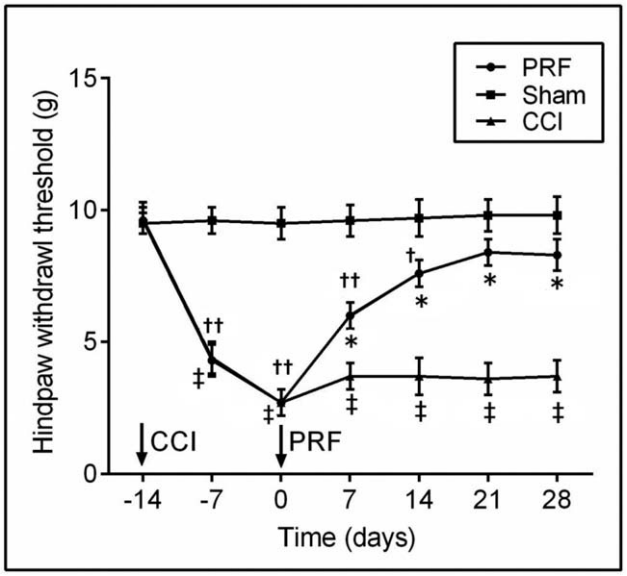

Figure 1: The effect of pulsed radiofrequency on the hindpaw withdrawal threshold after sciatic nerve ligation. $^{*}P < 0.01$, PRF group *vs.* CCI group; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, PRF group *vs.* Sham group; $^{\ddagger}P < 0.01$, CCI group *vs.* Sham group. Data are expressed as the mean ± standard error of the mean.

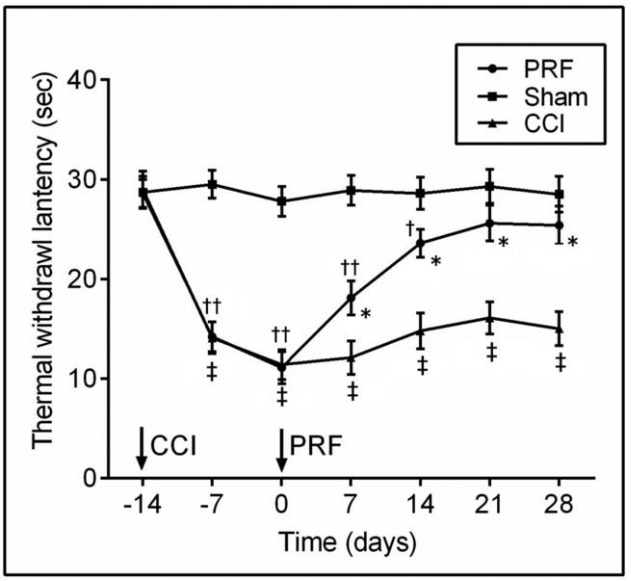

Figure 2: The effect of pulsed radiofrequency on the thermal withdrawal latency after sciatic nerve ligation. $^{*}P < 0.01$, PRF group *vs.* CCI group; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, PRF group *vs.* Sham group; $^{\ddagger}P < 0.01$, CCI group *vs.* Sham group. Data are expressed as the mean ± standard error of the mean.

remained significantly lower than that in Sham group (day 7: $P < 0.01$, day 14: $P < 0.05$). From day 21 to day 28 after PRF treatment, no significant difference in the HWT was observed between PRF and Sham groups ($P > 0.05$, Figure 1).

In PRF group, the TWL gradually increased after PRF treatment and was significantly higher than that in CCI group on day 7 after treatment ($P < 0.01$). This difference was even greater from day 14 to day 28 after treatment ($P < 0.01$). The TWL increased in PRF group by day 7 after treatment but remained significantly lower than that in Sham group ($P < 0.01$). By day 14 after treatment, the TWL increased further in PRF group but remained lower than that in Sham group ($P < 0.05$). From day 21 after PRF treatment through the end of the experiment; however, no significant difference was observed in the TWL between PRF and Sham groups ($P > 0.05$, Figure 2).

