Research Report

Interferential Therapy Produces Antinociception During Application in Various Models of Inflammatory Pain

Background and Purpose. Although interferential therapy (IFT) is used widely in the management of many painful conditions, the effectiveness and the mechanism of action of IFT in animal models of inflammatory pain have not been evaluated. The aim of this study was to evaluate the effectiveness of IFT in reducing inflammatory pain and edema in rats. Subjects. Sixty-nine male Wistar rats were used in the study. Methods. The effect of IFT application (4,000-Hz carrier frequency, 140-Hz amplitude-modulated beat frequency, pulse duration=125 milliseconds, current intensity=5 mA) for 1 hour on the formalin-induced nociceptive response and edema and on carrageenan-induced mechanical hyperalgesia and edema was evaluated. Results. Interferential therapy significantly reduced the formalin-evoked nociceptive response when applied to the paw immediately after but not before the formalin injection. Interferential therapy application at 2 hours after the carrageenan injection significantly prevented a further increase in carrageenan-induced mechanical hyperalgesia only immediately after discontinuation of the electrical current application. The antinociception induced by IFT was not attributable to a reduction in inflammation because IFT did not significantly reduce the edema induced by either formalin or carrageenan. Discussion and Conclusion. The results suggest that, despite its short-duration effect, IFT is effective in reducing inflammatory pain and should be considered primarily for use in the control of acute inflammatory pain. [Jorge S, Parada CA, Ferreira SH, Tambeli CH. Interferential therapy produces antinociception during application in various models of inflammatory pain. *Phys Ther.* 2006;86:800–808.]

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Inflammatory pain is a pervasive problem and usually results in both spontaneous pain and hyperalgesia. Hyperalgesia is characterized by a peripheral sensitization of pain receptors (nociceptors)—that is, an increase in neuronal membrane excitability—by inflammatory mediators.[1–7] Although the hyperalgesic state does not necessarily involve ongoing pain, the nociceptive threshold is lowered in this state, and the application of a nonnoxious mechanical, thermal, or chemical stimulus induces a nociceptive behavior response.[2,8] However, spontaneous inflammatory pain is characterized by a continuous endogenous stimulation of nociceptors caused by the release of inflammatory mediators that directly stimulate them. Postsurgical or traumatic pain usually is referred to as spontaneous pain in a hyperalgesic state.

The inflammatory mediators released at the site of tissue injury, such as prostaglandins, sensitize nociceptors.[2–4,6] The current focus of treatment is on the blockade of prostaglandin synthesis through the use of nonsteroidal anti-inflammatory drugs (NSAIDs) to prevent the peripheral sensitization of pain receptors.[1,5] Because many subjects are intolerant of prolonged treatment with NSAIDs, the use of electrotherapy in the management of inflammatory pain conditions has gained popularity.[9]

Compared with other methods of transcutaneous electrical nerve stimulation (TENS), interferential current is a form of electrical therapy that delivers currents to deep tissues through the use of kilohertz-carrier-frequency pulsed or sinusoidal currents to overcome the impedance offered by the skin. Because very-high-frequency currents are not uncomfortable for subjects, 2 currents can be delivered out of phase; these currents interfere with each other within tissues at the point at which the currents cross.[10,11] The resultant amplitude-modulated interference wave has beat frequencies of between 1 and 250 Hz, which have been reported to induce analgesia in humans.[12–15]

Although interferential therapy (IFT) is used widely in the management of many painful conditions, the effectiveness and the mechanism of action of IFT in animal models of inflammatory pain have not been evaluated yet.[16–18] Thus, the aim of the present investigation was to evaluate the effectiveness of IFT in reducing inflammatory pain and edema. The specific aims were to investigate the analgesic effect of IFT on carrageenan-induced mechanical hyperalgesia and formalin-induced spontaneous nociceptive behavior[6,19–23] and to investigate the anti-inflammatory effect of IFT on carrageenan- or formalin-induced edema.[21,23,24]

Method

Subjects

The study was carried out with male Wistar rats (150–250 g) maintained in a temperature-controlled room (23°±1°C [mean±SD]) with a 12-hour light-dark cycle. All experiments were approved by the Animal Care Committee at the University of Campinas and were conducted in accordance with ethical guidelines for investigations of experimental pain in conscious animals.[25]

