Analgesic Effects of Topical Amitriptyline in Patients With Chemotherapy-Induced Peripheral Neuropathy: Mechanistic Insights From Studies in Mice

Anne-Laure Genevois, Jérôme Ruel, Virginie Penalba, Séverine Hatton, Camille Petitfils, Myriam Ducrocq, Paola Principe, Gilles Dietrich, Céline Greco, Patrick Delmas

USASP
US ASSOCIATION FOR THE STUDY OF PAIN
PUBLISHED BY ELSEVIER

The Journal of Pain, Vol 22, No 4 (April), 2021: pp 440–453
Available online at www.jpain.org and www.sciencedirect.com

Analgesic Effects of Topical Amitriptyline in Patients With Chemotherapy-Induced Peripheral Neuropathy: Mechanistic Insights From Studies in Mice

Anne-Laure Genevois,[*,#] Jérôme Ruel,[†,#] Virginie Penalba,[†] Séverine Hatton,[*] Camille Petitfils,[‡] Myriam Ducrocq,[†] Paola Principe,[§] Gilles Dietrich,[‡] Céline Greco,[*,¶##] and Patrick Delmas[†,##]

[*]*Department of Pain and Palliative Care Unit, Necker Hospital for Sick Children, APHP, Paris, France,* [†]*Cognitive Neuroscience Laboratory, Aix-Marseille University, CNRS, UMR 7291, Marseille, France,* [‡]*IRSD, Toulouse University, INSERM, INRA, ENVT, UPS, Toulouse, France,* [§]*AlgoTherapeutix, Suresnes, France,* [¶]*Paris-Saclay University, INSERM, UMR-S935, Villejuif, France*

Abstract: Oral amitriptyline hydrochloride (amitriptyline) is ineffective against some forms of chronic pain and is often associated with dose-limiting adverse events. We evaluated the potential effectiveness of high-dose topical amitriptyline in a preliminary case series of chemotherapy-induced peripheral neuropathy patients and investigated whether local or systemic adverse events associated with the use of amitriptyline were present in these patients. We also investigated the mechanism of action of topically administered amitriptyline in mice. Our case series suggested that topical 10% amitriptyline treatment was associated with pain relief in chemotherapy-induced peripheral neuropathy patients, without the side effects associated with systemic absorption. Topical amitriptyline significantly increased mechanical withdrawal thresholds when applied to the hind paw of mice, and inhibited the firing responses of C-, Aβ- and Aδ-type peripheral nerve fibers in ex vivo skin-saphenous nerve preparations. Whole-cell patch-clamp recordings on cultured sensory neurons revealed that amitriptyline was a potent inhibitor of the main voltage-gated sodium channels (Nav1.7, Nav1.8, and Nav1.9) found in nociceptors. Calcium imaging showed that amitriptyline activated the transient receptor potential cation channel, TRPA1. Our case series indicated that high-dose 10% topical amitriptyline could alleviate neuropathic pain without adverse local or systemic effects. This analgesic action appeared to be mediated through local inhibition of voltage-gated sodium channels.

Perspective: ***Our preliminary case series suggested that topical amitriptyline could provide effective pain relief for chemotherapy-induced peripheral neuropathy patients without any systemic or local adverse events. Investigation of the mechanism of this analgesic action in mice revealed that this activity was mediated through local inhibition of nociceptor Nav channels.***

Key Words: *Amitriptyline, chemotherapy-induced peripheral neuropathy, analgesics, topical administration, voltage-gated sodium channels, nociceptive sensory neurons, transient receptor potential ankyrin 1, nociceptors; Nav1.8, Nav1.7 and Nav1.9 isoforms.*

Received July 1, 2020; Revised October 15, 2020; Accepted November 3, 2020.

[#]Joint first authors.
[##]G.C. and P.D. contributed equally to this work and wish to be recognized as joint last authors of this manuscript.

Funding sources: Part of the study was sponsored by AlgoTherapeutix.
Role of the sponsor: AlgoTherapeutix developed the protocols in conjunction with the clinical and preclinical investigators and provided the study drug. The sponsor had no role in the conduct of the study, the collection, analysis and interpretation of the results.
Conflicts of interest: C.G. is one of the holders of the patent related to repurposing amitriptyline for use in chemotherapy-induced neuropathic pain. P.P. was an employee of AlgoTherapeutix during the execution of the reported study. The other authors have no conflicts of interest to declare.

Address reprint requests to Céline Greco, MD, PhD, Department of Pain and Palliative Care Unit, Necker Hospital for Sick Children, APHP, 149 Rue de Sèvres, 75015, Paris, France. E-mail: celine.greco@aphp.fr, celine.greco@inserm.fr

1526-5900/$36.00

https://doi.org/10.1016/j.jpain.2020.11.002

Antidepressants are an essential component of the therapeutic strategy for treatment of many causes of persistent pain.[15, 49] Tricyclic antidepressants are the recommended first-line treatment for many forms of persistent neuropathic pain.[11,15,22] Amitriptyline hydrochloride (amitriptyline), a tricyclic antidepressant approved for the treatment of major depression, has been shown to be effective for the treatment of pain associated with a range of neuropathic pain conditions, including diabetic neuropathy, fibromyalgia and postherpetic neuralgia.[19,25,27] However, many patients are unable to achieve optimal pain relief with oral amitriptyline due to the occurrence of dose-limiting intolerable adverse events (AEs), including somnolence, anticholinergic effects and cardiotoxicity.[10,11,15]

As a potential alternative, 2 anecdotal case reports have indicated that topical administration of high-dose (10%) amitriptyline in creams may provide effective treatment for neuropathic pain, potentially allowing optimal treatment doses to be achieved while minimizing the risk of systemic AEs.[35,36] In particular, encouraging results have been reported with 10% topical amitriptyline in a pilot study of patients presenting with hand and foot chemotherapy-induced peripheral neuropathy (CIPN).[46]

At present, the precise mechanisms underlying the analgesic effects of amitriptyline remain unclear. A potential mechanism may involve reinforcement of the descending inhibitory pain pathways by the accumulation of norepinephrine and serotonin in central synapses, resulting in suppression of ascending pain messages in the spinal cord.[43] Amitriptyline also displays a range of other pharmacological activities that may contribute to its analgesic properties. Amitriptyline is an antagonist of the histamine, muscarinic, adrenergic, and serotonin receptors, and leads to blockade of voltage-gated sodium ion channels.[30,41] It also appears to interact with calcium ion channels.[37] Indirect studies using the forced swim test as a model of depressive-like behavior in rodents,[1,45] and functional assays in rat dorsal root ganglia (DRG) cell cultures,[44] have indicated that amitriptyline may also modulate members of the transient receptor potential (TRP) cation channel family. Dysregulation of Nav channels and TRP channels, such as TRP vanilloid 1 (TRPV1) and TRP ankyrin 1 (TRPA1), has been implicated in the pathophysiology of neuropathic pain,[8,14] including that of CIPN. Both sodium and TRP channels are viewed as therapeutic targets for the development of new neuropathic pain treatments.[52,56] Although many investigators have demonstrated the inhibitory effects of antidepressants on Nav channels as a possible mechanism of analgesia,[18] studies on the effects of amitriptyline on the activities of the TRP channels are limited and, to our knowledge, no one has tested whether amitriptyline applied to the skin improves pain by alleviating its peripheral somatosensory component.

Thus, we first investigated the potential effectiveness of topically applied 10% amitriptyline in a preliminary, uncontrolled, observational case series of patients with CIPN and assessed whether there were any local AEs, or AEs associated with systemic absorption of amitriptyline in these patients. We then aimed to gain insight into the mechanisms involved in the analgesic activity of topically applied amitriptyline by using experimental approaches in mice to assess the effects of amitriptyline on the nociceptive withdrawal threshold of hind paws, nociceptive afferent messages, TRP channel-mediated calcium ion mobilization, and Nav1.7, Nav1.8, and Nav1.9 channel activity.

Methods

Patient Case Series

Adult and pediatric patients with CIPN of the hands and/or feet were recruited after being referred to the pain management department at the Necker Hospital (Paris, France) by their oncologist or hematologist. Patients referred for severe CIPN that persisted after completion of anticancer therapy, and patients referred while still undergoing therapy were eligible for the study. For the patients still undergoing anticancer treatment, the aim of the referral was to determine if their CIPN could be managed before making any decisions to change or reduce the dose of their anticancer therapy.

Patients with open lesions on the hands or feet, and those with dementia or who were incapable of applying the cream themselves, were not eligible. This study was conducted within the framework of the ongoing management of the patients. Patients were informed of the aims of the study, the fact that any benefits of the treatment may not be observed before 1 month of use, and that the risk of systemic side effects was expected to be much lower than that associated with oral amitriptyline. Patients were also informed of the risk of skin irritation at application sites, and of safety issues regarding the need to avoid ingesting the cream and to prevent the cream from coming into contact with the eyes. All patients gave oral consent before being included in the study. The study was approved by the Research Ethics Committee of the Hôpitaux Universitaires Paris-Ouest (HUPO) and by the French Data Protection Authority (Commission Nationale de l'Informatique et des Libertés, CNIL).

Patients were instructed to apply 1 g (obtained using a measuring device) of 10% amitriptyline hydrochloride cream (oil-in-water emulsion), twice a day to each area with pain sensations such as burning, numbness, and tingling. Thus, the total amount of cream used per day varied between 2 and 4 g depending on the number of extremities (hands and/or feet) affected. Patients were told to apply the cream thinly and to rub it in gently, and to wait 30 minutes before handwashing. There was no need for foot washing after application of the cream. No other new or existing analgesics or other pain-relieving nonpharmacologic therapies (such as acupuncture, cryotherapy etc.) were to be used during the study period.