RT-PCR detection of the mRNA expression of the $P2X_3$ receptor

On day 28 after treatment, the relative mRNA expression of the $P2X_3$ receptor in the DRG was 1.12 times higher in CCI group than that in Sham group ($P < 0.05$). The relative mRNA expression of the $P2X_3$ receptor in the DRG in PRF group was 23.7% lower than that in CCI group ($P < 0.05$) and 60.3% higher than that in Sham group ($P < 0.05$). The relative mRNA expression of the $P2X_3$ receptor in the spinal dorsal horns was 1.21 times higher in CCI group than that in Sham group ($P < 0.05$), the relative mRNA expression of the $P2X_3$ receptor in the spinal dorsal horns in PRF group was 22.7% lower than that in CCI group ($P < 0.05$) and 71.3% higher than that in Sham group ($P < 0.05$).

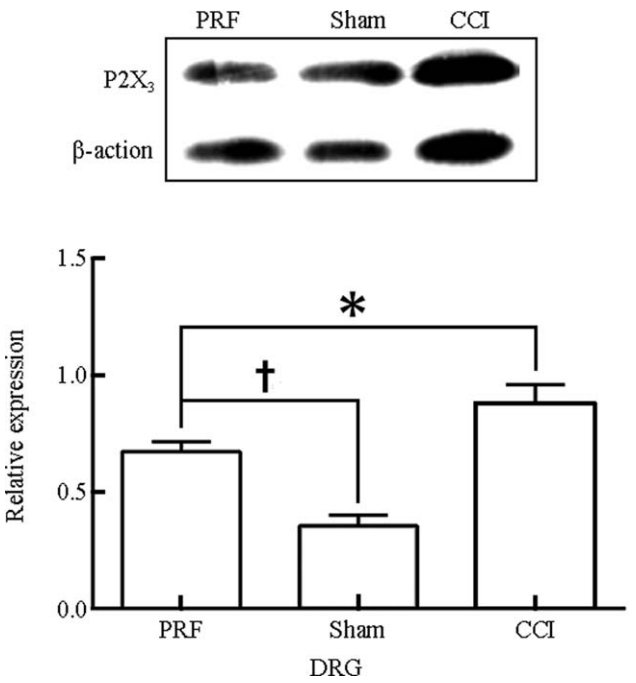

Figure 3: The relative protein expression of the $P2X_3$ receptor in the DRG on day 28 after treatment. $^{*}P < 0.05$, PRF group *vs.* CCI group; $^{\dagger}P < 0.05$, PRF group *vs.* Sham group. Data are expressed as the mean ± standard error of the mean.

Western blotting analysis of the protein expression of the $P2X_3$ receptor

On day 28 after sham treatment, the relative protein expression of the $P2X_3$ receptor in the DRG was 1.39 times higher in CCI group than that in Sham group ($P < 0.05$). On day 28 after PRF treatment, the relative protein expression

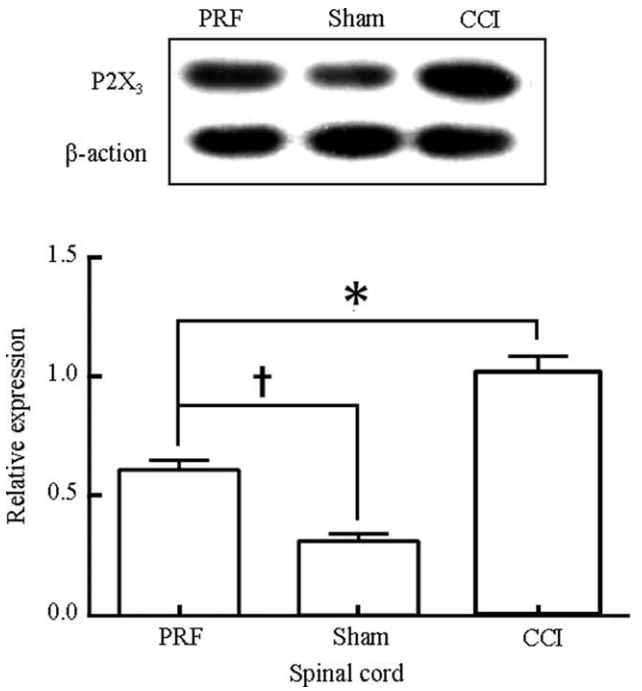

Figure 4: The relative protein expression of the $P2X_3$ receptor in the spinal cord on day 28 after treatment. $^{*}P < 0.05$, PRF group *vs.* CCI group; $^{\dagger}P < 0.05$, PRF group *vs.* Sham group. Data are expressed as the mean ± standard error of the mean.

of the $P2X_3$ receptor in the DRG in PRF group was 27.8% lower than that in CCI group ($P < 0.05$) and 89.6% higher than that in Sham group ($P < 0.05$) [Figure 3].