Procedure

Animals were anesthetized briefly by inhalation of 2.5% isoflurane for electrode placement. The distal half of the left hind limb was depilated, and a bipolar stimulating electrode consisting of 2 superficial 6-mm-diameter pregelled surface electrodes was applied, with the 2 electrodes located approximately 2 cm apart, between the lower third of the hind limb and the proximal portion of the dorsum of the hind paw. The electrodes were fixed in place with tape. Animals regained consciousness approximately 1 minute after discontinuation of the

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anesthetic. The stimulating electrodes were connected to a model ET9702 ENDOPHASYS-I electrical stimulator.* The stimulator was set to produce an interferential current with a 4,000-Hz carrier frequency, a 140-Hz premodulated beat frequency, and a pulse duration of 125 milliseconds.[26] Animals were treated at a sensory amplitude of 5 mA, which does not cause muscle contraction or discomfort in rats.[27] Methods used in nociceptive tests were similar to those previously described.[21–23,28,29]

Formalin-Evoked Nociceptive Behavior

Introduced by Dubuisson and Dennis in 1977, the formalin test is used widely to evaluate analgesic drugs, and it is well known as an animal model of tonic inflammatory pain.[20] Subcutaneous injection of formalin into the rat hind paw evokes an array of stereotyped behaviors. Among these behaviors, flinching (consisting of an elevation and shrinking back of the injected paw) is a reliable parameter of pain behavior.[30] The nociceptive response to formalin occurs in a biphasic pattern; there is an initial acute period (phase 1, duration of 7±10 minutes) and, after a short period of remission, phase 2 begins and consists of a longer period (1 hour) of sustained activity.[19,28–31] It has been demonstrated that the nociceptive behaviors evoked by formalin injection are associated with the local release of histamine and serotonin, which directly activate nociceptors.[22]

After a 30-minute habituation period in a test chamber (30×30×30 cm mirrored wood chamber with glass at the front side), each animal was given a subcutaneous injection of 50 μL of 1% formalin (1:100 dilution of stock formalin solution; 37% formaldehyde in 0.9% saline) in the dorsum of the hind paw and then was returned to the test chamber for a 1-hour observation period.

The recording time was divided into 12 blocks of 5 minutes, and a pain score was determined for each block by measuring the number of flinches of the affected limb during the observation time.[22,30] The formalin-evoked nociceptive flinching behavior was divided into phase 1 (0–10 minutes) and phase 2 (15–60 minutes).[7,22]

Because the administration of 1% formalin evokes prompt nociception for approximately 1 hour, IFT was applied for 1 hour immediately after the formalin injection. Additional experiments were performed to verify whether IFT application during the 1 hour before the formalin injection was able to reduce the nociceptive response. When the electrical current was applied before the formalin injection, the electrodes were kept in place for 2 hours (1 hour before and 1 hour after the formalin injection), and when it was applied immediately after the formalin injection, the electrodes were kept in place only during the 1 hour after the formalin injection. The same protocol was used for the control groups, except that the electrical stimulator was maintained in the off position. To control for the possibility that the electrodes could affect the formalin-evoked flinching behavior by themselves, subcutaneous injections of formalin also were given to animals that had no electrodes placed on the hind paw. All formalin tests were performed by the same experimenter, who was unaware of whether a rat had undergone IFT or not, and all rats were used only once.

Carrageenan-Induced Mechanical Hyperalgesia

Inflammation induced by carrageenan is acute, nonimmune, and highly reproducible. Cardinal signs of inflammation, such as edema, hyperalgesia, and erythema, develop immediately after the subcutaneous injection of carrageenan.[23]

