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ORIGINAL RESEARCH

Fire Acupuncture Combined with Pulse Radiofrequency Relieves Tactile Allodynia in Rats with Postherpetic Neuralgia

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Background: Postherpetic neuralgia (PHN) remains challenging to treat, with 40%–50% of patients experiencing inadequate pain relief despite comprehensive interventions. Neuromodulation techniques such as pulsed radiofrequency (RF) and traditional methods such as fire acupuncture (FA) are increasingly used for PHN. This study investigated the combined effect of FA and RF on tactile allodynia in a rat model of PHN and explored its underlying mechanisms.

Methods: Adult male rats were randomly divided into: Control, PHN, FA, RF, and combined FA_RF groups. PHN was induced by varicella-zoster virus inoculation. FA and/or RF interventions began on Day 15. Pain behavior was assessed via mechanical allodynia and thermal hyperalgesia tests. On Day 37, spinal cord tissues and cerebrospinal fluid were collected to evaluate astrocyte and microglial activation, neuronal apoptosis, expression of Insulin-Like Growth Factor 2 (IGF2), Peripheral Myelin Protein 2 (PMP2), Claudin-19 (CLDN19), Homeobox C8 (HOXC8), and levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor-α (TNF-α). The role of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway was verified using a PI3K/AKT agonist.

Results: The results revealed that mechanical allodynia thresholds decreased on Day 8 post-inoculation. Compared to the PHN group, FA_RF combination significantly increased mechanical thresholds and prolonged thermal hyperalgesia latency, with superior effects versus FA or RF alone. The FA_RF group showed increased spinal IGF2, PMP2, and CLDN19 expression, decreased HOXC8, reduced astrocyte activation, and modulated cytokine levels (IL-1β, IL-6, TNF-α, IL-10). The PI3K/AKT agonist reversed the analgesic effects of FA_RF.

Conclusion: Combined fire acupuncture and pulsed radiofrequency alleviates tactile allodynia in PHN rats, possibly by inhibiting spinal astrocyte activation, modulating inflammatory responses, and promoting nerve repair via IGF2, PMP2, and CLDN19 expression. The PI3K/AKT pathway may be critically involved in this analgesic mechanism.

Keywords: postherpetic neuralgia, P13K/AKT, fire acupuncture, pulse radiofrequency

Introduction

Postherpetic neuralgia (PHN) is a form of neuropathic pain that lasts 1 months or more after the onset of acute herpes zoster (AHZ),[1] and approximately 10% of AHZ patients develop PHN.[2] Varicella-zoster virus (VZV) is the most common infectious cause of neuropathic pain.[3] The treatment of postherpetic neuralgia is difficult, with 40%-50% of

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patients still having poor effects after comprehensive intervention measures,[4] leading to increased medical burden, so the prevention and treatment of postherpetic neuralgia is particularly important.[5] There is currently no consensus on the optimal methodology for studying neuralgia following herpes zoster virus infection.[6] The incidence of PHN increases with age, with more than 40% of PHN complications in zoster patients over 60 years of age and approximately 5 times greater in those aged 65 years and older than in other populations.[7] Currently, the pathogenesis of PHN has not been fully elucidated.[8] Multiple studies have confirmed that the cytokines and chemokines released by astrocytes can enhance and prolong the persistent pain state of the spinal cord.[9,10] These studies suggest that the activation of microglia and astrocytes is associated with postherpetic neuralgia.

The choice of medication for PHN needs to consider various factors, such as the efficacy of drugs, possible adverse effects, treatment of concomitant sleep and affective disorders, drug interactions, risk of drug abuse, and treatment costs.[11] Therefore, neuromodulation techniques such as pulse radiofrequency (RF) and traditional Chinese acupuncture techniques such as fire acupuncture are increasingly widely used to treat PHN. Compared with drug intervention, acupuncture has obvious advantages in relieving PHN and improving efficacy and safety, which makes it an ideal choice for patients who cannot tolerate the side effects of traditional analgesics or seek non-drug treatment.[12] Pulsed RF has no destructive effect on nerve fibre structure and can improve pain and quality of life. After treatment, hypoesthesia, pain, burning pain and motor nerve injury occur less frequently, and this modality is commonly used to treat postherpetic neuralgia.[13] Fire acupuncture is a type of Chinese acupuncture technology that has the dual effects of armature and acupuncture. However, the above studies did not include a comparable control group to verify the benefits of fire acupuncture and pulsed RF and the combination of both for neuropathic pain.

The combination of nonpharmacological Traditional Chinese Medicine (TCM) and Modern Medicine, such as the combination of fire acupuncture and pulsed radiofrequency, in the treatment of postherpetic neuralgia has not been reported in the literature. Therefore, this study mainly discusses whether the combination of fire acupuncture and pulsed radiofrequency can relieve tactile allodynia and the effects on spinal glial cells in rats with postherpetic neuralgia to provide more effective treatments.

Methods

Ethical Approval

All animal surgical procedures were approved by the Experimental Animal Welfare Ethics Committee of Nanjing University School of Medicine. The care and use of laboratory animals complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Guidelines for the Ethical Review of Laboratory Animal Welfare (GB/T 35892–2018, China). Rats (weighing 180 to 250 g, purchased from Nanjing Cavans Biotechnology Co., Ltd. (Nanjing, China) were maintained in 22–24°C environments with relative humidities ranging from 40% to 60%, a 12-hour light–dark cycle and ad libitum access to food and water.

Materials and Reagents

66 clean, healthy adult male Sprague–Dawley rats[14] (weighing 180 to 250 g, purchased from Nanjing Cavance Biotechnology Co., Ltd., Nanjing, China), Huatuo sterile disposable acupuncture pin (Suzhou Medical Supplies Factory Co., Ltd., batch No. 233004, Suzhou, China), radiofrequency instruments (RFG 1 B, Cossman Medical Company (Cosman Medical. LLC), trocar (CSK-10 set acupuncture, CSK-15 set, Cosman Medical. Inc)., radio frequency electrodes (CSK-TC10, CSK-TC15, Cosman Medical. Inc)., von Frey monofilament (Stoelting Co. Wood Dale, Illinois, USA), an IL-1 β ELISA Kit, an IL-6 ELISA Kit, a TNF-a ELISA Kit, an IL-10 ELISA Kit, a PMP2 polyclonal antibody, a PA-CLAUDIN 19 antibody, a PA-ANTI-IGF 2, ELISA kits and other antibodies were purchased from Nanjing Tusi Biotechnology Co., Ltd. (Nanjing, China).

Timeline

Schematic summary of the experimental protocol used in the study showed in Figure 1.

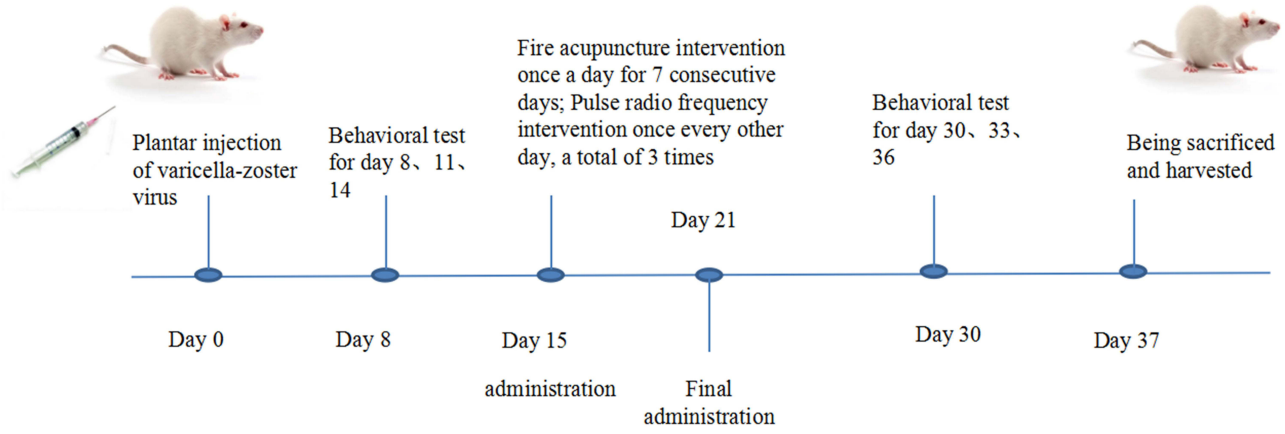

Figure 1 Schematic timeline of the experimental protocol. Behavioral tests (mechanical allodynia and thermal hyperalgesia) were conducted on days 8, 11, and 14 post-injection of varicella-zoster virus (VZV) into the right footpad of Sprague-Dawley rats. Therapeutic interventions commenced on day 15 post-VZV injection. Pulsed radiofrequency (RF) treatment was administered once per day for a total of 3 sessions. Fire acupuncture treatment was administered once per day for 7 consecutive days. A second set of behavioral tests was performed on days 30, 33, and 36 post-injection. All animals were euthanized on day 37 post-injection for specimen collection and subsequent laboratory analysis.

Varicella-Zoster Virus Extraction, Replication, Inoculation

Warner et al[14] found that varicella-zoster virus early infection but not complete replication is required for the induction of chronic hypersensitivity in rat models of postherpetic neuralgia. Patients who met the diagnostic criteria for postherpetic neuralgia[15] were selected and signed written informed consent. In accordance with the approval of Nanjing Drum Tower Hospital Medical Ethics Committee (No. 2023–059-03), under aseptic conditions, vesicular fluid from patients with herpes zoster was collected using a syringe and inoculated into MRC-5 human embryonic lung cell lines for culture. After approximately ten days of incubation, typical cytopathic effects (CPE), manifested as viral plaque formation, were observed under microscopy, indicating successful viral isolation and replication. The primary viral harvest was subsequently expanded in MRC-5 cells, and the viral titer was determined using a plaque-forming assay, expressed in plaque-forming units (PFU). Finally, a viral suspension with a titer of 2×10^5 PFU was inoculated into the left and right hind footpads of rats.[16] Successful viral replication was defined by the presence of typical varicella-zoster virus (VZV)-induced CPE in cell culture, including cell rounding, aggregation, and plaque formation. Furthermore, successful quantification of the viral titer via plaque assay confirmed effective viral replication and activity. All procedures were performed under inhalation anesthesia (sevoflurane, 5%, via face mask).

