Antihyperalgesic Effects of Clomipramine and Tramadol in a Model of Posttraumatic Trigeminal Neuropathic Pain in Mice

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Aims: *To develop a behavioral model in mice that is capable of mimicking some distinctive symptoms of human posttraumatic trigeminal neuropathic pain such as spontaneous pain, cold allodynia, and chemical/inflammatory hyperalgesia, and to use this model to investigate the antinociceptive effects of clomipramine and tramadol, two drugs used for the treatment of neuropathic pain.* ***Methods:*** *A partial tight ligature of the right infraorbital nerve by an intraoral access or a sham procedure was performed. Fourteen days later, mice were subcutaneously injected with saline or drugs and the spontaneous nociceptive behavior, as well as the responses to topical acetone and to formalin or capsaicin injected into the ipsilateral vibrissal pad, were assessed. Data were analyzed by ANOVA.* ***Results:*** *Neuropathic mice exhibited an increased spontaneous rubbing/scratching of the ipsilateral vibrissal pad, together with enhanced responses to cooling (acetone) and the chemical irritants (formalin, capsaicin). Clomipramine and tramadol produced an antihyperalgesic effect on most of these nociceptive responses, but tramadol was ineffective on capsaicin-induced hyperalgesia.* ***Conclusion:*** *Nociceptive responses in this neuropathic pain model in mice exhibited a pattern consistent with the pain described by posttraumatic trigeminal neuropathic patients. The selective antihyperalgesic effect obtained with two commonly used drugs for treating neuropathic pain confirms the validity of this preclinical model.* J OROFAC PAIN 2011;25:354–363

Key words: clomipramine, infraorbital nerve ligation, mice, neuropathic trigeminal pain, tramadol

Pain arising from the trigeminal territory is probably one of the most commonly reported pain conditions, constituting a significant public health problem.[1] Regardless of its origin, acute or chronic trigeminal pain typically exhibits a strong emotional component that is frequently very disabling.[2] Among the precipitating factors of chronic trigeminal pain, traumatic injury of trigeminal nerve branches is an increasingly recognized entity.[3,4] This posttraumatic trigeminal neuropathic pain can be induced by surgical and nonsurgical traumatic injuries, producing different degrees of nerve lesion or deafferentation. Orofacial traumas, endodontic therapy, extraction of teeth, dental implant placement, and maxillofacial surgical procedures have been implicated in the induction of posttraumatic trigeminal neuropathic pain.[3,5–7]

Clinical characteristics of posttraumatic trigeminal neuropathic pain include persistent, ongoing pain in the absence of noxious stimulation.[3] Sensory testing can also reveal mechanical allodynia, abnormal temporal summation of pain, and cold allodynia,[3,6] and responsiveness to chemical irritants can also be enhanced.[8] Indeed, a recent study reported chemical hyperalgesia to topical application of capsaicin in the oral mucosa of patients affected by trigeminal neuropathic pain.[9]

Unfortunately, effective treatment of trigeminal neuropathic pain represents a major therapeutic challenge, probably because the mechanisms underlying this type of pain are still poorly understood.[6] The pharmacological management of trigeminal neuropathic pain is based on extrapolations from guidelines for the treatment of neuropathic pain, which include several classes of drugs.[4] The first-line drugs for the treatment of trigeminal neuropathic pain are tricyclic antidepressants with balanced serotonin and norepinephrine reuptake inhibition, such as amitriptyline and clomipramine.[4,10] A second-line drug for the treatment of trigeminal neuropathic pain is tramadol, a synthetic compound acting as a μ-opioid agonist and as a monoamine reuptake inhibitor.[4,10,11]

Some behavioral tests[12–15] and pain models[16–18] have been developed in rats to study nociceptive mechanisms in the trigeminal region. On the other hand, the increasing use of transgenic mice in preclinical studies has greatly improved the understanding of the mechanisms underlying pain processing.[19] Therefore, the adaptation of trigeminal pain models originally developed in rats for use in mice would play a crucial role for this purpose; however, in the study of trigeminal neuropathic pain, few models have been developed in mice.[20–22] Although the posttraumatic trigeminal neuropathic pain exhibits many different clinical features, available preclinical models in mice have only assessed mechanical allodynia.[20–22] Furthermore, no pharmacologic validation of these models has yet been performed.