The sensitization of primary nociceptive neurons (hyperalgesia) is measured in animal tests either by a decrease in the nociceptive behavior threshold or by a shortening of the time to the induction of the behavioral end point. In the present work, the time until a typical reaction appeared after the application of constant pressure in normal or sensitized paws was measured as previously described.[32] Briefly, mechanical hyperalgesia was tested in rats by use of a constant pressure of 20 mm Hg, which was applied via a syringe piston moved by compressed air to a 15-mm^2 area on the dorsal surface of the hind paw and which was discontinued when the rat presented a typical "freezing reaction"; this reaction was signaled by brief apnea concomitant with a retraction of the head and forepaws and a reduction in the escape movements that animals frequently make to escape from the position imposed by the experimental situation. For each animal, the latency to the onset of the freezing reaction (from the time of the first pressure application) was measured before the carrageenan injection (zero time, defined as baseline) and at 2, 3, and 4 hours after the carrageenan injection. Carrageenan (100 μg, 50 μL) was injected subcutaneously into the hind paw immediately after the baseline measurements were obtained.

The intensity of mechanical hyperalgesia was quantified as the reduction in the reaction times and was calculated by subtracting the values measured at 2, 3, and 4 hours after carrageenan injection from the baseline value, that is, change in the reaction time = baseline value – posttreatment value.

Because carrageenan induces maximal hyperalgesia and edema 3 hours after its subcutaneous injection, IFT

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application was initiated 2 hours after the carrageenan injection. The same protocol was used for the control groups, except that the electrical stimulator was maintained in the off position.

Formalin- or Carrageenan-Induced Edema

In separate groups of animals, paw edema was determined by volume displacement of an electrolyte solution in a plethysmometer.[†] The hind paw was immersed to the hairy skin, and volumes were read from a liquid crystal display. Values were determined in triplicate before and 1 hour after the formalin injection and at 2, 3, and 4 hours after the carrageenan injection; these values were subtracted from baselines values to quantify edema as volumes (in milliliters). Because formalin induces spontaneous flinching behavior, rats were anesthetized by inhalation of 2.5% isoflurane immediately before the edema measurements were obtained.

Experimental Design

For nociceptive behavior tests, rats were divided into the following groups: group 1, IFT applied before formalin injection (n=7); group 2, IFT sham control applied before formalin injection (n=8); group 3, IFT applied immediately after formalin injection (n=6); group 4, IFT sham control applied immediately after formalin injection (n=6); group 5, no electrode placed on the hind paw treated with formalin (n=6); group 6, IFT applied 2 hours after carrageenan injection (n=6); and group 7, IFT sham control applied 2 hours after carrageenan injection (n=6).

To investigate the effect of IFT on edema, rats were divided into the following groups: group 8, IFT applied immediately after formalin injection (n=6); group 9, IFT sham control applied immediately after formalin injection (n=6); group 10, IFT applied 2 hours after carrageenan injection (n=6); and group 11, IFT sham control applied 2 hours after carrageenan injection (n=6).

The duration of IFT application was 1 hour in all experimental groups; however, IFT application was initiated 2 hours after the carrageenan injection and 1 hour before or immediately after the formalin injection for the reasons explained above. Immediately after the formalin test, rats were anesthetized by inhalation of 2.5% isoflurane to allow the edema measurements to be obtained.

Data Analysis

A *t* test was used to determine whether there were significant differences in formalin-evoked phases 1 and 2 of nociceptive flinching behavior between the IFT-treated group and the IFT sham control group when these treatments were applied before or immediately after the formalin injection; only the total number of flinches in each period (phase 1 and phase 2) of the formalin test was used for the statistical analysis.

A 1-way repeated-measures analysis of variance (ANOVA) was used to determine whether there were significant differences in phases 1 and 2 of formalin-evoked nociceptive flinching behavior among animals with electrodes kept in place for 1 hour, animals with electrodes kept in place for 2 hours, and animals without electrodes. When there was a significant difference among groups, the Tukey *post hoc* test was used to determine the basis of the significant difference.

A 2-way repeated-measures ANOVA with 1 between-subjects factor (ie, treatment) and 1 within-subject factor (ie, time) was used to determine whether there were significant differences in carrageenan-induced hyperalgesia or edema between the IFT-treated group and the IFT sham control group. Because the effect of time and the time × treatment interaction were not significant in virtually all cases, these results are not shown. When there was a significant between-subjects main effect of treatment group, a *t* test with an alpha level adjusted with a Bonferroni-type correction (eg, $P=.05/3=.01666$ for 3 time points) was used to identify the time points at which there were significant differences among groups. A P level of less than .05 was considered to indicate statistical significance. Data are plotted in figures as mean±SEM.