Treatment Groups and Design

Initially, 12 rats were inoculated with varicella-zoster virus (VZV) in a random hind paw to monitor behavioral changes. Mechanical allodynia and thermal hyperalgesia were assessed over 63 days to confirm model stability. Then, 30 rats were divided into 5 groups: Control, PHN, FA, RF and FA_RF, the treatment of each group is shown in Table 1. Finally, 24 rats were grouped into Control, PHN, FA_RF and FA_RF+740Y-P+SC79 to investigate Phosphatidylinostitol-3-kinase (P13K) / protein kinase B (AKT) pathway involvement. The FA_RF+740Y-P+SC79 group, after inoculation with VZV,

Table 1 Group Assignment and Treatment

Group	Fire Acupuncture	Pulse Radiofrequency	VZV sc
Control	–	–	–
PHN	–	–	+
FA	+	–	+
RF	–	+	+
FA_RF	+	+	+

Abbreviation: VZV sc, Varicella zoster virus subcutaneous injection.

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received combined intervention of fire acupuncture and pulsed radiofrequency. Additionally, they were intraperitoneally injected with the PI3K agonist 740Y-P and AKT activator SC79 (PI3K agonist 740Y-P, AKT activator SC79, MedChemExpress, NJ, USA) to investigate the role of the PI3K/AKT signalling pathway in the combined treatment of fire acupuncture and pulsed radiofrequency.[17]

Testing for Mechanical Allodynia (MA) and Thermal Hyperalgesia (TH)

Mechanical allodynia (MA) was evaluated via a calibrated von Frey monofilament. Throughout the procedure, the animals were distracted by the presence of fruit grains.[18] Response measurements were initiated by injecting a 10-g (rater size 5.07) filament 6 times through the metal grid phase on the hairless back pads of the ipsilateral side (opposite-lateral). Stimulation with the monofilament employed the "up and down" method.[19] The maximum weight of the von Frey monofilament was 180 g. Data were calculated as a weight threshold of 50% g and expressed as the mean withdrawal threshold in grams. Thermal hyperalgesia (TH) by Hargrave instrument (IITC, Woodland Hill, CA), the rats were placed on a 32°C glass platform and acclimated for 15 minutes before measurement.[20] Each footpad was evaluated four times, and the data were presented as the mean exit time of each footpad, in seconds.

Application of Fire Acupuncture

Application of fire acupuncture in the FA and FA_RF groups. Methods: Positioning was performed according to the group standards "Nomenclature and Location of Commonly Used Acupoints in Experimental Animals" (T/CAAM 0001–2020~0004-2020) issued by the China Association of Acupuncture-Moxibustion (issued on May 15, 2020; implemented on October 31, 2020): GB30 is located at the midpoint of the line connecting the highest point (most prominent part) of the greater trochanter of the femur and the sacral hiatus (corresponding to the depression at the base of the tail). EX-B2 is located on the dorsolumbar region of the rat (at the T13-L5 level), approximately 2–3 mm lateral to the posterior midline. Using inhalation anaesthesia (sevoflurane, 5%, mask), shaving, disinfecting, and the use of Huatuo disposable sterile acupuncture needles (Suzhou Medical Supplies Factory Co., LTD., batch No. 233004, Suzhou, China) were performed. One hand held an alcohol lamp, and one hand held the acupuncture needle. At the GB30 and EX-B2 acupoints, the skin epidermis was punctured by quick-puncture technique. The tip of the acupuncture needle was heated until red-hot before injection. Each needle was heated once and used for a total of three insertions. After the treatment, the puncture site was sterilized, and the rats were returned to their original residential environment. All acupuncture manipulations, were performed by a licensed and experienced TCM physician specializing in acupuncture with over 10 years of clinical practice, ensuring the safety and standardization of the procedures.

Application of the Pulsed RF

RF group and FA_RF group pulsed radiofrequency intervention method. Methods: Using inhalation anaesthesia (sevoflurane, 5%, mask). The acupuncture injection points were 4–5 mm to the left of the dorsal root ganglion area. After shaving the area, two injection points (A and B), less than 2 mm apart, were marked. The trocar was inserted vertically to a depth of approximately 3–5 mm and fixed in a space frame using a point B PRF trocar (CSK-15 set, Cosman Medical, Inc). Next, the core was removed and inserted into the RF electrode (CSK-TC10, CSK-TC15, Cosman Medical, Inc). The selection of RF parameters was based on established protocols from previous preclinical studies in neuropathic pain models,[21] the parameters of pulsed radiofrequency were modified as follows: on days 14, 16, and 18, rhythmic pulsations in the left foot were induced using a radiofrequency instrument (RFG 1 B; Cosman Medical, LLC) at a frequency of 2 Hz with a pulse duration of 20 ms, followed by a pulsed RF application at 2 Hz, with a target temperature of 42°C, and 45 V for 120 seconds.

Rat Cerebrospinal Fluid and Spinal Cord Tissue

On Day 37 after the subcutaneous injection of varicella-zoster virus in the rats, Under deep sevoflurane anesthesia, rats were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde, and cerebrospinal fluid and spinal cord tissues were harvested from the dorsal horn of the lumbar enlargement (L3-L5 segments). The CSF was centrifuged, and liquid supernatant was removed and stored at −20°C. Spinal cord tissue was extracted after perfusion with 4% neutral paraformaldehyde (PFA) and cryopreserved at −80°C.

Immunofluorescence (IF) Analysis

The tissue sections were fixed with paraformaldehyde for 30 min and washed three times with PBS. Then, infiltrate with 1%Triton X-100 (Beyotime Wuhan, China) for 15 minutes, wash with PBS: three times, seal with 5%BSA (Beyotime Biotech, Wuhan, China) for 1 hour, wash with PBS three times. The sections were then incubated overnight with primary antibodies at 4°C. The following primary antibodies were used: NF 200 (1:500), rat IgG, Abcam, CD68 (1:1000, Rabbit IgG, Abcam) and glial fiber acidic protein (GFAP, 1:500, rabbit IgM, UK). After incubating with the primary antibodies overnight, wash the slide with PBST three times, 5 minutes each time. After washing, the slide was incubated with the second antibody at room temperature for 2 hours. Nuclear dye 4', 6-diamino-2-benzoindole (DAPI; Beyotime Biotech, Wuhan, China) redye for 10 minutes, then wash with PBS three times for 5 minutes each time. Fluorescence images were obtained using the Zeiss LSM710 laser scanning confocal microscope (LSCM) (Zeiss, Jena, Germany). Image Premier software (Media Control, Silver Spring, USA) is used to determine dye strength.

Western Blot (WB) Analysis

Spinal cord tissues were placed in 1% RIPA lysis buffer and centrifuged at 18000×g at 4°C for 20 min. The supernatant was collected and the total protein concentration was quantified by BCA protein detection kit (Beyotime Biotech, Wuhan, China). A total of 20 μg of protein was loaded in each lane for either 8% or 12% of the SDS-PAGE. After electrophoresis, will be transferred to nitrocellulose membrane protein (Microwells, MA, USA), and then closed for 1 hour in 5% skimmed milk. The membrane is then incubated with the specified primary antibody at 4°C overnight and then washed three times with PBST for 5 minutes each time. Then incubated with the corresponding coupled secondary antibody at room temperature for 1 hour. Then wash three times with PBST. The main antibodies used include those targeting Calpain (1:1000 Arkham, UK), Bcl-2 (1:1000 Arkham, UK), Bax (1:1000 Arkham, UK), IGF2(1:2000 Tusaide Biotechnology, CHN), PMP2(1:1000 Tusaide Biotechnology, CHN), CLDN19(1:1000 Tusaide Biotechnology, CHN), HOXC8(1:1000 Tusaide Biotechnology, CHN), and GAPDH (1:500 Arkham, UK). After scanning, gray values were measured by ImageJ software (NIH, New York, USA), and finally statistical analysis was performed.

Enzyme-Linked Immunosorbent Assay

The expression levels of inflammatory factors, including IL-1β, IL-6, IL-10, and TNF-α, were determined via ELISA kits according to the manufacturer's protocol.

mRNA Sequencing Experimental Methods

Total RNA was extracted using the TRIzol reagent (Invitrogen, CA, USA). PCA was performed using R (v 3.2.0). The expression patterns of the genes in the different groups and samples were analyzed via R (v 3.2.0). Differential expression analysis was performed via DESeq2. Q-value <0.05 and fold change <0.5 or fold change> 2 were used as the threshold for significantly differentially expressed genes (DEGs). A radar map of the top 30 genes using the R package ggradar. GO, KEGG pathways, Reacome and Wiki pathways enrichment analysis were performed for KEGG s via R (v3.2.0). Gene set enrichment analysis (GSEA) was performed via GSEA software.

Statistical Methods

All the data are presented as the means ± SD. GraphPad Prism 9.5 (GraphPad software, Inc., CA, USA) was used for the statistical analysis. Tukey's multiple comparison test were used to compare data with a normal distribution of matching variance homogeneity. Nonparametric Mann–Whitney *U*-test was used to compare data that did not fit a normal distribution. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Development of Mechanical Allodynia and Thermal Hyperalgesia After Treatment with Varicella-Zoster Virus

To investigate the behavioural development of rats 0–63 days after varicella-zoster virus inoculation, mechanical allodynia and heat hyperalgesia were tested. The inoculum was administered in a random left–right manner to the hind foot pad as described previously, and the behavioural assessors were not aware of the paws or the nature of the inoculum. The development of mechanical abnormal pain and thermal hyperalgesia was evaluated over a period of 63 days (Figure 2).

