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ORIGINAL RESEARCH

Camphor Attenuates Hyperalgesia in Neuropathic Pain Models in Mice

Ziyuan Li[1,*], Yu Gan[1,*], Ting Kang[1,*], Yi Zhao[1], Tianguang Huang[2], Yuhao Chen[3], Jin Liu[1], Bowen Ke[1]

[1]Department of Anesthesiology, Laboratory of Anesthesia and Critical Care Medicine, National-Local Joint Engineering Research Centre of Translational Medicine of Anesthesiology, West China Hospital, Sichuan University, Chengdu, Sichuan, People's Republic of China; [2]Frontiers Science Center for Disease-Related Molecular Network, Sichuan University West China Hospital, Sichuan University, Chengdu, Sichuan, People's Republic of China; [3]West China School of Pharmacy, Sichuan University, Chengdu, Sichuan, People's Republic of China

*These authors contributed equally to this work

Correspondence: Bowen Ke, Department of Anesthesiology, Laboratory of Anesthesia and Critical Care Medicine, National-Local Joint Engineering Research Centre of Translational Medicine of Anesthesiology, West China Hospital, Sichuan University, No. 37 Guoxue Alley, Wuhou District, Chengdu, Sichuan, People's Republic of China, Tel +86-28-85422114, Fax +86-28-85582944, Email bowenke@scu.edu.cn

Background: The treatment of neuropathic pain is still a major troublesome clinical problem. The existing therapeutic drugs have limited analgesic effect and obvious adverse reactions, which presents opportunities and challenges for the development of new analgesic drugs. Camphor, a kind of monoterpene, has been shown anti-inflammatory and analgesic effects in traditional Chinese medicine. But we know little about its effect in neuropathic pain. In this article, We have verified the reliable analgesic effect of camphor in the neuropathic pain model caused by different predispositions.

Methods: The nociceptive response of mice was induced by transient receptor potential A1 (TRPA1) agonist to verify the effect of camphor on the nociceptive response. Multiple paclitaxel (PTX) injection models, Single oxaliplatin (OXA) injection models, Chronic constriction injury (CCI) models and Streptozotocin-induced (STZ) diabetic neuropathic pain models were used in this study. We verified the analgesic effect of camphor in mice by acetone test and conditioned place aversion test. At the same time, comparing the adverse reaction of nervous system between camphor and pregabalin at equivalent dose in locomotor activity test and rotarod test. Using patch clamp to verify the effect of camphor on dorsal root ganglion (DRG) excitability.

Results: In behavioral test, compared with vehicle group, camphor significantly reduced the spontaneous nociception caused by TRPA1 agonist-formalina and allyl isothiocyanate (AITC). Compared with vehicle group, camphor significantly reduced the flinching and licking time in neuropathic pain model mice, including PTX, OXA, STZ and CCI induced peripheral neuralgia models. Compared with vehicle group, pregabalin significantly increased the resting time and reduced the average speed without resting and distance in locomotor activity test, reduced the time stayed on rotarod in rotarod test. In patch clamp test, compared with vehicle group, camphor significantly reduced the action potential (AP) firing frequency of DRG.

Conclusion: Camphor can alleviate the symptoms of hyperalgesia in various neuropathic pain models, and has no obvious adverse reactions compared with pregabalin. This effect is related to the down-regulation of DRG neuron excitability.

Keywords: camphor, neuropathic pain, TRPA1, hyperalgesia

Introduction

According to statistics in USA, the annual total cost of pain has exceeded cardiovascular disease, cancer and diabetes, up to $600 million.[1] With the acceleration of global aging, neuropathic pain has become a chronic pain disease affecting 7–10% of the global population.[2] The burden of neuropathic pain will be further aggravated. Neuropathic pain has complex symptoms and poor prognosis. In addition, the effect of analgesic drugs on the market is not ideal, which greatly reduces the quality of life of patients. In view of the current market gap of analgesic drugs for neuropathic pain, screening molecules with analgesic activity from natural products has become a feasible idea to solve neuropathic pain.

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China has rich medicinal plant resources, and a large part of medicinal plants are monoterpenes. Monoterpenes which have been used for medical purposes for centuries are important raw material in medicine, food and cosmetics industry and often used as aromatics, preservatives, flavoring agents and disinfectants.[3] A ton of monoterpenoids, for instance, menthol and 1.8-cineole have been utilized for anesthetic, analgesic, anti-inflammatory.[4,5]

Camphor is a monoterpene molecule with long medicinal history. It is widely used in excitement, cardiotonic, anti-inflammatory, analgesic, antibacterial, cough relieving, promoting penetration, killing mites, etc.[6] The analgesic effects of camphor may be based on its inhibition of TRPA1, despite its long history of widespread use in medicine.[7]

TRPA1 is involved in a variety of neuropathic pain conditions, including diabetic neuropathy and chemotherapeutic-induced neuropathy,[8] and the abnormal expression of TRPA1 is significantly related to the mechanical and cold induced reactions in CIPN mice.[9] At present, the TRPA1 blockers developed have some problems in pharmaceutical performance and safety, It is not easy to achieve clinical translation.[10]

In this study, we revealed a new potential application of camphor in neuropathic pain. From the perspective of ion channels and neuronal excitability, we built animal models, conducted behavior test and whole-cell patch clamp detection. We hoped to lay a foundation for subsequent transformation research.

Materials and Methods

Animals

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Sichuan University West China Hospital and were performed in compliance with the recommendations of the Guide for Animal Experimentation of the International Association. SPF level male adult ICR mice weighing 20–24g were used. (Dashuo experimental animal corporation, Chengdu, Sichuan, China). The animals had free access to clean water and food and were housed at 22±2°C on a 12-hour light/dark cycle.