On day 28 after sham treatment, the relative protein expression of the $P2X_3$ receptor in the spinal dorsal horns was 1.75 times higher in CCI group than that in Sham group ($P < 0.05$). On day 28 after PRF treatment, the relative protein expression of the $P2X_3$ receptor in the spinal dorsal horns in PRF group was 35.6% lower than that in CCI group ($P < 0.05$) and 76.9% higher than that in Sham group ($P < 0.05$, Figure 4).

Discussion

PRF improved hyperalgesia after application to the ligated sciatic nerve in CCI rats

This study adopted a CCI rat model of the sciatic nerve, a classic NP model described in 1900 by Bennett *et al*.[15] The behavior features of this model are similar to the clinical manifestations of NP in humans and is thus widely used in NP research. After sciatic nerve ligation, CCI rats gradually exhibit limp and foot eversion, with persistent and continuous pain behaviors approximately 7 days after ligation. This study measured the HWT and TWL, two pain behavioral indicators, to investigate the mechanical and thermal pain thresholds of rats. As with previous studies,[11,16] the rats in CCI group and PRF group showed severe mechanical allodynia and thermal hyperalgesia by day 7 after sciatic nerve ligation. This condition further worsened by day 14, indicating the successful establishment of the CCI model.

In this study, PRF was administered at 42°C, a standard setting, to the ligated sciatic nerves of CCI rats. As in previous studies,[10,17,18] mechanical allodynia and thermal hyperalgesia were significantly improved 7 days after treatment and further improved by day 14. However, this study included a longer follow-up period than previous studies. The results showed that mechanical allodynia and thermal hyperalgesia continued to improve after day 14 and peaked on day 21 after PRF treatment in the NP rat model. This effect was maintained till at least day 28 after treatment; on the other hand, mechanical allodynia and thermal hyperalgesia did not improve in untreated CCI rats, suggesting that PRF had significant and lasting pain-relief effects, which is consistent with clinical experience in which PRF, a novel and minimally invasive interventional analgesic technique, showed significant and lasting pain-relief effects in patients with various pain conditions such as trigeminal neuralgia,[19] occipital neuralgia and migraine,[20] chronic inguinal neuralgia,[21] and post-herpetic neuralgia.[22]

The results showed that after one session of PRF treatment, pain relief peaked over 3 weeks, rather than immediately after treatment. This finding is consistent with clinical experience in which pain relief was gradual in patients with NP after PRF treatment.[4,19] We believe that PRF exerts long-lasting pain relief by regulating the expression of certain genes, thereby suppressing NP. This study did not show significant differences in mechanical allodynia or thermal hyperalgesia from day 21 to day 28 after PRF treatment between PRF group and Sham group. The mean thresholds were lower in PRF group, and additional research is needed to investigate how to improve the effect of PRF treatment on NP.

PRF likely exerts analgesic effects by reducing the expression of the $P2X_3$ receptor in the DRG and spinal dorsal horns

The $P2X_3$ receptor is expressed in the sensory neurons. Once activated, the $P2X_3$ receptor is involved in the plasticity of synapses in the spinal dorsal horns and promotes and maintains NP.[23] Dorn *et al*[24] showed that pain tolerance increases with a significant decrease in the mRNA level of the $P2X_3$ receptor in the DRG of mice. Yu *et al*[25] reported that the protein expression of the $P2X_3$ receptor was significantly up-regulated in the spinal dorsal horns of rats with chronic compression of dorsal root ganglion (CCD) and that suppressing the protein expression of the $P2X_3$ receptor alleviated mechanical allodynia. This study demonstrated that rats in CCI group exhibited severe mechanical allodynia and thermal hyperalgesia after sciatic nerve ligation, and this NP was sustained throughout the experiment while it was treated using PRF. Moreover, this study also indicated that the expression of $P2X_3$ receptor in the DRG and spinal dorsal horns on the ligated side was significantly higher in CCI group than in Sham group, which is in agreement with the findings of Barclay *et al* and Novakovic *et al*,[26,27] who found that the level of the $P2X_3$ receptor in the DRG and spinal dorsal horns is mediated by peripheral nerve injury and that the $P2X_3$ receptor plays an important role in NP.

