

Original Article

Paeoniflorin attenuates neuropathic pain through the regulation of Sirt1 in rats

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Abstract: Background and aim: Neuropathic pain is one of the most common types of pain. Paeoniflorin (PF) is known to protect against neuronal injury, although the underlying mechanism remains unclear. Our study aims to observe the effect of paeoniflorin on pain behavior in rats with chronic constriction injury. Methods: Sprague-Dawley rats were randomly divided into experimental groups as per the requirement. The behavior of rats was assessed by measuring the thermal withdrawal latency and mechanical contraction foot reflex threshold. We used HE staining to detect the changes in the number of neurons of the rats. Real-time PCR and western blot used to detect mRNA and protein level of Sirt1 and CREB. Results: The thermal withdrawal latency and mechanical contraction foot reflex threshold were lower in rats with chronic constriction injury (CCI) than in the sham group ($P < 0.05$). However, the thermal withdrawal latency and mechanical contraction foot reflex threshold were significantly higher with paeoniflorin than that in the CCI group ($P < 0.05$). The number of neurons cells in the CCI group was less than that in the sham operation group ($P < 0.05$). Real-time PCR and western blot analysis revealed that Paeoniflorin can affect the levels mRNA and protein of Sirt1, CREB, and BDNF in the spinal dorsal horn and dorsal root ganglion, with higher concentrations of Paeoniflorin leading to better regulation effects. Conclusion: Paeoniflorin can attenuate the pain caused by neuropathic disease, possibly through upregulation of Sirt1 in the spinal dorsal horn and the dorsal root ganglion.

Keywords: CCI, neuropathic disease, paeoniflorin, Sirt1

Introduction

Chronic peripheral neuropathic pain (NP) is caused by injury or disease in the peripheral or central nervous systems; the pain also shows long-term survival [1], and is difficult to treat with conventional methods. The common clinical forms of neuropathic pain include diabetic neuropathy, neuralgia related to herpes viral infection, trigeminal neuralgia, pain following stroke, spinal cord injury, or surgical trauma, and radioactive disease-related pain [2]. The present treatments for neuropathic pain include administration of drugs, minimally invasive procedures, or neural regulation as necessary. Several molecular biology studies have focused on identifying the mechanisms underlying neuropathic pain in the hope to find new treatments with fewer side effects.

Neuropathic pain has been found to be related to a variety of molecular pathways, such as the

MAPK pathway [3]. The silent information regulation factor-2 (Sir2) enzyme 1 (Sirt1) is a nuclear nicotinamide adenine dinucleotide (NAD⁺)-dependent histone deacetylase (HDAC) involved in the alkaline deacetylation of acetylated histones and other specific substrates, which can effectively relieve neuropathic pain [4]. Studies show that targeting Sirt1 may be effective in relieving neuropathic pain in rats with CCI [5]. The cyclic AMP-responsive element binding protein (CREB) is a nuclear transcriptional regulator from the CREB/ATF family. A study found that rats with CCI for 2 weeks had increased pCREB levels in the dorsal horn of the spinal cord and the dorsal root ganglion in addition to a decrease in pain sensitivity, indicating that CREB is involved in neuropathic pain related to central and peripheral sensitization of the formation [6]. MicroRNAs (miRNAs) are a class of non-coding RNAs, which play a decisive role in the post-transcriptional regulation of gene

expression and in the pathogenesis of a variety of pain diseases; studies have found that miRNAs are involved in inflammatory pain in the muscles. A week of neuralgia significantly changes the expression of miRNAs, including miR-34b [7], in the neural tissue [8]. We chose several proteins may relate to our research in the study, Sirt1 showed a very significant change of the proteins in this study, and we found that Sirt1 could affect cell survival through related proteins such as p53 [9]. This was in line with the purpose of our study that paeoniflorin exerted a role in cell apoptosis. So we regarded Sirt1 as the regulatory protein in this study. We performed the studies on the effect of paeoniflorin on rats' behavior, then we referred to the relevant literature and found that Sirt1 regulated nerve injury and cell survival [10], miR-34b also participated in occurrence and development of nerve injury [11]. Therefore, we further studied the expression of Sirt1 and miR-34b gene in the spinal dorsal horn and dorsal root ganglion after paeoniflorin treatment. Next we studied downstream protein of Sirt1 and miR-34b, and found CREB and BDNF protein changed in the effects of paeoniflorin, so we suspected that this pathway is one of the mechanisms that paeoniflorin affected nerve injury.

The effect of components used in traditional Chinese medicine on the prevention and treatment of neuropathic pain has gradually become a research hotspot. Many plants have been proven effective in the attenuation of chronic neuropathic pain [12]. Studies have shown that paeoniflorin has a protective effect on neurons [13] and our pre-experiment found that paeoniflorin can improve pain reactions in rats with CCI. Thus, the aim of our study was to verify that the effect of paeoniflorin on the alleviation of pain in rats with CCI is mediated by changes in the expression of Sirt1.