Drug

In the experiment, camphor (Sigma-Aldrich, St Louis, Missouri, USA) was dissolved in medium and long chain fat emulsion (C6~C24).[11] (Huarui Pharmaceutical Co., Ltd, Wuxi, Jiangsu, China). Pregabalin (Sigma-Aldrich, St Louis, Missouri, USA) was dissolved in deionized water and prepared into a solution of 50mg / mL. The administration volume for mice was 10g/0.1L.

Analgesic Effect Assessments

All behavioral experiments were carried out blindly by a single experimenter. The room where behavioral experiments are conducted to kept at constant temperature (22±2°C) and humidity (50%-60%).

Formalin Test

The mice were placed in clean resin boxes to adapt for at least 30 minutes. We dissolved 5% formalin (Sigma-Aldrich, St Louis, Missouri, USA) in saline. 20 minutes after intraperitoneal injection of vehicle or camphor, we injected 20 μL formalin into the left hind paw of mice. After the injection of formalin, the time Phase I (0–5 min) and Phase II (15–30 min) that the mice licked and flinched their left hind paw was record.[12]

Spontaneous Nociception Induced by capsaicin and AITC

In this test, the mice were placed in clear chamber for 60 min. After accommodation, camphor or vehicle was intraperitoneal injection to the mice. After 20 min, 20 μL of capsaicin (1 nmol/paw in DMSO:Tween-80:physiological saline = 1:1:8) or AITC (10 nmol/paw in 0.1% DMSO in PBS) (Sigma-Aldrich, St Louis, Missouri, USA) was injected to the left hind paw (i.pl.) of the mice. The nociception response of mice was recorded within 5 minutes after injection. The nociception response was measured by the sum of the time of licking and flicking the left hind paw.[13,14]

Cold Hyperalgesia Measurement

Cold hyperalgesia was measured by acetone test. 50μL acetone was splashed to the plantar of the hind-paw of the mice. The time of withdrawal, flinching or licking was counted during 1 min after acetone exposure. Mice with cold pain threshold of 1.5–3s were included in the experiment.[15]

Conditioned Place Aversion (CPA) Test

In this test, a three-chamber apparatus with different color wall and different texture floor were used. The three-chamber were call as start chamber, acetone chamber and contralateral chamber. In pre-stimulation stage, mice were put to explore three chambers for 10 min. An hour after receiving pregabalin (10mg/kg, i.p.) or 20 min after receiving camphor (50mg/kg, i.p.)/vehicle, each mouse was imprisoned to acetone chamber with 50μL acetone splashing for 10 min (stimulation stage). Then mice were allowed to run freely again between three chambers for 10 min (post-stimulation stage). The time mice stayed in each chamber were record by tracking system Smart v2.5 (Panlab, Barcelona, Catalunya, Spain).[16]

Models of Neuropathic Pain

Multiple Paclitaxel (PTX) Injection Models

Mice were injected with paclitaxel solution 2mg/kg for 4 times every other day. The first dose of paclitaxel solution was injected on day 0. On day 8, behavioral test was carried out to measure the cold pain threshold of mice. Paclitaxel (Yangzi Pharmaceutical Co., Ltd. Taizhou, Jiangsu, China) was diluted with a solvent (ethanol: hydrogenated castor oil: normal saline =4:4:17).[17] On day 9–14, We continued to inject corresponding drugs into each group of mice every day and conducted behavioral test on day 10 and day 14.

Single Oxaliplatin (OXA) Injection Models

Oxaliplatin (6 mg/kg) was injected intraperitoneally into the mice to induce the neuropathic pain. Oxaliplatin (Sigma-Aldrich, St. Louis, Missouri, USA) was dissolved in 5% dextrose (1 mg/mL). 10 days after oxaliplatin injection, the behaviors associated with neuropathic pain were test.

Chronic Constriction Injury (CCI) Models

Briefly, under anesthesia maintained by isoflurane, the left sciatic nerve was exposed. After the sciatic nerve trunk was isolated, two ligations were made with chrome catgut, and the tightness was suitable for the slight twitch of the left hind limb of mice during ligation. Seven days after the surgery, behavioral tests were conducted.[17]

Streptozotocin-Induced (STZ) Diabetic Neuropathic Pain Models

The diabetic peripheral neuropathy models were induced by STZ (Sigma-Aldrich, St Louis, Missouri, USA). The blood glucose baseline of mice was measured before administration of STZ (200 mg/kg, i.p.). Mice with fasting blood glucose of 3.7 ~ 6.9 mmol/L were included in this study. The STZ was dissolved in citrate buffer at pH 4.5. After a week, mice with serum glucose levels above 16.7 mmol/L were used as diabetic mice.[18]

Evaluation of Side Effects of Central Nervous System

Open Field Test

Before the experiment, the mice were adapted to the experimental room and chamber for an hour. Then the mice were administered either vehicle or test compounds (camphor 50mg/kg, i.p./pregabalin 10mg/kg, i.p.). After 20 min i.p. camphor or 60 min i.p. pregabalin the mice were placed into the chamber to evaluate the effect of compounds on autonomic activity in mice. The distance travelled, average speed, and resting time were recorded by Smart v2.5 video tracking system (Panlab, Barcelona, Catalunya, Spain).[19]