The aim of this study was to develop a behavioral model in mice that is capable of mimicking some distinctive symptoms of human posttraumatic trigeminal neuropathic pain, such as spontaneous pain, cold allodynia, and chemical/inflammatory hyperalgesia. This was made by partially ligating the infraorbital nerve via an intraoral surgical approach, as an adaptation of the extraoral surgical approach described by Xu et al.[22] Using this model, the antinociceptive effects of clomipramine and tramadol, two drugs used clinically for the treatment of neuropathic pain, were also investigated.

Materials and Methods

Animals

A total of 168 naïve outbred CF1 male adult mice weighing 27 to 33 g were used in the experiments. Animals were housed 10 per cage and kept in a temperature- and light-controlled environment (12:12 hour, light:dark lights on at 7 am) and had ad libitum access to food and water. Mice were allowed to habituate to the housing facility for 1 week before the experiments. The experiments were performed during the light phase between 9 am and 5 pm in a quiet room. The housing conditions and experimental procedures conformed to protocols approved by the Bioethics Committee of the Faculty of Medicine, Universidad de Chile, and were consistent with the ethical guidelines published by the International Association for the Study of Pain.[23] Every effort was made to minimize the number and suffering of animals used in the experiments. Each mouse was used only once and sacrificed at the end of experiments by cervical dislocation.

Drugs

Unless specifically stated, all chemicals used in the present study were obtained from Sigma-Aldrich. All drugs were dissolved in physiological saline (0.9% NaCl).

Surgical Procedures

After preliminary trials to identify the more appropriate surgical procedure, a partial nerve ligature injury of the right infraorbital nerve via an intraoral approach was performed. Briefly, mice were anesthetized with a solution of chloral hydrate 7% (400 mg/kg, ip) and the corneas were protected from desiccation by use of an ophthalmic ointment; mice were then secured to an operating table with adhesive tape and kept warm with a heat lamp. Using a stereomicroscope (Nikon) to provide direct vision, the investigator made a 4-mm-long incision in the gingivobuccal mucosa beginning just cranial to the first molar, and the infraorbital nerve was gently freed from surrounding muscles and connective tissue by blunt dissection. The lateral part of the infraorbital nerve (approximately one-half of the diameter) was tightly ligated with a single 5-0 silk ligature (Ethicon). After checking for hemostasis, the incision was closed with 5-0 silk suture. The sham procedure consisted of exposure of the infraorbital nerve, with care taken to avoid stretching the nerve or damaging the epineurium. Mice were allowed to recover under the heat lamp and treated with ketoprofen (5 mg/kg daily, subcutaneously [sc]) for 3 consecutive days. All experiments were performed 14 days after surgery.

Behavioral Assessment

The animals were acclimated to the experimental room 2 hours before starting any manipulation. For behavioral testing, mice were placed into an acrylic cylinder (25 cm high × 25 cm diameter) surrounded

by two mirrors placed perpendicularly to each other. Each mouse was first placed into this cylinder for 10 minutes before testing to acclimatize the mouse and minimize stress. The nociceptive behavior evaluated in this study was asymmetric orofacial grooming, ie, rubbing/scratching focused on the vibrissal pad and executed with ipsilateral fore- or hind-paw.[16,24,25] In each experiment, the time spent in this behavior was recorded with a stopwatch.

Spontaneous Nociceptive Behavior

To assess ongoing spontaneous pain, the rubbing and scratching behavior of mice was recorded during a period of 12 minutes in the absence of any external stimulus. For comparative purposes, the rubbing and scratching activity was expressed over a time base of 5 minutes.

Cold Allodynia

Mice were gently restrained and 20 µL of 90% acetone (diluted in distilled water) was topically applied to the vibrissal pad ipsilateral to the ligated infraorbital nerve by means of a customized 25-gauge needle (blunt and slightly bent) attached to a 100 µL microsyringe (Hamilton). Special care was taken to avoid any acetone leakage towards the ocular surface or the nose. Animals were immediately placed into the acrylic cylinder and the rubbing/scratching behavior was measured during a 5-minute period.

Chemical/Inflammatory Hyperalgesia

To evaluate chemical/inflammatory hyperalgesia, two different tests were used: the formalin and the capsaicin tests. Formalin and capsaicin target different transient receptor potential channels in nociceptors, the TRPA1 and TRPV1, respectively.[26] Thus, analgesic drugs may display differential efficacy according to the pain-producing substance.