Neuropathic pain was assessed before and after 1 month of treatment using a validated scale from the *Douleur neuropathique en 4 questions* (DN4, neuropathic pain in 4 questions) questionnaire.[51] Pain intensity was assessed at day 7, day 15 and month 1, using the Numeric Pain Rating Scale (NPRS[32]). Patients were also asked to report any local or systemic AEs at each visit.

Laboratory Animals

The mice used in this study were bred and housed in accredited facilities; they were adult males, 6 to 12 weeks old, and from a C57Bl/6J background. The animals were kept in standard laboratory conditions with 12-hour light/dark periods at a temperature of 22 $\pm$ 2°C and supplied with dry food and drinking water *ad libitum*. All animals were handled in compliance with the European Community guidelines for the care and use of animals (2010/63/EEC). Since pain might occur in these experiments, the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain[62] were followed. Furthermore, all procedures were approved by the institutional review board of the regional ethics committee (Région PACA).

Assessment of the Nociceptive Withdrawal Threshold in Mice Using the Randall-Selitto Test

The nociceptive withdrawal threshold was assessed using a Randall-Selitto electronic algesimeter (Ugo Basil, Italy) following a protocol similar to that described by Santos-Nogueira et al 2012.[48] During the test, the animal was placed into a pouch that provided access to the right hind paw (test paw). They were then carefully immobilized with the same hand used to hold the test paw. The test consisted of the application of an increasing mechanical force, in which pressure was applied onto the dorsal surface of the right hind paw. The mechanical threshold was defined as the force (in grams) at which the mouse withdrew its paw from the device. The cut-off value was fixed at 250 g in order to protect the paw from possible tissue damage. Mice were habituated during the four days before the test to get used to the manipulation.

The nociceptive withdrawal threshold was measured before and 5 minutes after the application of a control cream (Excipial hydrocreme) or a cream containing 10% amitriptyline to the test area (ie, the right hind paw). Creams were applied by an investigator wearing gloves, using their index finger to gently rub 300 μL of cream (approximately 30 μg of amitriptyline) over the dorsal surface of the test area for 5 minutes. Animals were randomly assigned to the treatment groups (n = 8 for each group) and measurements were made blind, that is, the investigator was not aware of the nature of the cream that had been applied. Each reported data point represents the mean of three consecutive measurements taken at 5-minute intervals.

Ex Vivo Single-Unit Recordings From Mouse Skin-Nerve Preparations

Adult male mice were anesthetized with isoflurane (4%) and sacrificed by severing of the carotid arteries. Skin-saphenous nerve preparations and single-fiber recordings were carried out according to the method described by Zimmerman et al (2009).[61] The saphenous nerve and the skin of the hind limb were carefully dissected and placed in a custom-designed organ chamber containing warm oxygenated synthetic interstitial fluid (SIF). The SIF buffer had the following composition (in mM): 120 NaCl, 3.5 KCl, 0.7 $MgSO_4$, 1.7 NaH_2PO4, 5 Na_2HCO_3, 2 $CaCl_2$, 9.5 Na-Gluconate, 5.5 glucose, 7.5 sucrose, and 10 HEPES. The pH was set to 7.4 and the osmolarity was maintained at 300 mOsm/L. The skin was placed corium side up in the organ bath, continuously superfused with SIF buffer and maintained at a temperature of 31°C using a CL-100 temperature controller (Harvard Apparatus; Warner Instruments, Holliston, MA).

The saphenous nerve was placed in an adjacent recording chamber filled with mineral oil, gently teased, and groups of nerve fibers were placed on a gold recording electrode in order to isolate single-unit activity. Extracellular action potentials from single nerve fibers were recorded with a DAM 80 AC differential amplifier (WPI) and digital outputs were acquired using the CED Spike2 system (sampling rate of 20 kHz; Cambridge Electronic Design Limited, UK). Spikes were discriminated off-line using the Spike2 software (Cambridge Electronic Design Limited, UK) and analyzed individually to avoid false positives. The mechanically evoked activity of single saphenous nerve fibers was first obtained in response to von Frey hair stimulations (Friedrich-Alexander University, Erlangen, Germany). Once a receptive field had been characterized, a patch of skin was isolated from the surrounding bath using a plastic minichamber (inner diameter: .8 cm) continuously superfused with buffer at 31°C. The receptive field was then stimulated using von Frey mechanical probes mounted on the arm of a computer-controlled piezoelectric stepper (E-861 NEXACT controller; PI, Germany). Conduction velocity was determined by electrically stimulating (Tungsten microelectrode: 10 mm ext/60 mm PI; FHC) identified receptive fields with square wave pulses (.1−.5 ms, 7 −15 V). Classically, axons are considered to be A-type when the conduction velocity is over 1 to 2 m/s.[60] However, to avoid ambiguity, axons in our study were only considered to be A-type when the conduction velocity was at least 4 m/s. Fibers with a conduction velocity below 1 m/s were classified as C fibers.

Recordings of mechanically induced firing of sensory fibers were carried out in the absence of amitriptyline (control) and after superfusion of the skin preparation with amitriptyline for at least 10 minutes and after a

30-minute washout. The amitriptyline was prepared as stock solutions in water and stored at −4°C until use. It was freshly prepared before each experiment and diluted to working concentrations in the SIF buffer. Dose-response was obtained by measuring firing activity after skin superfusion with increasing concentrations of amitriptyline (10−100 μM).

Culture of Mouse DRG Neurons

Cultures of DRG were prepared from male mice anesthetized with isoflurane (4%, Piramal Critical Care) and sacrificed by severing of the carotid arteries. Cultures of dissociated DRG neurons were established from thoracolumbar DRG as described previously.[26]

DRG for whole-cell patch-clamp recordings were incubated in Hanks Balanced Salt Solution (HBSS; Invitrogen, France) containing 2 mg/mL of collagenase IA (Sigma, France) for approximately 45 minutes at 37°C. DRG were washed at least 10 times and then triturated with a fired polished glass Pasteur pipette. Dissociated DRG neurons were cultured in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen) supplemented with 10% heat-inactivated fetal calf serum, 50 U/mL penicillin-streptomycin, 2 mM L-glutamine, 25 mM glucose, 25 ng/mL nerve growth factor, and 2 ng/mL glial-derived neurotrophic factor (Invitrogen).

For calcium mobilization measurements, 10 DRG cultures (2 mice per culture) were washed with HBSS (Thermo Fisher Scientific, MA), and incubated in HBSS containing 1 μg/mL papain (Sigma Aldrich, MO) and L-cysteine (pH 7.4, Sigma Aldrich) for 10 minutes at 37°C. DRG were rinsed in Leibovitz's L-15 Medium (Thermo Fisher Scientific) containing 1% penicillin–streptomycin and 10% Fetal Bovine Serum (FBS) (Thermo Fisher Scientific) and digested twice with 4 mg/mL dispase II (Sigma Aldrich) and 1 mg/mL collagenase type I (Sigma Aldrich) for 10 minutes at 37°C. Neurons were then mechanically dissociated. After centrifugation at 50 g, neurons were plated into an eight-well Nunc Lab-Tek II CC2 Chamber Slide System (Thermo Fisher Scientific) in DMEM containing 3% FBS, 1% penicillin-streptomycin and 10 μM of a cocktail of mitosis inhibitors including cytosine-β-D-arabinofuranoside, 5-fluorouracil, and uridine (Sigma Aldrich) for 24 hours.

Transient Transfection of HEK293T Cells With hNav1.7 cDNA

Human embryonic kidney 293T (HEK293T) cells were grown in DMEM containing 4.5 mg/mL glucose, 10% FBS, 50 U/mL penicillin and 50 μg/mL streptomycin. Cells were plated onto 12-mm round glass poly-D-lysine-coated coverslips placed in 24-well plates and transfected using lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. hNav1.7 and GFP plasmids were co-transfected at a concentration of 600 and 100 ng/mL, respectively. Experiments were conducted on cells 24 to 48 hours post-transfection.

Whole-Cell Patch-Clamp Recordings of Sodium Currents in DRG Neurons and Transfected Human Embryonic Kidney Cells

Patch-clamp recordings were made using borosilicate electrodes (Harvard Apparatus; Warner Instruments, Holliston, MA) with a resistance of 2 to 3 MΩ when filled with an intracellular solution consisting of (in mM): 130 CsCl, 10 Hepes, 8 NaCl, 0.4 NaGTP, 4 MgATP, 1 $MgCl_2$, 1 $CaCl_2$, and 10 EGTA (adjusted to pH 7.3 with CsOH, ~300 mOsm/L). The bath solution had a lower concentration of sodium (in mM): 60 NaCl, 110 Sucrose, 3 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose (pH 7.35, 300 mOsm/L). Voltage-gated sodium ion currents were leak-subtracted using a P/6 protocol and voltage errors were minimized using 75 to 80% series resistance compensation. Cultured cells were perfused with the bath solution at a flow rate of 5 mL/min. Recordings were made at room temperature (20−24°C) using an Axopatch 200B amplifier (Axon Instruments, Boston, MA), filtered at 1 to 2 kHz, and digitally sampled at 5 to 20 kHz.