Materials and methods

Animals

Healthy male Sprague-Dawley (SD) rats (weight, 220-260 g) were obtained from the experimental animal center at the Wenzhou Medical University. All the animal experiments were conducted in accordance with the institutional guide for the care and use of laboratory ani-

mals. The SD rats were maintained in a 12-hour light and 12-hour dark house at a temperature of $24 \pm 1^\circ\text{C}$ and humidity of $60 \pm 5\%$ throughout the course of the experiment, and the experiment was conducted one week after acclimatization to these conditions.

Drug treatment

The paeoniflorin (PF) used in the experiment was purchased from the Chinese Academy of Medical Sciences Institute of Medicine and had a purity of 99.99%. The remaining rats were randomly divided into 4 groups (12 rats per group): sham + vehicle (normal saline solution) group, CCI + vehicle group, CCI + PF group (50 mg/kg), and CCI + PF group (100 mg/kg). The drug injection dose was selected based on the results of previous studies. The PF or vehicle injections were administered intraperitoneally starting on the first day after the CCI operation, once a day for 14 days.

CCI of the sciatic nerve

The CCI group rats were designed as described earlier by Bennett and Xie [14]. Before the operation, the rats were injected with 10% hydrazine hydrate (according to weight), and the standard dose was ~30-40 mg/kg. A longitudinal incision was made along the back side of the left lower extremity under sterile conditions, and the exposed biceps muscle and the muscle tissue were separated using the blunt method. After the biceps muscle was exposed, the sciatic nerve was gently separated from the surrounding tissue. The 4 channels of 1 mm were separated by the silk thread, and then stitched together. Three days after the operation, the pain threshold decreased by 30%, and the operative side of the jaw adductor, foot mild valgus and limp. The performance of the rats showed that the CCI model was successful. Rats in the sham operation group did not undergo nerve ligation.

Mechanical withdrawal threshold and thermal paw withdrawal threshold testing

The behavioral data were measured 1 day before the operation, and 3 days, 7 days and 14 days after the operation. The data were collected at the same time (11:00 to 14:00) each day and the room temperature was maintained at 25°C .

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Table 1. Primers sequences used for quantitative real-time PCR

Gene	Sense primer (5'→3')	Antisense primer (5'→3')
U6	TGCGGGTGCCTCGCTCGGCAGC	CCAGTGCAGGGTCCGAGGT
β-actin	CTCCATCCTGGCCTCGCTGT	GCTGTCACCTCACCCTCC
Sirt1	GGATCCTTCATTATCAGAGTTGCCACC	CTTCGAGGTTCTTAAACTTGGACTCTGG
CREB	GAGAAGCCGAGTGTTGGT	GGATACCTGGGCTAATGTG
miR-34b	TTTAGTTACGCGTGTGTGC	ACTACAACCTCCGAACGATC

Thermal withdrawal latency detection (TWL): To use the jaw/tail of 336 to perform the pain test to detect rat TWL. The laser emission intensity was set at 20% when the heating head was idle and at 60% when the heating head was working. The cutting time was set to 25 s, and the temperature of the heating head did not exceed 30°C in order to prevent it from causing damage to the animal. The rats were placed on the test bench and allowed to adapt for a moment using the heating head irradiation at the operative side of the bottom of the foot. The leg avoidance time at this moment is defined as the TWL. The inter-stimulus interval was 5 min; the data were collected as triplicates and the average values were calculated.

Mechanical withdrawal threshold (MWT) detection: The rat was placed in a transparent organic glass box with a 1 cm × 1 cm barbed wire at the bottom. The rat was allowed 15 min in the glass box to adapt to the environment before the experiment began. A dolorimeter was used to vertically stimulate the lateral plantar smooth part of the skin in the rats. The intensity of the stimulation was gradually increased while recording the foot retraction response (e.g., licking events) to the stimulation (this was defined as MWT). The inter-stimulus interval was 10 s; the data were collected as 5 replicates and the average values were calculated.

Hematoxylin-eosin staining

The CCI rats were anesthetized with 1% sodium in accordance with the 60 mg/kg standard concentration. The rats were perfused with pre-cooled saline through the ascending aorta catheter and then reperfused with 4% polyformaldehyde (PFA). The L4-L5 spinal cord segments ipsilateral to the nerve injury site were removed and fixed for 24 h with formalin, rinsed, dehydrated and embedded in paraffin, sectioned, and stained with HE. The number of spinal cord neurons in each animal was

observed under a light microscope using a 200 × amplification factor.

Real-time PCR

The total RNA was extracted from the tissue at the indicated time points using the TRIzol reagent (Invitrogen, USA) as per the manufacturer's instructions. Next, it was subsequently reverse-transcribed using the AMV Reverse Transcription System (Takara, Shiga, Japan). Real-time PCR was performed using the SYBR Green PCR mix on an ABI Prism 7900HT (Applied Biosystems, CA, USA) machine. The cDNA for miRNA quantitation was reverse transcribed from the total RNA samples using specific miRNA primers using reagents from the TaqMan MicroRNA assays and the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems, CA, USA). The cDNA was amplified by PCR using the TaqMan MicroRNA Assay primers with the TaqMan Universal PCR Master Mix. The relative miRNA expression levels were calculated from the relevant signals through normalization with the U6 snRNA expression level. The details of the primers and their sequences are outlined in **Table 1**.