Results

Effect of IFT on Formalin-Induced Nociceptive Behavior and Edema

The electrical current application during the 1 hour after the formalin injection (Fig. 1A) but not before the formalin injection (Fig. 1B) significantly ($P<.05$, *t* test) reduced both phases 1 and 2 of the formalin-evoked flinching behavior compared with the results obtained for the sham-stimulated control group. There was no significant difference ($P>.05$, Tukey test) in phases 1 and 2 of the formalin-evoked nociceptive flinching behavior between animals with electrodes kept in place for 1 hour (mean±SEM for phases 1 and 2, respectively: 69.0±12.5 and 299.0±46.8), animals with electrodes kept in place for 2 hours (60.4±15.9 and 261.8±12.3), and animals without electrodes (56.2±7.5 and 246±34.4). These results rule out the possibility that the electrodes by themselves could have affected the formalin-induced nociceptive flinching behavior. The electrical stimulation did not change the behavior of naive rats (rats that were not challenged with inflammatory agents), evaluated by licking behavior associated with self-cleaning (data not shown).

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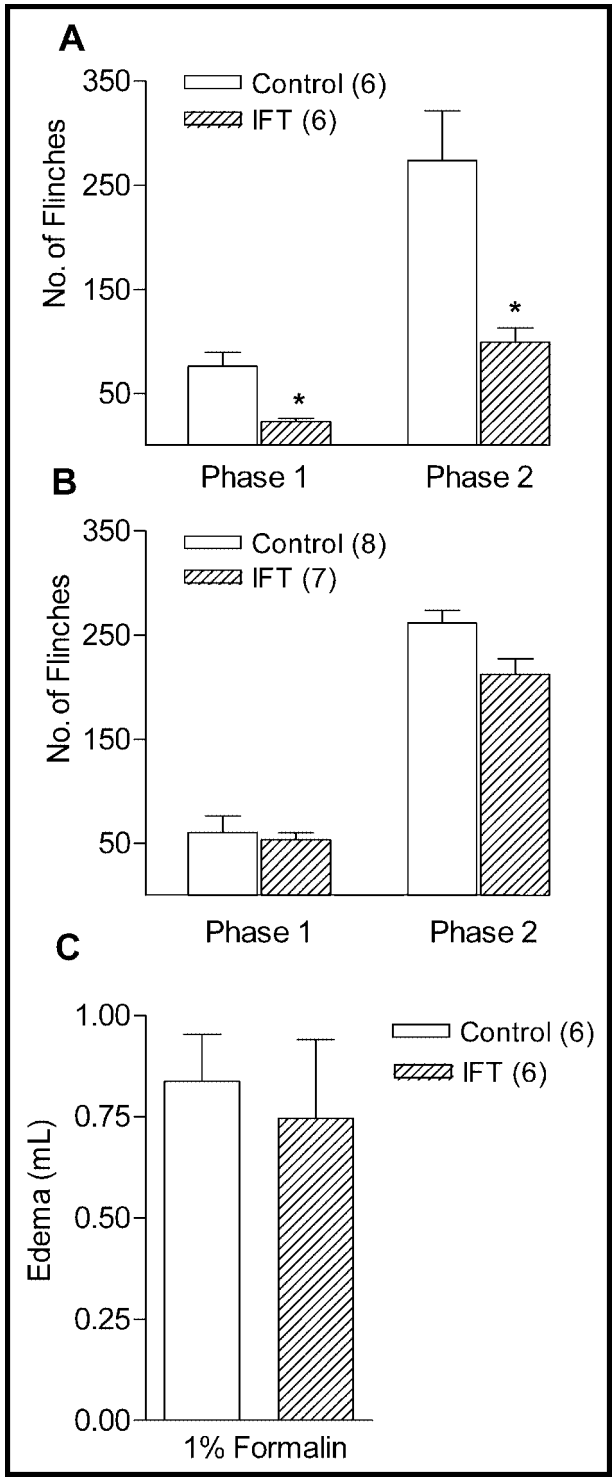