Chemical Hyperalgesia to Formalin. The orofacial formalin test was conducted following the method proposed by Luccarini et al,[25] with minor modifications. Briefly, mice received a 20-µL injection (sc) of 0.25% formalin into the center of the right vibrissal pad. Formalin was injected subcutaneously through a 27-gauge × 4-mm needle attached to a 100-µL microsyringe (Hamilton) as quickly as possible, with only minimal animal restraint. This formalin solution was prepared from commercially available stock formalin (35% formaldehyde, Merck) diluted in physiological saline (0.25% v/v). This concentration of formalin was chosen because it is much lower than concentrations that induce a robust nociceptive response in mice,[25] thereby allowing observation of increased nociceptive responses. The authors' preliminary data confirmed that this concentration produces only a very small nociceptive response in naive mice (data not shown). Following injection, each mouse was immediately placed back in the acrylic cylinder for a 42-minute observation period. The observation time was divided into seven blocks of 6 minutes, and the cumulated time spent grooming the injected area was recorded.

Chemical Hyperalgesia to Capsaicin. The orofacial capsaicin test in mice was adapted from the procedure previously described by Pelissier et al.[13] Briefly, capsaicin was dissolved in a lipidic emulsion for venous perfusion (Endolipide; Braun Medical SA) to a concentration of 10 µg/mL. Mice received an injection of 20 µL of this solution (total dose of 0.2 µg of capsaicin, sc) into the center of the right vibrissal pad, using a 27-gauge × 4-mm needle attached to a microsyringe (Hamilton). This dose of capsaicin was chosen since in the rat it is almost devoid of nociceptive effects,[13] allowing an eventual chemical hypersensitivity to be assessed. Preliminary data confirmed that this concentration only produces a modest nociceptive response in naive mice (data not shown). Following capsaicin injection, each mouse was immediately placed back in the acrylic cylinder for a 42-minute observation period, and the time spent in grooming the injected area was recorded.

Pharmacological Treatments

The antinociceptive effects of clomipramine and tramadol were assessed in mice receiving the trigeminal nerve injury and in respective sham controls. Forty-five minutes before testing the spontaneous nociceptive behavior, cold allodynia, and chemical hyperalgesia, mice were injected subcutaneously into the nape of the neck with 1.5 mg/kg of either clomipramine or tramadol. This time period was chosen because the maximum effect for clomipramine[27,28] and tramadol[29–31] in rats or mice shows a plateau between 30 to 120 minutes and 30 to 60 minutes, respectively, after SC or IP administration.

The dose chosen for clomipramine and tramadol was well below doses that produce nonspecific psychomotor effects in mice.[27,32–35] As control treatment, the same volume of saline was injected subcutaneously (0.1 mL per 10 g mouse). All experiments were performed blind by the same experimenter.

Statistical Analysis

The results were expressed as mean ± SEM, and all statistical analyses were performed with GraphPad

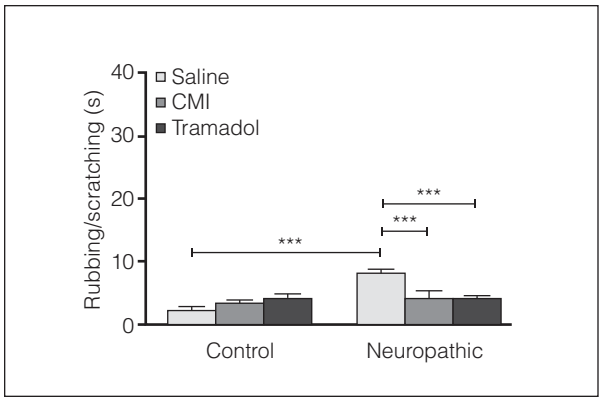

Fig 1 Effects of clomipramine and tramadol on spontaneous rubbing/scratching behavior in control and trigeminal neuropathic mice. Each bar represents the time spent in rubbing/scratching during a 5-minute period (mean ± SEM, n = 7 for each group). Statistical comparisons were made by means of a two-way ANOVA followed by the Bonferroni's multiple comparisons post-hoc test. CMI: clomipramine; ***$P < .001$.

