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ORIGINAL RESEARCH

Efficacy and Spinal Noradrenergic Mechanisms of Contralateral Melittin Acupuncture Against Paclitaxel-Induced Peripheral Neuropathic Pain in Rats

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Background: Paclitaxel-induced peripheral neuropathy is a leading cause of premature discontinuation of taxane-based chemotherapeutic regimens. Studies have affirmed the analgesic properties of bee venom-containing pharmacoacupuncture, demonstrating that anti-nociceptive effects occur following melittin treatment at the ipsilateral ST36 (Zusanli acupoint). However, current understanding of the therapeutic potential of melittin-based approaches for the contralateral side is limited.

Objective: This study comprehensively explored the analgesic potential and central mechanisms of melittin pharmacoacupuncture using behavioral, in vivo electrophysiological, and neuropharmacological techniques in rats with paclitaxel-induced peripheral neuropathy, focusing on the contralateral limb.

Methods: Neuropathic signs following intraperitoneal paclitaxel regimens were quantified on the right-hind paw of rats using acetone drop and von Frey filament experiments. In vivo electrophysiological single-cell recordings of spinal wide-dynamic-range (WDR) neurons were made from the right-dorsal horn extracellularly (n=9–10/group). Melittin was administered subcutaneously at the ST36 acupoint on the left-hind limb (n=7/group). For neuropharmacological analysis, prazosin (an α1-adrenoceptor antagonist) or idazoxan (an α2-adrenoceptor antagonist) was administered before apitherapy (n=6/group).

Results: Following contralateral melittin treatments, a marked attenuation of peripheral cold and mechanical hypersensitivities, along with a sustained reversal of central sensitization in WDR neurons in response to peripheral cutaneous stimuli, was observed in neuropathic rodents after apitherapy. Melittin-induced analgesia involved central noradrenergic mechanisms: its effects on mechanical allodynia and hyperalgesia were counteracted by spinal α2-adrenoceptor antagonism, and its effects on cold allodynia were dependent on activation of spinal α1- and α2-adrenoceptors.

Conclusion: Applications of melittin to ST36 modulated spinal α1- and α2-adrenoceptors. This modulation induced a significant reorganization of nociceptive processing within dorsal horn pain-transmitting neurons, leading to attenuated neuropathic signs in the contralateral limb of rats. Collectively, our behavioral and neurophysiological findings provide pre-clinical evidence supporting melittin-based pharmacoacupuncture as a potential therapy for paclitaxel-induced neuropathic pain.

Keywords: chemotherapy-induced peripheral neuropathy, apitherapy, analgesia, dorsal horn, in vivo recording, adrenoceptor

Introduction

Paclitaxel, a natural derivative of *Taxus brevifolia*, is a life-saving key countermeasure for non-small cell lung cancer, breast cancer, and gynecologic cancers.[1] Nonetheless, like vinca alkaloids and platinum-based antineoplastic (eg,

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vincristine and oxaliplatin), paclitaxel frequently causes symmetrical neuropathic allodynia or hyperalgesia in the digits, which can ultimately compromise limb function.[2,3] In clinical settings, these dose-limiting peripheral toxicities induce premature chemotherapy discontinuation, which eventually compromises the survival prognoses of patients already experiencing immense mental stress due to a cancer diagnosis.[4,5] The American Society of Clinical Oncology (ASCO) has issued guidelines stating that serotonin and noradrenaline reuptake inhibitors (SNRIs), such as duloxetine, are the only class of medication that has been proven to provide modest relief for those experiencing paclitaxel-induced neuropathic pain.[6–8] Conventional pharmaceuticals against neuropathic pain, such as anticonvulsants and tricyclic antidepressants (TCAs), are hampered by adverse effects, such as confusion, dizziness, and tremors, and by limited analgesic properties, which prevent their wide use against chemotherapy-induced peripheral neuropathy (CIPN).[6,9,10] Undoubtedly, implementing optimal avenues to address these neuropathic challenges is paramount in oncological practice.

Bee venom acupuncture (BVA, also known as *apipuncture*), which is administered by stimulating specific acupoints with venom extract obtained from honey bees, has been used as a classic mainstay of pain relief in traditional medicine for millennia.[11–13] Recently, we reported that subcutaneous administration of melittin (a bioactive peptide constituting 50% of BV's weight, s.c., 0.5 mg/kg) at ST36 could alleviate paclitaxel-induced cold and mechanical hypersensitivity in the treated hind limb via restoring the noradrenergic inhibitory tone.[14] In the central nervous system (CNS), pain signals are inhibited by the endogenous noradrenergic pathway, mediated by the activation of spinal α-adrenoceptors by noradrenaline (NA).[15,16] In vivo neurophysiological recordings revealed that after melittin treatment, this analgesia was prominently accompanied by a marked decrement in the spinal sensitization of wide-dynamic-range (WDR) neurons in the dorsal horn.[14] While previous studies have shed light on the analgesic properties of apitherapy on the treated side in CIPN rodent models,[14,17,18] direct research on the potential and suppressive mechanisms underlying melittin administration at acupoints contralateral to the pain assessment site is still lacking.

In clinical practice, contralateral acupuncture treatment is often performed, which has been reported to be more clinically effective in a number of conditions.[19,20] Optimal contralateral analgesic strategies may open up new avenues for patients with contraindications to ipsilateral apitherapy, such as severe epidermal infection in the ipsilateral acupoint area and ipsilateral sciatic nerve injury.[21,22] These contraindications may stem from chemotherapy-induced immunosuppression as well as direct action on normal neurons within the peripheral nervous system (PNS).[23] In addition to its location inferior to the knee joint, ST36 lies adjacent to the ascending peripheral nerve pathways that innervate the extremities.[24] Anatomically, NA-containing nuclei within the pons and midbrain project bilaterally to the spinal cord through the dorsolateral funiculus.[25,26] Within this context, we hypothesize that melittin acupuncture at ST36 may alleviate neuropathic pain in the contralateral limb by activating the spinal α-adrenoceptors.

To further develop melittin-based therapeutic strategies for CIPN, an in-depth understanding of the following is required: (1) the curative potential of melittin on the contralateral side and (2) its potential to modulate spinal pain-transmitting neurons and central anti-nociceptive mechanisms. In this study, we first demonstrated whether administration of melittin at ST36 could attenuate neuropathic signs in the contralateral hind limb of paclitaxel-treated rats. Afterwards, we performed in vivo single-cell recordings to examine the suppressive potential of melittin on paclitaxel-induced spinal sensitization of pain-transmitting neurons in the contralateral dorsal horn. Finally, using spinal pharmacological antagonism of adrenoceptors, we aimed to probe the involvement of specific adrenergic mechanisms underlying the analgesic activity of melittin on paclitaxel-induced neuropathic manifestations.