Figure 1.
Effect of interferential therapy (IFT) on the formalin-induced nociceptive response and edema. Results are shown as mean±SEM. (A and C) Application of IFT for 1 hour after the formalin injection significantly reduced formalin-induced flinching (A) but not edema measured at 1 hour after IFT (C). The asterisk indicates a significant reduction in phases 1 (P=.010, t test) and 2 (P=.017, t test) of the formalin-induced nociceptive response compared with the results obtained for the control group. (B) Application of IFT for 1 hour before the formalin injection did not significantly affect phases 1 (P=.703, t test) and 2 (P=.346, t test) of the formalin-induced nociceptive response.

Although IFT significantly reduced formalin-induced nociception, the same current parameters applied for 1 hour immediately after the formalin injection did not significantly (P>.05, t test) change the edema measured immediately after discontinuation of the electrical current application (Fig. 1C).

Effect of IFT on Carrageenan-Induced Mechanical Hyperalgesia and Edema

The subcutaneous injection of carrageenan (100 μg per paw) induced mechanical hyperalgesia and edema that were maximal between 3 and 4 hours after the carrageenan injection. The 1-hour application of IFT at 2 hours after the carrageenan injection significantly (P=.012, t test) prevented a further increase in mechanical hyperalgesia measured immediately after but not 1 hour after discontinuation of the electrical current application (Fig. 2A) and did not significantly (P>.05, 2-way repeated-measures ANOVA) prevent the development of edema (Fig. 2B). There were no changes either in the baseline reaction times in naive animals after IFT application or in carrageenan-induced mechanical hyperalgesia when IFT was applied to the contralateral hind paws (data not shown).

Discussion and Conclusion

In the present investigation, we were able to demonstrate, for the first time, that interferential electrical stimulation is effective in producing antinociception in 2 experimental models used to mimic human inflammatory pain states: carrageenan-induced mechanical hyperalgesia and formalin-induced nociceptive behavior. From a clinical perspective, the experimental use of carrageenan or formalin as an inflammatory agent satisfies the criteria for mimicking inflammatory pain in humans. Nonsteroidal anti-inflammatory drugs inhibit carrageenan-induced hyperalgesia and partially reduce formalin-induced nociception. Furthermore, both models of inflammatory pain are completely blocked by morphine.[20,33–37] Thus, these models serve as valid screens for testing the efficacy of pharmacological agents and similarly can be used to test the efficacy of nonpharmacological agents.[23,38]

Although the local administration of carrageenan causes neutrophil migration, edema, and NSAID-sensitive hyperalgesia, the local administration of 1% formalin causes ordinary mast cell degranulation.[22,23,39] Thus, the major inflammatory mediators involved in the inflammatory pain induced by carrageenan are eicosanoids and sympathomimetic amines, whereas the major inflammatory mediators involved in the inflammatory pain induced by formalin are serotonin and histamine, both of which directly activate sensitized nociceptors.[22] This fact explains why NSAID pretreatment only partially