The results revealed that the ipsilateral foot pads of rats inoculated with varicella-zoster virus began to develop mechanical allergies on Day 8. Compared with the contralateral uninoculated viral foot pad, hypersensitivity was significantly at multiple time points, and the TRPE rats inoculated without VZV showed no significant hypersensitivity during the study (Figure 2a). Importantly, this mechanical hypersensitivity lasted for several weeks and began to weaken at 50 dpi, and by 63 dpi, the hypersensitivity of the inoculated viral footpad was no longer significant and gradually returned to baseline values (Figure 2a). Rats inoculated with VZV or the TRPE equivalent of the contralateral footpad showed no significant hypersensitivity reactions throughout the study (Figure 2c). Interestingly, thermal hypersensitivity showed the same trend, with varicella-zoster virus-inoculated rats exhibiting thermal hyperalgesia beginning on Day 7 and gradually returning to baseline values on Day 52 (Figure 2b). By 11 dpi, significant differences were detected in the measurements of these rats compared with those of the contralateral foot pad (Figure 2b). In the 63-day study, the mean withdrawal time from the varicella-zoster virus rats was less than 7 s, whereas the response to the virus-free TRPE or

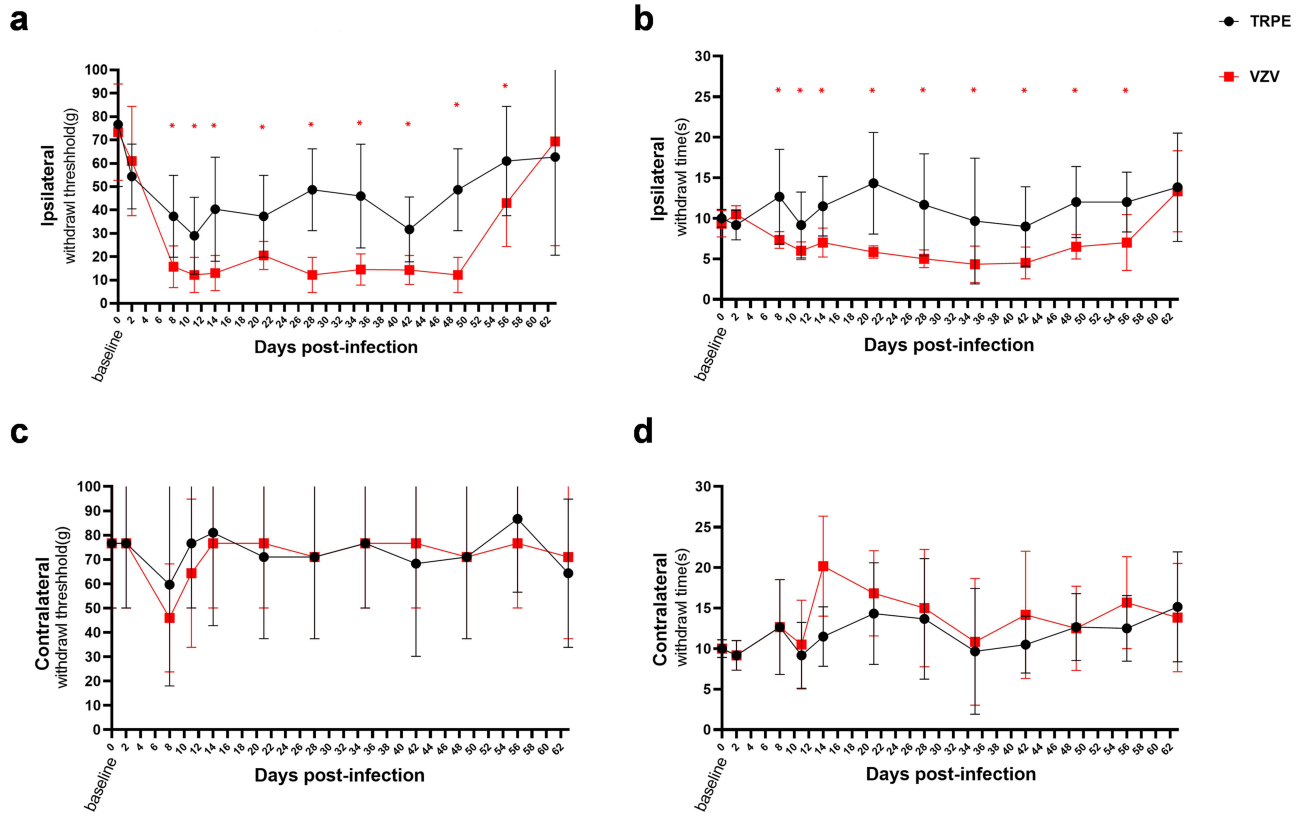

Figure 2 Development of mechanical allodynia and thermal hyperalgesia following varicella-zoster virus (VZV) inoculation. Male Sprague-Dawley rats were inoculated in the right footpad with either VZV-infected (2×10^5 PFU) or uninfected telomerase-immortalized human fibroblast (TREP) cell equivalents. Baseline responses were established prior to inoculation. (**a**) Mechanical allodynia of the ipsilateral (inoculated) paw over time, presented as the 50% paw withdrawal threshold (g) measured using von Frey filaments. (**b**) Thermal hyperalgesia of the ipsilateral paw over time, presented as the paw withdrawal latency (s) measured using a Hargreaves apparatus. (**c**) Mechanical allodynia of the contralateral (non-inoculated) paw over time. (**d**) Thermal hyperalgesia of the contralateral paw over time. Data are presented as mean ± SD (n=6 per group). Data points: VZV-infected group (red squares); uninfected control group (black circles). Statistical significance between groups at each time point was determined by Mann–Whitney *U*-tests (*$p < 0.05$).

uninoculated lateral foot pads remained greater than 10s (Figure 2d). Thermal hypersensitivity also decreased during the later stages of the research and gradually returned to baseline values. These results indicate that tactile allodynia lasts at least, indicating the success of the animal model of postherpetic neuralgia and providing a good time node reference for the intervention effect of fire acupuncture and pulsed radiofrequency.

The Combination of Fire Acupuncture and Pulsed Radiofrequency Relieves Tactile Allodynia in Rats with Postherpetic Neuralgia

To explore the effects of fire acupuncture and pulsed radiofrequency intervention, mechanical allodynia and thermal hyperalgesia were assessed. Male Sprague–Dawley rats adapted to the experimental conditions were divided into control, PHN, FA, RF, and FA_RF groups. Control rats were inoculated with TRPE cell equivalents (dark blue rectangle), and PHN rats were hypodermic injected of varicella-zoster virus (brown rectangle). The FA group of rats were hypodermic injected with 2×10^5 PFU of varicella-zoster virus and subjected to fire acupuncture intervention (gray rectangle). RF rats were hypodermic injected with 2×10^5 PFU of varicella-zoster virus and subjected to pulsed radiofrequency intervention (light blue rectangle). Similarly, the rats in the FA_RF group were subcutaneous injected with 2×10^5 PFU of varicella-zoster virus and subjected to pulsed radiofrequency intervention (black rectangle). As mentioned above, mechanical allodynia and thermal hyperalgesia were evaluated on Days 8, 11, 13, 30, 33, and 36 after inoculation with varicella-zoster virus (Figure 3).

The results indicated that on the 8th, 11th and 14th days after inoculation with VZV, there were statistically significant differences in mechanical allodynia and thermal hyperalgesia between Control and the other four groups ($P < 0.05$) (Figure 3a and b), indicating that the postherpetic neuralgia rat model was successfully established. Next, the same method was used to detect the development of tactile heterodynia in each group of rats after fire acupuncture and/or pulsed radiofrequency intervention. After fire acupuncture and/or pulsed radiofrequency intervention, at Days 30, 33 and 36 after inoculation with varicella-zoster virus, there was a statistically significant in mechanical allodynia between Control and PHN group ($P < 0.05$) (Figure 3c), and there was a statistically significant difference in mechanical allodynia between PHN and RF groups ($P < 0.05$) (Figure 3c). Notably, compared with the FA group, there was a statistically significant difference in mechanical allodynia between PHN group and FA group on Day 30 ($P < 0.05$) (Figure 3c), whereas there was no statistically significant difference in mechanical allodynia between PHN and FA group at Days 33 and 36 ($P> 0.05$) (Figure 3c). These results indicate that the effect of fire acupuncture intervention on mechanical allodynia in varicella-zoster virus-infected rats was unclear. Similarly, after fire acupuncture and/or pulsed radiofrequency intervention, there was a statistically significant difference between Control and PHN group ($P < 0.05$) (Figure 3d), and there was a statistically significant difference between PHN and FA_RF groups ($P < 0.05$) (Figure 3d). Notably, compared with the FA or RF groups, there was no statistically significant difference in thermal hyperalgesia between PHN and FA or RF group at Days 30 and 36 ($P > 0.05$) (Figure 3d). On Day 33, there was a statistically significant difference between PHN and FA group ($P <0.05$) (Figure 3d), indicating that the response to fire acupuncture or pulsed radiofrequency intervention for thermal hyperalgesia in postherpetic neuralgia rats was not obvious. These results suggest that the combined effects of fire acupuncture and pulsed radiofrequency alleviate tactile allodynia in rats with postherpetic neuralgia, whereas the effects of fire acupuncture or pulsed radiofrequency alone on tactile allodynia in rats inoculated with varicella-zoster virus are unclear.

Fire Acupuncture Combined with Pulsed Radiofrequency Can Inhibit Spinal Astrocyte Activation, Alleviate Neuronal Apoptosis and Promote Neuronal Repair After Herpes Zoster Virus Infection in Rats

To investigate the anatomical basis of spinal cord repair after varicella-zoster virus infection, we examined the activation of astrocytes and microglia, and the distribution/status of neurons in spinal cord tissue. The effects of fire acupuncture and pulsed radiofrequency intervention on spinal astrocytes, microglia, and neurons were investigated via the astroglial activation marker GFAP, the microglial activation marker CD68, and immunofluorescence staining of NF200, which reflects the health status of the neurons. On Day 37 after rat footpad inoculation with varicella-zoster virus, spinal cord tissue was collected for immunofluorescence staining and Western blotting to evaluate the expression of apoptosis-related proteins in each group (Figure 4).