Methods

Animal Preparation

In this study, the analgesic potential and mechanisms of melittin apitherapy on the contralateral side were investigated in a series of randomized double-blind trials involving 78 male Sprague-Dawley (SD) rats. Male rodents were selected to minimize differences in pain responses resulting from sex hormones.[14,27,28] Animals (5 weeks old, 160–180g, purchased from Vital River Laboratory Animal Technology Co., Ltd, Beijing, China) were housed in a specific pathogen-free (SPF) environment with water and chow available *ad libitum* (n=2–3/cage).[14] The animal facility was maintained at 22 ± 1 °C,

with a humidity of 60–70%, and with a fixed artificial light-dark cycle (lights off from 20:00 to 08:00). Animal research was reviewed and approved by the Ethical Committee of Capital Medical University (Nos. AEEI-2024-119; approved in May 2024, and AEEI-2025-511; approved in September 2025) and was performed following the ethical guidelines of the International Association for the Study of Pain (IASP).[29]

Study Design and Groups

In this study, n refers to the number of spinal neurons recorded during the in vivo electrophysiological experiments. For behavioral assays (ie, assessing analgesia and neuropharmacology), n denotes the number of rodents. Based on published studies using chemotherapy-induced neuropathic pain (CIPN) models in rodents, a sample size of 6–7 rats per group was used for the behavioral assays.[17,18,30–32] For extracellular single-unit recordings, a sample size of 9–10 neurons per group was set, with each recording yielding one single-cell waveform trace.[17,18,31,33]

Animals were randomly assigned to each treatment group using an online program (https://www.graphpad.com/quickcalcs/randomize1/). This randomization was performed by an independent researcher. Experimenters who conducted behavioral testing or in vivo recording remained blinded to group assignment as well as the specific drugs (eg, intrathecal antagonisms) and interventions administered.[14] The order of apitherapy treatment was randomized across all groups.

In the analgesic behavioral investigation (n=7/group): Control (saline) group: Rats with paclitaxel-induced neuropathic pain were treated with saline at the left ST36. Melittin group: Rats with paclitaxel-induced neuropathic pain were treated with melittin apitherapy at the left ST36.

In the in vivo spinal recording (to confirm spinal desensitization): Control (saline, n=9) group: Rats with paclitaxel-induced neuropathic pain were treated with saline at the left ST36 during recording. Melittin (n=10) group: Rats with paclitaxel-induced neuropathic pain were treated with melittin apitherapy at the left ST36 during recording. In the subsequent in vivo spinal recording (to determine central noradrenergic mechanisms; n=9/group): Control (saline) group: Rats with paclitaxel-induced neuropathic pain were treated with saline at the spinal cord during recording. Prazosin group: Rats with paclitaxel-induced neuropathic pain were treated with prazosin at the spinal cord during recording. Idazoxan group: Rats with paclitaxel-induced neuropathic pain were treated with idazoxan at the spinal cord during recording.

In the neuropharmacological behavioral trial (n=6/group): Control (saline) group: Rats with paclitaxel-induced neuropathic pain were treated with intrathecal saline. Prazosin group: Rats with paclitaxel-induced neuropathic pain were treated with intrathecal prazosin. Idazoxan group: Rats with paclitaxel-induced neuropathic pain were treated with intrathecal idazoxan.

Behavioral Evaluation

The rats were habituated to the experimental environment and handled by the experimenters to establish stable familiarity 7 days before behavioral testing (days 1 to 7, Figure 1A).[10,17,18] On each evaluation day, the animals were placed in an inverted, transparent acrylic chamber (15 × 19×28 cm) over a metal mesh floor and allowed 30 min to acclimate to the environment. After acclimation, peripheral hypersensitivity was assessed using the acetone drop test and the von Frey filament (VFF, RWD Life Science Co., Ltd, Sugar Land, TX, USA) test, following established methods.[14,18]

Neuropathic signs were evaluated by stimulating the right-hind paw in the following order: VFFs with bending forces of 4 g (for innocuous stimuli) and 15 g (for noxious stimuli), and acetone cooling stimuli.[14] To quantify peripheral mechanical hypersensitivity, VFF stimuli were applied perpendicularly to the mid-plantar surface of the hind limb 10 times at 10-s intervals.[18] The frequencies of hind paw brisk withdrawal responses elicited by calibrated filaments with bending forces of 4 g and 15 g were calculated as the response percentage and used for statistical analysis.[34] These responses represented mechanical allodynia and hyperalgesia, respectively.[14,35]

Rapid withdrawal frequencies of the right-hind paw in response to evaporative acetone drops were used to assess cold allodynia. In brief, 50 μL of acetone was applied topically to the ventral surface of the hind paw using a micropipette three times, once every 10 min.[36] Characteristic withdrawal behaviors, including shaking, flicking, and licking of the