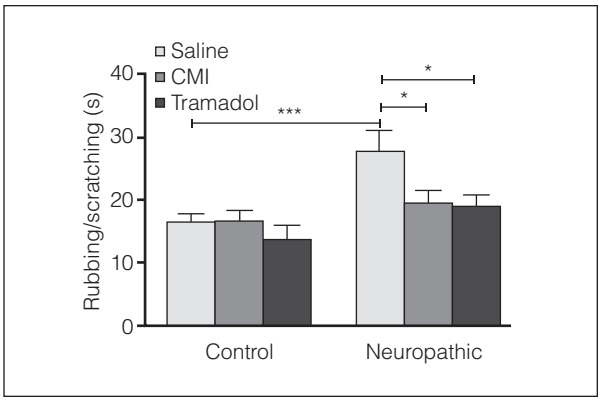

Fig 2 Effects of clomipramine and tramadol on cold allodynia testing in control and trigeminal neuropathic mice. Each bar represents the time spent in rubbing/scratching behavior induced by the facial instillation of acetone (20 µL) during a 5-minute period of observation (mean ± SEM, n = 7 for each group). Statistical comparisons were made by means of a two-way ANOVA followed by the Bonferroni's multiple comparisons post-hoc test. *$P < .05$; ***$P < .001$.

Prism software (GraphPad Software). To analyze the time-course of saline and drug effects in control or neuropathic mice submitted to formalin testing, a two-way repeated measures ANOVA was performed. To assess the effects of the pharmacological treatment (saline, clomipramine, or tramadol) and the influence of nerve injury on spontaneous nociceptive behavior, cold allodynia, formalin, and capsaicin testing, a two-way ANOVA was made. In all cases, the Bonferroni's multiple comparisons was applied as a post-hoc test. Statistical significance (ie, the α-level) was set at $P < .05$.

Results

Mice recovered uneventfully from the surgical intervention, with those submitted to a partial injury of the infraorbital nerve being visibly indistinguishable from mice in the control (sham-operated) group.

Spontaneous Nociceptive Behavior

Once in the acrylic cylinder, mice exhibited typical behaviors of exploration, walking, and rearing and being attentive to environmental stimuli. All the mice engaged spontaneously in grooming activity in the form of face-wash strokes involving the vibrissal pad area. Neuropathic mice receiving saline, however, showed a significantly increased rubbing/scratching behavioral activity compared to sham-operated controls (8.24 ± 0.64 s and 2.29 ± 0.57 s, respectively, n = 7 in each group) ($P < .001$, Fig 1). Systemic administration of clomipramine did not modify the spontaneous grooming in control mice. In contrast, clomipramine induced a significant inhibition of the rubbing/scratching activity in neuropathic mice compared to animals receiving saline injection ($P < .001$, Fig 1). As with clomipramine, systemic administration of tramadol also reduced the spontaneous behavioral activity only in neuropathic mice ($P < .001$, Fig 1).

Cold Allodynia

After the application of 20 µL of acetone to the vibrissal pad, control mice receiving saline exhibited an immediate rubbing/scratching behavior focused to the site of application of the stimulus (16.43 ± 1.21 s, n = 7). This behavioral response to acetone in neuropathic mice with saline was significantly greater (27.3 ± 2.9 s, n = 7) than in control mice ($P < .001$, Fig 2). Systemic administration of clomipramine did not change the nociceptive responses to acetone instillation in control mice (16.71 ± 1.48 s, n = 7), whereas it significantly inhibited such responses in neuropathic mice (19.43 ± 1.73 s, n = 7) ($P < .05$, Fig 2). As with clomipramine, systemically administered tramadol did not modify the behavioral responses to acetone in control animals (13.79 ± 2.30 s, n = 7) while significantly reducing the responses in the neuropathic mice group (18.86 ± 2.16 s, n = 7) ($P < .05$, Fig 2).