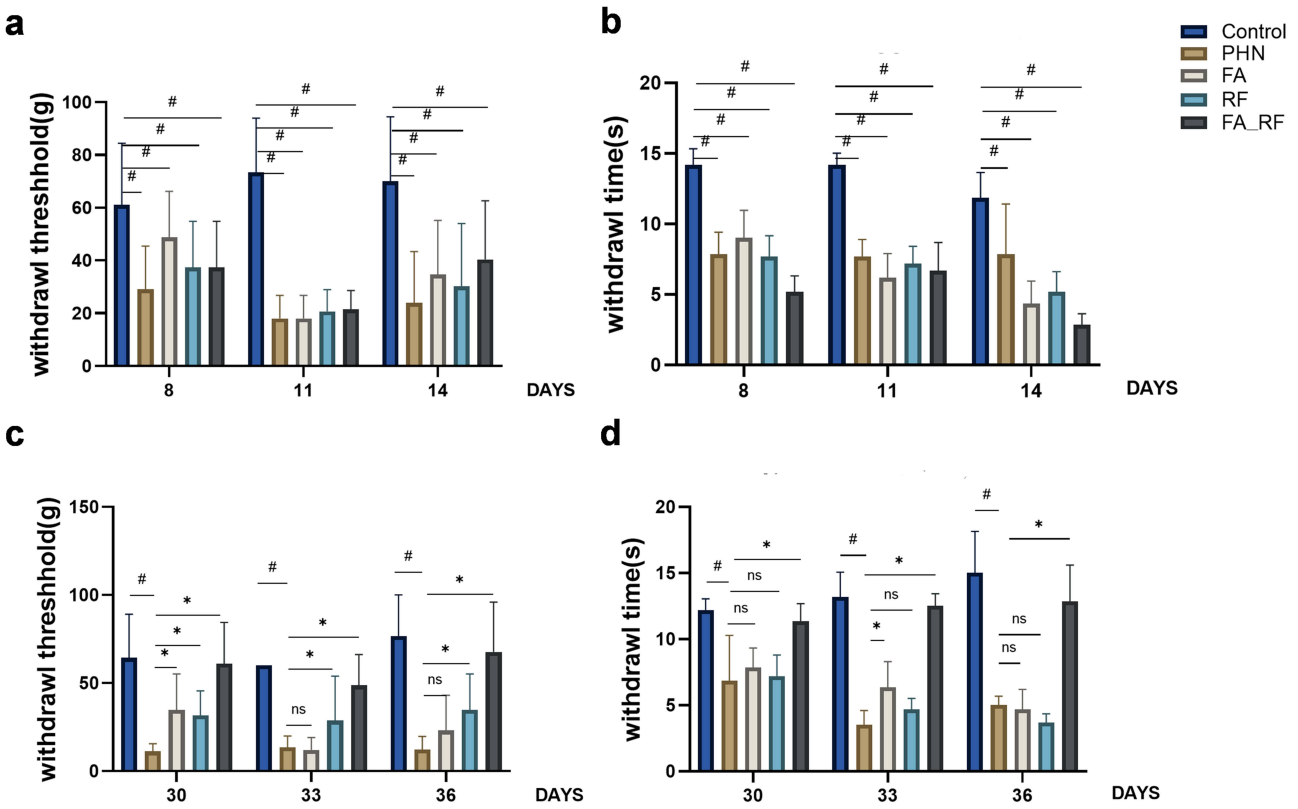

Figure 3 Combined therapy of fire acupuncture and pulsed radiofrequency alleviates tactile allodynia in a rat model of postherpetic neuralgia. Rats were inoculated in the right footpad with either uninfected telomerase-immortalized retinal pigment epithelial (TRPE) cell equivalents (Control group) or varicella-zoster virus (VZV, 2×10^5 PFU) to induce PHN. VZV-inoculated rats were then divided into the following treatment groups: PHN (no treatment), fire acupuncture (FA), pulsed radiofrequency (RF), or combined FA and RF (FA_RF). (**a**) Mechanical allodynia (50% paw withdrawal threshold in grams) of the ipsilateral (inoculated) paw over time. (**b**) Thermal hypersensitivity (paw withdrawal latency in seconds) of the ipsilateral paw over time. (**c**) Mechanical allodynia of the contralateral (non-inoculated) paw over time. (**d**) Thermal hypersensitivity of the contralateral paw over time. Data are presented as mean ± SD (n=6 per group). Statistical significance was determined by Tukey's multiple comparison test. Symbols and groups: Control (dark blue rectangle), PHN (brown rectangle), FA (gray rectangle), RF (light blue rectangle), FA_RF (black rectangle). `#` indicates comparison of the PHN group to the Control group (`#$p < 0.05$). `*` indicates comparison of the FA, RF, or FA_RF groups to the PHN group (`*$p < 0.05$). **Abbreviation**: ns, not significant.

On Day 37 after varicella zoster virus inoculation, GFAP immunoreactivity was significantly greater in PHN than in Control group, and multiple $GFAP^+$ astrocytes were present (Figure 4a and b). The GFAP intensity was significantly lower in FA_RF than in PHN group (Figure 4a and b). Furthermore, the GFAP intensity was lower in FA and RF than in PHN group (Figure 4a and b). To assess the effect of varicella-zoster virus infection on microglial activation in the spinal cord, we quantitatively analysed the number of $CD68^+$ microglia in the spinal cord. Varicella-zoster virus infection, fire acupuncture and pulsed radiofrequency intervention had no effect on spinal microglia (Figure 4a and d). These findings suggest that infection of rats with varicella-zoster virus caused only astrocyte activation in the spinal cord, with little effect on spinal microglia.

On Day 37 after infection with varicella-zoster virus, the NF200 level in PHN was significantly lower than in Control group, the NF200 level was greater than in the PHN group, and the NF200 immunoreactivity in FA and RF groups was also greater than that in the PHN group (Figure 4a and c). Next, we evaluated the expression of apoptosis-related proteins, including the proapoptotic proteins Bax and calpain and the antiapoptotic protein Bcl-2, in each spinal cord dorsal horn by Western blot analysis (Figure 4e–h). Compared with Control group, Bax and calpain were significantly augmented, whereas Bcl-2 was significantly decreased in PHN group. However, the levels of calpain and Bax were lower than in PHN group, and the expression of Bcl-2 was also augmented. In the present study, intervention with fire acupuncture and pulsed radiofrequency increased the ratio of Bcl-2 to Bax, thereby inhibiting neuronal apoptosis. These findings indicate that fire acupuncture and pulsed radiofrequency can reduce neuronal apoptosis and promote damaged neuronal repair. Interestingly, the combination of fire acupuncture and pulsed radiofrequency can further promote the repair of damaged neurons caused by varicella-zoster virus.

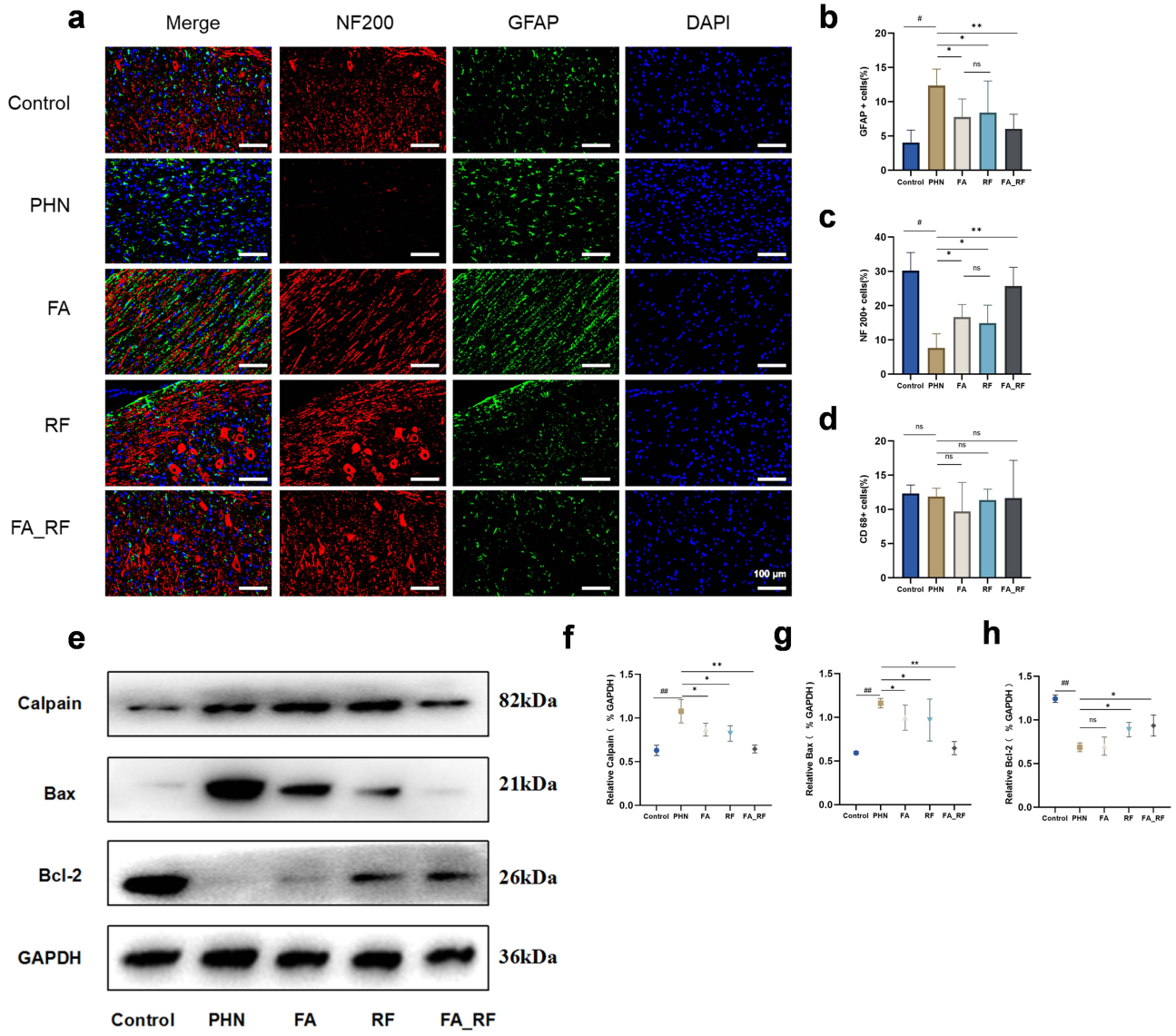

Figure 4 Combined therapy of fire acupuncture and pulsed radiofrequency inhibits spinal cord astrocyte activation, reduces neuronal apoptosis, and promotes neuronal repair. (**a**) Representative immunofluorescence staining images showing the expression of NF-200 (neuronal marker, red) and GFAP (astrocyte marker, green) in the spinal dorsal horn across different treatment groups on day 37 post-inoculation. Scale bar = 100 μm. (**b**) Quantitative analysis of the number of GFAP-positive astrocytes in the spinal cord lesion area. (**c**) Quantitative analysis of the number of CD68-positive microglia in the spinal cord lesion area. (**d**) Quantitative analysis of the number of NF-200-positive neurons in the spinal cord lesion area. (**e**) Representative Western blot bands showing protein expression levels of Calpain, Bax, and Bcl-2.(**f-h**) Relative mRNA expression level of Calpain, Bax and Bcl-2. Data are presented as mean ± SD (n=6 per group). Statistical significance was determined by Mann–Whitney *U*-tests. Symbols: `#` indicates comparison of the PHN group to the Control group (`#*p*`< 0.05, `##*p*` < 0.01). `*` indicates comparison of the FA, RF, or FA_RF treatment groups to the PHN group (`**p*`< 0.05, `**p*`< 0.01).
Abbreviation: ns, not significant.