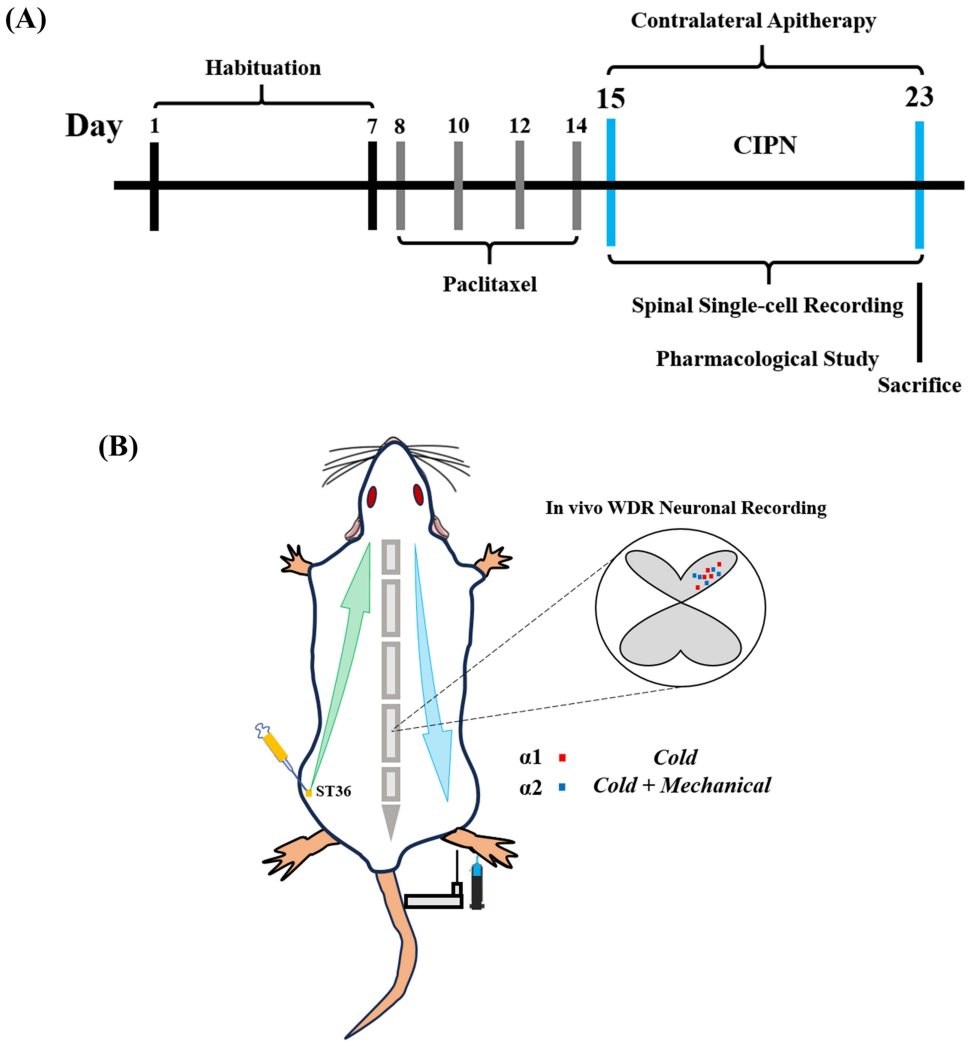

Figure 1 The reporting of this study follows the recommendations in the animal research: reporting of in vivo experiments (ARRIVE) guidelines. Animals were habituated to the testing environment for 7 days prior to behavioral evaluations. Paclitaxel was administered on days 8, 10, 12, and 14. From days 15 to 23—the period when all three types of CIPN symptoms were most pronounced—we conducted spinal extracellular electrophysiological and neuropharmacological studies, as well as behavioral experiments involving melittin-based pharmacoacupuncture (**A**). (**B**) illustrates that in vivo single-unit recordings were obtained from the right dorsal horn WDR neurons. Acetone and von Frey filament tests were performed on the right hind limb before and 30 min after apitherapy (melittin, 0.5 mg/kg) at the left ST36. In mechanical allodynia and hyperalgesia, the spinal α2-adrenoceptor antagonism, but not the α1-adrenoceptor antagonism, abolished melittin-induced analgesia in the contralateral limb. Furthermore, the therapeutic property of contralateral melittin apitherapy in cold allodynia requires the activation of spinal α1- and α2-adrenoceptors (**B**).
Abbreviations: CIPN, chemotherapy-induced peripheral neuropathy; WDR neuron, wide-dynamic-range neuron; ST36, Zusanli acupoint.

hind limb, were monitored over 30s.[14] The total responses were averaged over the three trials and used as read-outs for the severity of cold allodynia.[10,18]

Recently, we affirmed that neuropathic symptoms were evident one day after the paclitaxel regimen and persisted for up to nine days (ie, from day 15 to day 23).[14] Melittin apitherapy (0.5 mg/kg) demonstrated limited efficacy in alleviating mechanical allodynia 60 min post-intervention.[14] However, at 30 min post-treatment, melittin apitherapy ameliorated all three types of neuropathic signs (cold allodynia, mechanical allodynia, and mechanical hyperalgesia).[14] Based on these previous findings, behavioral evaluations were performed at three time points: (1) before the initiation of chemotherapy on day 8; (2) pre-treatment (at a time point when both cold and mechanical hypersensitivity were significant); and (3) 30 min after melittin treatment.

Chemotherapeutic Regimen

To elicit tri-modal peripheral neuropathy, rats received paclitaxel treatment (i.p., 2 mg/kg/day; Macklin Biochemical Technology Co., Ltd., Shanghai, China) as described in detail elsewhere.[37–39] Paclitaxel was dissolved to a concentration

of 6 mg/mL with a mixture of Cremophor EL (Macklin Biochemical Technology Co., Ltd., Shanghai, China) and absolute ethanol, and then further diluted with sterile saline (SAL; China Resources Double-Crane Pharmaceutical Co., Ltd., Beijing, China) to a final concentration of 2 mg/mL (ie, vehicle formula was Cremophor EL: ethanol: saline, 1:1:4, v/v/v).[14] Paclitaxel was administered 4 times, once every other day (days 8, 10, 12, and 14).[14,38]

Melittin Treatment

Melittin (Macklin Biochemical Technology Co., Ltd., Shanghai, China) was dissolved in 50 μL of sterile SAL and administered subcutaneously (s.c.) using a 30-gauge insulin syringe to a depth of approximately 5 mm at ST36 of the left-hind limb (ie, contralateral to the locations of the behavioral and recording investigations), anatomically defined as in the tibialis anterior muscle, 5 mm lateral and distal to the anterior tibial tubercle, at 0.5 mg/kg.[17,18] This optimum dose was chosen from previous dose-response studies showing therapeutic action against chemotherapy-induced neuropathic hypersensitivity in rodent models.[14,17,38,40] Post-apitherapy, the treated limbs were smoothly massaged before returning the animals to their cage.[27]

In vivo Spinal Extraneural Recording

In vivo electrophysiological recordings of WDR neurons were made extracellularly in the right-dorsal horn of the spinal cord.[33,41] Briefly, before thoracolumbar laminectomy (T13-L2), rats were anesthetized with urethane (1.2–1.5 g/kg, i.p). The dorsal surface of the spinal L3 to L5 regions (ie, the lumbar enlargement) was exposed, and the dura mater was then removed.[42] The rats were secured in a stereotaxic apparatus (MA-1, Narishige, Tokyo, Japan) and maintained in a horizontal prone position on a warm plate to allow superfusion of the exposed spinal cord with Krebs solution.[18,43] Following this, the pia-arachnoid membranes over the target insertion site were carefully incised to make a window.[33] An insulated tungsten electrode (impedance 10 MΩ; 243860, FHC, ME, USA) was then inserted at a 30-degree angle.[31] A series of cutaneous stimuli was sequentially applied to the peripheral receptive field restricted to the plantar surface of the right-hind paw corresponding to the identified WDR neuron, as follows: (i) A 4-s brush stimulus was made by stroking the surface five times using a camel brush;[17,31] (ii) A pinch stimulus was delivered by slightly pinching the skin for 4 s with toothed forceps (Fine Science Tools, Heidelberg, Germany);[8,31] (iii) An innocuous evaporative cold stimulus was applied by spraying a 50-μL drop of acetone.[14] The action potentials of isolated WDR neurons were high-pass filtered (250 to 7500 Hz) and digitized at 30 kHz using a Digital Headstage Processor (Plexon, Dallas, TX, USA).[14] Recorded extracellular signals were stored using OmniPlex Software and further sorted with Offline Sorter V4.0 (both from Plexon, Dallas, TX, USA).[44] Each basic analog trace of firings was obtained using the NeuroExplorer program (Nex Technologies, Colorado Springs, CO, USA).[14]

Spinal Administration of Adrenoceptor Antagonist

To clarify which spinal α-adrenoceptor subtype mediates the analgesia of melittin, either prazosin (α1-adrenoceptor antagonist; 30 μg) or idazoxan (α2-adrenoceptor antagonist; 50 μg) was administered before apitherapy.[17,18] Both antagonists were diluted in 10 μL of SAL.[30] In the in vivo neurophysiological recordings, the Krebs solution superfusing the spinal cord was aspirated, and the respective antagonist was topically applied to the site of electrode insertion using a Hamilton syringe. Melittin (0.5 mg/kg) was then injected into ST36 of the left-hind limb 1 min after spinal application. The exposed tissue was perfused again with Krebs solution 1 min after antagonist administration. Neuronal spikes from extraneural recordings of the WDR neuron were monitored before spinal application and 30 min after apitherapy.[14,33] In the behavioral measurements, antagonists were administered intrathecally (i.t.) 1 min before melittin treatment.[17,33] In a prone position and under brief isoflurane anesthesia, rats were subjected to insertion of a Hamilton syringe needle into the subarachnoid space at the L3–L4 intervertebral level.[17,18] Peripheral sensitivities were measured twice: immediately before the administration of antagonists and 30 min after apitherapy.[17,18,33] At the study endpoint, animals were humanely euthanized via CO2 inhalation using the gradual displacement method, in accordance with the AVMA Guidelines for the Euthanasia of Animals (2020 Edition).[27]

Statistics

Rats were excluded if they developed paclitaxel-induced toxicity (eg, alopecia, motor dysfunction), severe subcutaneous infection or swelling at the ST36 following apitherapy, or if they died due to urethane overdose or substantial spinal hemorrhage during the in vivo recording of WDR neurons.[14,18] No animals met these exclusion criteria for the subsequent statistical analysis.