Western blot analysis

After the protein was extracted from the tissue using the Radio Immunoprecipitation Assay buffer (Beyotime, China), the proteins were separated by 10% SDS-PAGE and subsequently transferred on to a PVDF membrane (Millipore, USA). The membranes were probed with the following antibodies: Sirt1, CREB (Cell Signaling Technology, USA), and β-actin (Sigma, USA). The results were visualized in the X-Ray film developer from Kodak (Fujifilm, Japan).

Statistical analysis

The data are expressed as means (± SD) of the results derived from three independent experi-

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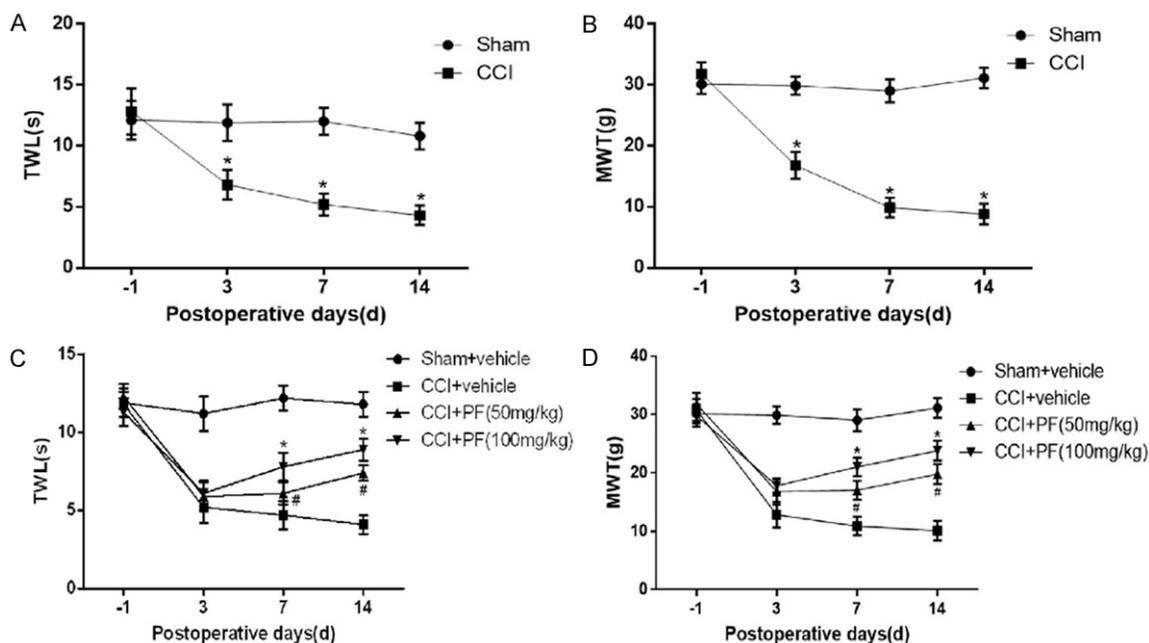


Figure 1. Effects of surgery and Paeoniflorin on Mechanical withdrawal threshold and Thermal withdrawal latency detection in rats. A: TWL of surgical treatment of rats were detected using the Plantar Test Apparatus. B: MWT of surgical treatment of rats were determined using a thermal pain stimulator. C: TWL of surgical and Paeoniflorin treatment of rats were detected using the Plantar Test Apparatus. D: MWT of surgical and Paeoniflorin treatment of rats were determined using a thermal pain stimulator. n=4 rats/group * $P < 0.05$.

ments performed in triplicates. Statistical analysis of comparisons was performed using the two-tailed Student's t-test, and simple fact analysis of variance was used in different groups. The results with P values less than 0.05 were considered statistically significant.

Results

PF upregulated the value of TWL and MWT caused by CCI

We first measured and compared the behavioral changes in rats from the CCI and the sham groups. The baseline TWL and MWT values were not significantly different from those measured after the surgery in the sham group (Figure 1A and 1B). However, the TWL and MWT values in the CCI group were lower on the 3rd, 7th, and 14th day after the surgery compared to those in the sham group ($P < 0.05$). This result confirmed our success in developing a rat model for CCI with an obvious and long-lasting thermal hyperalgesia and mechanical allodynia. Furthermore, the TWL and MWT values measured in the other groups treated with either vehicle or PF are shown in Figure 1C and

1D. The results demonstrate that the TWL and MWT values in the PF groups were significantly higher on the 7th and 14th day after the surgery compared to those in the CCI + vehicle group ($P < 0.05$). In addition, the 7- and 10- day (after surgery) values for TWL and MWT were significantly higher with a PF dose of 100 mg/kg than with a dose of 50 mg/kg ($P < 0.05$).