Sodium currents were recorded individually. To record the tetrodotoxin (TTX)-resistant Nav1.8 and Nav1.9 currents in relative isolation, DRG neurons were bathed in a standard bath solution containing 300 nM TTX (to block TTX-sensitive Na^+ currents) and 50 μM La^{3+} and 1 mM amiloride (to block voltage-activated Ca^{2+} currents).[12] The Nav1.8 current was recorded in Nav1.9$^{-/-}$ DRG neurons, whereas the Nav1.9 current was recorded in Nav1.8$^{-/-}$ DRG neurons. Nav1.8$^{-/-}$ and Nav1.9$^{-/-}$ mice were generated from a C57Bl/6J background as previously described by Bonnet et al.[9] Fast activating/inactivating TTX-sensitive Na^+ currents (Nav1.1 and Nav1.6) in putative non-nociceptors were recorded in wild-type DRG neurons that had large cell membrane capacitance (Cm >60 pF) and lacked any TTX-resistant Na^+ current components. Nav1.7 recordings were made using the transfected HEK cells. All Na^+ currents were recorded before and after cumulative addition of amitriptyline (1 μM−1 mM).

Treatment of Dorsal Root Ganglion Neurons for Measurements of Calcium Mobilization

DRG neurons were incubated with HBSS containing 20 mM Hepes, 1 mM fluo-4 acetoxymethyl ester (Thermo Fisher Scientific) and .02% pluronic acid for 30 minutes at 37°C followed by an additional incubation for 30 minutes at room temperature. The medium was then discarded and replaced by HBSS. Calcium mobilization in the DRG neurons was measured in response to increasing concentrations of amitriptyline (.01−1 mM) to obtain a dose-response curve. The effects of TRP channel agonists and antagonists on amitriptyline-induced calcium ion mobilization were then assessed as follows: neurons were incubated with either an antagonist for TRPV1 (10 μM AMG 9810, Sigma Aldrich) or an antagonist for TRPA1 (HCO30031; Sigma Aldrich), or with the vehicle (.001% DMSO HBSS) for 5 minutes

before adding increasing amounts of amitriptyline or an agonist either for TRPV1 (62 nM capsaicin, Sigma Aldrich) or for TRPA1 (50 μM allyl isothiocyanate, AITC; Sigma Aldrich).

Calcium Imaging

Neurons were imaged using an inverted microscope (Zeiss) and a 10 × .5 NA objective. Images were acquired using a CCD camera (Zeiss) and Zen software (Zeiss). Acquisition parameters were kept constant during each experiment. Kinetic analysis was performed using 85 recordings (one per second). Baseline fluorescence was determined for 0 to 10 seconds, and then fluorescence was measured from the 10th to the 65th second after the neurons were incubated with the drugs or controls. At 65 seconds, 50 mM KCl was added to the cells in order to allow discrimination between neurons and glial cells. Variations in fluorescence intensity measured in each neuron were identified using Image J2 software.

Statistics

Data are presented as numbers and percentages, or means ± the standard deviation (SD) or standard error of the mean (SEM). Depending on the sample size, statistical analyses were performed using the paired two-tailed t-test, the Kruskal-Wallis test (with subsequent Dunn's multiple comparison tests) or the Wilcoxon test as appropriate. Differences were considered significant if $P < .05$.

Results

Effectiveness of 10% Amitriptyline Hydrochloride Cream in Patients With Chemotherapy-Induced Peripheral Neuropathy

In total, 25 patients (23 adults, and 2 children aged 8 and 12 years) with CIPN of the hands (n = 1, 4%), feet (n = 9, 36%), or hands and feet (n = 15, 60%) were included in the case series and treated for 1 month with a cream containing 10% amitriptyline. Among the 25 patients, 7 were referred for onset of CIPN while responding well to ongoing anticancer treatment. The remaining 18 patients were cancer survivors who had long-standing CIPN that persisted after they had completed their chemotherapy. The baseline demographic and clinical characteristics of the patients are shown in Table 1. The most common neuropathic chemotherapeutic agents were bortezomib (n = 6; 24%), oxaliplatin (n = 4, 16%), and taxanes (n = 4, 16%).

All the patients were experiencing severe pain at baseline with a mean NPRS score of 7/10 and a mean DN4 score of 6/10 (Table 2). After the 1-month treatment with topical amitriptyline, the mean DN4 score had decreased to 3/10 (Table 2 and Fig 1A; $P < .001$). In addition, reported mean pain intensity decreased from severe at baseline to mild after the 1-month treatment (NPRS score: 3: Table 2 and Fig 1B; $P < .001$). Large improvements in pain scores were observed in association with the topical amitriptyline treatment for the 7 patients with CIPN whose anticancer therapy was ongoing, and these patients were able to continue their effective cancer treatment without any changes to the dose or regimen being needed.

Table 1. Demographic and Clinical Characteristics of Patients at Baseline

Demographic and Clinical Characteristics	Patients N = 25
Gender, n (%)	
Male	10 (40)
Female	15 (60)
Age (y)	
Mean ± SD	58 ± 18.3
Median (min–max)	63 (8–82)
Primary disease, n (%)	
Lymphoma	5 (20)
Myeloma	7 (28)
Breast cancer	6 (24)
Colon/rectum cancer	3 (12)
Leukemia	2 (8)
Sinus cancer	1 (4)
Rhabdomyosarcoma	1 (4)
Neurotoxic agent, n (%)	
Bortezomib	6 (24)
Oxaliplatin	4 (16)
Vincristine	4 (16)
Taxanes	4 (16)
Trastuzumab emtansine	2 (8)
Cisplatin	1 (4)
Cyclophosphamide	1 (4)
Doxorubicin	1 (4)
Lenalidomide	1 (4)
Vindesine	1 (4)
Time to CIPN onset (d)	
Mean ± SD	106 ± 94.0
Median (min–max)	90 (1–365)*
Duration of CIPN before treatment (mo)	
Mean ± SD	18 ± 32.4
Median (min–max)	3 (0–120)
Area affected by CIPN, n (%)	
Feet	9 (36)
Hands	1 (4)
Hands and feet	15 (60)

Abbreviations: SD, standard deviation; CIPN, chemotherapy-induced peripheral neuropathy.
*Two patients had onset of CIPN on the same day as receiving their first chemotherapy treatment (day 1).

Table 2. Pain Scores at Baseline and After 1 Month of Treatment

Pain Scores	Baseline N = 25	1 m N = 25
DN4 score (0–10)		
Mean ± SD	6 ± 1.6	3 ± 1.8
Median (min–max)	6 (4–9)	2 (0–7)
NPRS score (0–10)		
Mean ± SD	7 ± 2.0	3 ± 2.1
Median (min–max)	8 (3–10)	2 (0–7)

Abbreviations: DN4, *Douleur Neuropathique 4 Questions* (Neuropathic pain in 4 questions); NPRS, Numeric Pain Rating Scale; SD, standard deviation.

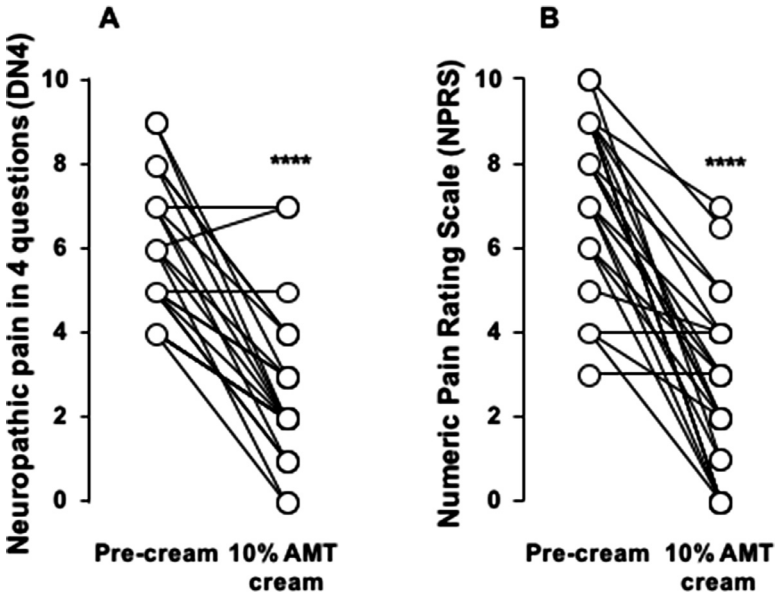

Figure 1. Chemotherapy-induced hand and/or foot pain is alleviated by local 10% amitriptyline application. Twenty-five patients with chemotherapy-induced neuropathic pain were treated with 10% amitriptyline hydrochloride cream, applied twice a day to each area with pain, for 1 month. Pain was assessed before and 1 month after treatment using both the Neuropathic pain in 4 questions (Douleur Neuropathique 4 Questions; DN4) questionnaire (**A**) and the Numeric Pain Rating Scale (**B**). Circles represent patient scores and each line represents one patient. Statistical analysis was performed using the Wilcoxon matched pairs test. ****$P < .0001$.

No local AEs (such as skin irritation), nor any of the frequent systemic AEs associated with oral amitriptyline use (notably xerostomia, dizziness, or somnolence) were reported following application of the cream by the patients in our case series.

Amitriptyline Administered Topically to the Skin Increases the Nociceptive Withdrawal Threshold in Mice

We tested whether a cream containing 10% amitriptyline had analgesic/antinociceptive effects on the response thresholds to mechanical pressure stimulation in mice using the Randall-Selitto test with an electronic algesimeter. The nociceptive withdrawal threshold was assessed before and after a 5-minute gentle massage with a control cream or a cream containing 10% amitriptyline. No difference in nociceptive withdrawal threshold was observed following treatment with the control cream (Fig 2A), whereas paw surfaces were significantly less sensitive to noxious stimuli following application of the 10% amitriptyline cream (Fig 2B), with the mean nociceptive threshold value increasing by approximately 12% following application of the 10% amitriptyline cream.