Rotarod Test

The rotarod test is divided into training stage and testing stage. On the first training day, mice were placed on the rotarod (ENV-575M, MED Associates, Fairfax, Vermont, USA) for 2 min with a low-speed rotation (4 rpm). Mice that could not stay on the rotarod for two minutes were excluded from this study. On the second day of training, the speed of rotarod system was set to start at 4 rpm to the maximal at 40 rpm. Mice were placed to five rounds of training every day for 4

consecutive days. Mice that could not stay on the rotor for 250s were excluded from the study. On testing days, mice were placed on the rotarod before and after camphor (20 min) or pregabalin (60 min) injection.[20]

Electrophysiological Study

DRG Neurons Preparation

The DRG neurons (< 25 mm) in mice which on the 8th day after PTX injection were isolated from the spinal canals. We put them in HBSS (Hank's Balanced Salt Solution) and cut the nerve fibers. Next 30 minutes, they were digested by 2mL collagenase IV (2–3mg/mL) and 2mL 1% trypsin. After stopping the digestion process with 200μL FBS (fatal bovine serum), we gently blow them until the tissue blocks disappeared. We put the cell suspension into a centrifuge (300g,4 min) and removed the supernatant after centrifugation. We resuspended the cell sedimentation with 2mL Neurobasal. Then the cell suspension we got were plated on glass coverslips with polyornithine and laminin-coated. Neurons were fed with 2mL Neurobasal (contained:1%Glutamax^{TM}, 0.5% penicillin and streptomycin) + B27 Additive for neuron culture (with final concentration 1X) and put into the incubator for 1h. Incubator conditions: 5% CO_2-95% O_2,37°C.[17]

Drug Preparation

Camphor was prepared to 2M stock solution in DMSO. Before adding into the external solution, camphor solutions were shaken 20 min and warmed to 37°C. Camphor solutions in 660 μM could be completely dissolved to use in electrophysiological recordings.[7]

Electrophysiological Recordings

The pipette solution contained (in mM): 2 Na-ATP, 10 EGTA, 126 KCl, 1 MgCl2, 10 NaCl and 0.1 Mg-GTP, adjusted to pH 7.3 with KOH. The external solution included (in mM): 10 glucose, 10 HEPES, 1 $MgCl_2$, 2 CaCl2, 5 KCl, 140 NaCl, adjusted to pH 7.4 with NaOH.[21] The digitized current was sampled at 10 kHz and filtered at 0.1 kHz for analysis.[22] Under the inverted microscope, giving negative pressure suction to get GΩ sealing. After GΩ sealing, a rapid capacitance compensation was carried out, and then the negative pressure was continued to break the cell membrane, forming a whole cell recording mode. Cells with a stable resting potential were used in this study. At the beginning of the rapid upward rise of the depolarizing phase, the action potential threshold was measured. The rheobase was recorded as the threshold current that triggered the action potential. Firing frequency of action potentials was measured in 600 ms at 2-fold rheobase injection.[17,23]

Statistical Analysis

Data were analyzed by GraphPad 8.0. Data are presented as mean ± SEM. Values of $P < 0.05$ were considered statistically significant. Data of behavioral analgesic assessments and electrophysiological study were analyzed using one-way ANOVA followed by Tukey post tests. Safety evaluation were analyzed using Student's *t*-test. N.S. indicates no significance. *,** and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Results

Camphor Specifically Inhibits AITC-Induced Pain in vivo

In order to confirm that camphor plays an anti-injury role by inhibiting transient receptor potential (TRP) channel in vivo, we initially investigated the effect of camphor in spontaneous nociception induced by the exogenous nonspecific agonist formalin, specific TRPA1 agonists AITC and specific TRPV1 agonists Capsaicin. Intraperitoneal injection of camphor decreased the total time of flinching and licking observed in formalin test phases I (Flicking and licking time: camphor 50mg/kg 84.17s vs vehicle 156.83s p<0.001,) and II (Flicking and licking time: camphor 50mg/kg 82.33s vs vehicle 147s p<0.001,) (Figure 1A). Similarly, camphor also dose-dependently reversed an AITC-induced pain (Flicking and licking time: camphor 25mg/kg 51.30s vs vehicle 89.84s, p<0.01. Camphor 50mg/kg 20.52s vs vehicle 89.84s p<0.001,) (Figure 1B). Compared with the vehicle group, administration of camphor in advance had no significant effect on capsaicin induced spontaneous nociception in mice. (Figure 1C) (Flicking and licking time: camphor 50mg/kg 76.31s vs