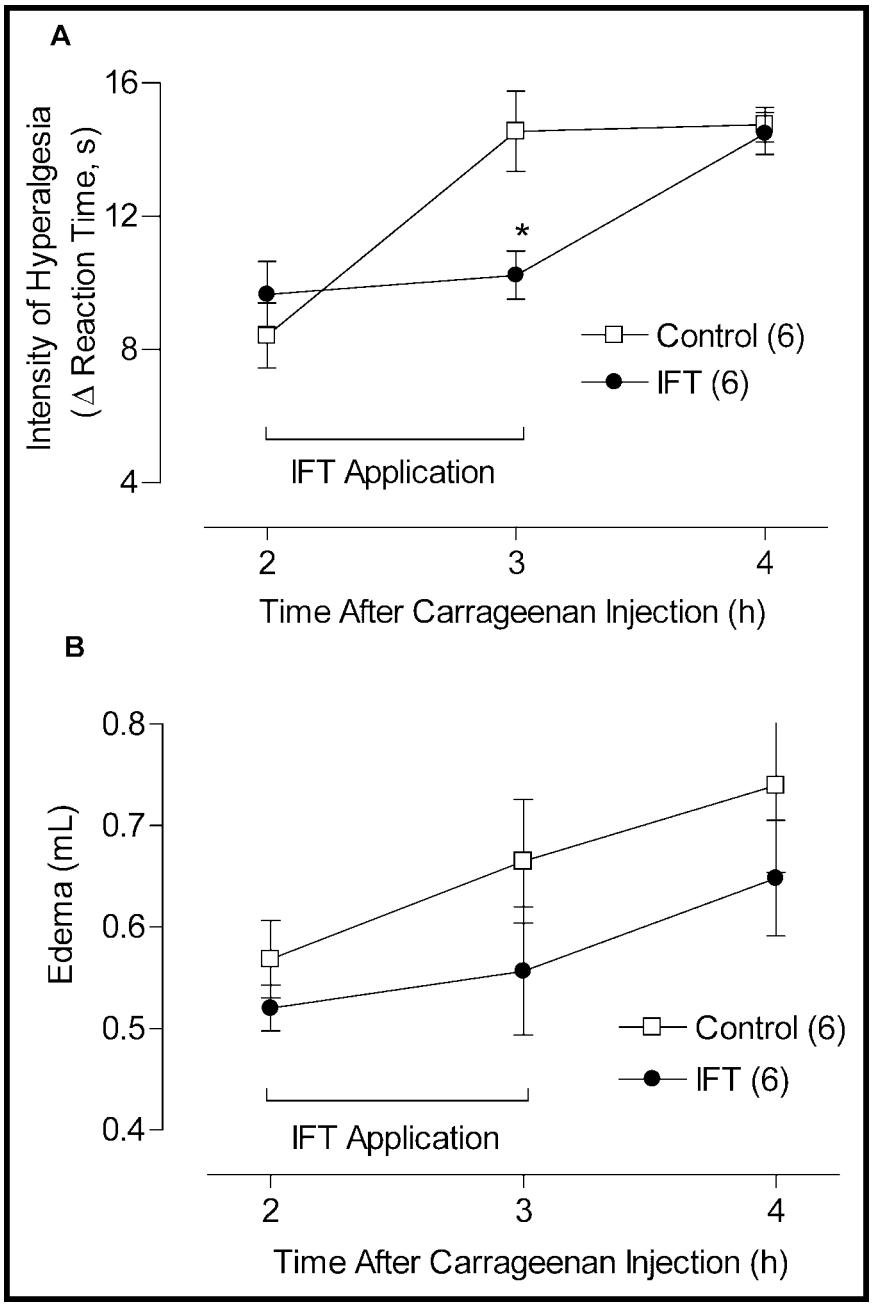

Figure 2.
Effect of interferential therapy (IFT) application on carrageenan-induced mechanical hyperalgesia and edema. Results are shown as mean±SEM. Interferential therapy was applied for 1 hour beginning 2 hours after the carrageenan injection. (A) The asterisk indicates a significant reduction in carrageenan-induced mechanical hyperalgesia ($P=.012$, *t* test) compared with the results obtained for the control group. Δ=change in. (B) There was no significant difference in carrageenan-induced edema between the groups ($P>.05$, 2-way, repeated-measures analysis of variance).

reduces phase 2 of formalin-induced nociceptive behavior.[21,35,40–43]

Although the involvement of serotonin and histamine in carrageenan-induced inflammation and of eicosanoids in formalin-induced nociception has been reported, the relative levels of importance of these inflammatory mediators in the inflammatory pain models are not the same.[22,44,45] In fact, the relative participation of inflammatory mediators, including cytokines, depends on the inflammatory stimulus and on the affected tissue.[5,46]

Importantly, different inflammatory mediators may produce different pain responses. In line with this idea, the subcutaneous injection of formalin induces "overt" pain, characterized by nociceptive behaviors, such as flinching and licking the injected paw, whereas the subcutaneous injection of carrageenan increases the pain response induced by noxious stimulation or induces pain in response to an ordinarily nonnoxious stimulation of peripheral tissue. Thus, the pain response induced by formalin mimics postsurgical or traumatic pain, which is usually referred to as spontaneous pain, and the pain response induced by carrageenan mimics the hyperalgesia that usually follows postsurgical or traumatic pain.