Fire Acupuncture and/or Pulsed Radiofrequency Intervention Reduced the Inflammatory Response in CSF Supernatants

To assess the inflammatory response in the spinal cord after varicella-zoster virus inoculation and after fire acupuncture and/or pulsed radiofrequency intervention, we used an ELISA to measure the release of pro-inflammatory and anti-inflammatory cytokines in the CSF supernatants to assess the inflammatory response (Figure 5).

The results showed that compared with Control group, the expression levels of IL-6, TNF-αand IL-1β in PHN were higher, while the expression levels of pro-inflammatory cytokines in FA_RF were lower than PHN group ($P < 0.01$). Fire acupuncture and pulsed radiofrequency intervention also reduced the levels of IL-6, TNF-α, and IL-1β (Figure 5a–c). Anti-inflammatory

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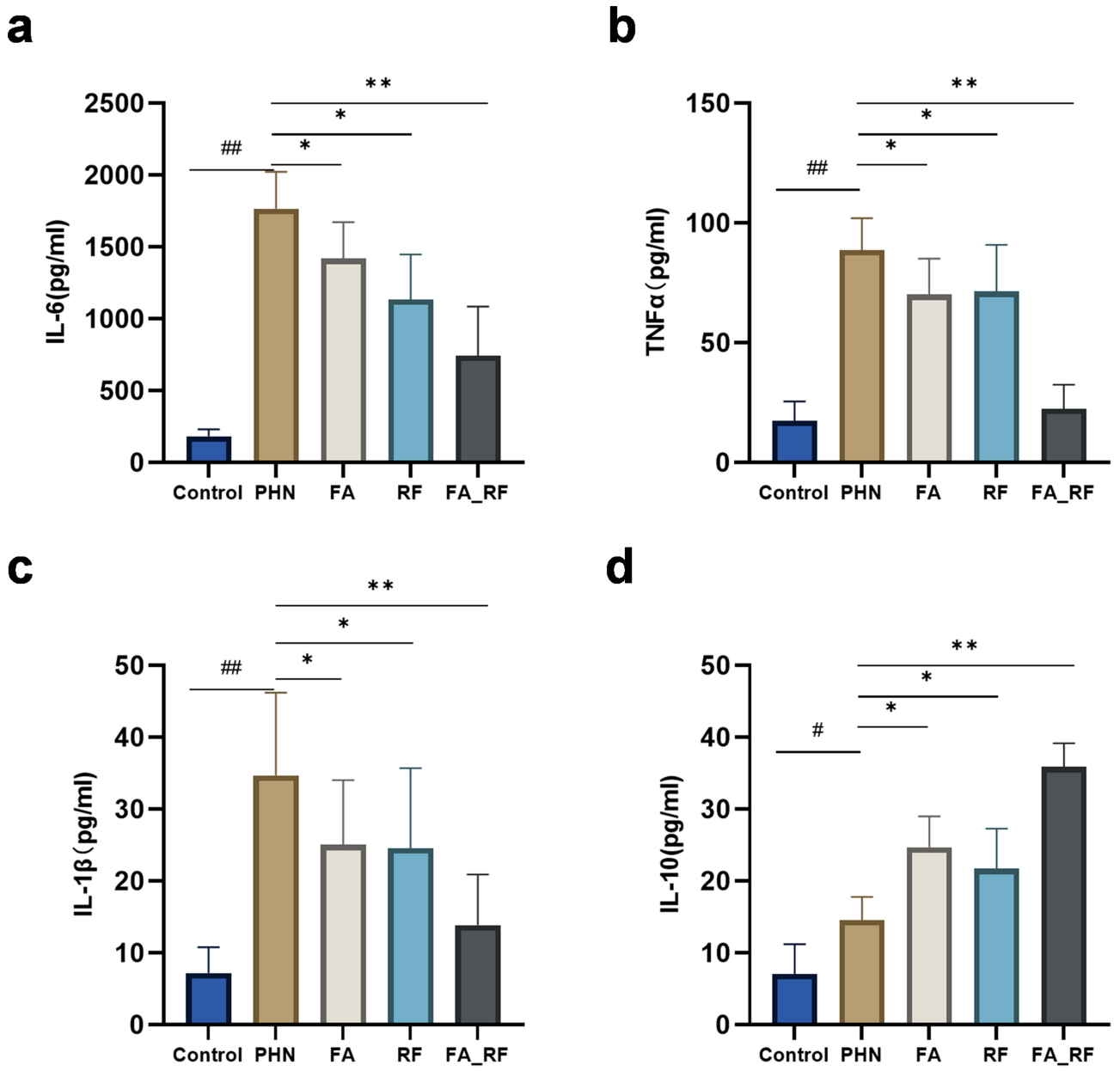

Figure 5 Fire acupuncture and pulsed radiofrequency intervention reduces the neuroinflammatory response in spinal cord tissue. The levels of inflammatory cytokines in the supernatants of spinal cord tissue cultures were determined by enzyme-linked immunosorbent assay (ELISA). (**a**) Concentration of tumor necrosis factor-α (TNF-α). (**b**) Concentration of interleukin-1β (IL-1β). (**c**) Concentration of interleukin-6 (IL-6). (**d**) Concentration of interleukin-10 (IL-10). Data are presented as mean ± SD (n=6 per group). Statistical significance was determined by Mann–Whitney *U*-tests. Symbols: `#` indicates comparison of the PHN group to the Control group (`#*p*` < 0.05, `##*p*` < 0.01). `*` indicates comparison of the treatment groups (FA, RF, or FA_RF) to the PHN group (`**p*` < 0.05, `**p*` < 0.01).

cytokine IL-10 levels were significantly greater in FA_RF than in PHN group ($P < 0.01$, Figure 5d), and fire acupuncture and pulsed radiofrequency intervention also increased IL-10 levels. These data support the anti-inflammatory effects of fire acupuncture and pulsed radiofrequency, and, notably, the combination of fire acupuncture and pulsed radiofrequency has more significant effects on IL-6, TNF-α, IL-1β and IL-10 than single-fire acupuncture or pulsed radiofrequencyalone, indicating that the combination of both has a synergistic effect on the inflammatory response in spinal cord tissue.

Fire Acupuncture Combined with Pulsed Radiofrequency Intervention Altered the Expression of Genes in Rats with Postherpetic Neuralgia

To determine the symphyogenetic of fire acupuncture and pulsed radiofrequency on gene expression in the spinal cord of rats with postherpetic neuralgia and explore the underlying molecular mechanisms, we performed mRNA sequencing analysis of spinal cord tissue from the PHN and FA_RF groups on Day 37 after varicella-zoster virus inoculation.

In total, 597 and 99 genes were upregulated and 99 genes were downregulated in FA_RF compared with PHN group (Figure 6a). Group cluster heatmaps were used to analyse the mRNA levels of the top 10 genes (Figure 6b). After the differentially expressed genes were obtained, we performed GO enrichment analysis of the biological processes and molecular functions of the differentially expressed genes associated with the TOP30 entries to explore the molecular mechanism of the combined effect of fire acupuncture and pulsed radiofrequency in the spinal cords of the varicella-zoster virus-infected rats. The results showed that after fire acupuncture combined with pulsed radiofrequency intervention, the gene set related to the tight connection of the nervous system barrier, insulin-like growth factor and extracellular matrix structure components was significantly enriched (Figure 6c), which is a feature of nervous system repair and demonstrated that the combined effect of fire acupuncture and pulsed radiofrequency can promote the repair of the nervous system after injury. The KEGG results revealed that the P13K/AKT signalling pathway was significantly