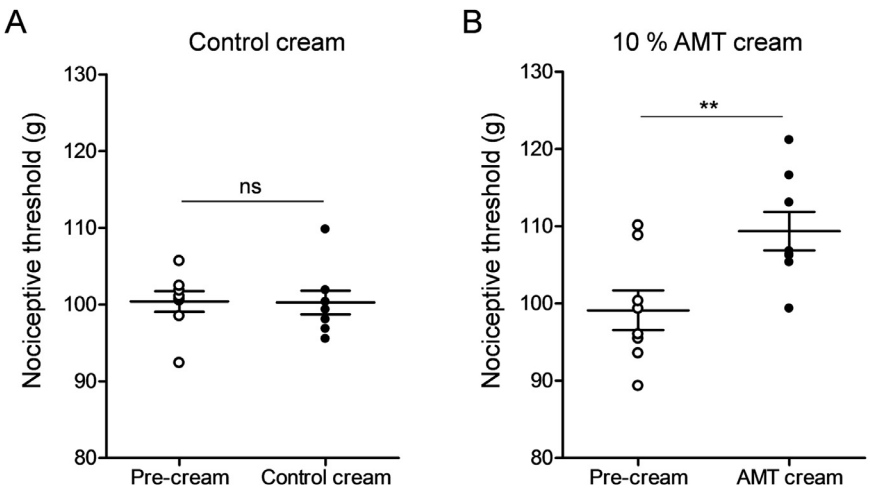

Figure 2. Effects of a single, 5-minute skin application of a cream containing 10% amitriptyline on the nociceptive withdrawal threshold in mice. Nociceptive withdrawal thresholds were measured using a Randall-Selitto electronic algesimeter. Values were obtained for individual mice before and 5 minutes after application of a control cream (**A**) or a 10% amitriptyline (AMT) cream (**B**). Data are the means ± SEM of measurements conducted in triplicate on 8 mice from each group. ns, not significant; **$P < .01$, Wilcoxon test.

Amitriptyline Inhibits the Activities of Both Nociceptive and Nonnociceptive Nerve Fibers in Skin-Nerve Preparations

The effects of amitriptyline on nerve fibers were evaluated by recording single-unit activity from skin-saphenous nerve preparations from adult mice (Fig 3). Low-threshold Aβ mechanoreceptors were identified as showing low mechanical thresholds ($\leq$1 mN, n = 6) with high conduction velocities (>20 m/s) and were identified as slowly adapting based on responses to constant force mechanical stimuli. C-type mechanonociceptive fibers were recognized as displaying high mechanical thresholds (range = 11–32 mN, n = 13) with low conduction velocities ($\leq$1 m/s) and tonic activity. Aδ mechanonociceptive fibers were identified by their very high mechanical thresholds (>80 mN, n = 6) and relatively high conduction velocities (3–14 m/s).

Exposure of the receptive field of both C-type mechanonociceptors and of low-threshold Aβ mechanoreceptors to 100 μM amitriptyline inhibited mechanically induced firing (Fig. 3A and 3B). Mean inhibition of evoked firing after a 10-minute application of 100-μM amitriptyline to the skin preparation was 95.6%, 87.5% and 82.5% in C, Aδ and Aβ fibers, respectively (Fig 3C). Amitriptyline inhibition was dose-dependent with IC_{50} values of 15, 16 and 26 μM for C, Aδ and Aβ fibers, respectively (Fig 4) and reversible within 30 to 35 minutes of washout.

Amitriptyline Inhibits Sodium Ion Channels in Sensory Neurons With Greater Potency Towards Nociceptor Channel Isoforms

We investigated the effects of amitriptyline on Nav channels in sensory neurons from mouse DRG neurons recorded using the patch-clamp technique. Nav channels including the Nav1.7, Nav1.8, and Nav1.9 isoforms are key to nociception, whereas other TTX-S Nav channel isoforms (Nav1.1 and Nav1.6) transmit non-noxious information[29] We found that amitriptyline applied at increasing concentrations inhibited these channels (Fig 5). Inhibition by 100-μM amitriptyline was typically reversible within 20 $\pm$ 2 minutes. Plotting the inhibitory effect versus amitriptyline concentration (Fig 6A) yielded IC_{50} values of 4.25 $\pm$ .4, 6.97 $\pm$ .3, and 15.6 $\pm$ .5μM for Nav1.7, Nav1.8, and Nav1.9, respectively (Fig 6B). Amitriptyline was 10-fold less potent (62 $\pm$ .5 μM) at inhibiting TTX-S Nav currents of touch mechanoreceptors than the Nav1.8, Nav1.7, and Nav1.9 currents of nociceptive neurons (Fig 6B).

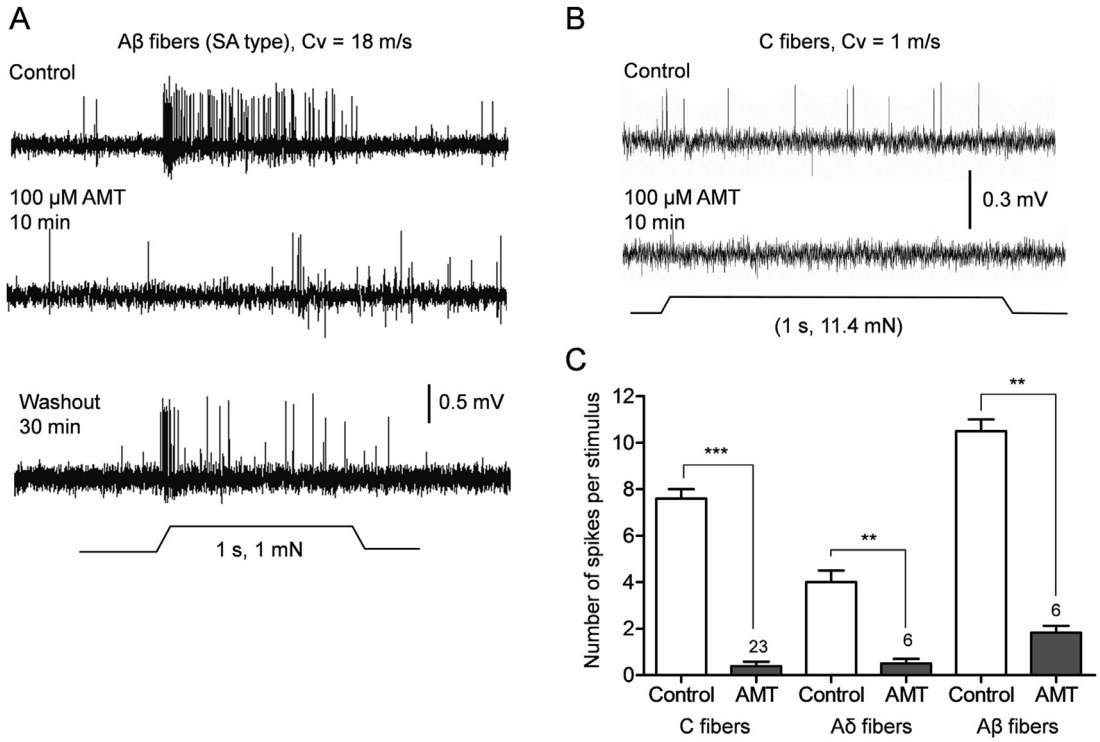

Figure 3. Amitriptyline applied to skin preparations reduces the mechanically induced activities of sensory fibers from the saphenous nerve. **(A)** Discharge of a low-threshold Aβ (slow-adapting) mechanoreceptor in response to a 1 mN mechanical stimulus applied to the surface of the skin in the absence of amitriptyline (control), and after 10 minutes of skin superfusion with amitriptyline (100 μM). Note that inhibition was not complete and partially reversed after a 30-minute washout of amitriptyline. **(B)** Firing in a C-type mechanonociceptor fiber in response to 11.4 mN mechanical stimulation in the absence of amitriptyline (control), and full inhibition after 10 minutes of superfusion of the receptive field with amitriptyline (100 μM). Data presented are representative of 6 and 13 Aβ- and C-type fiber recordings, respectively. **(C)** Mean firing discharge of C, Aδ and Aβ sensory fibers before and after 10 minutes of skin superfusion with 100 μM amitriptyline. The number of recordings is indicated. Recordings for the C, Aδ and Aβ fibers were conducted using 7 skin-saphenous nerve preparations from 7 mice. Data are expressed as means $\pm$ SEM. AMT, amitriptyline; SA, slow-adapting; Cv, conduction velocity; $^{**}P < .01$, Wilcoxon test $^{***}P < .001$, paired two-tailed t-test.

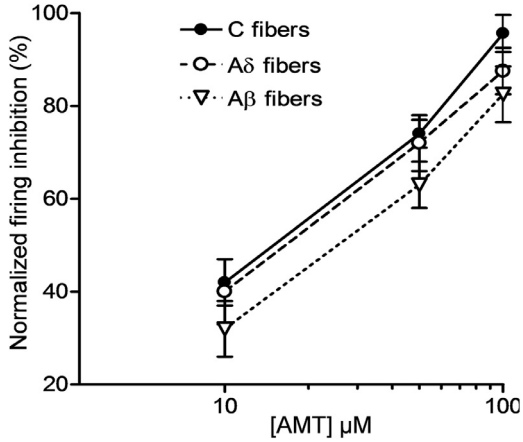

Figure 4. Nociceptive C and Aδ fibers are more susceptible to amitriptyline block than low-threshold Aβ fibers. Dose-dependent effect of amitriptyline (AMT) on firing responses of single saphenous C, Aδ and Aβ sensory fibers (n = 5 for each concentration). Each bar represents the mean inhibition expressed as a percentage of the control response. Approximate IC_{50} values were 15, 16, and 26 μM for C, Aδ and Aβ sensory fibers, respectively.