At present, no studies investigating how PRF alleviates NP have focused on the changes in the expression of the $P2X_3$ receptor.

Previous research has shown that the analgesic effect of PRF involves changes in neuronal synaptic transmission, neuromodulation, and the biological effects of cytokines, rather than thermal damage.[6,28] Huang *et al* administered PRF to the dorsal roots of rats with diabetic NP and showed that PRF reduced diabetes-induced hyperalgesia, suggesting that PRF alleviates NP by inhibiting the release of the excitatory neurotransmitters associated with noxious stimuli.[29] However, it is difficult to administer PRF to dorsal roots. Several recent studies have attempted to administer PRF to damaged nerves. Yeh *et al* administered PRF to the nerve near the injured site in rats with spared nerve injury (SNI) and showed that PRF prevented NP while also inhibiting the activity of extracellular signal-regulated kinase (ERK).[9] Lee *et al* administered PRF to the injured sciatic nerves of CCI rats and suggested that PRF alleviates NP by inhibiting the expression of tumor necrosis factor-α (TNF-α).[10] Consistent with Yeh *et al*,[9,10] the current study showed that administering PRF to the injured nerve was a safe and effective method for relieving hyperalgesia in the NP model. It is simple and easy to administer PRF to compressed nerves, and future research should investigate optimal treatment targets by examining the effect of PRF at different sites to provide a basis for clinical treatment.

This study showed that the mRNA and protein expression levels of the $P2X_3$ receptor in the DRG and spinal dorsal horns of the ligated side of CCI rats were significantly lower in PRF group than in the untreated CCI group on day 28 after PRF treatment; moreover, mechanical allodynia and thermal hyperalgesia were improved in PRF group. We hypothesize that PRF alleviates NP in CCI rats by down-regulating the expression of the $P2X_3$ receptor on the pain pathway, reducing or inhibiting the transmission of pain signals to the central nervous system, and modulating pain transmission. This study showed that despite PRF treatment, the expression of the $P2X_3$ receptor in the DRG and spinal dorsal horns remained significantly higher and pain thresholds were lower in PRF group than in Sham group without sciatic nerve ligation, suggesting that one session of PRF treatment with the standard setting has limited effects. Additional research is needed to investigate the effect and mechanism of multiple PRF sessions or PRF treatments with different parameters on NP. Honore *et al* adopted a $P2X_3$ receptor knockout rat model and McGaraughty *et al* performed an intrathecal injection of a selective $P2X_3$ receptor antagonist to confirm that suppressing the expression of the $P2X_3$ receptor alleviated NP.[30,31] PRF treatment combined with other methods to reduce the expression of the $P2X_3$ receptor on the pain pathway should improve the efficacy further.

The expression of the $P2X_3$ receptor in the DRG and spinal dorsal horns at 28 days after PRF treatment was investigated in the study. However, the relationship between the expression of the $P2X_3$ receptor and behavioral indicators at different time points after treatment was not discussed. In addition, the quantity of $P2X_3$ receptor was analyzed but not the function of $P2X_3$ receptor, and other potential substances related with neuropathic pain were not investigated. Moreover, this study did not investigate the effect of PRF in a $P2X_3$ receptor gene knockout model or making use of specific $P2X_3$ receptor antagonist. Therefore, we can only speculate that PRF alleviates NP by regulating the expression of the $P2X_3$ receptor. Additional researches need to explore the effect of different PRF parameters, cycles and treatment targets on the efficacy and the relationship between effect and the expression of the $P2X_3$ receptor. The effect of $P2X_3$ receptor antagonist application needs to be verified in the future. In addition, in-depth research is needed to investigate how PRF alleviates NP to provide a theoretical basis for improving the efficacy of PRF.

In conclusion, PRF effectively alleviated NP after its administration to the compressed peripheral nerve, with a long-lasting effect. The mechanism might involve down-regulating the expression of the $P2X_3$ receptor in the DRG and spinal dorsal horns, subsequently reducing the transmission of pain signals to the central nervous system.

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Conflicts of interest

None.

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