We present data as mean ± standard deviation (SD). Data were analyzed by a statistician blinded to all treatments. Normality was assessed using the Shapiro–Wilk test, and a parametric method was applied. Statistical analysis was performed using GraphPad Prism version 10.0 (GraphPad Software, La Jolla, USA), with two-way ANOVA, followed by Bonferroni's multiple-comparison test. Effect sizes (η^2) and 95% confidence intervals (CI) were calculated. A p-value < 0.05 was considered statistically significant. Figure 1 illustrates the study flowchart and schematic.

Results

Melittin at ST36 Dampened Paclitaxel-Induced Cold and Mechanical Hypersensitivities in the Contralateral Hind Limb of Rats

First, we investigated the ameliorative properties of melittin therapy on the contralateral side. Using our established neuropathic models,[14] we arbitrarily assigned rats with confirmed paclitaxel-induced peripheral hypersensitivity (i.p., total dose 8 mg/kg; assessed on days 15–23, Figure 1A) to two groups, receiving either a subcutaneous (s.c.) injection of melittin (0.5 mg/kg) or saline (SAL, 50 μL) at ST36 of the left-hind limb. Both baseline sensitivity before chemotherapy (BL; mechanical allodynia, 95% CI: −16.18 to 14.75; mechanical hyperalgesia, 95% CI: −16.00 to 18.86; cold allodynia, 95% CI: −0.6970 to 0.9827, all $p > 0.05$, Figure 2A–C) and the degree of neuropathic responses measured immediately prior to apitherapy (T0; mechanical allodynia, 95% CI: −10.46 to 20.46; mechanical hyperalgesia, 95% CI: −18.86 to 16.00; cold allodynia, 95% CI: −0.6018 to 1.078, all $p > 0.05$, Figure 2A–C) did not differ markedly between the groups. In the mechanical sensitivity assessments, withdrawal frequencies in response to 4 or 15 g VFF stimulation were noticeably eased 30 min after apitherapy (mechanical allodynia, 95% CI: 6.679 to 37.61, $p < 0.01$; mechanical hyperalgesia, 95% CI: 1.855 to 36.72, $p < 0.05$, Figure 2A and B). Likewise, in the acetone drop trial, the worsening of cold abnormalities in the right-hind paw was substantially impeded by melittin treatment compared to the controls (95% CI: 0.7316 to 2.411, $p < 0.001$, Figure 2C). In summary, melittin apitherapy markedly reduced cold and mechanical hypersensitivities in the contralateral limbs. Based on these results, we subsequently sought to explore alterations in neuronal hyperexcitability in spinal neurons following melittin application at the left ST36, 30 min post-application.

Melittin at ST36 Suppressed the Spinal Neuronal Hyperexcitation of the Contralateral Dorsal Horn Following Systemic Paclitaxel Regimens

In our recent work,[14] following paclitaxel regimens, in addition to intense behavioral hypersensitivities in the hind limb, the excitability of spinal WDR neurons was markedly heightened. Since contralateral melittin therapy substantially dampened cold and mechanical disturbances in the right-hind limbs (Figure 2), we further recorded stimulus-elicited acute spikes of dorsal horn WDR neurons in neuropathic rodents to examine whether and how melittin injections at the left ST36 could constrain paclitaxel-induced spinal sensitization. Melittin treatment (s.c., 0.5 mg/kg) conspicuously reduced mechanical stimuli-evoked discharges of WDR neurons located in the right-dorsal horn in response to stimulation of their peripheral receptive fields (specifically, the ventral surface of the right-hind paw) (brush, 95% CI: 3.397 to 21.57, $p < 0.01$; pinch, 95% CI: 5.979 to 21.91, $p < 0.001$, Figure 3A and B). Additionally, melittin potently reduced firing in WDR neurons in response to cutaneous application of evaporative acetone (50 μL) 30 min after treatment (95% CI: 0.7862 to 10.21, $p < 0.05$, Figure 3C). In rats with neuropathic signs, the neuronal discharge frequency elicited by mechanical and cold stimuli remained unchanged by SAL administration (brush, 95% CI: −8.192 to 10.96; pinch, 95% CI: −2.505 to 14.28; acetone, 95% CI: −4.302 to 5.635, all $p > 0.05$, Figure 3A–C). A representative raw trace illustrating the decline in isolated WDR neurons' responses to the peripheral brush, pinch, and acetone cooling stimulation 30 min after melittin treatment at ST36 is shown in Figure 3D–F. These in vivo electrophysiology results are consistent with the ability of melittin to counteract peripheral allodynia and hyperalgesia in the contralateral hind limb (Figure 2), indicating