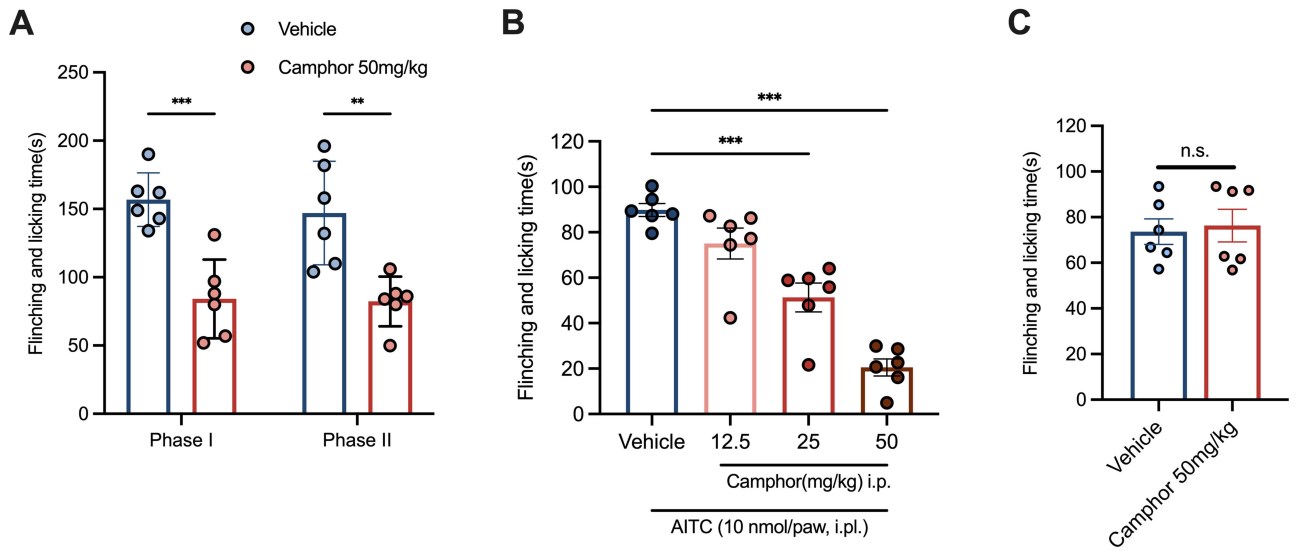

Figure 1 Effect of camphor on inhibiting acute pain. After injection formalin, AITC and capsaicin into the left hind paw of mice, camphor can decrease the flinching and licking time in both formalin-induced (**A**) and AITC-induced (**B**) pain but not in capsaicin-induced pain (**C**). n=6 per group. **p<0.01, ***p<0.001.

vehicle 73.64s, p=0.599,) The results presented here indicated that the camphor blocked the pain responses mediated by TRPA1 instead of TRPV1.

Camphor Reverses Cold Hyperalgesia in PTX Models

On the 8th day after continuous paclitaxel injection, we defined the dose effect relationship of camphor in PTX models. In acetone test, compared with pregabalin which is the first-line drug for the treatment of neuropathic pain,[24] mice in the high-dose camphor group can achieve greater max cold pain threshold than in pregabalin group. (flinching and licking time: camphor 50mg/kg 4.125s vs pregabalin 6.3675s) (Figure 2A). The area under the curve (AUC) of camphor in high-dose group was similar to that of pregabalin (AUC: camphor 50mg/kg 50.55 vs pregabalin 10mg/kg 46.83, p=0.74) (Figure 2B). We further administered the drug continuously within two weeks after modeling, and repeated the behavioral test on the 10th and 14th days. The results showed that the cold pain threshold of each dose group mice did not decrease significantly under the condition of continuous daily injection (Figure 2C).

Pain could induce aversion. So, we investigated whether camphor produced analgesia using a three-chamber conditioned place aversion (CPA) assay (Figure 2D). After pre-conditioning, the PTX modeled mice were treated with camphor or vehicle 20min before testing, and then gave the repeated stimulation with 50ul acetone to the left hind paw of mice. The result showed that acetone produced marked aversion in vehicle group and less in the camphor-treated group. (Time in acetone chamber: camphor 50mg/kg 132.8s vs vehicle 52.8s, p<0.001) (Figure 2E and F).

Camphor Reverses Cold Hyperalgesia in Multiple Neuropathic Pain Models

We further investigated the effect of camphor on various neuropathic pain models which associated with TRPA1 sensitization in previous reports.[25] The models of pathological pain we used include Single oxaliplatin (OXA) injection models, (Figure 3A and B), Chronic constriction injury (CCI) models (Figure 3C and D) and Streptozotocin-induced (STZ) diabetic neuropathic pain models (Figure 3E and F). In acetone test of all these models, the analgesic effect of camphor was close to that induced by pregabalin. In conclusion, camphor showed definite, analgesic effect in a variety of neuropathic pain models.

Camphor Had No Obvious Adverse Reaction at Analgesic Dose

We compared the effects of camphor and pregabalin on motor function in mice by locomotor activity test and rotarod test. In locomotor activity test, after giving the equivalent analgesic dose, significant sedative effects were observed in