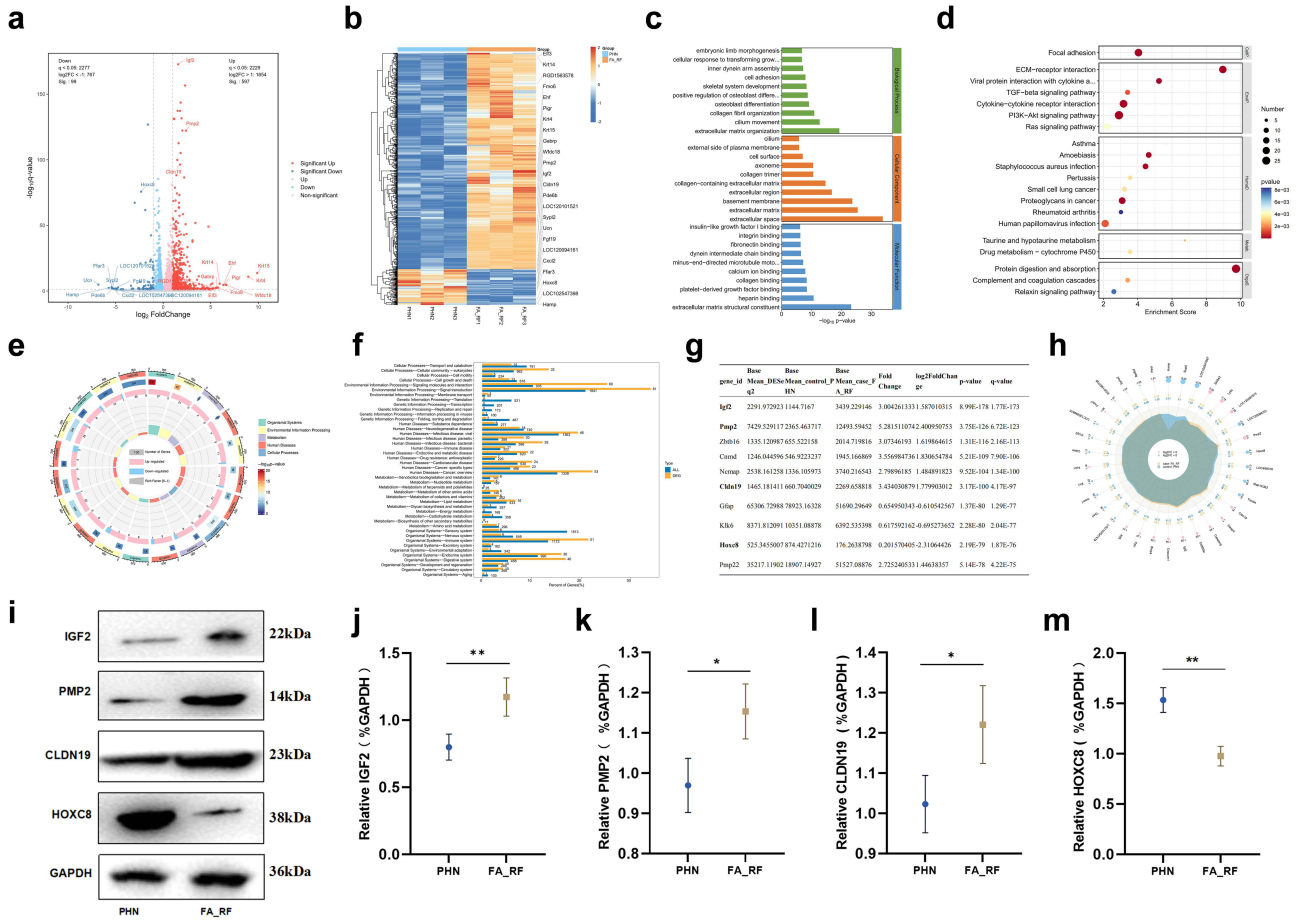

Figure 6 Combined therapy of fire acupuncture and pulsed radiofrequency alters gene expression profiles in a rat model of postherpetic neuralgia. (**a**) Volcano plot displaying transcripts that were significantly upregulated (red) or downregulated (blue) in the FA_RF group compared to the PHN group. (**b**) Heatmap of differentially expressed genes associated with neural repair processes. (**c**) Enrichment analysis of Gene Ontology (GO) for biological processes and molecular functions. (**d**) Enrichment analysis of differentially expressed genes. (**e**) GO enrichment circle diagram comparing differentially expressed genes to all genes. (**f**) Classification of enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.(**g** and **h**) Top 10 genes with the most significant expression levels in the spinal dorsal root of the PHN group.(**i**) Representative Western blot images showing protein expression levels of IGF2, PMP2, CLDN19, and HOXC8. (**j–m**) Relative mRNA expression level of IGF2, PMP2, CLDN19 and HOXC8. Data are presented as mean ± SD (n=6 per group). Statistical significance for panels (**j–m**) was determined by Mann–Whitney *U*-tests (*$p < 0.05$, **$p < 0.01$). Bioinformatics analyses (**a–i**) were performed using GSEA software.

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enriched and that other involved pathways, such as the ECM receptor interaction, the TGF-β signalling pathway, and the Ras signalling pathway, were also regulated (Figure 6d). The results of the GO enrichment analysis of the differentially expressed genes and all the genes revealed that in terms of biological processes, they were significantly enriched in nervous system development, the neural myelin tight connection, mRNA homeostasis regulation, apoptosis, the immune response, neuroinflammation, and cell proliferation, differentiation and survival (Figure 6e). Moreover, the KEGG pathway classification focused mainly on signal transduction, viral diseases, cancer, and the immune system (Figure 6f). We further analysed the top 10 genes with the most significant variation after fire acupuncture combined with pulsed radiofrequency intervention, combined with the 30 top/downregulated genes with the least differential gene radar map q value or p value, and found that the expression levels of genes related to neurological function, such as insulin-like growth Factor 2 (IGF2), peripheral myelin protein 2 (PMP2), and nervous system barrier tight junction protein (CLDN19), were significantly upregulated and that homeobox protein 8 (HOXC8) levels were significantly downregulated (Figure 6g–m), confirming that the combined effect of fire acupuncture and pulsed radiofrequency could alter the levels of IGF2, PMP2, CLDN19 and HOXC8 in spinal cord tissue.

P13K/AKT Agonists Reversed the Effects of the Combination of Fire Acupuncture and Pulsed Radiofrequency on Tactile Allodynia in Rats with Postherpetic Neuralgia

24 Male Sprague–Dawley rats adapted to the measured conditions were divided into 4 groups: Control, PHN, FA_RF, and FA_RF + 740Y-P + SC79 groups. Control rats were inoculated with a TRPE cell equivalent (dark blue rectangle), and PHN rats were subcutaneous injected with 2×10^5 PFU of varicella-zoster virus (brown rectangle). FA_RF rats were subcutaneous injected with 2×10^5 PFU of varicella-zoster virus and subjected to fire acupuncture combined with pulsed radiofrequency intervention (gray rectangle). FA_RF + 740Y-P + SC79 rats were subcutaneous injected with 2×10^5 PFU with 2×10^5 PFU of varicella-zoster virus, subjected to intraperitoneal injection of 740Y-P and SC79, and subjected to fire acupuncture combined with pulsed radiofrequency intervention (light blue rectangle). As mentioned above, mechanical allodynia and thermal hyperalgesia were evaluated on Days 30, 33 and 36 after inoculation with varicella-zoster virus. Moreover, the immunoreactivity for NF-200 and GFAP was also assessed. The levels of IL-1β, IL-6, TNF-α, and IL-10, as well as the levels of IGF2, PMP2, CLDN19, and HOXC8, which reflect the inflammatory response in the spinal cord, were assessed (Figure 7).

Compared with those in the FA_RF + 740Y-P + SC79 group, the effects of mechanical allodynia and thermal hyperalgesia were reversed in FA_RF + 740Y-P + SC79 compared with FA_RF group (Figure 7a and b). Compared with FA_RF group, GFAP immunoreactivity was significantly greater in the FA_RF + 740Y-P + SC79 group (Figure 7c and d), indicating that P13K/AKT agonists reversed the activation effect of fire acupuncture combined with pulsed RF on astroglia. We also found greater NF200 immunoreactivity in FA_RF + 740Y-P + SC79 group than in FA_RF group (Figure 7e), indicating that P13K/AKT agonists reversed the protective effects of fire acupuncture and pulsed RF on neuronal health. Moreover, the changes in the IL-1β, IL-6, TNF-α, and IL-10 levels in the CSF were reversed by P13K/AKT agonists (Figure 7f–i). Notably, spinal cord IGF2, PMP 2, CLDN19 and HOXC 8 levels were also reversed by P13K/AKT agonists and corresponded to significantly decreased IGF2, PMP 2, CLDN19 and HOXC 8 levels compared with FA_RF group (Figure 7j–n). These results suggest that the P13K-AKT signalling pathway may play an important role in the alleviation of postherpetic neuralgia.

Discussion

PHN is a refractory neuropathic pain syndrome that, although not a threat to patient safety, results in a severe decline in patient quality of life.[22] Herpes zoster viruses have neurotropic properties. After herpes zoster virus infection invades the skin, it can sensitize the peripheral and central nervous systems, enhance the synaptic transmission of sensory neurons (especially pain-related neurons), and induce neuralgia after herpes zoster virus infection.[23] Here, by detecting changes in mechanical allodynia and thermal hyperalgesia in rats with postherpetic neuralgia, we found that the combination of fire acupuncture and pulsed radiofrequency can reduce tactile allodynia in rats with postherpetic neuralgia. It was worth noting that, as mentioned above, fire acupuncture alone or pulse radiofrequency alone did not clearly relieve tactile

allotopic pain in rats with postherpetic neuralgia. It proves that the combined application of traditional Chinese medicine technology such as fire acupuncture and western medicine technology such as pulsed radiofrequency has advantages in the treatment of refractory neuropathic pain such as postherpetic neuralgia.

Postherpetic neuralgia belongs to the category of "Bi Zheng" in traditional Chinese medicine. "Su Five Zang generation" refers to "blood coagulation in the skin, for bi". Its aetiology is mostly congestion and collateral obstruction, Yang deficiency and cold coagulation, and treatment should focus on promoting blood circulation, removing blood stasis, dispersing cold and relieving pain.[24] Acupuncture stimulation at the GB30 (Huantiao) and EX-B2 (Jiaji) acupoints is frequently used to relieve postherpetic neuralgia in rat models.[25,26] In this experiment, the fire acupuncture acts EX-B2 and GB30 on the ipsilateral side of the inoculated viral foot pad, which is located with the "odd points outside" and the "foot shaoyang gallbladder meridian", and it is the gathering place of the body. The fire acupuncture points along the spinal nerve and neuralgia-related area activate the warm passage of the fire acupuncture such that the "Qi" and blood can fill the four ends. Fire acupuncture therapy involves heating sterilized needles before rapid insertion into specific acupoints or skin areas. Postherpetic neuralgia, the heat from the needles not only stimulates acupoints but also enhances local blood circulation, accelerates healing, and reduces inflammation. According to evidence-based medical research, fire acupuncture are frequently used in acupuncture treatments for PHN.[27] In animal model studies, acupuncture's potential therapeutic effects on postherpetic neuralgia have been extensively validated. Li et al[28] established a rat PHN model by administering varicella-zoster virus for 21 consecutive days, followed by daily fire needle therapy starting from day 7. At the end of the experiment, the treatment group demonstrated significantly higher mechanical abnormal pain thresholds compared to the control group, along with reduced expression levels of inflammatory factors in spinal cord tissues. The analgesic mechanism of pulsed radiofrequency is complex and involves both changes in tissue