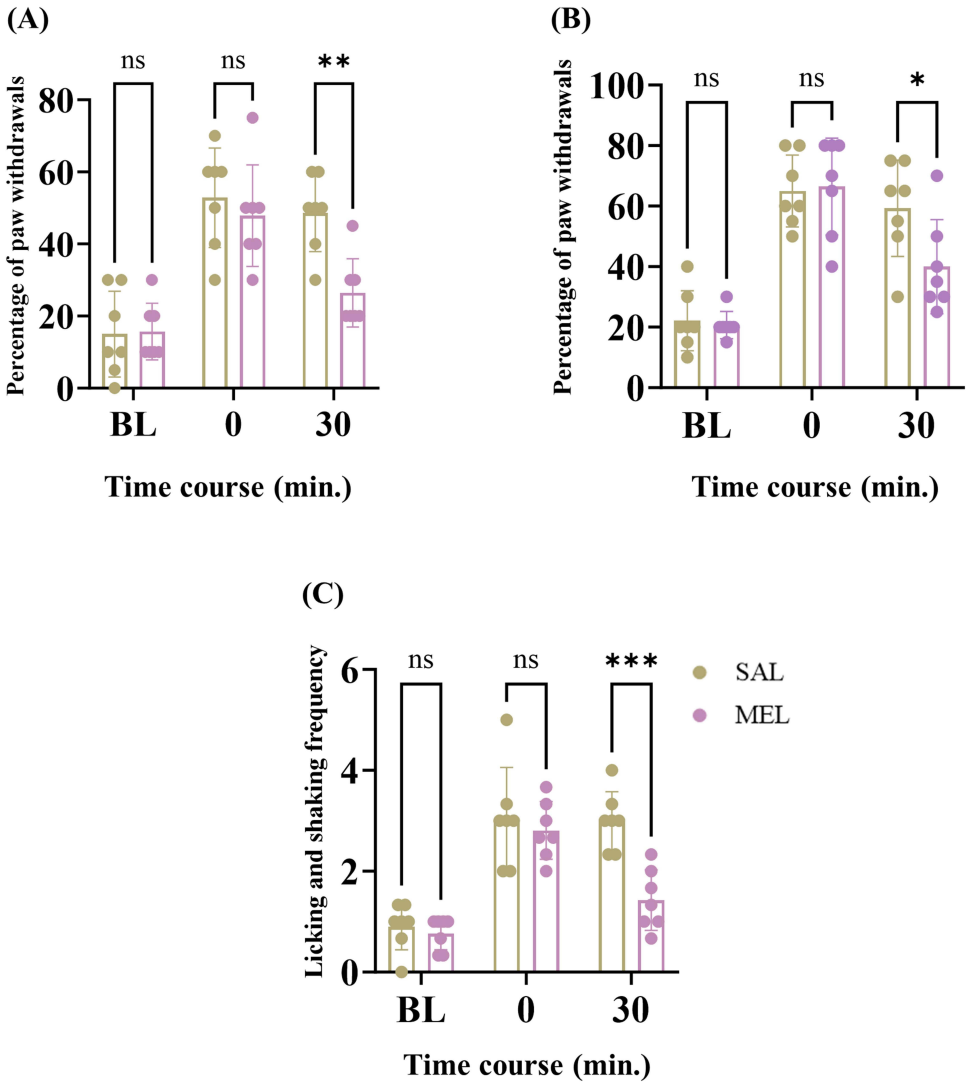

Figure 2 Neuropathic rats were randomly assigned to receive either melittin or SAL at ST36 (n = 7/group). Mechanical allodynia (**A**), mechanical hyperalgesia (**B**) and cold allodynia (**C**) were determined at three time points: baseline, before treatment, and 30 min after dosing at ST36 (timeline labels: BL, 0, and 30). Bars represent the mean ± SD; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, vs SAL ((**A**), η^2 =0.7127, F (2, 24) = 60.76, $p < 0.0001$; (**B**), η^2 =0.7285, F (2, 24) = 42.99, $p < 0.0001$; (**C**), η^2 =0.5800, F (2, 24) = 52.06, $p < 0.0001$). ns, no significance ($p > 0.05$).
Abbreviations: SAL, saline; MEL, melittin.

its significant analgesic properties following intervention at ST36. Furthermore, the specificity of the contralateral acupoint location and the normalization of spinal sensitization underscore that central mechanisms may predominantly mediate its analgesic action against paclitaxel-induced neuropathic conditions.

Effects of Antagonism of Spinal Adrenoceptors on the Melittin-Induced Dorsal Horn Neuronal Suppressive Properties

Subsequent neurophysiological analyses aimed to gauge the specific role of α-adrenoceptors underlying the analgesic features of contralateral melittin therapy. To achieve this, we pretreated rats exhibiting pain behaviors by microinjecting prazosin (30 μg) or idazoxan (50 μg) at the recording site in the right-dorsal horn before administering melittin. Neurophysiological single-cell recordings of WDR neurons were done twice: first, prior to the spinal microinjection, and second, 30 min after melittin intervention at ST36 of the left-hind limb. Pretreatment with prazosin did not affect the inhibitory effects of melittin on peripheral mechanical stimulus-induced neuronal spikes (brush, 95% CI: 3.262 to 19.27, $p < 0.01$; pinch, 95% CI: 9.275 to 28.00, $p < 0.0001$, Figure 4A and B). Conversely, melittin-induced neuronal inhibitions

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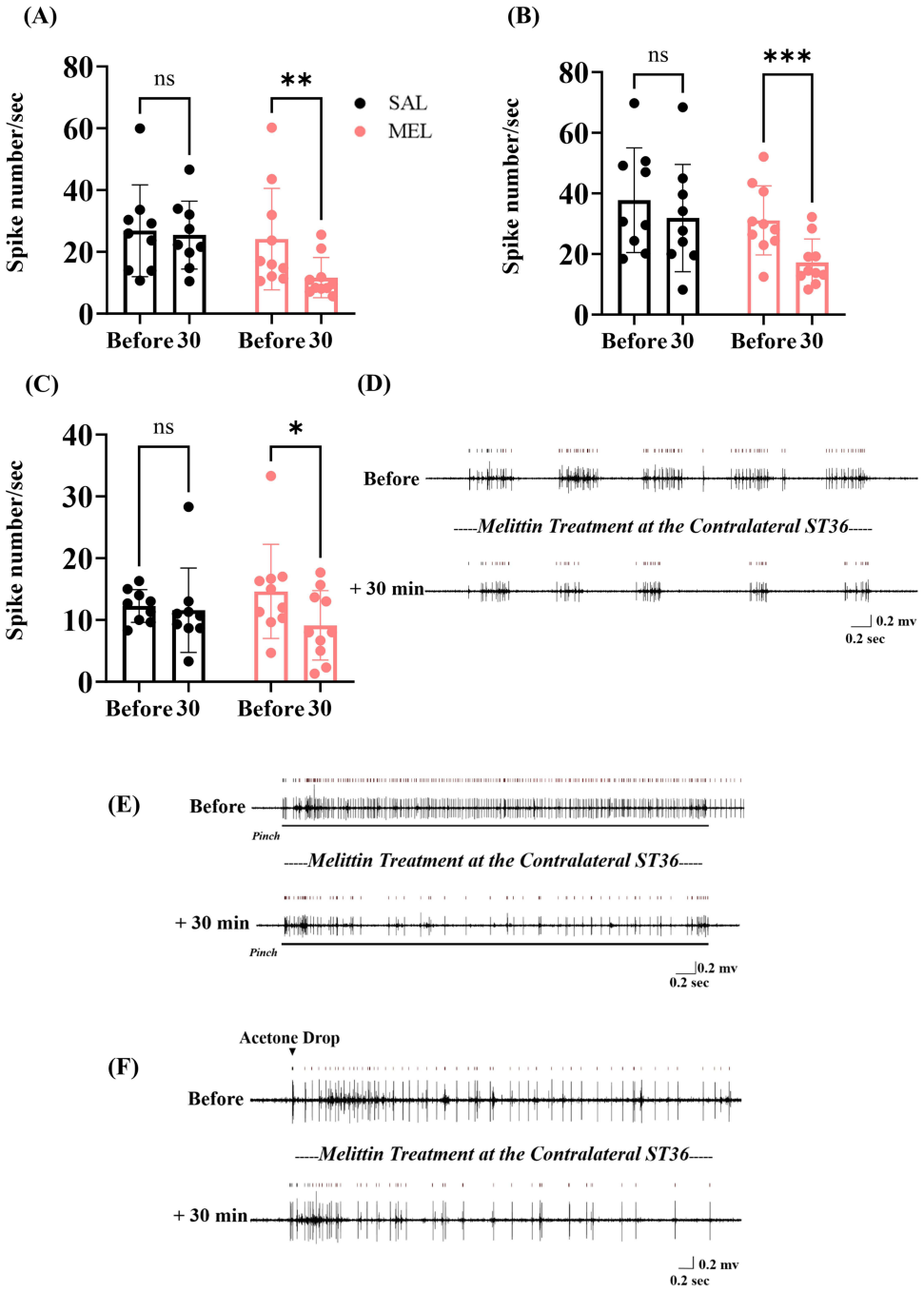

Figure 3 Extracellular recordings in response to cutaneous brush (**A**), pinch (**B**), and acetone (**C**) stimuli were obtained from WDR neurons before and 30 min after melittin (n=10 neurons) or SAL (n=9 neurons) treatment (time points labels: Before and 30). Typical discharge profiles show neuronal responses to brush (**D**), pinch (**E**), and acetone (**F**) stimulation were attenuated 30 min after apitherapy. Bars represent the mean ± SD; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, vs Before ((**A**), η^2 =0.1784, F (1, 17) = 6.667, p = 0.0194; (**B**), η^2 =0.3049, F (1, 17) = 17.75, p = 0.0006; (**C**), η^2 =0.1967, F (1, 17) = 4.898, p = 0.0409). ns, no significance ($p > 0.05$).