Amitriptyline Activates Dorsal Root Ganglion Neurons Through TRPA1 Channels

The effect of amitriptyline on calcium ion mobilization was investigated in primary cultures of mouse DRG neurons. Exposure to amitriptyline led to increases in the concentration of intracellular calcium ions within 10 seconds (Fig 7A) and this effect was dose-dependent with an IC_{50} of .05 mM (Fig 7B). The intracellular calcium ion amplitude curve reached a plateau after around 20 seconds of amitriptyline exposure (Fig 7A), indicating that the response was associated with calcium ion channel opening. We therefore assessed whether amitriptyline-induced calcium ion mobilization was dependent on amitriptyline binding to TRPV1 or TRPA1 by pretreating the cells with antagonists of these receptors. Pretreatment of the DRG neurons with an excess of TRPA1 antagonist (HC30301) led to inhibition of amitriptyline-induced calcium ion mobilization (Fig 7C). In contrast, the pretreatment of neurons with an antagonist for TRPV1 (AMG9810) had no effect (Fig 7C). We then assessed the ability of amitriptyline to facilitate the response of DRG neurons to stimulation. Addition of suboptimal doses of amitriptyline did not potentiate the activation of the DRG neurons induced by either a TRPV1 agonist (capsaicin) or a TRPA1 agonist (AITC) (Fig 7D).

Discussion

Our preliminary case series suggested that a 1-month topical treatment with 10% amitriptyline could lead to pain relief in a series of patients with CIPN, without any

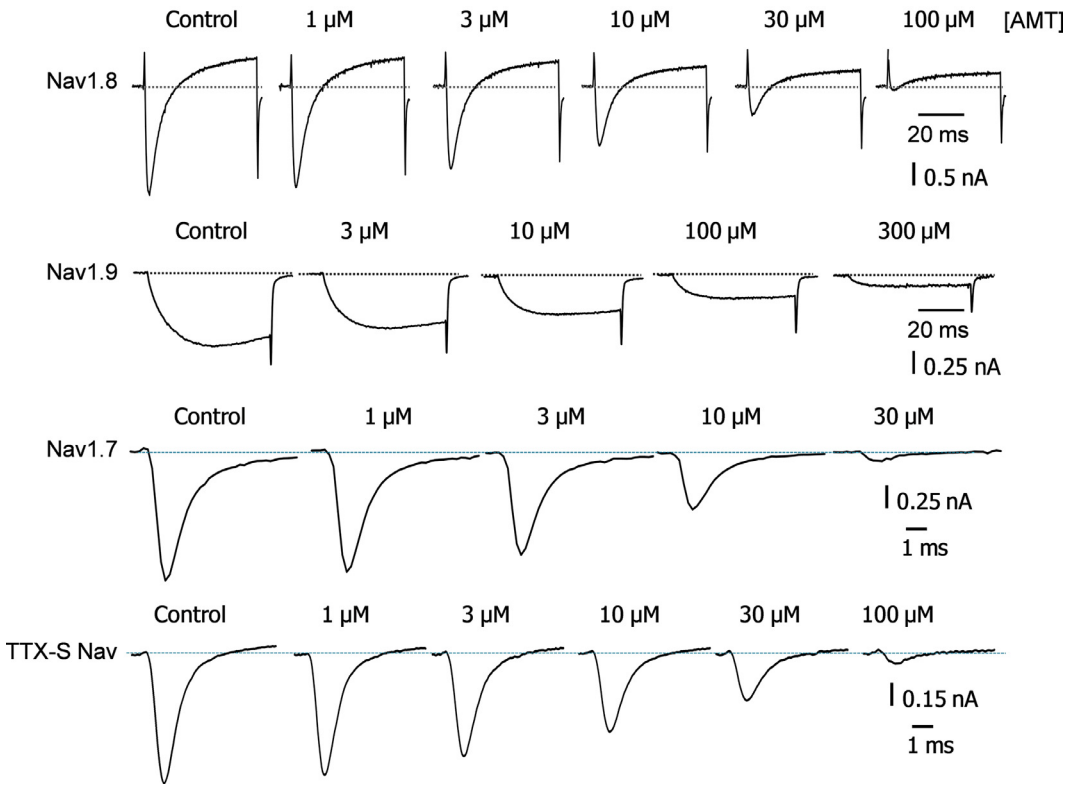

Figure 5. Amitriptyline inhibits all sodium channel isoforms from sensory neurons. Effect of cumulative application of amitriptyline (AMT) on the current through Nav channels expressed in nociceptors (Nav1.8, Nav1.9, and Nav1.7) and the tetrodotoxin (TTX)-sensitive fast activating/inactivating Nav channels (TTX-S: mixture of Nav1.2 and Nav1.6) expressed in non-nociceptive (large diameter) sensory neurons. Currents were elicited by voltage steps from a holding potential of −100 mV (Nav1.8 and Nav1.9) or −80 mV (Nav1.7 and TTX-S Nav). Nav1.9 current was recorded in Nav1.8$^{-/-}$ DRG neurons, whereas Nav1.7 was recorded in hNav1.7-expressing human embryonic kidney cells. Data presented are representative of the recordings from 7 measurements, except for the Nav1.9 current (n = 5).

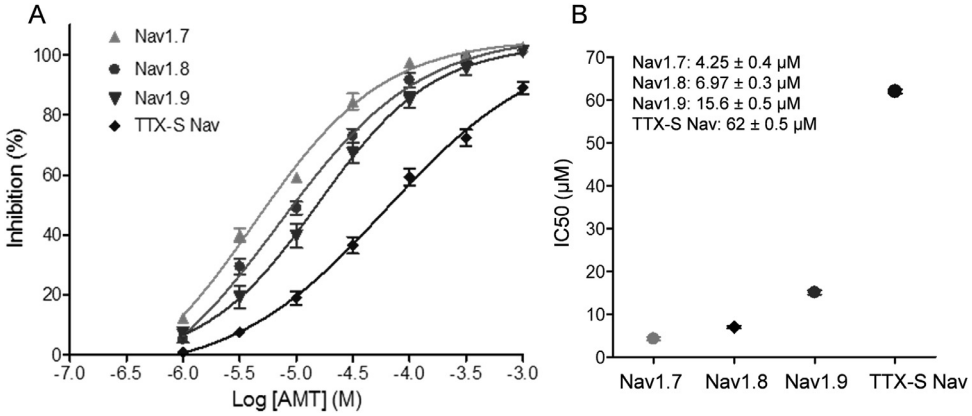

Figure 6. Amitriptyline shows a 10-fold potency for Nav1.8 and Nav1.7 over Nav1.1 and Nav1.6. (**A**) Dose-response curves for amitriptyline (AMT) block of Nav currents. Dose-response curves were obtained by plotting the percentage inhibition at steady-state against the drug concentration. Data are expressed as means ± SEM (n = 5–7). (**B**) IC_{50} values for Nav1.7, Nav1.8, Nav1.9 and TTX-S Nav currents derived from the dose-response Hill curves shown in **A**. Note that amitriptyline shows a greater potency for nociceptor Nav channel isoforms (Nav1.7, Nav1.8, and Nav1.9) than for the TTX-S Nav channels (Nav1.1 and Nav1.6) present in non-nociceptive neurons.

local or systemic AEs. We also demonstrated that amitriptyline inhibited nocifensive behavior when applied to the skin and dampened nociceptive C and Aδ afferent signaling in a skin-nerve preparation from mice. Our findings indicate that topically applied amitriptyline acts through local peripheral inhibition of a variety of Nav channel subtypes, preferentially those expressed in nociceptors, and that amitriptyline induces calcium ion influx via TRPA1 channel activation.

Effectiveness of 10% Amitriptyline Hydrochloride Cream in Patients With CIPN

Our exploratory case series involving 25 patients with CIPN of the hands and/or feet suggested that topical 10% amitriptyline cream, administered twice daily for 1 month, could be effective at relieving the neuropathic pain induced by chemotherapy. This treatment allowed CIPN patients to continue their anticancer therapy without any adjustments to their chemotherapy dose or regimen. However, the major limitation of this case series was that it was carried out on 25 patients as part of their ongoing management, and thus this design did not allow for inclusion of a control arm. Further, larger scale and controlled studies are needed to confirm the effectiveness of the treatment.