Changes in the number of neurons cells in the dorsal horn and the dorsal root ganglion

The changes in the number of neurons cells in the dorsal horn of the spinal cord are shown in Figure 2A. The number of neurons cells in animals from the CCI + vehicle group was significantly less than that in animals from the sham + vehicle group ($P < 0.05$). The number of neurons cells increased significantly in response to administration of PF (50 mg/kg) in the CCI + vehicle group. The number of neurons cells further increased when the concentration of PF was increased to 100 mg/kg ($P < 0.05$), indicating that PF could increase the number of neurons cells in the injured nerves, and that higher concentrations of PF exhibited better effects. Administration of PF demonstrated the same

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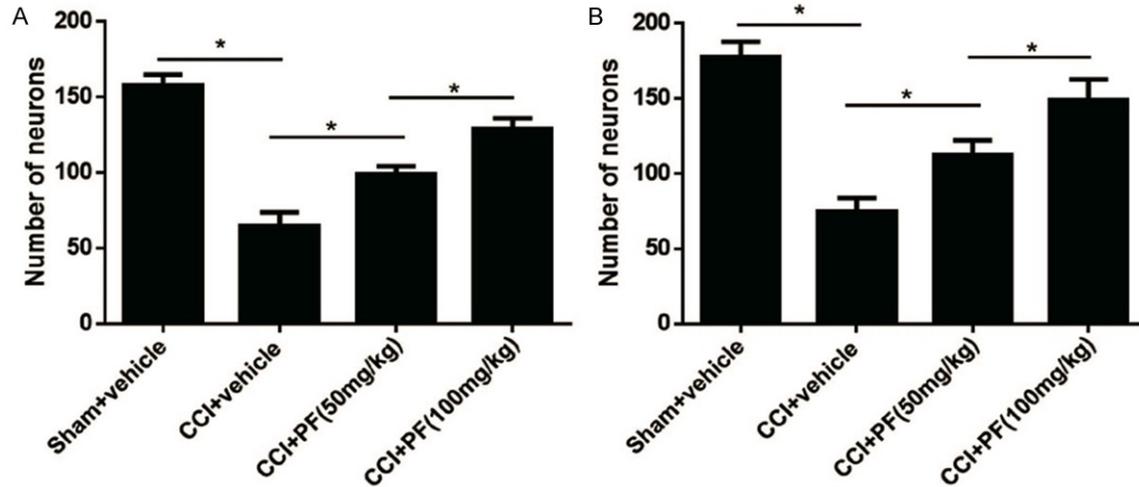


Figure 2. The changes in the number of neuronal cells on the 14th day. A: Neuronal cells in the dorsal horn detected by HE staining and counted using a light microscope. B: Neuronal cells in the dorsal root ganglion detected by HE staining and counted using a light microscope. n=4 rats/group, *P<0.05.

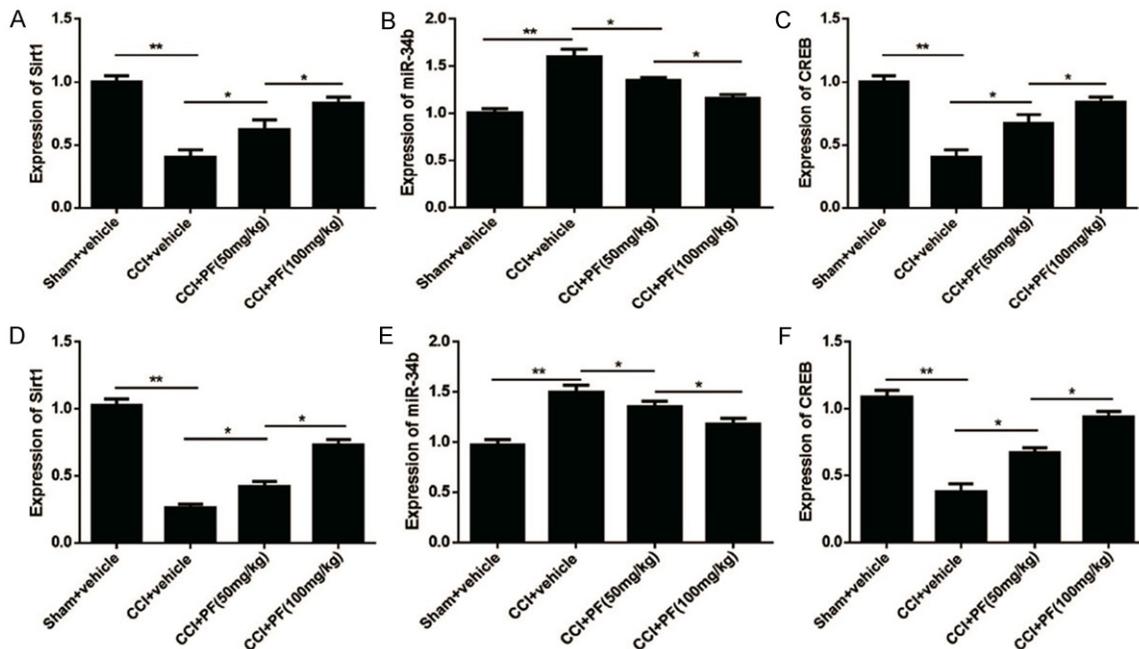


Figure 3. Changes in Sirt1, miR-34b, and CREB expression levels in the dorsal horn and the dorsal root ganglion on the 14th day. Expression of Sirt1 (A), miR-34b (B), and CREB (C) in the dorsal horn detected by real-time PCR. Expression of Sirt1 (D), miR-34b (E), and CREB (F) in the dorsal root ganglion detected by real-time PCR. n=4 rats/group, *P<0.05, **P<0.01.

effect on the neuron number in the spinal dorsal root ganglia (Figure 2B).