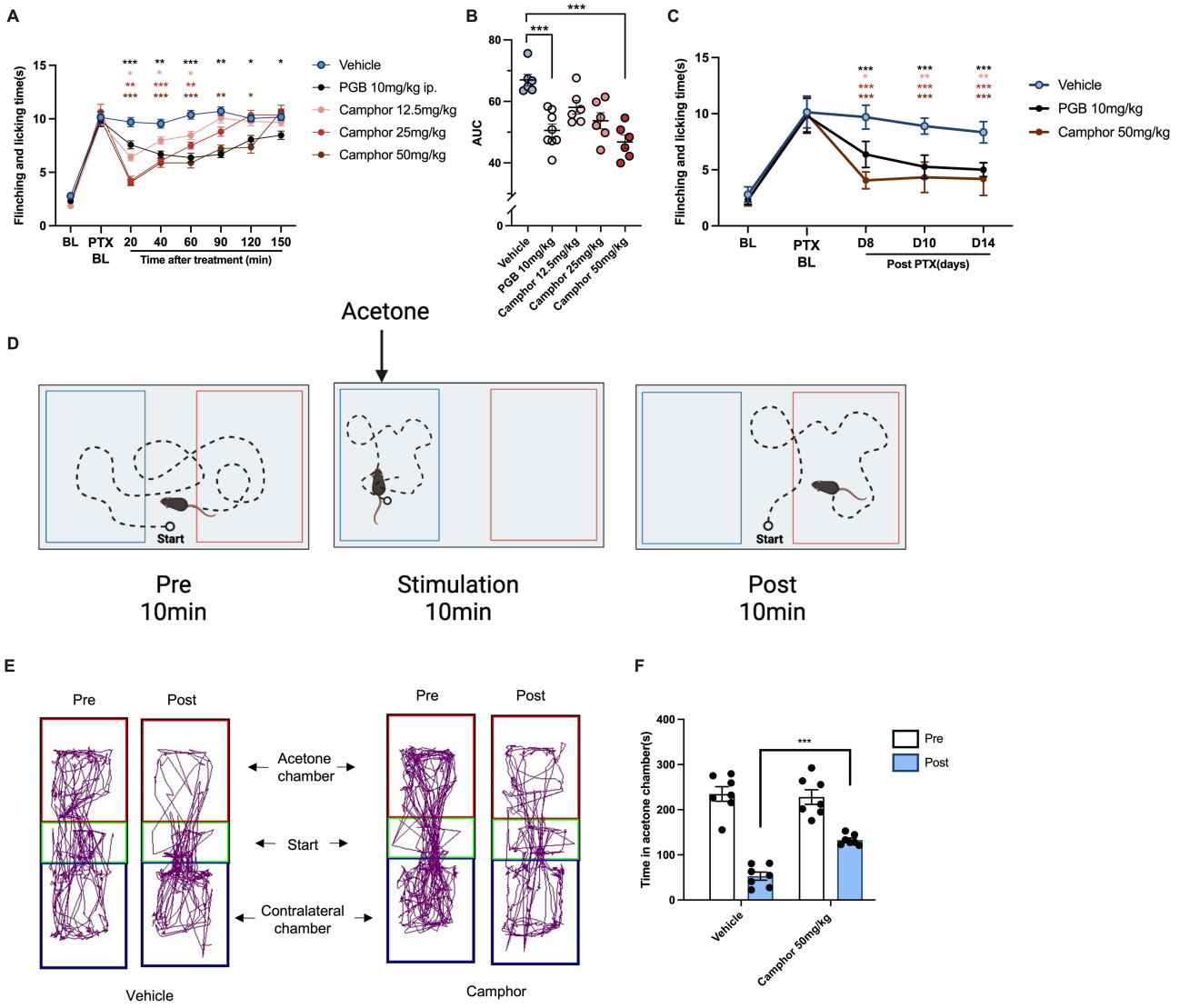

Figure 2 Effect of camphor on reversing hyperalgesia in PTX model. Intraperitoneal (i.p.) injection of camphor at 12.5, 25, or 50 mg/kg induces dose-dependent cold hyperalgesia (**A**) and AUC (**B**). (**C**) 50 mg/kg camphor did not produce significant tolerance. Brief protocol of conditioned preference aversion (CPA) test (**D**) and representative traces in two chambers following vehicle and camphor (i.p., 50mg/kg) treatment (**E**). (**F**) CPA test indicates that acetone produces aversive behavior in vehicle-treated mice but not camphor-treated mice. n=6-8 per group. *p<0.05, **p<0.01, ***p<0.001.

pregabalin group but not in camphor (Figure 4A). The sedative effect of pregabalin in mice included the decreasing of the total distance of action (Distance: Baseline 17520 cm vs After treatment 11,455.1 cm, p<0.01) (Figure 4B), the average moving speed (Average speed without resting: Baseline 31.68 cm/s vs After treatment 23.92 cm/s, p<0.001) (Figure 4C) and the increasing of the resting time in open field. (Resting time: Baseline 48.98s vs After treatment 121.4s, p<0.001) (Figure 4D). Notably, in rotarod test, pregabalin at 10 mg/kg attenuated the ability of mice to maintain their position on an accelerating rotarod. However, camphor at 50mg/kg did not show any ataxic effect. (Time on rotarod: Baseline 286.38s vs After treatment 167.75s, p<0.001) (Figure 4E).

Camphor Inhibits PTX-Induced Hyperexcitability of DRG Nociceptive Neurons

Since peripheral nociceptors in DRG transduce PTX-induced neuropathic pain, we next recorded action potentials from dissociated DRG nociceptors in mice and found that acute camphor perfusion significantly reduced action potential firing (Figure 5A and B). So, we deduce that camphor can suppress PTX-induced hyperexcitability of DRG nociceptors.