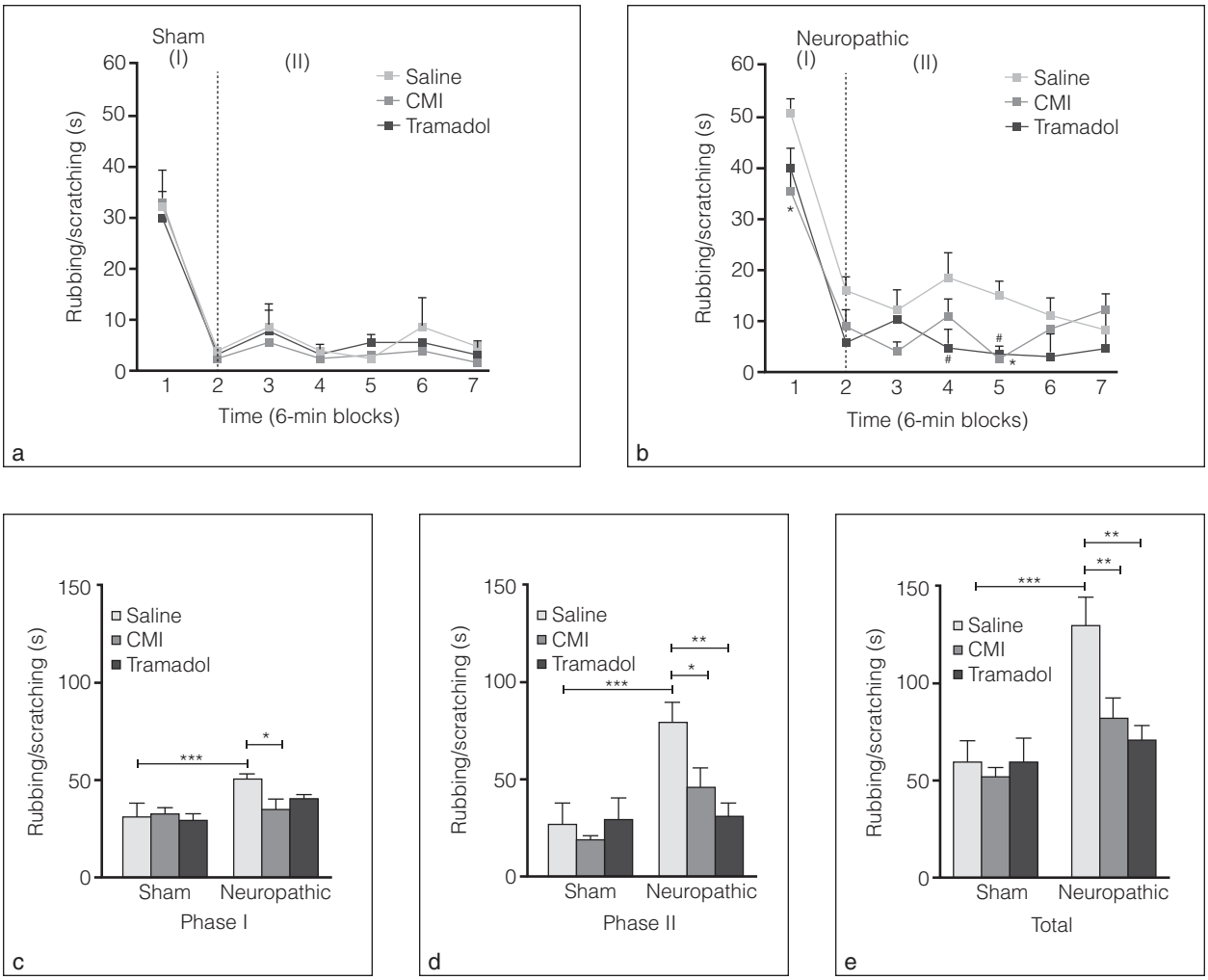

Fig 3 Effects of clomipramine and tramadol on chemical hyperalgesia induced by facial 0.25% formalin in control and neuropathic mice. *(a and b)* Temporal evolution: each symbol represents the mean ± SEM of cumulated nociceptive behavior in 6-minute blocks during 42 minutes after formalin injection (n = 7 for each group). Statistical comparisons were made by means of a two-way repeated measures ANOVA followed by the Bonferroni's multiple comparisons post-hoc test. **P* < .05 clomipramine versus saline; #*P* < .05 tramadol versus saline. *(c to e)* Time spent in rubbing/scratching behavior. Each column represents the mean ± SEM of cumulated nociceptive behavior during *(c)* phase I, *(d)* phase II, and *(e)* total time of the formalin test (n = 7 for each group). Statistical comparisons were made by means of a two-way ANOVA followed by the Bonferroni's multiple comparisons post-hoc test. **P* < .05; ***P* < .01; ****P* < .001.