Expression of Sirt1, miR-34b, and CREB in the dorsal horn and the dorsal root ganglion

The expression level of the Sirt1 gene in the spinal dorsal horn as measured by real-time

quantitative PCR detection in the CCI + vehicle group was significantly lower than that in the sham + vehicle group of spinal dorsal horn (P<0.01). Moreover, the expression of Sirt1 markedly increased with the administration of PF (50 mg/kg) in the CCI + vehicle group (P<0.05); the Sirt1 expression further increased

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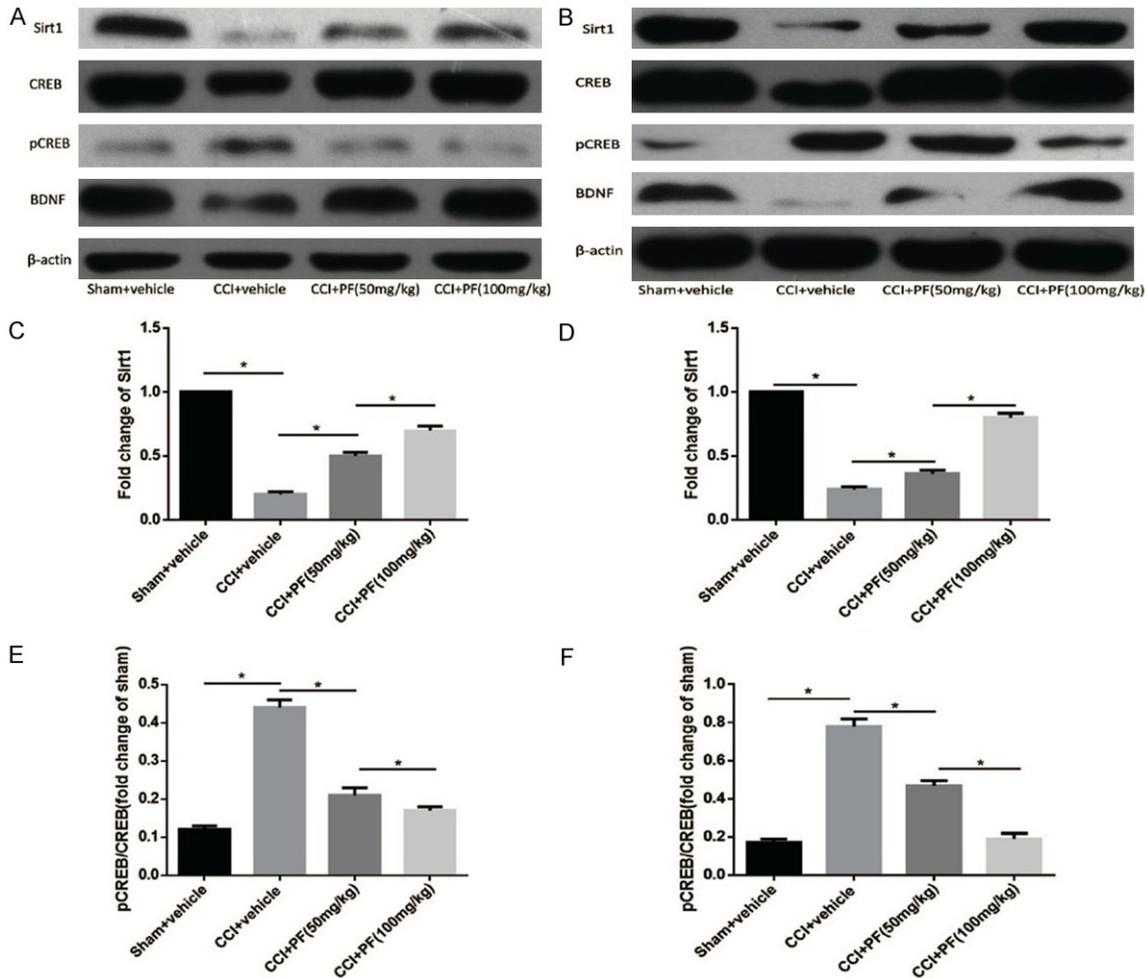


Figure 4. Detection of protein levels in the spinal cord using western blots. Detection of different protein levels in the dorsal horn of the spinal cord (A) and the dorsal root ganglion (B) by western blot. Sirt1 expression in different groups was detected using the gray value in the dorsal horn (C) and the dorsal root ganglia (D) of the spinal cord. (E, F) Demonstrate the pCREB/CREB ratio in the dorsal horn and the dorsal root ganglia. $n=4$ rats/group, $*P<0.05$.