CIPN is a common AE of cancer therapy and is characterized by spontaneous or evoked pain reported as electric shock-like sensations, burning, tingling, paresthesia, dysesthesia, allodynia, or hyperalgesia.[57] Symptoms persist or worsen with successive rounds of chemotherapy, and may continue for months or even years after chemotherapy. Indeed, among the patients in our case series, around 70% were referred for severe CIPN that persisted after completion of chemotherapy. Treatment options for relieving CIPN are very limited.[31] In the absence of an FDA-approved therapy, duloxetine, a serotonin, and norepinephrine dual reuptake inhibitor, is the only treatment option for CIPN recommended by the American Society of Clinical Oncology.[28] As onset of CIPN has a major impact on long-term quality of life and may lead to early dose reduction or early discontinuation of chemotherapy, there is an urgent need to develop new effective treatment strategies to improve outcomes for CIPN patients.[28]

A randomized controlled study evaluating the effectiveness of oral amitriptyline as a CIPN treatment found that amitriptyline had no significant effect on pain; however, the amitriptyline dose used in this study was kept low to limit the risk of AEs.[33] Similarly, randomized clinical trials evaluating the effectiveness of low-dose (<5%) topical amitriptyline in combination with 2% ketamine, or with 1.5% ketamine and .8% baclofen, revealed a trend toward the reduction of sensory symptoms and improvement of motor neuropathy, but no significant effect on pain.[7,23] The findings of our case series are in agreement with those reported by Rossignol et al (2019) showing that a 1-month treatment with high dose (10%) topical amitriptyline led to a significant reduction in pain scores.[46]

Skin irritation has been reported as a local AE of creams containing topical amitriptyline in some previous reports[54]; however, no local AEs were reported in our case series. In addition, some case reports have indicated that high-dose amitriptyline creams are associated with an increased risk of systemic absorption and AEs.[35,36] No systemic AEs were reported in our study or in the 44 CIPN patients treated with the 10% amitriptyline cream reported by Rossignol et al (2019).[46] In addition, preliminary analyses of blood samples from 12 patients in our study found no detectable levels (limit 40 ng/mL by immunoassay; Abbot laboratories) of amitriptyline, or its metabolite nortriptyline, in the serum. Although these analyses need to be conducted on a larger group of patients using a more sensitive detection method, these initial findings—taken together with the absence of systemic AEs in patients treated with the 10% amitriptyline cream—suggest that topical amitriptyline exerts an analgesic action by having a direct, local effect on the peripheral neuropathy.

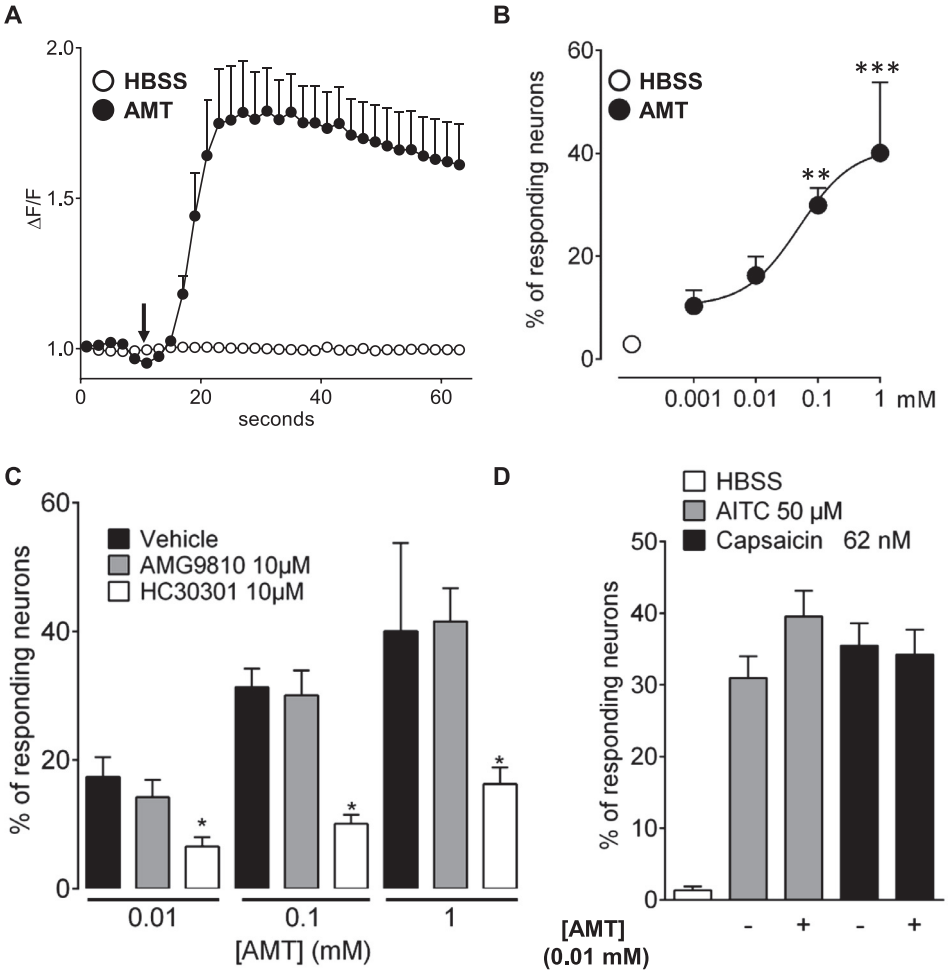

Figure 7. Amitriptyline induces an increase in intracellular [Ca^{2+}] in mouse sensory neurons through TRPA1. (**A**) Representative Ca^{2+} flux measurements in DRG neurons exposed to .1 mM amitriptyline (AMT). ΔF/F was calculated as maximal fluorescence/baseline fluorescence. The baseline fluorescence was measured over a 10-second period before the addition (indicated by the arrow) of either AMT (black circles) or the vehicle (HBSS; white circles). (**B**) Dose-response curve showing the percentage of DRG neurons displaying intracellular calcium mobilization in response to increasing amounts of amitriptyline (black circles). As a control, the response to exposure to HBSS alone is also shown (white circle). The percentage of responding neurons was calculated as the number of DRG neurons in which AMT induced calcium mobilization, relative to the total number of neurons identified by their ability to respond to 50 mM KCl. ***$P < .001$ and **$P < .01$, as compared to a control with the vehicle alone, according to the Kruskal-Wallis test and subsequent Dunn's multiple comparison tests. (**C**) Effects of a 5-minute incubation with 10 μM AMG9810 (TRPV1 antagonist) or HC30301 (TRPA1 antagonist) on amitriptyline-induced Ca^{2+} mobilization in mouse DRG neurons. Data are expressed as means ± SEM of 3 independent experiments with 2 wells per condition and 60 to 80 neurons per well. Statistical analysis was performed using the Kruskal-Wallis test and subsequent Dunn's multiple comparison tests. *$P < .05$ for the difference between antagonist-treated cells compared with cells incubated with the vehicle alone. (**D**) Effects of a 5-minute incubation with .01 mM amitriptyline on Ca^{2+} mobilization induced by 62 nM capsaicin (TRPV1 agonist) or 50-μM allyl isothiocyanate (AITC; TRPA1 agonist) in mouse sensory neurons. Data are expressed as means ± SEM of 3 independent experiments with 2 wells per condition and 60 to 80 neurons per well. Statistical analysis was performed using the Kruskal-Wallis test and subsequent Dunn's multiple comparison tests.

This preliminary study provided promising data that led us to investigate the mechanism of action of topical amitriptyline in detail in mice.

Antinociceptive Action of Amitriptyline Cream in Mice

Our in vivo experiments in mice demonstrated that amitriptyline cream could act on the peripheral nervous system, and was efficient at raising the nociceptive withdrawal threshold. These findings are in agreement with previous studies showing that amitriptyline applied to rodent paws can alleviate pain in animal models of neuropathic (nerve constriction injury) and inflammatory (formalin test) pain.[50]

Amitriptyline Inhibits Nociceptive Afferent Messages by Targeting Sodium Channels

Our experiments on sensory fibers in isolated mouse skin-saphenous nerve preparations indicate that amitriptyline, dose-dependently and reversibly, inhibited the evoked firing responses of the low-threshold Aβ-mechanosensory fibers and high-threshold C- and Aδ-sensory fibers that innervate the superficial layers of

the mouse skin. Thus, as expected from its wide-ranging action on sodium ion channels, amitriptyline suppressed the activities of sensory afferents regardless of their sensory modality. However, the degree of block appeared to be slightly greater in nociceptive fibers than in Aβ-mechanosensory fibers. Use-dependent block is classically associated with preferential binding of amitriptyline to inactivated and/or open-state Nav channels.[5] Use-dependent block is particularly advantageous for reducing ectopic discharge and pain transmission. At appropriate concentrations, these properties therefore predispose amitriptyline to preferentially target active nociceptive fibers over touch mechanoreceptors.

Patch−clamp-derived mechanistic analysis demonstrated that amitriptyline is a potent inhibitor of both TTX-sensitive and TTX-resistant Nav channels in DRG neurons, providing a potential mechanism of analgesia. Like many Nav inhibitors—including local anesthetics, anticonvulsants, antiarrhythmics, and analgesics—amitriptyline exerts most of its effects by stabilizing the channels in an inactivated conformation.[18,42] Through its inhibitory action on Nav channels, amitriptyline dampens the excitability of sensory neurons and abolishes the firing activity of cutaneous sensory nerve fibers. The inhibitory effects were more potent for Nav1.7, Nav1.8. and Nav1.9, distributed in DRG nociceptors, than for Nav1.1 and Nav1.6, which are primarily found in non-nociceptive sensory neurons.[47] The effect of amitriptyline was most potent on Nav1.7 and Nav1.8, with half-maximal inhibitory concentrations of $4.25 \pm .4$ and $6.97 \pm .3$ μM, respectively. Nav1.7 is an essential component of nociception and produces a rapidly activating and inactivating current involved in subthreshold depolarization and action potential generation. Gain-of-function mutations of the Nav1.7 gene, *SCN9A,* are associated with pain disorders such as inherited erythromelalgia and paroxysmal pain disorder[17,21]; whereas loss-of-function mutations are linked to the complete insensitivity to pain that may be accompanied by anosmia.[13,24] In addition, increased Nav1.7 expression has been implicated in paclitaxel- and oxaliplatin-induced CIPN.[58] The Nav1.8 channel is also associated with clear pain phenotypes in humans, with gain-of-function mutations causing painful peripheral neuropathy[20] and gene polymorphisms appearing to be associated with an increased incidence of oxaliplatin-induced CIPN.[4] Amitriptyline was also a very potent inhibitor of Nav1.9, which is strongly expressed in nociceptors and which contributes to the generation of a persistent inward current at subthreshold voltages.[16] Nav1.9 is associated with diverse clinical disorders including familial episodic limb pain,[59] congenital insensitivity to pain,[40] and small fiber neuropathy.[34] Thus, the broad potency of amitriptyline toward the nociceptor channels Nav1.7, Nav1.8, and Nav1.9 may prove to be a valuable characteristic, allowing topical amitriptyline to be an effective analgesic for a range of conditions. However, it should be noted that these studies were carried out using wild-type mice measuring evoked pain, and it may be of interest to perform further studies, if technically feasible, to investigate the efficacy of topical amitriptyline in an animal model of CIPN.