The clinical literature demonstrates IFT to be either effective or ineffective, depending on the pain condition. In line with this idea, IFT has been reported to be effective for cold-induced pain and experimentally induced ischemic pain but ineffective for recurrent jaw pain[47] and for the RIII nociceptive and H reflexes in humans.[15,48–50] However, it is important to emphasize that different types of pain may respond to IFT in very different ways.

Despite the distinct mechanisms involved in the inflammatory pain induced by carrageenan and that induced by formalin, IFT was effective in decreasing both, even when the current was applied after the initiation of the inflammatory pain (Figs. 1A, 2A). Although some *in vitro* experiments have shown that IFT can modulate the release of inflammatory mediators, the mechanism underlying the effect of IFT on inflammatory pain remains unclear.[51,52] Interferential therapy may produce analgesia via the pain gate mechanism.[53] According to this theory, the stimulation of large-diameter afferent fibers inhibits input from

small-diameter afferent fibers. This theory is in line with the idea that high-frequency stimulation (>100 Hz) tends to stimulate A fibers but tends to have relatively little impact on C fibers.[50] Furthermore, under the same experimental conditions as those used to verify the IFT effect on the nociceptive response induced by formalin and on mechanical hyperalgesia induced by carrageenan, IFT did not significantly reduce the edema induced by the local administration of formalin (Fig. 1C) or carrageenan (Fig. 2B). These results suggest that its analgesic effect is not mediated by an anti-inflammatory action. This factor has clear clinical value, in that analgesia does not always include decreased inflammation. For example, opioids, such as morphine, promote analgesia not through an anti-inflammatory action like that of an NSAID that inhibits the cyclooxygenase enzyme but by directly counteracting the sensitization of the primary afferent nociceptors or by blocking nociceptive transmission in the spinal cord. It is well established that opioids administrated either peripherally or in the subarachnoid space block inflammatory hyperalgesia.[54–57]

In agreement with our findings, it has been demonstrated that TENS applied at a high frequency (130 Hz) similar to that used in this study induces transient inhibition of carrageenan-induced hyperalgesia. In contrast, TENS applied at a low frequency (10 Hz) induces a long-lasting and opioid-mediated inhibition of inflammatory hyperalgesia.[58] In addition, neither high- nor low-frequency TENS is able to reduce carrageenan-induced edema in rats.[58,59] Although the analgesia induced by low-frequency TENS is associated with mu opioid receptor activation, it has been suggested that high-frequency TENS reduces the release of glutamate via the activation of presynaptic delta opioid receptors associated with the transmission of thermal hyperalgesia in the spinal cord.[60–62] However, this mechanism may not be the only one involved in the analgesic effect of high-frequency TENS, because the delta opioid receptor antagonist naltrindole does not reverse its analgesic effect in carrageenan-induced mechanical hyperalgesia.[58]

The antinociceptive effect documented in this study with only 1 session of 1 hour of IFT application occurred during and immediately after stimulation, similar to that of a 20-minute high-frequency TENS application.[48] In contrast, some clinical investigations have demonstrated that IFT is an effective method for producing pain relief for up to 1 week and for up to 6 months when used with a twice-daily exercise program in some subjects with osteoarthritic knee pain.[63,64] These studies failed, however, to include controls for placebo effects, and subjects received approximately 10 sessions of IFT. Nevertheless, despite the duration of its effects, it is important to emphasize that, in contrast to the traditional pharmacological approaches used to manage inflammatory pain, IFT produces immediate and strong analgesia. The lack of a long-lasting effect in this study could be attributable either to its mechanism of action or to the parameters and application time used.

In summary, we provide evidence that IFT is effective in reducing inflammatory pain in controlled animal models. This effect, which does not appear to be the result of an anti-inflammatory action, warrants future studies to better understand the mechanism by which this commonly used nonpharmacological treatment reduces pain. Furthermore, despite its short-duration effect, IFT is effective in immediately reducing inflammatory pain and should be considered for use in the control of acute inflammatory pain.

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