when the concentration of PF was increased to 100 mg/kg ($P<0.05$) (Figure 3A). The miR-34b expression in the spinal dorsal horn was much lower in the sham + vehicle group compared to the CCI + vehicle group ($P<0.01$). Administration of PF (50 mg/kg) led to the downregulation of miR-34b compared to the CCI + vehicle group ($P<0.05$), with a better effect when the concentration of PF was increased to 100 mg/kg ($P<0.05$) (Figure 3B). The expression of the CREB gene in the spinal dorsal horn tissue of animals in the CCI group was significantly lower than for the animals in the sham + vehicle group ($P<0.01$). The CREB expression significantly increased in response to the administration of PF (50 mg/kg) in the CCI + vehicle group ($P<0.05$). The expression of CREB further

increased when the concentration of PF was increased to 100 mg/kg ($P<0.05$) (Figure 3C). The changes in the levels of Sirt1, miR-34b, and CREB were similar in the dorsal root ganglion ($P<0.01$) (Figure 3D-F).

Detection of protein levels in the spinal cord

As shown in Figure 4A, the Sirt1 level in the dorsal horn of the spinal cord of animals in the CCI + vehicle group was lower than that for the sham + vehicle group. The Sirt1 expression level in the CCI + PF (50 mg/kg) group was higher than that in the CCI + vehicle group. However, the CCI + PF (100 mg/kg) group showed a higher expression of Sirt1 than the CCI + PF (50 mg/kg) group. On the contrary, the

CREB and pCREB demonstrated an opposite trend, in that the CREB levels in the CCI + vehicle group were lower than in the sham + vehicle group, while the pCREB levels were higher than in the sham + vehicle group. Furthermore, the expression of CREB (pCREB) increased (decreased) with an increase in the PF concentration in the CCI + PF (50 mg/kg) group ($P < 0.05$). The BDNF levels in the dorsal horn on the spinal cord showed a trend similar to the one observed for Sirt1 in the CCI + vehicle group, in that the gradual decrease in the expression of BDNF improved with an increase in the concentration of PF. The fold change in the Sirt1 levels is shown in **Figure 4C**; the use of different concentrations of PF markedly improved the expression level of the Sirt1 gene in the CCI + vehicle group ($P < 0.05$). The ratio of the levels of CREB and pCREB reflects the influences of PF and CCI (**Figure 4E**). The administration of PF reduces the current CREB/pCREB ratio, which increases in response to the CCI surgery ($P < 0.05$). The administration of PF at a concentration of 100 mg/kg was more effective in the regulation of CREB/pCREB than a concentration of 50 mg/kg ($P < 0.05$). The expression levels of Sirt1, CREB, pCREB, and BDNF in the dorsal root ganglion of the spinal cord were similar to those seen in the dorsal horn (**Figure 4B, 4D, 4F**).

Discussion

The current research related to neuropathic pain is heavily dependent on animal models. Despite its shortcomings, the current experimental model was able to provide instruments for us to understand and explore human neuropathic disease. Bennett and Xie [14] successfully designed the CCI model for the first time in 1988; the model is easy to produce and the neuropathic pain exists for 14 after the pain reaction reaches its peak, thereby meeting the current international standard. Therefore, we created animal models according to the classical method in this study. We found decreased TWL and MWT values, the claw inward convergence, and a mild valgus of rats in the CCI group [15], indicating that our animal model can be used in further experiments.

Studies have indicated that there is a close relationship between neurons and pain [16]. An increase in the occurrence of hyperalgesia has

been suggested to be associated with the inhibition of reduced excitability of the nociceptive neurons in the spinal dorsal horn [17], and the spontaneous discharge of neurons mediated by the opening and closing of ion channels in order to realize the transmission of pain [18]. We found that the number of neurons at the level of L4-5 in the spinal dorsal horn and the dorsal root ganglion was significantly lower in the CCI group than that in the sham group, indicating that this decrease in the number of neurons may underlie the neuropathic pain transmission changes. However, there is a significant increase in the number of neurons with the administration of PF, such that the higher the concentration of the peony, the more the number of neurons. One possible mechanism for this effect is the increase in the number of neurons by repairing the injured neurons, for which higher concentration of PF are more effective.

A previous study showed that miRNAs can regulate the expression of inflammatory pain-related transcription factors [8]; our results showed that the miR-34b levels in the CCI rats were downregulated in the spinal dorsal horn and the dorsal root ganglion. Moreover, a higher concentration of the herbaceous peony showed a better regulation effect of miR-34b. Since the role of miRNAs is to inhibit the transcription of the target gene, the expression of the target CREB gene increased which is consistent with previous studies and indicates that miRNAs are closely related to the occurrence and maintenance of pain. Sirt1 has a wide distribution in the brain and plays an important role in the induction of gene silencing [19]. It can also be involved in the programmed cell death [20] and energy metabolism of neurons in the nucleus and cytoplasm [21]. The CREB protein could bind to the promoters I and IV of the BDNF gene so as to increase the transcription of BDNF in the absence of Sirt1 catalytic activity. The binding of CREB and BDNF promoters was lower in mutant mice lacking the catalytic activity of hippocampal Sirt1, while the CREB protein was able to regulate the transfer function of the nerve synapses [21]. These changes in the protein lead to the development of neuropathic pain transmission.

