

Effect of Moxibustion at Acupoints Ren-12 (*Zhongwan*), St-25 (*Tianshu*), and St-36 (*Zuzanli*) in the Prevention of Gastric Lesions Induced by Indomethacin in Wistar Rats

ANAFLÁVIA O. FREIRE, MD, MS,* GISELE C. M. SUGAI, MD, MS,* MIRIAM M. BLANCO, MS,*
ANGELA TABOSA, MD, PhD,* YSAO YAMAMURA, MD, PhD,*
and LUIZ EUGÊNIO A. M. MELLO, MD, PhD†

This study was aimed at assessing the physical characteristics underlying the action of moxibustion at acupoints Ren-12 (*Zhongwan*), St-25 (*Tianshu*), and St-36 (*Zuzanli*) in preventing acute injuries of the gastric mucous membrane induced by indomethacin in Wistar rats. Induction of gastric lesions, by means of intragastric administration of indomethacin (100 mg/kg), in adult male Wistar rats was followed by treatment with moxibustion using *Artemisia vulgaris* dried leaves at 60 or 45°C, heating with *Artemisia vulgaris* charcoal at 50°C, heating with a regular tobacco cigar at 50°C, and heating with a regular water pad at 50°C. The effects of the different heating protocols over the gastric lesions were then compared. In addition, another group of animals was pretreated with capsaicin (100 mg/kg, s.c.), in order to lesion C fibers and, 15 days later, subjected to indomethacin administration and moxibustion treatment. Moxibustion was significantly more efficient at 60°C than at 45°C in preventing gastric lesions triggered by indomethacin. Moxibustion applied in acupoints provided a significant reduction of the lesion area, which was two times less than that of animals stimulated in a nonacupoint (sham group). Comparing the therapeutic effects provided by different forms of heating over the gastric lesions, the burning of dry leaves of *Artemisia vulgaris* was significantly more efficient in preventing gastric lesions than moxibustion made with *Artemisia* charcoal or tobacco (cigar) or by heating the animal with a water pad. Desensitization of the afferent sensory C fibers by capsaicin significantly diminished the ability of moxibustion to block the lesions in the gastric mucous membrane. Moxibustion can efficiently prevent indomethacin-induced gastric lesions in rats and this effect is dependent on the temperature, the material used for moxibustion, the use of acupuncture points, and the integrity of C fibers.

KEY WORDS: gastric lesions; indomethacin; moxibustion; acupoints; neuronal C fibers.

Moxibustion, the localized heating of specific acupoints, is a basic therapeutic technique used in acupuncture in its

broader sense (1, 2). Moxibustion stimulation is done by means of burning a rod comprised of dried leaves of a plant species (*Artemisia*) in the close vicinity of the same set of points (acupoints) used for needle insertion in the better known form of acupuncture (3). It has been suggested that moxibustion not only cures diseases, but also acts as a preventive agent and strengthens physical resistance of the body (4–7). Moxibustion (*moxa*) has been used for the treatment of a wide variety of infectious, inflammatory,

Manuscript received June 1, 2004; accepted August 12, 2004.

From the *Division of Chinese Medicine and Acupuncture, Department of Orthopedics and Traumatology, and †Departments of Physiology and Psychobiology, Universidade Federal de São Paulo (UNIFESP), São Paulo/SP, Brazil.

Address for reprint requests: Prof. Dr. Luiz Eugênio A. M. Mello, Department of Physiology, Rua Botucatu 862, 04023-062 São Paulo/SP, Brazil; lemello@ecb.epm.br.

allergic, and febrile conditions in both humans and other animals (8–10). The effects of moxibustion are influenced by heat stimulation and the formation of chemical compounds when moxibustion rods are burned. The mechanisms involved in these effects are being studied and some of them have been shown to be based on the marked biochemical and histological influence on cutaneous tissues exerted by the application of moxibustion, depending on the acupoints selected (11). It is also suggested that moxa itself and the combustible product of moxa also inhibit the formation of superoxide (12). In addition, coffee tannins, isolated from leaves of *Artemisia* species, have an inhibitory effect on the radical scavenging mechanism (13).