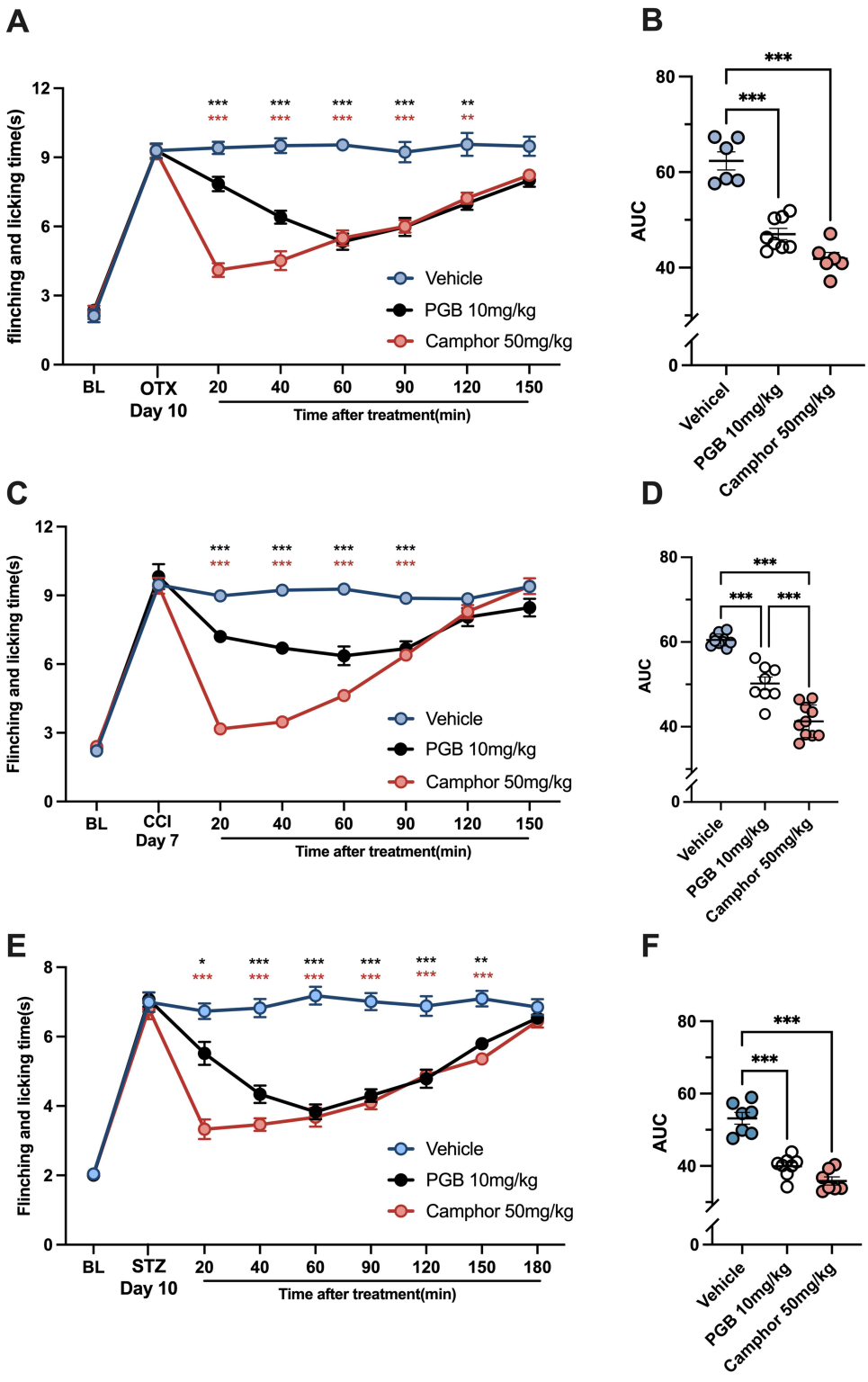

Figure 3 Effect of camphor on reversing cold hyperalgesia in multiple neuropathic pain models. Intraperitoneal (i.p.) injection of camphor at 50 mg/kg induces cold hyperalgesia (**A**) and AUC (**B**) in OXA model, CCI model (**C** and **D**) and STZ model (**E** and **F**). n=6-8 per group. *p<0.05, **p<0.01, ***p<0.001.

Discussion

In this study, we reported a new indication for camphor, a natural product. Camphor can produced an effective anti-hyperalgesic effect by blocking TRPA1 with fewer central nervous system adverse reactions.