Amitriptyline Induces Calcium Ion Influx by Activation of TRPA1

Our study of the effect of amitriptyline on calcium ion mobilization revealed that amitriptyline increases the intracellular calcium concentration in sensory neurons via TRPA1 channel activation. TRPA1 is predominantly expressed in nociceptive C- and Aδ-type sensory neurons in DRGs and the trigeminal ganglia.[53] It plays a key role in chemonociception and is activated in response to a broad range of exogenous stimuli found in many plants, food, cosmetics, and pollutants, and by endogenous stimuli such as bradykinin, prostaglandins and hydrogen peroxide.[55] In addition, TPRA1 activation plays a role in diabetic neuropathy and CIPN induced by oxaliplatin, vincristine and paclitaxel.[8]

The precise role played by amitriptyline-induced TRPA1 activation in the action of amitriptyline remains to be determined. On the one hand, pungent natural compounds (mustard, clove, cinnamon, ginger etc.) that induce burning sensations are known to activate TRPA1,[6] and burning skin irritation has been reported in a few studies as a local side effect of amitriptyline cream use.[54] Sensitization of sensory neurons via activation of TRPA1 has also already been implicated in injection site and postoperative inflammation and pain after use of local anesthetics.[38,39] However, no local AEs were reported by the 25 patients in our case series or by the 44 CIPN patients treated with the 10% amitriptyline cream in the study by Rossignol et al (2019).[46] On the other hand, the TRPA1 activation could also be involved in the analgesic action of amitriptyline. Agonist-induced intracellular accumulation of calcium has already been shown to lead to TRPA1 desensitization in sensory neurons[2] and a mechanism involving initial activation of TRPA1, followed by desensitization and sustained inhibition, has already been implicated in the antinociceptive action of acetaminophen.[3] Thus, amitriptyline-induced activation of the TRPA1 channel may be implicated in the onset of local AEs, such as the burning sensations and skin irritation at cream application sites reported previously in a few cases, whereas its subsequent desensitization may contribute to the analgesic activity of topical amitriptyline.

Conclusions

The results of our case series provided an indication that 10% topical amitriptyline had an analgesic action that was mediated by local, rather than systemic, effects on neuropathic pain in patients with severe CIPN. Thus, topical amitriptyline will be expected to have a much better safety profile compared to oral and parenteral routes. The results of our studies in mice, and on sensory small fibers from the peripheral nervous system, provided insight into the mechanisms underlying the potential pain-relieving effects of 10% amitriptyline

cream in the patients with CIPN. Taken together these findings suggested that the high-dose topically administered amitriptyline displayed antinociceptive action in the peripheral nervous system through potent inhibition of nociceptor Nav channels and nociceptive fiber firing.

Acknowledgments

The authors would like to thank Drs Emma Pilling and Marielle Romet (Santé Active Edition) for language editing and writing assistance and Pharmacie Delpech (Paris) for preparation of the amitriptyline hydrochloride cream used in this study. Céline Greco would also like to thank the Ecole de l'Inserm Liliane-Bettencourt and Fondation Bettencourt-Schueller for their support.

References

1. Abdelhamid RE, Kovacs KJ, Nunez MG, Larson AA: Depressive behavior in the forced swim test can be induced by TRPV1 receptor activity and is dependent on NMDA receptors. Pharmacol Res 79:21-27, 2014

2. Akopian AN, Ruparel NB, Jeske NA, Hargreaves KM: Transient receptor potential TRPA1 channel desensitization in sensory neurons is agonist dependent and regulated by TRPV1-directed internalization. J Physiol 583:175-193, 2007

3. Andersson DA, Gentry C, Alenmyr L, Killander D, Lewis SE, Andersson A, Bucher B, Galzi JL, Sterner O, Bevan S, Hogestatt ED, Zygmunt PM: TRPA1 mediates spinal antinociception induced by acetaminophen and the cannabinoid Delta(9)-tetrahydrocannabiorcol. Nat Commun 2:551, 2011

4. Argyriou AA, Cavaletti G, Antonacopoulou A, Genazzani AA, Briani C, Bruna J, Terrazzino S, Velasco R, Alberti P, Campagnolo M, Lonardi S, Cortinovis D, Cazzaniga M, Santos C, Psaromyalou A, Angelopoulou A, Kalofonos HP: Voltage-gated sodium channel polymorphisms play a pivotal role in the development of oxaliplatin-induced peripheral neurotoxicity: Results from a prospective multicenter study. Cancer 119:3570-3577, 2013

5. Bagal SK, Marron BE, Owen RM, Storer RI, Swain NA: Voltage gated sodium channels as drug discovery targets. Channels (Austin) 9:360-366, 2015

6. Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A: Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 41:849-857, 2004

7. Barton DL, Wos EJ, Qin R, Mattar BI, Green NB, Lanier KS, Bearden JD 3rd, Kugler JW, Hoff KL, Reddy PS, Rowland KM Jr., Riepl M, Christensen B, Loprinzi CL: A double-blind, placebo-controlled trial of a topical treatment for chemotherapy-induced peripheral neuropathy: NCCTG trial N06CA. Support Care Cancer 19:833-841, 2011

8. Basso L, Altier C: Transient receptor potential channels in neuropathic pain. Curr Opin Pharmacol 32:9-15, 2017

9. Bonnet C, Hao J, Osorio N, Donnet A, Penalba V, Ruel J, Delmas P: Maladaptive activation of Nav1.9 channels by nitric oxide causes triptan-induced medication overuse headache. Nat Commun 10:4253, 2019

10. Brooks KG, Kessler TL: Treatments for neuropathic pain. Pharm J 9:1-12, 2017

11. Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D, Freeman R, Truini A, Attal N, Finnerup NB, Eccleston C, Kalso E, Bennett DL, Dworkin RH, Raja SN: Neuropathic pain. Nat Rev Dis Primers 3:1-19, 2017

12. Coste B, Crest M, Delmas P: Pharmacological dissection and distribution of NaN/Nav1.9, T-type Ca2+ currents, and mechanically activated cation currents in different populations of DRG neurons. J Gen Physiol 129:57-77, 2007

13. Cox JJ, Reimann F, Nicholas AK, Thornton G, Roberts E, Springell K, Karbani G, Jafri H, Mannan J, Raashid Y, Al-Gazali L, Hamamy H, Valente EM, Gorman S, Williams R, McHale DP, Wood JN, Gribble FM, Woods CG: An SCN9A channelopathy causes congenital inability to experience pain. Nature 444:894-898, 2006

14. Devor M: Sodium channels and mechanisms of neuropathic pain. J Pain 7:S3-S12, 2006

15. Dharmshaktu P, Tayal V, Kalra BS: Efficacy of antidepressants as analgesics: A review. J Clin Pharmacol 52:6-17, 2012

16. Dib-Hajj SD, Black JA, Waxman SG: NaV1.9: A sodium channel linked to human pain. Nat Rev Neurosci 16:511-519, 2015

17. Dib-Hajj SD, Rush AM, Cummins TR, Hisama FM, Novella S, Tyrrell L, Marshall L, Waxman SG: Gain-of-function mutation in Nav1.7 in familial erythromelalgia induces bursting of sensory neurons. Brain 128:1847-1854, 2005

18. Dick IE, Brochu RM, Purohit Y, Kaczorowski GJ, Martin WJ, Priest BT: Sodium channel blockade may contribute to the analgesic efficacy of antidepressants. J Pain 8:315-324, 2007

19. Edelsberg JS, Lord C, Oster G: Systematic review and meta-analysis of efficacy, safety, and tolerability data from randomized controlled trials of drugs used to treat postherpetic neuralgia. Ann Pharmacother 45:1483-1490, 2011

20. Faber CG, Lauria G, Merkies IS, Cheng X, Han C, Ahn HS, Persson AK, Hoeijmakers JG, Gerrits MM, Pierro T, Lombardi R, Kapetis D, Dib-Hajj SD, Waxman SG: Gain-of-function Nav1.8 mutations in painful neuropathy. Proc Natl Acad Sci U S A 109:19444-19449, 2012

21. Fertleman CR, Baker MD, Parker KA, Moffatt S, Elmslie FV, Abrahamsen B, Ostman J, Klugbauer N, Wood JN, Gardiner RM, Rees M: SCN9A mutations in paroxysmal extreme pain disorder: Allelic variants underlie distinct channel defects and phenotypes. Neuron 52:767-774, 2006

22. Finnerup NB, Attal N, Haroutounian S, McNicol E, Baron R, Dworkin RH, Gilron I, Haanpää M, Hansson P, Jensen TS, Kamerman PR, Lund K, Moore A, Raja SN, Rice ASC, Rowbotham M, Sena E, Siddall P, Smith BH, Wallace M: Pharmacotherapy for neuropathic pain in adults: A systematic review and meta-analysis. Lancet Neurol 14:162-173, 2015