At present, the treatment of neuropathic pain is predominantly drug treatment and then combined with minimally invasive treatments or

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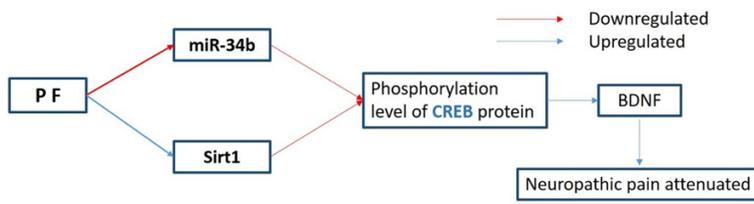


Figure 5. The schematic shows how PF in the spinal dorsal horn and the dorsal root ganglion can regulate hyperalgesia in neuropathic pain.

neural regulation treatments. With the recent deepening of the research related to natural solutions, the effective ingredients in many Chinese herbs or their extracts are used to treat neuropathic disease. For example, a recent study found that chelerythrine and matrine alleviate the TWL and MWT (related to allergies) in the CCI rat model, but also inhibit the secretion of inflammatory cytokines [22]. Previous studies have demonstrated that yellow baicalin can effectively reduce allergy-related symptoms of pain caused by the spinal nerve ligation [23]. Many studies of the effect of such traditional Chinese medicine compositions have also found the molecular mechanism of action. For example, one of the mechanisms proposed for the relief of neuropathic pain by the intraperitoneal injection of curcumin is through reducing the expression of the CX3CR1 gene in the spinal dorsal horn and dorsal root ganglia [24]. Other studies have found that intraperitoneal injections of emodin in CCI rats led to a clear remission of pain symptoms accompanied by a decrease in the expression of P2X3 receptor in the spinal dorsal root ganglia [25]. These studies indicate that Chinese herbs can effectively alleviate neuropathic pain through molecular mechanisms that have been investigated.

A previous study demonstrated that miR-34b negatively regulates CREB expression [26]. In this experiment, we found that the expression of miR-34b was negatively correlated with the expression of CREB as well as the expression of Sirt1 in the CCI group. On the other hand, the CREB protein has been confirmed to be a protein downstream to the Sirt1 protein and regulate its expression [27]. Our results also demonstrate that the expression of the Sirt1 and CREB proteins are positively correlated at the gene and protein levels. Therefore, as shown in **Figure 5**, this study found that the PF can regulate the expression of Sirt1 and the phosphory-

lation level of the CREB protein; the phosphorylation status of CREB determined its interaction with BDNF. In addition, we found that PF regulates miR-34b so that CREB is also a target gene of miR-34b, leading to a modification in the binding of CREB and BDNF genes. All of these changes can mediate the attenuation

of pain related to neuropathic disease caused by the administration of PF.

In conclusion, our study indicated that Paeoniflorin can attenuate the pain caused by neuropathic disease, possibly through upregulation of Sirt1 in the spinal dorsal horn and the dorsal root ganglion.

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Disclosure of conflict of interest

None.

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References

- [1] Ochoa JL. Neuropathic pain: redefinition and a grading system for clinical and research purposes. *Neurology* 2009; 72: 1282-1283.
- [2] Haanpaa ML, Backonja MM, Bennett MI, Bouhassira D, Cruccu G, Hansson PT, Jensen TS, Kauppila T, Rice AS, Smith BH, Treede RD, Baron R. Assessment of neuropathic pain in primary care. *Am J Med* 2009; 122 Suppl: S13-21.
- [3] Qu YJ, Jia L, Zhang X, Wei H and Yue SW. MAPK pathways are involved in neuropathic pain in rats with chronic compression of the dorsal root ganglion. *Evid Based Complement Alternat Med* 2016; 2016: 6153215.
- [4] Shao H, Xue Q, Zhang F, Luo Y, Zhu H, Zhang X, Zhang H, Ding W and Yu B. Spinal SIRT1 activation attenuates neuropathic pain in mice. *PLoS One* 2014; 9: e100938.
- [5] Gui Y, Li A, Chen F, Zhou H, Tang Y, Chen L, Chen S and Duan S. Involvement of AMPK/