Abbreviations: SAL, saline; MEL, melittin.

were conspicuously reversed by spinal α2-adrenoceptor blockade (ie, microinjections of idazoxan; brush, 95% CI: −7.941 to 8.065; pinch, 95% CI: −8.084 to 10.64, all $p > 0.05$, Figure 4A and B). For acetone stimulation, neuronal activity exhibited no considerable decrease at 30 min after melittin therapy compared to baseline (detected prior to antagonist microinjection), in rodents pretreated with either prazosin (95% CI: −4.298 to 6.965, $p > 0.05$, Figure 4C) or idazoxan (95% CI: −5.668 to 5.595, $p > 0.05$, Figure 4C). In the control group, spinal microinjection of SAL failed to

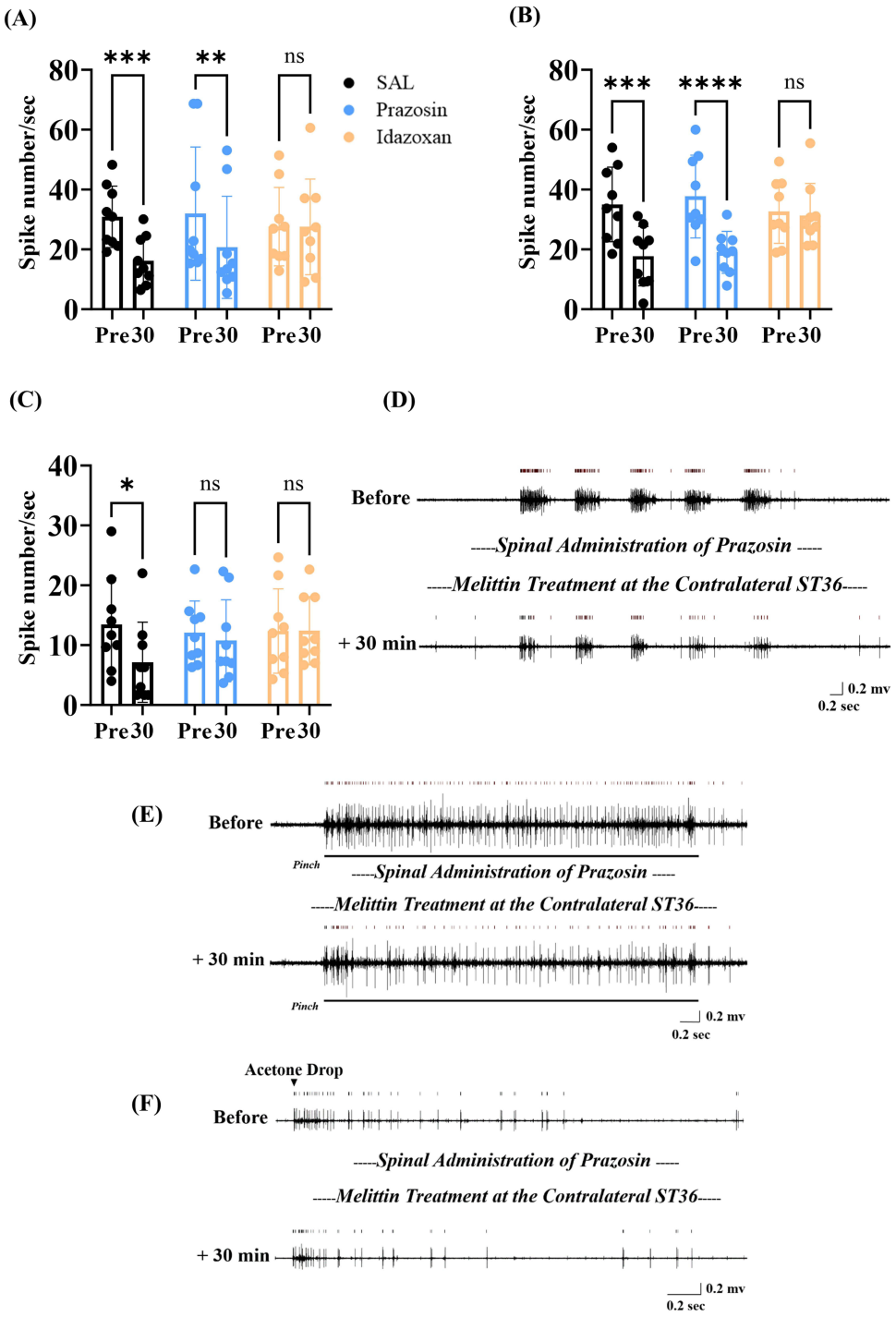

Figure 4 In vivo recordings in response to cutaneous brush (**A**), pinch (**B**), and acetone (**C**) stimuli were conducted before microinjection and 30 min after apitherapy at ST36 (n=9 neurons/group, time points labeled: Pre and 30). Typical discharge profiles detected from WDR neurons in response to brush (**D**), pinch (**E**), and acetone (**F**) stimulation, both before antagonism of spinal α1-adrenoreceptors and 30 min after apitherapy. Bars represent the mean ± SD; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, vs Pre ((**A**), η^2 =0.3711, F (1, 24) = 23.42, $p < 0.0001$; (**B**), η^2 =0.4476, F (1, 24) = 34.84, $p < 0.0001$; (**C**), η^2 =0.1164, F (1, 24) = 4.014, $p = 0.0565$). ns, no significance ($p > 0.05$).
Abbreviation: SAL, saline.

diminish the inhibitory effects of melittin on neuronal firings 30 min post-intervention at the left ST36 (brush, 95% CI: 6.735 to 22.74, $p < 0.001$; pinch, 95% CI: 7.911 to 26.64, $p < 0.001$; acetone, 95% CI: 0.6647 to 11.93, $p < 0.05$, Figure 4A–C). Together, microinjections of prazosin and idazoxan counteracted the suppressive effect of melittin on dorsal horn neuronal sensitization in neuropathic rats. Figure 4D–F illustrate representative analog waveforms of the

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WDR neuronal firings to peripheral brush, pinch, and acetone stimuli before spinal microinjection and 30 min after melittin-based pharmacoacupuncture.

Spinal Blockade of α-Adrenorceptors Reversed Melittin-Induced Analgesic Action on the Contralateral Peripheral Neuropathy

To examine the central noradrenergic mechanism of melittin treatment in the contralateral limb, either 30 μg of prazosin or 50 μg of idazoxan was administered intrathecally before s.c. injection of melittin (0.5 mg/kg at the left ST36) in neuropathic rats. Data revealed that pretreatment with idazoxan (mechanical allodynia, 95% CI: −9.209 to 19.21; hyperalgesia, 95% CI: −8.624 to 25.29, all $p > 0.05$, Figure 5A and B), but not prazosin (mechanical allodynia, 95% CI: 2.458 to 30.88; hyperalgesia, 95% CI: 1.376 to 35.29, all $p < 0.05$, Figure 5A and B) or vehicle (SAL, allodynia, 95% CI: 4.958 to 33.38, $p < 0.01$; hyperalgesia, 95% CI: 3.042 to 36.96, $p < 0.05$, Figure 5A and B), abolished the melittin-induced decrease in withdrawal frequency of the right-hind paw in response to VFFs. In contrast, melittin treatment failed to result in substantial mitigation of cold hypersensitivity in both the prazosin-injected and idazoxan-injected rats