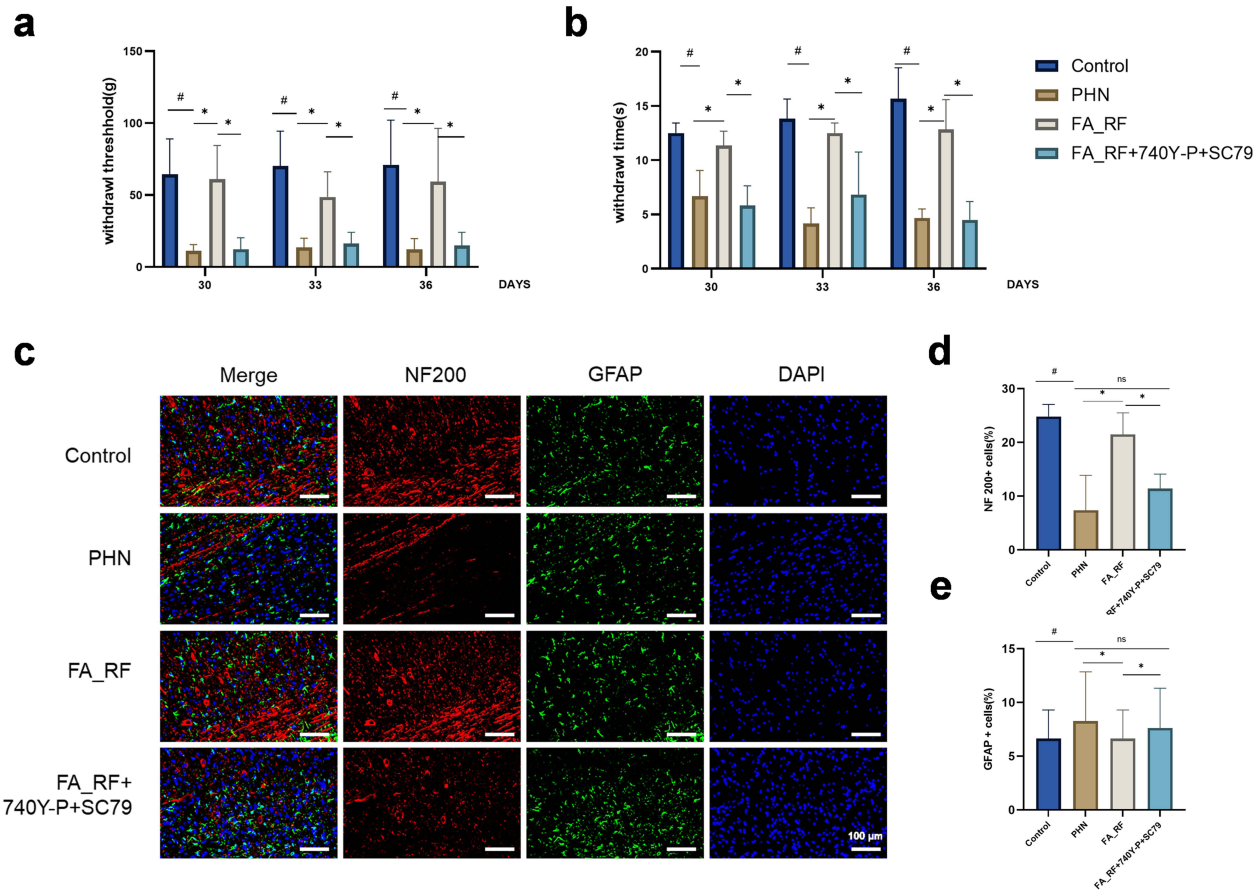

Figure 7 Continued.

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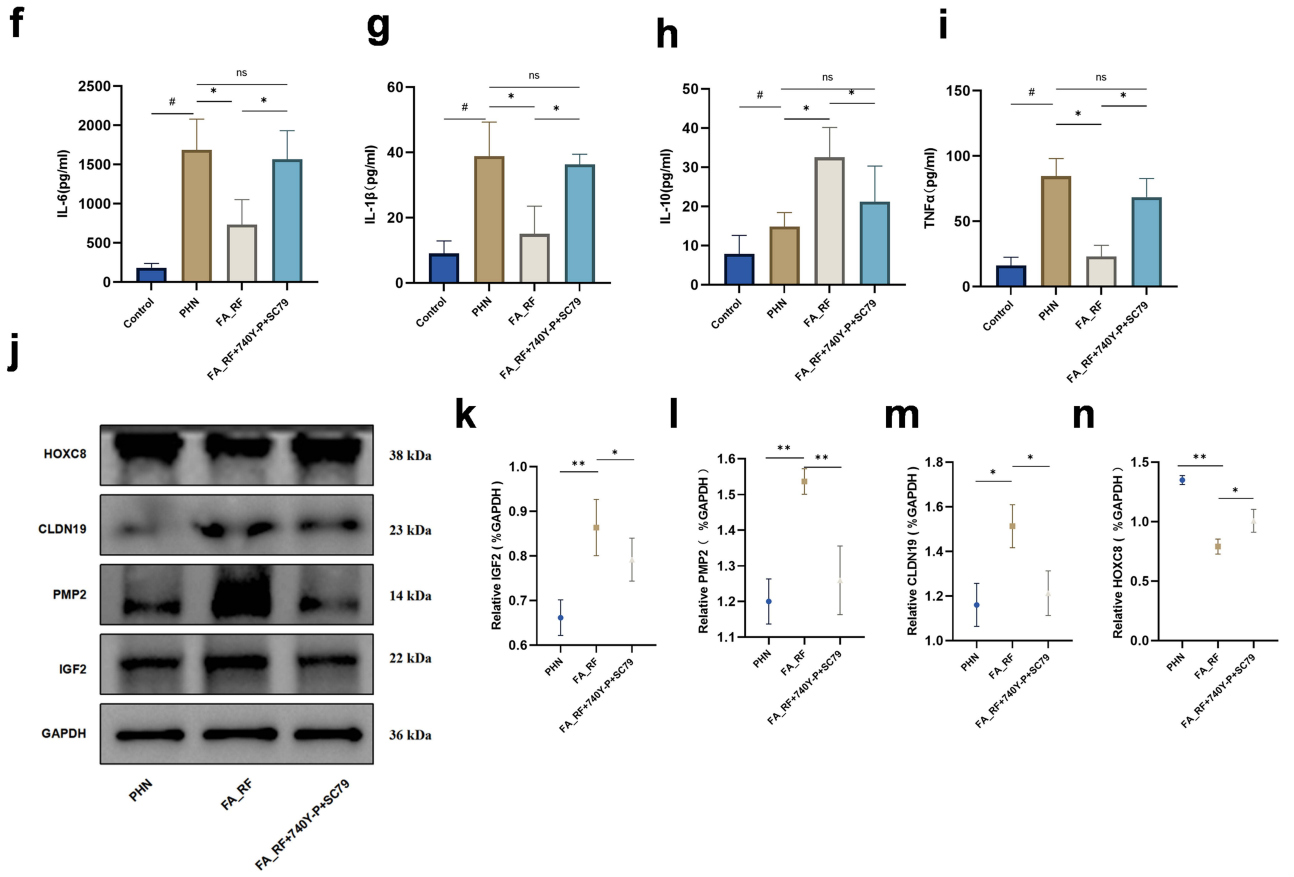

Figure 7 PI3K/AKT agonists reverse the therapeutic effects of combined fire acupuncture and pulsed radiofrequency by modulating gene expression and neuroinflammation in postherpetic neuralgia rats. Male Sprague-Dawley rats were divided into four groups: Control (inoculated with uninfected TRPE cell equivalents), PHN (inoculated with 2×10^5 PFU varicella-zoster virus, VZV), FA_RF (VZV-inoculated and treated with combined fire acupuncture and pulsed radiofrequency), and FA_RF + 740Y-P + SC79 (VZV-inoculated, treated with FA_RF, and intraperitoneally injected with PI3K/AKT agonists 740Y-P and SC79). (**a**) Mechanical allodynia of the ipsilateral paw, presented as the 50% paw withdrawal threshold (g). (**b**) Thermal hyperalgesia of the ipsilateral paw, presented as the paw withdrawal latency (s). (**c**) Representative immunofluorescence staining images showing the expression of NF-200 (neuronal marker, red) and GFAP (astrocyte marker, green) in the spinal dorsal horn. Scale bar = 100 μm. (**d**) Quantitative analysis of the number of $NF\text{-}200^+$ neurons in the spinal cord lesion area. (**e**) Quantitative analysis of the number of $GFAP^+$ astrocytes in the spinal cord lesion area. (**f–i**) Concentration of interleukin-1β (IL-1β), Concentration of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10) in spinal cord tissue, detected by ELISA.(**j**) Representative Western blot images showing protein expression levels of IGF2, PMP2, CLDN19, and HOXC8.(**k–n**) Relative mRNA expression level of IGF2, PMP2, CLDN19, HOXC8. Data are presented as mean ± SD (n=6 per group). Statistical significance was determined by Mann–Whitney *U*-tests. Symbols: `#` indicates comparison of the PHN group to the Control group (`#p` < 0.05). `*` indicates comparison of the FA_RF group to the PHN group or the FA_RF+Agonists group to the FA_RF group (`*p`< 0.05, `**p` < 0.01).

Abbreviation: ns, not significant.

and cell microstructures and the expression of multiple ion channels and molecular proteins. Pulsed radiofrequency can reduce the expression levels of inflammatory factors in the sciatic nerve, such as TNF-α and IL-6, and prolonged application of pulsed radiofrequency may improve analgesia and not increase tissue damage.[29] The above results were consistent with our results and showed that the combination of fire acupuncture and pulsed radiofrequency can inhibit spinal astrocyte activation; reduce IL-1β, IL-6, and TNF-α levels in spinal cord tissue; and increase IL-10 levels, thus affecting tactile allodynia in postherpetic neuralgia model rats. Besides, our results also show that the inflammatory response in spinal cord astrocytes and spinal cord tissue plays an important role in PHN.

The latest studies indicate that the PI3K/Akt signalling pathway plays important roles in the inflammatory pain induced by capsaicin, thermal hyperalgesia, and mechanical hyperalgesia[30] and in the neuropathic pain induced by nerve ligation[31] and nerve growth factor.[32] PI3K is an important member of the growth factor receptor superfamily during signal transduction. It is ubiquitous in the body and can be activated by various cytokines and physicochemical factors. The Akt pathway is located downstream of PI3K, and AKT, which is mainly responsible for the conduction of biological information initiated by PI3K, is at the central link of this pathway. P13K/AKT agonists affect the P13K/AKT activation

of spinal astrocytes, and the levels of PMP2, IGF2, CLDN19 and HOXC8, including spinal cord-related inflammatory factors such as IL-1β, IL-6, TNF-α, and IL-10, are also affected. Similarly, Tanabe et al[33] performed proteomic analysis of in vitro cultured astrocyte cell lines from mice treated with pulsed radiofrequency. They found that the expression of 2431 genes increased, whereas that of 209 genes decreased, and the PI3K/AKT signalling pathway was associated with pain behaviour. The KEGG pathway enrichment results indicated that the P13K / AKT signaling pathway plays a crucial role in the intervention. These results suggest that the P13K/AKT signalling pathway is involved in the role of fire acupuncture and that pulsed radiofrequency altered tactile allodynia in postherpetic neuralgia rats.