Chemical Hyperalgesia to Formalin

The injection of 0.25% formalin into the vibrissal pad of control mice induced an initial period of vigorous rubbing/scratching activity, followed by a period of more discrete and scattered episodes of this nociceptive behavior until the end of the testing (42 minutes) (Fig 3). Thus, in order to simplify the analysis, two phases were defined: I, from 0 to 6 minutes, and phase II, from 6 to 42 minutes, after formalin injection (Fig 3a). During the phase I, control mice spent 32.29 ± 6.83 s (n = 7) in rubbing/scratching behavior, whereas during phase II this nociceptive response lasted 27.43 ± 10.51 s (n = 7), leading to a total time (phase I plus phase II) of the nociceptive response of 59.71 ± 11.30 s (n = 7) (Fig 3c to 3e). In control mice, the systemic administration of clomipramine neither modified the phase I (33.00 ± 1.89 s, n = 7), phase II (18.71 ± 2.38 s, n = 7), nor the total time (51.71 ± 4.11 s, n = 7) spent in rubbing/scratching behavior (Fig 3c to 3e). Systemically administered tramadol was equally ineffective in inhibiting the phase I (29.57 ± 2.85 s, n = 7), phase II (28.57 ± 11.00 s, n = 7), or total time (58.14 ± 13.63 s, n = 7) in this group (Fig 3c to 3e).

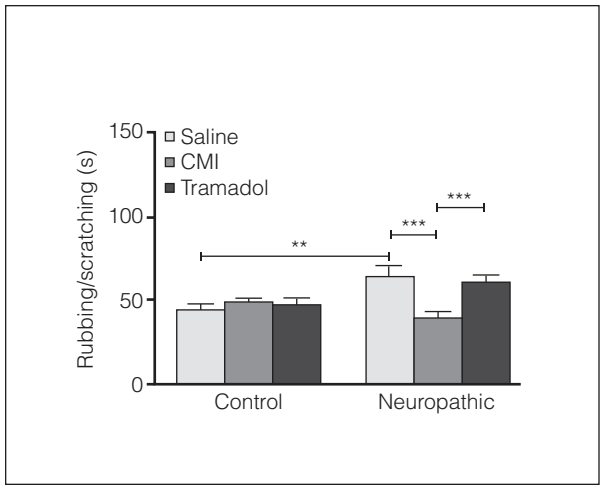

Fig 4 Effects of clomipramine and tramadol on chemical hyperalgesia induced by orofacial capsaicin in control and trigeminal neuropathic mice. Each bar represents cumulated time spent in rubbing/scratching behavior during a 42-minute period after a 0.2 µg capsaicin injection (mean ± SEM, n = 7 for each group). Note that the hyperalgesic effect of capsaicin is evident only in neuropathic mice. This effect was attenuated by clomipramine but not by tramadol. Statistical comparisons were made by means of a two-way ANOVA followed by the Bonferroni's multiple comparisons post-hoc test. $^{**}P < .01$, $^{***}P < .001$.

Neuropathic mice receiving saline exhibited a marked enhancement of the nociceptive behavior evoked by facial formalin injection compared to control mice with saline (Fig 3b). In these neuropathic mice, the time spent in rubbing/scratching was significantly increased for phase I, phase II, and total time, amounting to 50.29 ± 2.89 s (n = 7, $P < 0.01$), 79.57 ± 10.34 s (n = 7, $P < .001$) and 129.43 ± 14.84 s (n = 7, $P < .001$), respectively (Fig 3c to 3e). Systemic clomipramine administered to neuropathic mice inhibited the nociceptive responses in phase I (35.00 ± 4.11 s, n = 7, $P < .05$), phase II (45.71 ± 9.49 s, n = 7, $P < .05$), and total time (80.71 ± 10.54 s, n = 7, $P < .01$) compared to neuropathic mice receiving saline (Fig 3c to 3e). Although systemic tramadol did not affect nociceptive responses in phase I (39.71 ± 3.61 s, n = 7), it significantly reduced the time spent in nociceptive behavior in phase II (31.29 ± 7.04 s, n = 7, $P < .01$) and total time (70.00 ± 7.06 s, n = 7, $P < .01$) in neuropathic mice (Fig 3c to 3e).