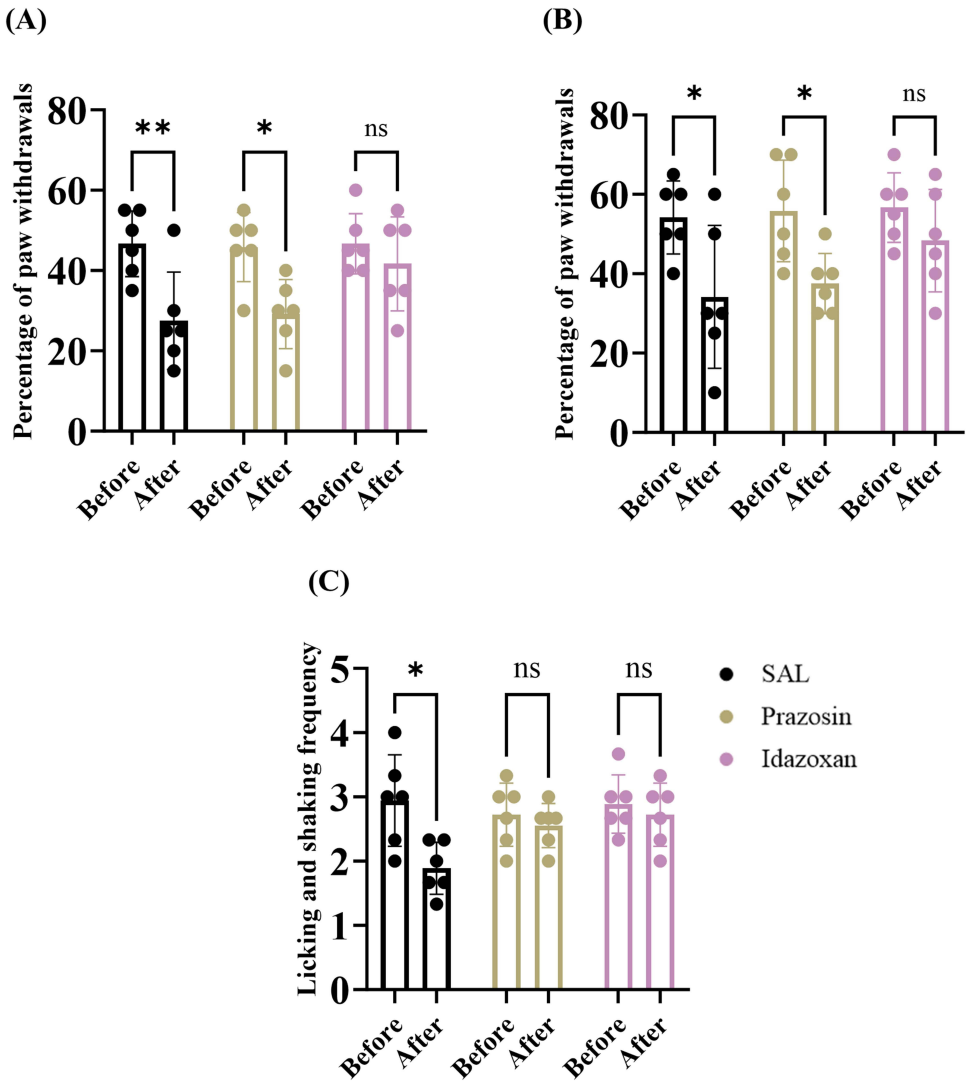

Figure 5 Neuropathic rodents were randomly allocated into three groups (n = 6/group). Mechanical allodynia (**A**), mechanical hyperalgesia (**B**), and cold allodynia (**C**) were determined in the right hind limb twice: just before the antagonist administration (Before) and 30 min after apitherapy (After). Bars represent the mean ± SD; * $p < 0.05$, ** $p < 0.01$, vs Before ((**A**), η^2 =0.4578, F (1, 15) = 19.98, $p = 0.0004$; (**B**), η^2 =0.4695, F (1, 15) = 18.32, $p = 0.0007$; (**C**), η^2 =0.2025, F (1, 15) = 5.672, $p = 0.0309$). ns, no significance ($p > 0.05$).
Abbreviation: SAL, saline.

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(prazosin, 95% CI: −0.7403 to 1.074; idazoxan, 95% CI: −0.7403 to 1.074, all $p > 0.05$, Figure 5C). Our data indicated that melittin-induced analgesia involved central noradrenergic mechanisms. Specifically, its effects on mechanical allodynia and hyperalgesia were counteracted by spinal α2-adrenoceptor antagonism, while its effects on cold allodynia were dependent on the activation of both spinal α1- and α2-adrenoceptors.

Discussion

Paclitaxel-induced peripheral neuropathy, a dose-dependent side effect with considerable medical and socio-economic costs, arguably remains a leading cause of premature non-adherence to scheduled chemotherapy.[6,23] Known risk factors include older age, higher total dosage, and chronic alcohol abuse, among others.[45,46] This painful disorder persists as a long-lasting and prominent challenge, affecting up to 90% of survivors even years after completing paclitaxel-containing cytostatic prescriptions.[1,4] Consequently, intensified research into reliable methods for managing disabling paclitaxel-induced neuropathic comorbidities is strongly warranted worldwide. Pure bioactive ingredients derived from flora and fauna serve as a natural source of analgesics.[13,47,48] Modern processing methods for natural BV allow the production of its primary bioactive elements, such as melittin, with enhanced purity.[49] As expected, these technical breakthroughs have further driven pharmaceutical research on melittin-based apitherapy for various pain conditions.[11,47] As reported in previous studies, melittin has been demonstrated to exert anti-viral, anti-cancer, anti-arthritis, and anti-nociceptive properties.[50,51] Our team and other researchers have investigated the therapeutic activities of varying paradigms of apitherapy using 1.0 mg/kg of BV and 0.5 mg/kg of melittin.[14,17,18,38,40] These optimum doses, chosen based on dose–response evaluation, were administered at ST36 in rodents with chemotherapy (paclitaxel, oxaliplatin, or vincristine)-induced neuropathic pain.[18,38,40] Among these approaches, we recently demonstrated that ipsilateral melittin intervention (s.c., ST36, 0.5 mg/kg) apparently dampened paclitaxel-induced neuropathic pain, with its underlying mechanisms specifically involving the noradrenergic inhibitory process.[14] Nevertheless, the current understanding of whether central mechanisms underlie the analgesic potential of melittin-based apitherapy on the contralateral side remains limited.

ST36 is certainly one of the most frequently utilized acupoints in research on peripheral neuropathic disorders.[14,17,18,21,38,40,52] It has been proven that BVA at this acupoint exerts a particularly curative effect against paclitaxel-induced allodynia, in contrast to interventions at non-acupoint sites.[38] In conventional East Asian medicine, acupuncture can be applied both proximally to the affected area and, at times, to more remote localizations (eg, on the extremities), or even on the contralateral side.[22,52,53] Emerging evidence supports the potential of contralateral acupuncture in ameliorating shoulder pain, post-stroke pain, and acute lumbar sprain; this evidence encompasses diverse assessments, including the severity of nociception, mobility of the affected limbs, and other relevant parameters.[54–59] This pre-clinical study is the first to establish the analgesic potential of melittin intervention at ST36 in reducing paclitaxel-induced neuropathic pain on the contralateral side. We observed that melittin (s.c., 0.5 mg/kg) administered at the left ST36 largely impeded three modalities of neuropathic signs in the right-hind paw of rats (Figure 2). Indeed, treating the contralateral ST36 with moxibustion or electrostimulation in models with inflammatory or neuropathic conditions has been shown to exert potent suppressive action.[21,52,60] Our previous data and current observations have highlighted that therapeutic intervention applied both ipsilaterally and contralaterally, specifically at ST36 in rodents, alleviates hind limb painful hypersensitivity, a finding consistent with earlier outcomes in several pain conditions.[14,52,53] While another practice revealed somewhat different results, experimental apitherapy generated negligible improvements in the mechanical allodynia in the contralateral limbs.[61] We speculated that distinctions in chemotherapeutic agents and intervention protocols could partially account for this (paclitaxel 8 mg/kg vs oxaliplatin 10 mg/kg; 0.5 mg/kg of melittin vs 0.1 mg/kg of BV).