A1

Heating of wounds with inflammatory processes in the abdominal cavity for alleviating pain and providing faster healing by means of a heating pad are part of broad therapeutic measures used since ancient times (14). In a world dominated by therapies based on the use of chemical compounds, little attention has been devoted to the understanding of the efficacy of, or which detailed mechanisms underlie, the therapeutic effects provided by warming a restricted body part. The similarities between warming of specific skin points by means of moxibustion and warming of a broader skin area by means of a heating pad prompted us to investigate whether these two techniques would provide a similar effect under experimental conditions. The aim of the present study was to investigate (a) if the protective effect of moxibustion in acupoints Ren-12 (*Zhongwan*), St-25 (*Tianshu*), and St-36 (*Zuzanli*) in the prevention of gastric lesions induced by indomethacin in Wistar rats is merely associated with plain thermal stimulation or if other characteristics of how the heat is applied are also important and (b) if C fibers play a role in the mechanism of action of these acupoints in preventing indomethacin-induced lesions of the gastric mucous.

METHODS

Animals

One hundred sixty-six adult, male *Wistar* rats from the Central Vivarium (CEDEME) at our university, weighing 250–300 g at the beginning of the experiment, were used in the current experiments. The rats were housed in groups of five per cage, in a controlled environment with a constant temperature (22°C) and a 12/12-hr light/dark cycle with lights on 0700 hr, and fed with rat chow pellets (Nuvilab, Purina) and water *ad libitum*. The present work was comprised of five different experiments. All experiments were subjected to and approved by the Animal Care and Use Committee of the University.

Induction of Gastric Lesions

The animals were fasted for 12 hr (15), with free access to water, and kept in cages preventing coprophagy. By the time of the

experiment the animals received inhaled ether (Quimesp, Brazil) and were weighed and randomized into the different groups. The immobilized rats received gastric administration of a dose of 100 mg/kg indomethacin (Prodome Química—Farmacêutica, Brazil) dissolved in 5% sodium bicarbonate (Merck, Brazil) to a final concentration of 5 mg/mL, through an orogastric metal probe BD 16 (8 cm × 1 mm; Yall Stainless) (16, 17). The control group received an equivalent volume of saline. Approximately 10 min after the injection of indomethacin the animals were divided into the following groups.

Experiment 1. Here we compared the effects of the application of a moxibustion stick made of dry leaves of *Artemisia vulgaris* 0.7 cm in diameter (W.L. Co. Imp. Exp., China) at acupoints Ren-12 (*Zhongwan*), St-25 (*Tianshu*), and St-36 (*Zuzanli*) at two different temperatures, 45°C ($n = 12$) and 60°C ($n = 12$), on the prevention of gastric lesions induced by indomethacin (indo group; $n = 7$). Heating the point to the temperature of 45°C took 50 sec, and to a temperature of 60°C took 150 sec, with the moxibustion stick at a distance of 5 cm from the skin and using up-and-down movements of the rod, traditionally referred to as “bird drinking water” (1). The temperature was assessed with a thermometer for high temperatures (BD G05; Brazil) placed in the rat’s skin. Upon reaching the desired temperature for a given acupoint stimulation was terminated. Stimulation of acupoints was always performed in the following order: Ren-12 (unilateral), St-25 (bilateral), and St-36 (bilateral). Accordingly, moxibustion stimulation lasted from approximately 5 to 13 min for each animal.

A2

Experiment 2. Here we assessed the effects of application of moxibustion at 50°C at acupoints ($n = 10$) Ren-12 (*Zhongwan*), St-25 (*Tianshu*), and St-36 (*Zuzanli*), compared to the same stimulation applied to nonacupoints (sham) near the used acupoints ($n = 10$), in the prevention of gastric lesions induced by indomethacin (indogroup; $n = 10$). These results were compared to those for animals administered saline rather than indomethacin and stimulated with moxibustion at the above-described acupoints (control; $n = 10$). On average, it took approximately 1 min for the stimulation to reach 50°C at each acupoint. Stimulating procedures were performed in the same manner as described above.

Experiment 3. The effects of the application of moxibustion at 50°C at acupoints Ren-12 (*Zhongwan*), St-25 (*Tianshu*), and St-36 (*Zuzanli*) using three types of materials to heat the points—a rod of dry leaves ($n = 10$) of *Artemisia* (the same as used in clinical practice), a rod of *Artemisia* charcoal ($n = 10$) 11 cm long and 0.7 cm in diameter (which might also be used in clinical practice), and a regular tobacco cigar ($n = 10$) 9 cm long and 0.7 cm in diameter (Chaba-charutos da Bahia, Alagoinhas-BA, Brazil)—were compared with regard to effectiveness in the prevention of gastric lesions induced by indomethacin ($n = 10$).

Experiment 4. Here we investigated the application of moxibustion ($n = 10$) at 50°C at acupoints ($n = 10$) Ren-12 (*Zhongwan*), St-25 (*Tianshu*), and St-36 (*Zuzanli*), compared to the application of moxibustion at nonpoints ($n = 10$) and to the application of a regular water pad at 50°C on the abdomen ($n = 10$) of rats, for prevention of gastric lesions induced by indomethacin ($n = 10$).