Paeoniflorin attenuates neuropathic pain

- SIRT1 pathway in anti-allodynic effect of troxerutin in CCI-induced neuropathic pain. *Eur J Pharmacol* 2015; 769: 234-241.
- [6] White DM, Walker S, Brenneman DE and Gozes I. CREB contributes to the increased neurite outgrowth of sensory neurons induced by vasoactive intestinal polypeptide and activity-dependent neurotrophic factor. *Brain Res* 2000; 868: 31-38.
- [7] Aldrich BT, Frakes EP, Kasuya J, Hammond DL and Kitamoto T. Changes in expression of sensory organ-specific microRNAs in rat dorsal root ganglia in association with mechanical hypersensitivity induced by spinal nerve ligation. *Neuroscience* 2009; 164: 711-723.
- [8] Zhao J, Lee MC, Momin A, Cendan CM, Shepherd ST, Baker MD, Asante C, Bee L, Bethry A, Perkins JR, Nassar MA, Abrahamsen B, Dickenson A, Cobb BS, Merckenschlager M and Wood JN. Small RNAs control sodium channel expression, nociceptor excitability, and pain thresholds. *J Neurosci* 2010; 30: 10860-10871.
- [9] Lu H and Wang B. SIRT1 exerts neuroprotective effects by attenuating cerebral ischemia/reperfusion-induced injury via targeting p53/microRNA-22. *Int J Mol Med* 2017; 39: 208-216.
- [10] Yang H, Gu ZT, Li L, Maegle M, Zhou BY, Li F, Zhao M and Zhao KS. SIRT1 plays a neuroprotective role in traumatic brain injury in rats via inhibiting the p38 MAPK pathway. *Acta Pharmacol Sin* 2017; 38: 168-181.
- [11] An J, Cai T, Che H, Yu T, Cao Z, Liu X, Zhao F, Jing J, Shen X, Liu M, Du K, Chen J and Luo W. The changes of miRNA expression in rat hippocampus following chronic lead exposure. *Toxicol Lett* 2014; 229: 158-166.
- [12] Gao T, Hao J, Wiesenfeld-Hallin Z, Wang DQ and Xu XJ. Analgesic effect of sinomenine in rodents after inflammation and nerve injury. *Eur J Pharmacol* 2013; 721: 5-11.
- [13] Kapoor S. Neuroprotective effects of paeoniflorin: an emerging concept in neurology. *Folia Neuropathol* 2013; 51: 92.
- [14] Bennett GJ and Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 1988; 33: 87-107.
- [15] Andrew D. Sensitization of lamina I spinoparabrachial neurons parallels heat hyperalgesia in the chronic constriction injury model of neuropathic pain. *J Physiol* 2009; 587: 2005-2017.
- [16] Baba H, Petrenko AB and Fujiwara N. Clinically relevant concentration of pregabalin has no acute inhibitory effect on excitation of dorsal horn neurons under normal or neuropathic pain conditions: an intracellular calcium-imaging study in spinal cord slices from adult rats. *Brain Res* 2016; 1648: 445-58.
- [17] Mao J, Sung B, Ji RR and Lim G. Neuronal apoptosis associated with morphine tolerance: evidence for an opioid-induced neurotoxic mechanism. *J Neurosci* 2002; 22: 7650-7661.
- [18] Monteiro C, Lima D and Galhardo V. Switching-on and -off of bistable spontaneous discharges in rat spinal deep dorsal horn neurons. *Neurosci Lett* 2006; 398: 258-263.
- [19] Tanno M, Sakamoto J, Miura T, Shimamoto K and Horio Y. Nucleocytoplasmic shuttling of the NAD⁺-dependent histone deacetylase SIRT1. *J Biol Chem* 2007; 282: 6823-6832.
- [20] Fulco M, Schiltz RL, Iezzi S, King MT, Zhao P, Kashiwaya Y, Hoffman E, Veech RL and Sartorelli V. Sir2 regulates skeletal muscle differentiation as a potential sensor of the redox state. *Mol Cell* 2003; 12: 51-62.
- [21] Messersmith DJ, Kim DJ and Iadarola MJ. Transcription factor regulation of prodynorphin gene expression following rat hindpaw inflammation. *Brain Res Mol Brain Res* 1998; 53: 260-269.
- [22] Wu GJ, Wen ZH, Chang YC, Yang SN, Tao PL and Wong CS. Protein kinase C inhibitor chelerythrine attenuates the morphine-induced excitatory amino acid release and reduction of the antinociceptive effect of morphine in rats injected intrathecally with pertussis toxin. *Life Sci* 2006; 78: 1801-1807.
- [23] Cherng CH, Lee KC, Chien CC, Chou KY, Cheng YC, Hsin ST, Lee SO, Shen CH, Tsai RY and Wong CS. Baicalin ameliorates neuropathic pain by suppressing HDAC1 expression in the spinal cord of spinal nerve ligation rats. *J Formos Med Assoc* 2014; 113: 513-520.
- [24] Zheng J, Zheng C, Cao H, Li J and Lian Q. Curcumin down-regulates CX3CR1 expression in spinal cord dorsal horn and DRG in neuropathic pain rats. *Zhongguo Zhong Yao Za Zhi* 2011; 36: 2552-2556.
- [25] Gao Y, Liu H, Deng L, Zhu G, Xu C, Li G, Liu S, Xie J, Liu J, Kong F, Wu R, Li G and Liang S. Effect of emodin on neuropathic pain transmission mediated by P2X_{2/3} receptor of primary sensory neurons. *Brain Res Bull* 2011; 84: 406-413.
- [26] Sandoval S, Pigazzi M and Sakamoto KM. CREB: a key regulator of normal and neoplastic hematopoiesis. *Adv Hematol* 2009; 2009: 634292.
- [27] Li J, Dou X, Li S, Zhang X, Zeng Y and Song Z. Nicotinamide ameliorates palmitate-induced ER stress in hepatocytes via cAMP/PKA/CREB pathway-dependent Sirt1 upregulation. *Biochim Biophys Acta* 2015; 1853: 2929-2936.