Chemical Hyperalgesia to Capsaicin

The injection of 0.2 µg of capsaicin into the vibrissal pad area evoked an immediate rubbing/scratching response of the injected area. The duration of this nociceptive response during the 42-minute period of observation was 44.71 ± 3.25 s (n = 7) in sham mice receiving saline, while the duration significantly increased to 64.14 ± 6.42 s in neuropathic mice with saline (n = 7) ($P < .01$, Fig 4). The systemic administration of clomipramine did not produce any effect in control mice, as compared to mice receiving saline (48.86 ± 1.98 s, n = 7, Fig 4). In neuropathic mice, however, clomipramine significantly inhibited the nociceptive response to facial capsaicin injection compared to saline (39.29 ± 2.53 s, n = 7, $P < .001$, Fig 4). Systemic administration of tramadol did not significantly affect the nociceptive responses to capsaicin either in control mice (47.00 ± 4.89 s, n = 7) or in neuropathic mice (61.00 ± 4.459 s, n = 7) (Fig 4).

Discussion

The present study sought to develop a preclinical model of posttraumatic trigeminal neuropathic pain in mice. Results show that this model not only mimics spontaneous pain, cold allodynia, and increased responsiveness to irritants observed in patients, but also is sensitive to two clinically relevant drugs, clomipramine[10,35] and tramadol.[4,10]

Nociceptive Responses in Posttraumatic Trigeminal Neuropathic Mice

Consistent with spontaneous pain observed in posttraumatic trigeminal neuropathic pain patients, neuropathic mice exhibited a persistent spontaneous nociceptive behavior directed to the territory innervated by the injured infraorbital nerve. This kind of nociceptive behavior has also been observed in rats subjected to a chronic constriction of the infraorbital nerve[16] and it might also be related to other symptoms observed in patients with trigeminal neuropathy, such as paresthesias or dysesthesias.[16] Xu and colleagues[22] also reported an enhancement of the spontaneous nociceptive behavior after partial ligature of the infraorbital nerve in mice, but

only during day one after surgery. However, these authors assessed this behavior only for the first 7 days postsurgery, and the changes were probably related to acute nerve damage.[22] The persistent spontaneous nociceptive behavior observed here, however, is unlikely to result from acute nerve damage or incisional pain. Indeed, instead of a conventional cutaneous access to the underlying nerves, an intraoral surgical approach of the infraorbital nerve was used, which had been previously described in rats.[17] In addition, the use of postoperative ketoprofen and a 14-day recovery period helped to minimize the contribution of the incisional/inflammatory pain to the behavioral responses analyzed, a well-established factor for cutaneous hyperalgesia in mice.[36] Moreover, given the importance of the hair on the snout and the vibrissae for normal trigeminal somatosensory processing,[16,37] the present procedure allowed these important structures to remain intact.

Cold allodynia is a common symptom in posttraumatic trigeminal neuropathic pain patients. The application of acetone in the vibrissal pad ipsilateral to the injured infraorbital nerve also revealed the existence of cold allodynia in trigeminal neuropathic mice. Cold allodynia is also observed in models of neuropathic pain induced by tight ligation of spinal nerves in rats[38] and in mice.[39] In the orofacial area, loose ligature of the infraorbital nerve in rats also produces allodynia and hyperalgesia to a cooling spray.[14]

Consistent with enhanced pain ratings to topical irritants in patients with trigeminal nerve injury,[9] the injection of mild concentrations of formalin or capsaicin in trigeminal neuropathic mice produced increased nociceptive responses indicative of general chemical hyperalgesia. These results are consistent with those obtained by Bennett and Xie,[5] who reported increased nociceptive responses to mustard oil in sciatic neuropathic rats. In addition, enhanced behavioral responses to formalin injection have been observed in rats rendered neuropathic by a tight ligation of the L5 spinal nerve.[40] Contrasting with these data, however, Anderson and colleagues[41] reported that the orofacial injection of 3% formalin in trigeminal neuropathic rats increased several nociceptive behaviors but reduced the time spent in orofacial rubbing/scratching activity. This rather "paradoxal" effect has already been reported when such high concentrations of formalin are employed. For instance, the orofacial injection of formalin at concentrations beyond 2% can induce in rats a strong expression of defensive behaviors, such as freezing, in detriment of the time spent in rubbing/scratching.[24]

Effects of Clomipramine

At the dose used here, clomipramine exhibited a selective antihyperalgesic effect, ie, while reducing hyperalgesic responses in neuropathic mice, clomipramine was devoid of inhibitory effects on nociceptive responses in control mice. In agreement with these observations, acute administration of similar or even higher doses of clomipramine has been shown to be ineffective in the formalin test in naïve mice.[42–44] Also, there was no antinociceptive effect in the hot plate test at doses close to that used here,[42] the antinociceptive effect appearing only at higher doses in naïve mice.[27,43] However, clomipramine can inhibit nociceptive responses in mice subjected to the writhing test, a visceral pain assay that is very sensitive to mild analgesic drugs.[33] Finally, experiments performed in rats with the paw-pressure test seem to confirm the low antinociceptive efficacy of clomipramine in naïve animals.[45]