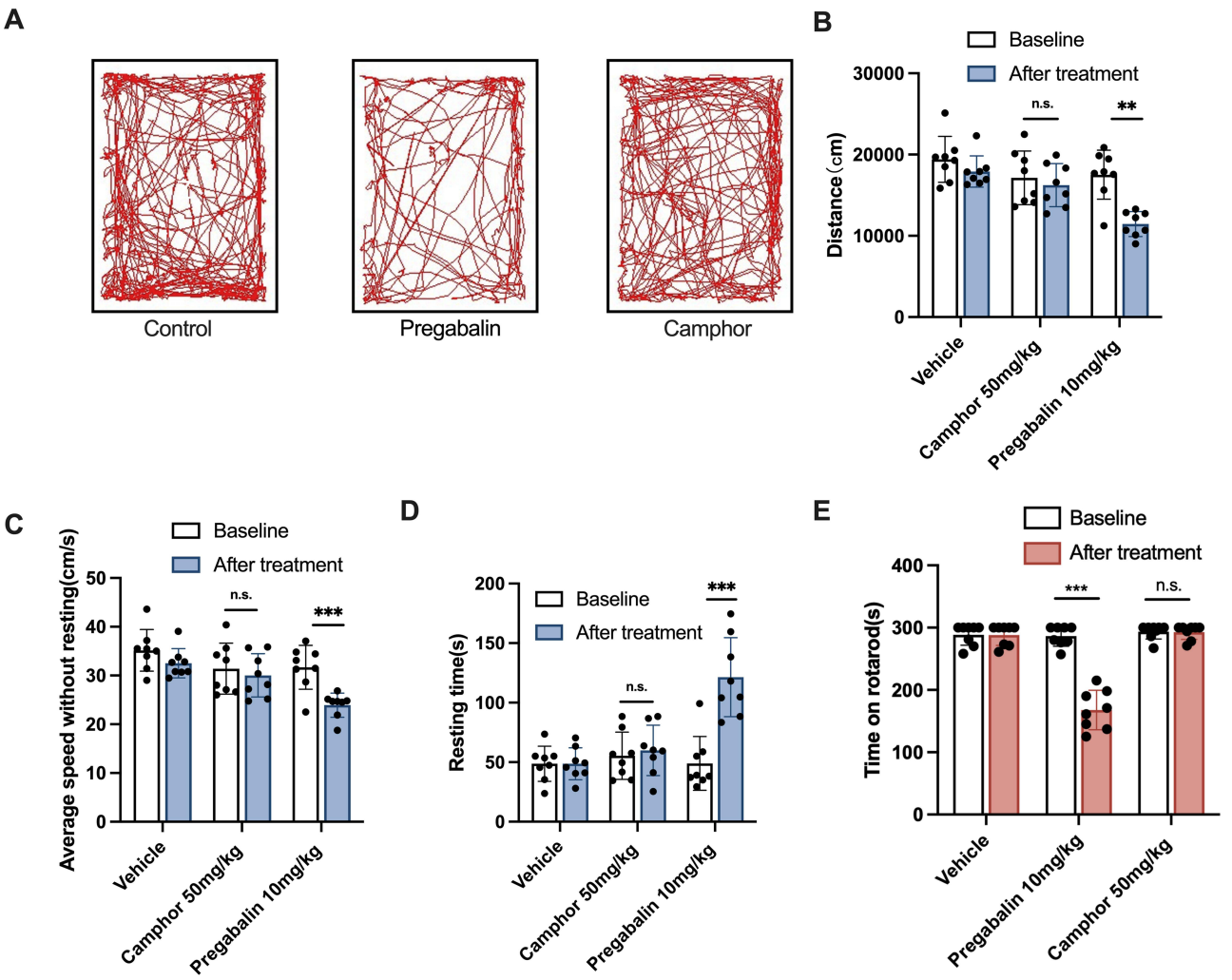

Figure 4 Evaluation of neurological adverse reactions to camphor. Motor functions were compared the effects of camphor and pregabalin on in mice by locomotor activity test and rotarod test. (**A**) Representative track plots in control, pregabalin and camphor group. Total distance of action (**B**), average moving speed (**C**) and resting time (**D**) were in open field. And we evaluated the ability of mice to maintain their position on an accelerating rotarod (**E**). n=6-8 per group. **p<0.01, ***p<0.001.

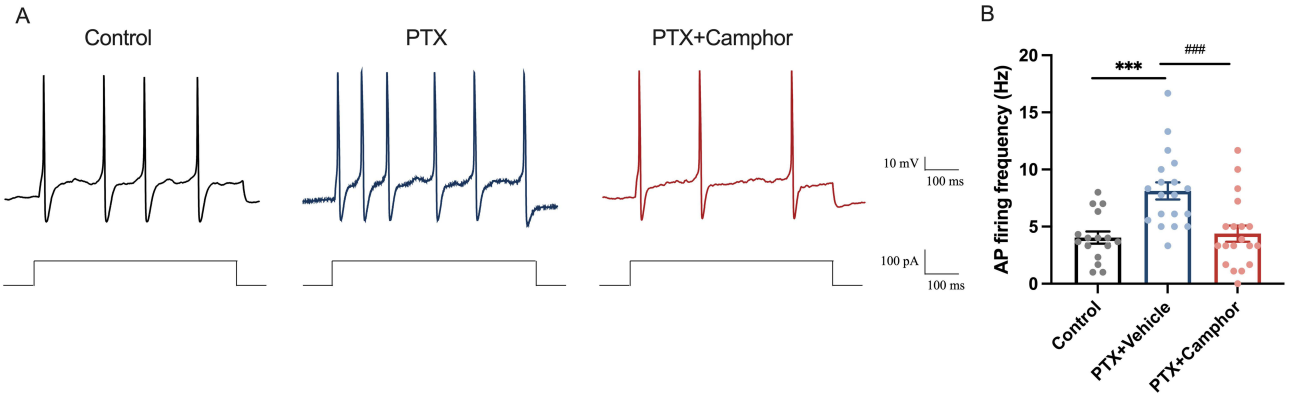

Figure 5 Ex vivo camphor reduces neuronal hyperexcitability of DRGs from PTX-induced pain mice. Traces (**A**) and bar graph (**B**) showing the effects of camphor on firing frequency in nociceptive neurons. n=12 cells in six mice. ***p<0.001, ###p<0.001.

Although camphor as an active component of essential oil has been reported to have analgesic effect in nociceptive pain and inflammatory pain models,[26] this study has found its application potential in neuropathic pain for the first time.

Small diameter C or A sensory neurons, such as dorsal root ganglion (DRG) and trigeminal nerve cells, are the ordinary positions of TRPA1 expression.[27] According to recent reports, TRPA1 extensively express positions included large cortical vessels, cerebral cortex and hippocampus.[28] TRPA1 sometimes coexpressed with TRPV1 channels. In previous studies, camphor has been proven to block TRPA1 and desensitize TPRV1 after stimulation.[7] According to previous studies, formalin is a nonspecific agonist of TRPA1, which can induce an increase in calcium influx in nociceptors, thus inducing two-phase pain.[29] Phase I was believed to be induced by chemical substances directly stimulating peripheral nociceptive sensory neurons, and phase II was due to central sensitization and peripheral inflammation.[30] Camphor has a dose-dependent inhibitory effect on pain in both phases.(Figure 1A) Therefore, we verified the effect of camphor on two targets at the animal level in vivo. After the nociceptive response induced by the plantar injection of TRPV1 and TPRA1 agonists in mice, camphor can significantly inhibit the pain induced by AITC, a TRPA1 agonist. And this analgesic effect is dose-dependent (Figure 1B). Correspondingly, camphor has no significant effect on the nociceptive response induced by TRPV1 agonist- capsaicin (Figure 1C).