23. Gewandter JS, Mohile SG, Heckler CE, Ryan JL, Kirshner JJ, Flynn PJ, Hopkins JO, Morrow GR: A phase III randomized, placebo-controlled study of topical amitriptyline and ketamine for chemotherapy-induced peripheral neuropathy (CIPN): A University of Rochester CCOP study of 462 cancer survivors. Support Care Cancer 22:1807-1814, 2014

24. Goldberg YP, MacFarlane J, MacDonald ML, Thompson J, Dube MP, Mattice M, Fraser R, Young C, Hossain S, Pape T, Payne B, Radomski C, Donaldson G, Ives E, Cox J, Younghusband HB, Green R, Duff A, Boltshauser E, Grinspan GA, Dimon JH, Sibley BG, Andria G, Toscano E, Kerdraon J, Bowsher D, Pimstone SN, Samuels ME, Sherrington R, Hayden MR: Loss-of-function mutations in the Nav1.7 gene underlie congenital indifference to pain in multiple human populations. Clin Genet 71:311-319, 2007

25. Griebeler ML, Morey-Vargas OL, Brito JP, Tsapas A, Wang Z, Carranza Leon BG, Phung OJ, Montori VM, Murad MH: Pharmacologic interventions for painful diabetic neuropathy: An umbrella systematic review and comparative effectiveness network meta-analysis. Ann Intern Med 161:639-649, 2014

26. Hao J, Padilla F, Dandonneau M, Lavebratt C, Lesage F, Noel J, Delmas P: Kv1.1 channels act as mechanical brake in the senses of touch and pain. Neuron 77:899-914, 2013

27. Häuser W, Bernardy K, Üçeyler N, Sommer C: Treatment of fibromyalgia syndrome with antidepressants: A meta-analysis. JAMA 301:198-209, 2009

28. Hershman DL, Lacchetti C, Dworkin RH, Lavoie Smith EM, Bleeker J, Cavaletti G, Chauhan C, Gavin P, Lavino A, Lustberg MB, Paice J, Schneider B, Smith ML, Smith T, Terstriep S, Wagner-Johnston N, Bak K, Loprinzi CL: Prevention and management of chemotherapy-induced peripheral neuropathy in survivors of adult cancers: American Society of Clinical Oncology clinical practice guideline. J Clin Oncol 32:1941-1967, 2014

29. Ho C, O'Leary ME: Single-cell analysis of sodium channel expression in dorsal root ganglion neurons. Mol Cell Neurosci 46:159-166, 2011

30. Horishita T, Yanagihara N, Ueno S, Okura D, Horishita R, Minami T, Ogata Y, Sudo Y, Uezono Y, Sata T, Kawasaki T: Antidepressants inhibit Nav1.3, Nav1.7, and Nav1.8 neuronal voltage-gated sodium channels more potently than Nav1.2 and Nav1.6 channels expressed in Xenopus oocytes. Naunyn Schmiedebergs Arch Pharmacol 390:1255-1270, 2017

31. Hou S, Huh B, Kim HK, Kim KH, Abdi S: Treatment of chemotherapy-induced peripheral neuropathy: Systematic review and recommendations. Pain Physician 21:571-592, 2018

32. Jensen MP, McFarland CA: Increasing the reliability and validity of pain intensity measurement in chronic pain patients. Pain 55:195-203, 1993

33. Kautio AL, Haanpaa M, Saarto T, Kalso E: Amitriptyline in the treatment of chemotherapy-induced neuropathic symptoms. J Pain Symptom Manage 35:31-39, 2008

34. Kleggetveit IP, Schmidt R, Namer B, Salter H, Helas T, Schmelz M, Jorum E: Pathological nociceptors in two patients with erythromelalgia-like symptoms and rare genetic Nav 1.9 variants. Brain Behav 6:e00528, 2016

35. Kopsky DJ, Hesselink JM: High doses of topical amitriptyline in neuropathic pain: Two cases and literature review. Pain Pract 12:148-153, 2012

36. Kopsky DJ, Liebregts R, Keppel Hesselink JM: Central neuropathic pain in a patient with multiple sclerosis treated successfully with topical amitriptyline. Case Rep Med 2012:471835, 2012

37. Lawson K: A brief review of the pharmacology of amitriptyline and clinical outcomes in treating fibromyalgia. Biomedicines 5, 2017

38. Leffler A, Fischer MJ, Rehner D, Kienel S, Kistner K, Sauer SK, Gavva NR, Reeh PW, Nau C: The vanilloid receptor TRPV1 is activated and sensitized by local anesthetics in rodent sensory neurons. J Clin Invest 118:763-776, 2008

39. Leffler A, Lattrell A, Kronewald S, Niedermirtl F, Nau C: Activation of TRPA1 by membrane permeable local anesthetics. Mol Pain 7:62, 2011

40. Leipold E, Liebmann L, Korenke GC, Heinrich T, Giesselmann S, Baets J, Ebbinghaus M, Goral RO, Stodberg T, Hennings JC, Bergmann M, Altmuller J, Thiele H, Wetzel A, Nurnberg P, Timmerman V, De Jonghe P, Blum R, Schaible HG, Weis J, Heinemann SH, Hubner CA, Kurth I: A de novo gain-of-function mutation in SCN11A causes loss of pain perception. Nat Genet 45:1399-1404, 2013

41. Liang J, Liu X, Zheng J, Yu S: Effect of amitriptyline on tetrodotoxin-resistant Nav1.9 currents in nociceptive trigeminal neurons. Mol Pain 9:31, 2013

42. Nau C, Seaver M, Wang SY, Wang GK: Block of human heart hH1 sodium channels by amitriptyline. J Pharmacol Exp Ther 292:1015-1023, 2000

43. Obata H: Analgesic mechanisms of antidepressants for neuropathic pain. Int J Mol Sci 18, 2017, 2483

44. Olah Z, Josvay K, Pecze L, Letoha T, Babai N, Budai D, Otvos F, Szalma S, Vizler C: Anti-calmodulins and tricyclic adjuvants in pain therapy block the TRPV1 channel. PLoS One 2:e545, 2007

45. Reyes-Mendez ME, Castro-Sánchez LA, Dagnino-Acosta A, Aguilar-Martínez I, Pérez-Burgos A, Vázquez-Jiménez C, Moreno-Galindo EG, Álvarez-Cervera FJ, Góngora-Alfaro JL, Navarro-Polanco RA, Alamilla J: Capsaicin produces antidepressant-like effects in the forced swimming test and enhances the response of a sub-effective dose of amitriptyline in rats. Physiol Behav 195:158-166, 2018

46. Rossignol J, Cozzi B, Liebaert F, Hatton S, Viallard ML, Hermine O, Greco C: High concentration of topical amitriptyline for treating chemotherapy-induced neuropathies. Support Care Cancer 27:3053-3059, 2019

47. Rush AM, Cummins TR, Waxman SG: Multiple sodium channels and their roles in electrogenesis within dorsal root ganglion neurons. J Physiol 579:1-14, 2007

48. Santos-Nogueira E, Redondo Castro E, Mancuso R, Navarro X: Randall-Selitto test: A new approach for the detection of neuropathic pain after spinal cord injury. J Neurotrauma 29:898-904, 2012

49. Sawynok J: Antidepressants as analgesics: An introduction. J Psychiatry Neurosci 26:20, 2001

50. Smith H: Topical analgesic agents. Current Therapy in Pain. Philadelphia, PA, Saunders Elsevier, 2009, pp 501-507

51. Spallone V, Morganti R, D'Amato C, Greco C, Cacciotti L, Marfia GA: Validation of DN4 as a screening tool for neuropathic pain in painful diabetic polyneuropathy. Diabet Med 29:578-585, 2012

52. Stevens EB, Stephens GJ: Recent advances in targeting ion channels to treat chronic pain. Br J Pharmacol 175:2133-2137, 2018

53. Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, Earley TJ, Hergarden AC, Andersson DA, Hwang SW: ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 112:819-829, 2003

54. Thompson DF, Brooks KG: Systematic review of topical amitriptyline for the treatment of neuropathic pain. J Clin Pharm Ther 40:496-503, 2015

55. Viana F: TRPA1 channels: Molecular sentinels of cellular stress and tissue damage. J Physiol 594:4151-4169, 2016

56. Waxman SG, Zamponi GW: Regulating excitability of peripheral afferents: Emerging ion channel targets. Nat Neurosci 17:153-163, 2014

57. Yoon SY, Oh J: Neuropathic cancer pain: Prevalence, pathophysiology, and management. Korean J Intern Med 33:1058-1069, 2018

58. Zajaczkowska R, Kocot-Kepska M, Leppert W, Wrzosek A, Mika J, Wordliczek J: Mechanisms of chemotherapy-induced peripheral neuropathy. Int J Mol Sci 20, 2019, 1451

59. Zhang XY, Wen J, Yang W, Wang C, Gao L, Zheng LH, Wang T, Ran K, Li Y, Li X, Xu M, Luo J, Feng S, Ma X, Ma H, Chai Z, Zhou Z, Yao J, Zhang X, Liu JY: Gain-of-function mutations in SCN11A cause familial episodic pain. Am J Hum Genet 93:957-966, 2013

60. Zimmerman A, Bai L, Ginty DD: The gentle touch receptors of mammalian skin. Science 346:950-954, 2014

61. Zimmermann K, Hein A, Hager U, Kaczmarek JS, Turnquist BP, Clapham DE, Reeh PW: Phenotyping sensory nerve endings in vitro in the mouse. Nat Protoc 4:174-196, 2009

62. Zimmermann M: Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16:109-110, 1983