There is a body of electrophysiological data suggesting that chemotherapy exacerbates evoked discharges in dorsal horn WDR neurons, where somatosensory inputs, as well as ascending and descending signals, converge and are heavily integrated.[14,18,31,33] This spinal sensitization of pain-transmitting neurons can serve as a measurable read-out of neuropathic progressions within the CNS.[62–66] It is accepted that somatosensory stimulation-based interventions, such as electro-acupuncture or BV-containing acupuncture, can alleviate pathological nociception by reorganizing and normalizing central nociceptive circuitry in pain states.[17,62,64] Reflecting the behavioral outcomes observed peripherally, marked

hypoactivity of stimulus-evoked neuronal discharges in the dorsal horn was recorded 30 min following the peripheral ST36 melittin intervention in vivo (Figure 3). Our electrophysiological finding is consistent with a previous study showing that duloxetine markedly attenuated oxaliplatin-induced spinal sensitization via noradrenergic mechanisms in a rodent model of neuropathy.[8] The integrity of the functional ascending tracts from the periphery that transmit acupuncture-induced input to the CNS is proposed to be a vital prerequisite for pain-relieving outcomes.[22] It has been reported that electro-acupuncture was ineffective on the ipsilateral side, while interventions at the contralateral side protected against allodynic signs resulting from pre-clinical chronic constriction injury (CCI) procedures.[67] Furthermore, after acute transection of the thoracic spinal cord, the anti-nociceptive effects of acupuncture applied at the contralateral ST36 were totally reversed.[21] These previous findings, together with the fact that the locations of pain evaluations and apitherapy interventions are contralateral in this validation (ie, right-hind paw vs left ST36), suggest that rearrangements in nociceptive processing within the CNS likely account for the analgesic feature observed with contralateral apitherapy.

Using an in vivo spinal extraneural recording approach, we gauged, for the first time, the effects of pretreating the right-dorsal horn recording area with prazosin or idazoxan before apitherapy. Our neuropharmacological results (Figures 4 and 5) demonstrated that: (1) the dorsal horn α1- and α2-adrenoceptors are activated following acupuncture, and these noradrenergic tones are responsible for mitigating cold allodynia and (2) activation of the dorsal horn α2-adrenoceptors is essential for the analgesic properties of melittin in mechanical neuropathy. Although our current findings do not allow us to definitively explain this difference, we speculate that it may stem from differences in anatomical underpinnings: peripheral cold sensation is primarily mediated by C fibers, whereas mechanical sensory input is transmitted to the CNS by both A and C fibers.[16,32,68] Further fiber-type-specific validations are needed to elucidate the detailed mechanisms. A similar anti-nociceptive pattern was observed: the suppressive effects of activating spinal α1-adrenoceptors by phenylephrine (an α1 agonist) and spinal α2-adrenoceptors by clonidine (an α2 agonist) on peripheral cold allodynia and central sensitization in oxaliplatin-induced neuropathic rats were simultaneous and reliable.[33] As previously revealed, specific antagonism of α2-adrenoceptors rekindled pre-apitherapy mechanical hypersensitivity in behavioral trials.[18,61] Established anti-nociceptive mechanisms within the spinal noradrenergic pathway include engagement of α2-adrenoceptors at the central terminals of primary afferent neurons (presynaptic inhibition) and at spinal pain-relay neurons (postsynaptic inhibition).[15,16,69] Additionally, noradrenergic signaling via spinal α1-adrenoceptors on interneurons contributes to pain relief by further releasing inhibitory substances (eg, glycine).[16,70,71] This study sought to determine which adrenoceptor subtype predominates in mediating melittin-induced analgesia on the contralateral side and observed that spinal α2-adrenoceptors play a more tonic role than α1-adrenoceptors in both cold and mechanical neuropathy (compared to α1-adrenoceptors, which only influence cold sensitivity; Figures 4 and 5). In part, this aligns with clinical evidence in volunteers with reflex sympathetic dystrophy, where clonidine provided considerable analgesia, whereas administration of α1-adrenoceptor agonists even exerted an opposing pro-nociceptive action.[72] Apitherapy at ST36 may act as a noxious stimulus. In fact, diffuse noxious inhibitory control (DNIC), a fundamental analgesic paradigm induced by heterotopic conditioning stimuli, was completely absent when spinal α2-adrenoceptors were antagonized.[69] It will be interesting to further investigate the detailed connections between *apipuncture*-induced analgesia and DNIC facilitation in the CIPN condition.

A thorough understanding of the efficacy and central mechanisms of melittin administered to the contralateral limb is crucial for developing melittin-based analgesic therapies for CIPN. Our recent study demonstrated that depletion of NA with the neurotoxin N-(2-Chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) abolished melittin-induced analgesia in the ipsilateral limb.[14] Furthermore, melittin's analgesic effects in the contralateral limb were mediated by spinal α1- and α2-adrenoceptors (Figures 4 and 5). Collectively, these findings indicate that endogenous noradrenergic pathways critically mediate the suppression of paclitaxel-induced neuropathic pain by melittin treatment at ST36.

Limitations

One limitation of the current study is that it focused solely on the analgesic properties following a single treatment of melittin. In the future, the longer-lasting, and potentially prophylactic, effects of repetitive melittin pharmacoacupuncture should be comprehensively studied. Another limitation is the use of male animals only, which precludes the assessment of potential sex-specific effects. Therefore, the generalizability of our findings to females requires further investigation.

Conclusion

At present, CIPN cannot be cured. Our findings demonstrated that melittin applied at ST36 activates spinal α1- and α2-adrenoceptors. This activation modulates nociceptive processing in WDR neurons of the dorsal horn, resulting in significant analgesia in the contralateral hind limb of rats with paclitaxel-induced neuropathic pain. The potential value of our results for the future management of CIPN is considerable. Clinically, CIPN symptoms often present in a symmetrical "glove-and-stocking" distribution.[9,45] Previous articles have shed light on the ipsilateral analgesic properties of melittin treatments in CIPN rodents. In this context, our current findings address a significant gap in the literature. They provide valuable insights for decision-making in integrative pain management and support theoretical evidence for using apitherapy against paclitaxel-induced neuropathy.

Abbreviations

CIPN, chemotherapy-induced peripheral neuropathy; WDR neuron, wide-dynamic-range neuron; ST36, Zusanli acupoint; BVA, bee venom acupuncture; SNRIs, serotonin and noradrenaline reuptake inhibitors; ASCO, American Society of Clinical Oncology; TCAs, tricyclic antidepressants; SPF, specific pathogen-free; IASP, International Association for the Study of Pain; VFF, von Frey filament; CNS, central nervous system; DSP-4, N-(2-Chloroethyl)-N-ethyl-2-bromobenzylamine; PNS, peripheral nervous system; NA, noradrenaline.

Data Sharing Statement

The data generated for the present study are available from the corresponding author, Tianlong Wang: w_tl5595@hotmail.com, upon reasonable request.

Ethical Statement

This study was ratified by the Ethical Committee of Capital Medical University (Nos. AEEI-2024-119; approved in May 2024, and AEEI-2025-511; approved in September 2025).

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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

The authors declare that they have no conflicts of interest in this work.

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