Paclitaxel (PTX) is one of the most common drugs that cause chemotherapy induced neuropathic pain. TRPA1 channels have been linked to PTX toxicity and other chemotherapy agents like oxaliplatin in a variety of preclinical models. In recent studies, PTX induced CIPN model is associated with increased TRPA1 activity. This regulatory effect has been proved to be associated with Through PKA and PKC.[31] Finally, the CIPN model mice are characterized by hypersensitivity. In contrast, TRPV1 seems to mainly contribute to the heat-induced hyperalgesia, whereas TRPA1 to cold allodynia.[32] We hypothesized that TRPA1 mediates the cold hypersensitivity caused by PTX. Because TRPA1 is a sensor which be activated by oxidative stress and low temperature. Based on the previous blocking effect of camphor on TRPA1, we successfully verified the good inhibitory effect of camphor on cold stimulation hyperalgesia in the PTX model. (Figure 2A and B) After the analgesic effect was confirmed on the 8th day after PTX models were established, we daily administrated camphor for mice during the whole course of the PTX models and repeated the behavioral test every two days. The analgesic effect of camphor did not decline because of inducing tolerance in two weeks (Figure 2C).

The toxicity of chemotherapy drug, chronic mechanical compression, and the disorder of glucose metabolism are common inducing factors of nerve injury.[33] So in this study, more neuropathic pain models were used to demonstrate the analgesic effect of camphor in neuropathic pain, which corresponds to the neuropathic pain caused by different causes in clinic. We have further confirmed the analgesic effect of camphor on models related to TRPA1 overexpression in previous studies, including OXA, CCI and STZ models. The analgesic effect was time and dose dependent. Compared with the current clinical first-line drug pregabalin, Camphor's analgesic effect peaked faster. (Figure 3A, C and E and Figure 3A, C and E and Figure 3A, C and E) From the area under the curve, we can conclude that the overall benefit of camphor taking was not weaker than pregabalin (Figure 3B, D and F).

Pregabalin may cause side effects of sedation and ataxia, which limits its clinical improvement on the overall quality of life of patients with neuropathic pain.[34] In locomotor activity test and rotarod test, we confirmed the neurological side effects of pregabalin at the therapeutic dose. However, camphor had no similar adverse reactions at doses with similar analgesic effects. When camphor exerted analgesic effect, there are no ataxia (Figure 4E) and sedation (Figure 4B-D) were observed in mice. We believe that camphor has the potential to greatly improve the quality of life of patients with neuropathic pain. The lethal dose of camphor is 50 to 500 mg/kg in a-dults, 0.5 to 1 g in children or 70 mg/kg in infants. A dose of 2 g or more may lead to toxic effect.[35] According to the conversion of body surface area, the maximum dose (50 mg/kg) we used in mice for our study was equal to about 4 mg/kg in adults.[36] The doses we used were far less than that may lead to toxic effects in humans. As a natural product of traditional Chinese medicine with long history, its safety was proof by many prescriptions. Meanwhile, we will verify the safety of camphor for analgesia in humans by further clinical experiments.

Previous studies have reported that the expression of TRPA1 is significantly up-regulated in chemotherapy-induced neuropathic mice, and this phenomenon participated in mediating the hypersensitivity of the models.[37] In addition to Ca^{2+} influx, TRPA1 activating can also directly triggered vesicle exocytosis and calcitonin gene-related peptide release in lysosome-like organelles. These will greatly enhance the excitability of DRG.[38] It provided a theoretical basis for the treatment of hyperalgesia in neuropathic pain that down-regulating the excitability of neurons by inhibiting TRPA1. To verify the mechanism of camphor in neuropathic pain, we collected primary dorsal root ganglion cells from paclitaxel induced neuropathic pain model

mice. Compared with normal mice, PTX model mice had significantly higher action potential firing frequency, which was consistent with the phenomenon of hyperalgesia caused by abnormally increased neuronal excitability. After the application of camphor, the firing frequency of action potential of neurons in PTX model mice decreased significantly, suggesting that the abnormal firing frequency of primary afferent neurons was reduced. (Figure 5A and B) These results confirmed that camphor indeed reduce the excitability of DRG which increased in chemotherapy-induced neuropathic pain.

Camphor has been showed the inhibition of the nociceptive response induced by TRPA1 agonist in vivo and the inhibition on the current of TRPA1 channels in vitro.[7] We preliminarily speculated that TRPA1 channel may be involved in mediating the analgesia of camphor in mice with neuropathic pain. But the role of other TRP family members and targets in other families cannot be excluded at present. Further research is needed to verify that.

TRPA1 is highly conservative in the animal kingdom, and its evolutionary origin can be traced back to the sensory system of lower animals. TRPA1 from planarian and human both can recover the thermal escape of drosophila which accepted TRPA1 mutation.[39] It provides a greater possibility of clinical translation by using model animals that the conservation of TRPA1 from planarian to human.

Conclusion

In short, this study revealed the potential application of camphor which is a natural product with a long medicinal history in neuropathic pain for the first time. Camphor can significantly inhibit the nociceptive response mediated by TRPA1 agonist, which suggested that the analgesic effect of camphor may be related to TRPA1. In various neuropathic pain models related to TRPA1, camphor can attenuate cold hyperalgesia as the first-line drug pregabalin. At equivalent dose, there is no side effect related to the sedation and ataxia. Whole-cell patch clamp recordings confirmed that camphor could significantly reduce anomalous neuronal excitability. The analgesia of camphor in mice with neuropathic pain suggested a potential new choice to treat neuropathic pain, but more studies are needed.

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Disclosure

The authors report no conflicts of interest in this work.

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