Experiment 5. Here we investigated the application of moxibustion at 50°C at acupoints Ren-12 (*Zhongwan*), St-25 (*Tianshu*), and St-36 (*Zuzanli*) for prevention of gastric lesions induced by indomethacin ($n = 5$) in rats submitted to

GASTRIC RECOVERY BY MOXIBUSTION

desensitization of afferent sensory C fibers by capsaicin ($n = 5$), compared to animals treated only with vehicle ($n = 5$).

Sensory Desensitization

The method of Tramontana and colleagues was adopted for ablation of sensory neurons (18). Rats were divided into capsaicin- and vehicle-treated groups. The capsaicin-treated group was treated subcutaneously (s.c.) with a total dose of 100 mg/kg E-capsaicin 99% (Tocris, Bristol, UK), which was given in two equal doses (50 mg/kg) over 2 consecutive days. Another group received equal volumes of vehicle (sterile saline). All injections of capsaicin and vehicle were given under light ether anesthesia (19, 20). On day 15 following the injections, rats received indomethacin and were submitted to treatment as described above.

Procedure for Heating the Acupoints

All of the rats were submitted to tricotomy in selected areas and acupoints were located based on rat anatomical references. The choice of all acupoints used in this paper was based on their reported abilities to stimulate gastrointestinal activity (3, 21). A number of papers on different animal species have clearly shown that the location of acupuncture points follows a similar distribution in different mammals (22–24). Acupoints and neighboring nonacupoints were further characterized by measuring skin conductance for a 5-V pulse applied 10 mm from an acupoint (or nonacupoint). A nonacupoint corresponding to a given acupoint was chosen on the basis of its signals being less than half the conductance measured for that acupoint (25), and the distance we used in this study was 1.5 cm from the real point (sham group). The skin over the acupoint (moxa group) and the nonpoint (sham) determined in this way was marked with a felt pen for subsequent heating.

Measurement of Gastric Lesions

At the end of the experimental procedures (moxibustion stimulation) animals were returned to their cages and 6 hr later were sacrificed by ether asphyxiation (15). The abdomen was immediately opened, the stomach removed, opened along the greater curvature, carefully rinsed in saline, pinned on a cork board, and photographed with a camera (Asahi Pentax Spotmatic, Japan) with two adapted lenses (SQK 49 mm +3 and +4) (15). Then each specimen was fixed in 10% formalin (DGL Chemical Co., Brazil) for 2 days for further detailed evaluation of the lesions. The stomachs were coded in a manner such that the examiner had no knowledge of the treatment given. Assessments of the lesions were performed with a $\times 2$ binocular magnifier and the number of erosions, ulcerations, and petechiae was counted (15, 26). Stomachs were examined macroscopically for the presence of petechiae and ulcers. Ulcers were divided into those larger than 1 mm and those smaller than 1 mm. Lesions were rated systematically in rank order of anatomopathological and clinical significance (15). The number of ulcers was counted and the average number of ulcers per stomach was calculated for each group. Each animal was also rated according to a severity scale. Petechiae received the lowest score: stomachs with fewer than 10 petechiae scored 2 points, and stomachs with more than 10 petechiae scored 3 points. Ulcers smaller than 1 mm were scored as number of ulcers $\times 2$ points, and ulcers larger than 1 mm were scored as number of lesions $\times 3$ points. For most experiments, in addition to the severity scale, we also measured

the area occupied by the lesions. For this, microphotographs of each stomach were scanned (scanner TCE MK 300s connected to a SyncMaster 3 computer) with a zoom of 150% of the original photograph. The scanned images were then printed on paper (75 g/m²), the lesions were carefully cut with fine scissors and weighed on an analytical balance (Mettler AE 200), and the results in grams converted to area values.

Statistics

All values are expressed as mean \pm SEM. The data were evaluated with a one-way analysis of variance (ANOVA). To further establish significance, the Fisher PLSD test was used when the ANOVA showed significant differences. A P value $< 5\%$ was considered significant.

RESULTS

Development of Gastric Lesions by Indomethacin

Orogastric administration of indomethacin produced constant and uniform gastric lesions that were hemorrhagic and linear or dotted in shape (Figures 1 and 2). The lesion index and all other parameters were always higher for the indo group compared to the other groups in all experiments. Gastric lesions were not found in the control group in the second experiment, showing that the application of moxibustion per se did not induce stress-related gastric ulcers.