In neuropathic mice, however, clomipramine significantly inhibited nociceptive spontaneous behavior as well as cold and chemically evoked nociception, confirming its selective antihyperalgesic effect. These results are consistent with earlier reports showing significant antinociceptive effects of clomipramine in rats with a mononeuropathy of the sciatic nerve.[27,45] In rats subjected to a chronic constriction injury of the infraorbital nerve, however, clomipramine did not affect mechanical allodynia.[46] Although there is no definitive explanation for this discrepancy, it might be related to differential antinociceptive effects of clomipramine on different pain modalities.

Many mechanisms of action have been proposed for the antinociceptive effect of clomipramine.[47] Strong evidence indicates, however, that antinociceptive effects of clomipramine depend on noradrenergic/serotoninergic bulbospinal inhibitory pathways.[48–51] Although clomipramine is often considered as a preferential inhibitor of serotonin reuptake, it has clinically relevant serotonin and noradrenaline reuptake inhibition capacity.[52] Indeed desmethyl-clomipramine, a product of clomipramine metabolism, has a substantial effect as inhibitor of noradrenaline reuptake[53] and produces antinociceptive effects in mice.[51] In addition, clomipramine can act as an inhibitor of glial activation in vitro.[54] Since glial activation is observed in many rodent models of posttraumatic trigeminal neuropathic pain,[20,22,55] this mechanism may play an important role in the antihyperalgesic effects of clomipramine observed here.

Effects of Tramadol

Data presented here showed that a low dose of tramadol is devoid of antinociceptive effect in naïve mice. Consistent with this result, comparable or even higher doses of tramadol have been shown to be ineffective in the first phase of the formalin test in naïve mice or rats.[56,57] For the second phase of the formalin test, only higher doses than that used here were reported to be effective.[56,57] Again, only in the writhing test does such a low dose of tramadol produce antinociceptive effects in naïve mice.[33]

In neuropathic mice, however, tramadol displayed marked antihyperalgesic effects on spontaneous nociceptive behavior, cold allodynia, and the second phase of the formalin test. This is in good agreement with previous reports where responses induced by mechanical and cold stimuli were consistently inhibited by tramadol in rat models of mononeuropathic pain.[58] Furthermore, tramadol inhibits menthol-induced cold hyperalgesia in humans and cold allodynia in neuropathic pain patients.[59] Tramadol was, however, unable to attenuate capsaicin-induced hyperalgesia in trigeminal neuropathic mice. Although there is no satisfactory explanation for this result, the recent discovery that tramadol is an agonist of the capsaicin receptor TRPV1[60] may shed some light on this matter. Indeed, this mechanism seems to account for the diminution of potency of peripherally administered tramadol in glutamate-induced allodynia in rats.[61] It is thus tempting to speculate that in a context of enhanced responsiveness to capsaicin, the agonistic properties of tramadol on TRPV1 might counteract its own antinociceptive effects.

The antinociceptive effects of tramadol are related to the inhibition of serotonin and norepinephrine reuptake by its (+) and (–) enantiomers, respectively.[62] Additionally, the active metabolite M1 (*O*-desmethyl-tramadol) acts as a high-affinity μ opioid agonist.[11] The inhibitory effects of tramadol on the phase II of the formalin-induced hyperalgesia observed here suggest an action on endogenous opioidergic systems. Indeed, naloxone-treated wild type mice or homozygous μ opioid receptor knockout mice exhibited enhanced nociceptive responses in the second phase, but not in the first phase, of the formalin test.[63] Finally, the well-established action of tramadol on serotoninergic and noradrenergic systems also seems to contribute to its antinociceptive effects during the second phase of the formalin test.[57]

In conclusion, a preclinical model has been developed in mice that mimics some features of human posttraumatic trigeminal neuropathic pain, which has proven elusive in previous trigeminal pain animal models. One important advantage of this model is that the intraoral surgical approach of the infraorbital nerve minimizes the contribution of the incisional pain to the behavioral responses analyzed. The pharmacological data obtained with this approach further support its reliability as a preclinical model of posttraumatic trigeminal neuropathic pain.

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