Comparative analysis of the protein expression of PMP2, IGF2, CLDN19 and HOXC8 in the rat spinal lumbar region revealed significant differences in mRNA expression between animals in the PHN and control groups. In the PHN rats, the mRNA expression of the genes PMP2, IGF2, and CLDN19 decreased by 96.5%, 88.4%, and 91.2%, respectively; that of HOXC8 increased by 76.9% ($P < 0.05$); that of the mRNAs PMP2, IGF2, and CLDN19 increased by 86.5%, 77.3%, and 81.4%, respectively; and that of HOXC8 decreased by 77.1% ($P < 0.05$). The data on myelin-specific gene expression levels are consistent with the results of our mRNA-sequencing analysis of the rat spinal cord. PMP2 is a small protein composed of 132 amino acids located on the cytoplasmic side of the peripheral nervous system (PNS), and the PMP2 protein is required for stabilizing the myelin protein.[34] IGF2 plays a role in neuroplasticity, learning, and memory.[35] During neuronal development, IGF2 causes nerve budding,[36] neurite outgrowth,[37] and spine maturation in cultured hippocampal neurons.[38] CLDN19 plays an important role in maintaining the neural barrier.[39] CLDN19 is located close to Schwann cells and promotes the recovery of sensory and motor transmission in the nervous system.[40] Homeobox protein (HOX) is a transcription factor that regulates the coordinated expression of multiple genes during development, differentiation and malignant transformation. HOXC8 can mediate the interaction between pigment epithelium-derived factor (PEDF) and the NF-κB pathway and stimulate the transcription of neuroprotective genes in the nervous system, thus exerting neuroprotective effects.[41] In LMK235-treated mice, HOXC8 is an important pain-related upregulated gene.[42] These findings suggest that PMP2, IGF2, CLDN19 and HOXC8 levels are closely related to nerve function and pain perception, and these studies are consistent with our results. The results of Western blot study showed that the expression levels of PMP 2, IGF2, CLDN19 and HOXC8 changed significantly after fire acupuncture and pulsed radiofrequency intervention. Therefore, we believe that the combination of fire acupuncture and pulsed radiofrequency can promote the recovery of tactile allodynia in rats with postherpetic neuralgia by increasing the levels of PMP2, IGF2, and CLDN19 and downregulating HOXC8 levels.

The lack of reliable animal models of VZV reactivation and postherpetic neuralgia hinders studies of neuropathic pain induced after VZV infection. However, experiments in multiple groups of rats demonstrated that prolonged signs of pain in Wistar and Sprague–Dawley rat strains subcutaneous injected into the footpad could be used as preclinical models to explore the mechanisms and therapeutic strategies of PHN.[43] These models involve the subcutaneous inoculation of cells associated with the VZV into the rat hind foot pad[44] or the nearest whisker pad.[45,46] Although animals show no external signs of skin infection, an inflammatory response, or disease, they develop harmless behaviours that persist for several weeks. VZV has been shown to induce subtle changes in host gene expression in infected ganglia.[47] These studies suggest that in rats inoculated with varicella-zoster virus, the virus can infect and replicate in ganglia, causing behavioural changes that last for several weeks and mimic postherpetic neuralgia. In this study, we observed persistent mechanical allodynia and thermal hyperalgesia within 0–63 days after varicella-zoster virus inoculation, indicating the successful establishment of the animal model.

Due to the lack of suitable in vitro models for fire acupuncture and pulsed radiofrequency techniques, their effects on cellular molecular mechanisms remain unclear. Secondly, while a PI3K/AKT agonist has been used to confirm the role of the PI3K/AKT signaling pathway in alleviating tactile allodynia in rats with zoster-associated neuropathic pain, the underlying molecular mechanisms of this pathway and its crosstalk with the downstream NF-κB signaling pathway require further elucidation. Thirdly, the therapeutic effect of fire acupuncture combined with pulsed radiofrequency (FA_RF) on tactile allodynia has not been validated in aged animal models, which represents an important direction for future research. Moreover, the molecular experiments in this study were not independently replicated multiple times, implying that the results may be influenced by chance or bias and should be verified through repeated experiments in future studies. Subsequent research should focus on developing appropriate cellular models and employing PI3K/AKT inhibitors to further investigate the mechanism by which FA_RF alleviates tactile allodynia in rats with postherpetic neuralgia.

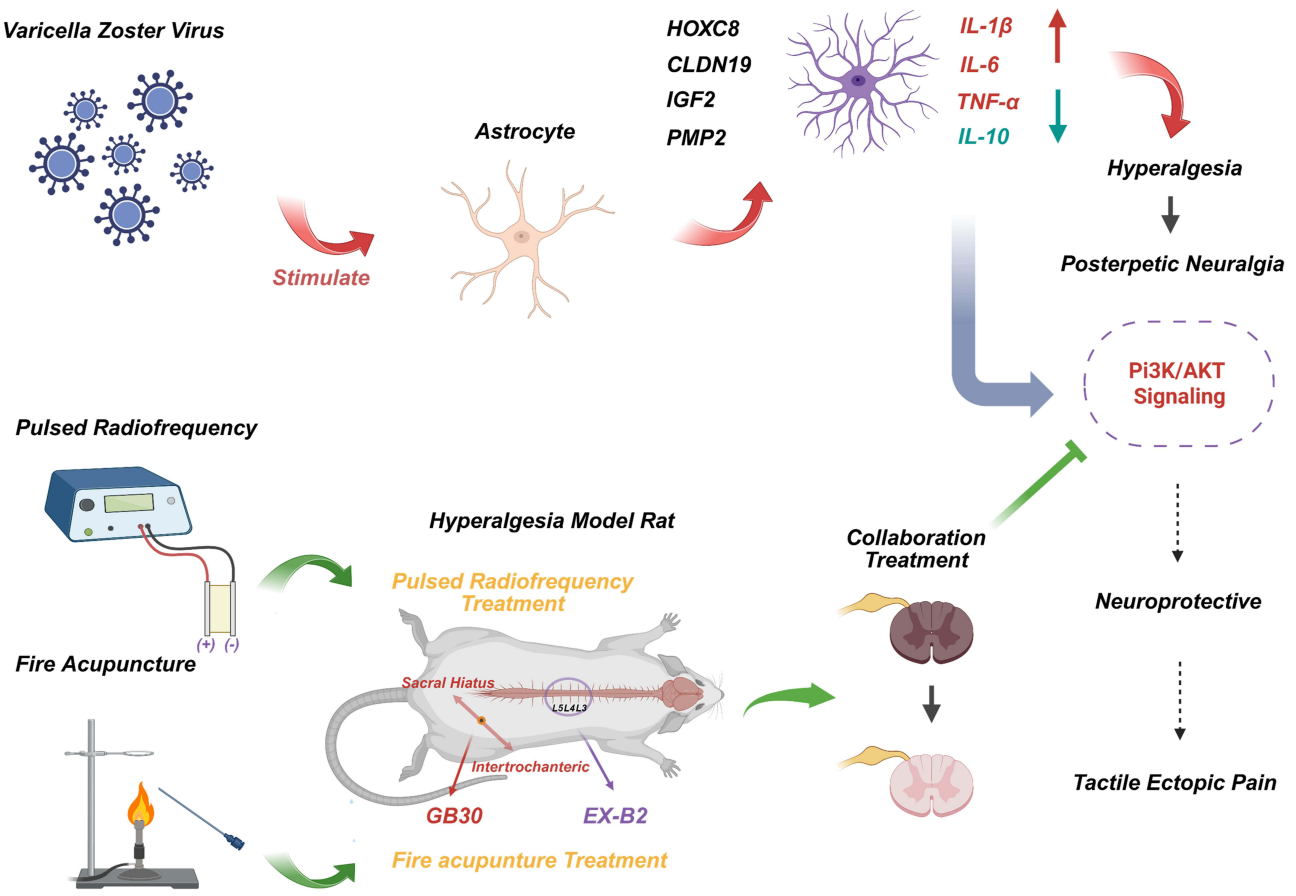

Figure 8 Proposed mechanism underlying the analgesic effect of combined fire acupuncture and pulsed radiofrequency therapy in a rat model of postherpetic neuralgia. Schematic diagram illustrating the hypothesized pathway through which combined fire acupuncture and pulsed radiofrequency (FA_RF) therapy alleviates tactile allodynia. The proposed mechanism involves two key processes:1) Inhibition of neuroinflammation and glial activation: FA_RF therapy inhibits the activation of spinal cord astrocytes and reduces the release of pro-inflammatory cytokines (eg, IL-1β, IL-6, TNF-α), thereby attenuating central sensitization and pain.2) Promotion of neuronal repair: FA_RF therapy upregulates the expression of spinal genes associated with neural repair (IGF2, PMP2, CLDN19) and downregulates HOXC8, promoting the repair of neurons damaged by varicella-zoster virus (VZV) infection. The analgesic effects of FA_RF therapy are potentially mediated through the PI3K/AKT signalling pathway.

Conclusion

Our results suggest that the combination of fire acupuncture and pulsed radiofrequency can alleviate tactile allodynia in rats after herpes zoster neuralgia. The mechanism may involve the inhibition of spinal astrocyte activation and the reduction of spinal cord tissue and inflammation. Moreover, the combination of fire acupuncture and pulsed RF can regulate spinal cord IGF2, PMP2, and CLDN19; downregulate HOXC8 levels; and promote the recovery of damaged nerve function caused by varicella-zoster virus infection, thus exerting an analgesic effect. The signalling pathway involved may be related to P13K/AKT signalling (Figure 8).

Data Sharing Statement

The datasets used and analyzed during the current study are available from the corresponding author (Manlin Duan) on reasonable request.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

Each author certifies that he or she, or a member of his or her immediate family, has no commercial association (ie, consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted manuscript.

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