Effect of the Application of Moxibustion in the Prevention of Gastric Ulcers at Two Temperatures

Moxibustion applied at a temperature of 60°C was significantly more effective in preventing gastric lesions

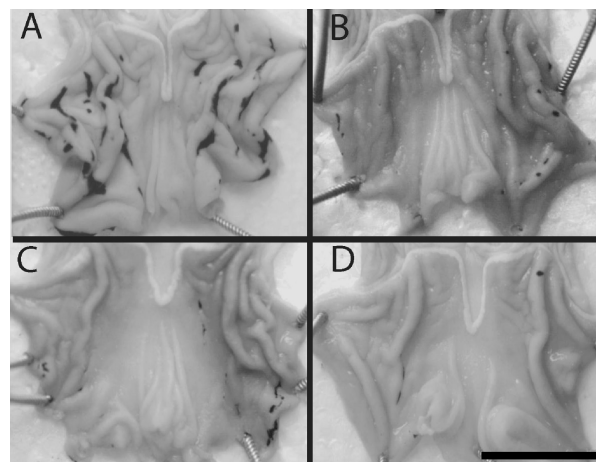


Fig 1. Comparative analysis of lesions in the gastric lining of rats subjected to treatment with indomethacin and later subjected to application of heat at 50°C by moxibustion of dried *Artemisia vulgaris* leaves compared to animals subjected to the same stimulation by means of a water pad at the same temperature. (A) Indo group; (B) water pad group; (C) sham group; (D) moxa group. Black spots are the gastric lesions. Calibration bar = 1 cm.

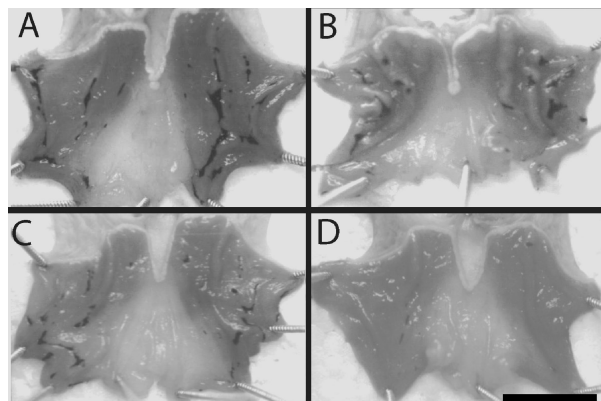


Fig 2. Comparative analysis of lesions in gastric lining of rats subjected to treatment with indomethacin and later subjected to application of heat at 50°C by means of different kinds of materials (regular tobacco cigar, moxibustion with a charcoal of *Artemisia vulgaris*, moxibustion with dried leaves of *Artemisia vulgaris*). (A) Indo group; (B) cigar group; (C) moxa-charcoal group; (D) moxa-*Artemisia* group. Calibration bar = 1 cm.

compared to moxibustion at 45°C (Figure 3). Indeed, the 60°C group was significantly different from the indo group on all of the evaluated parameters ($P < 0.05$). In contrast, for the 45°C group, only the number of petechiae was significantly reduced compared to that for the indo group. Skin lesions associated with the heating process were later observed in the abdomen and hindlimbs of all rats in the 60°C group. For that reason the temperature was adjusted to 50°C in subsequent experiments.

Comparative Effects of the Application of Moxibustion at Acupoints and Nonpoints

Application of moxibustion at acupoints significantly decreased the degree of lesion compared to moxibustion

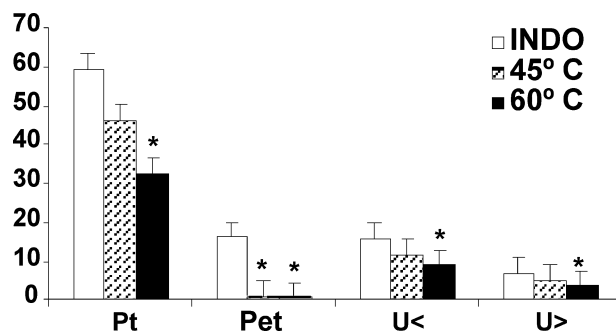


Fig 3. Comparative analysis of lesions in the gastric lining of rats subjected to treatment with indomethacin and later subjected to application of moxibustion at 45 or 60°C. Pt, lesion score; Pet, number of petechiae; U<, number of ulcers smaller than 1 mm in diameter; U>, number of ulcers greater than 1 mm in diameter. Data on lesions expressed as mean \pm SE. * $P < 0.05$ vs indo (Fisher PLSD).

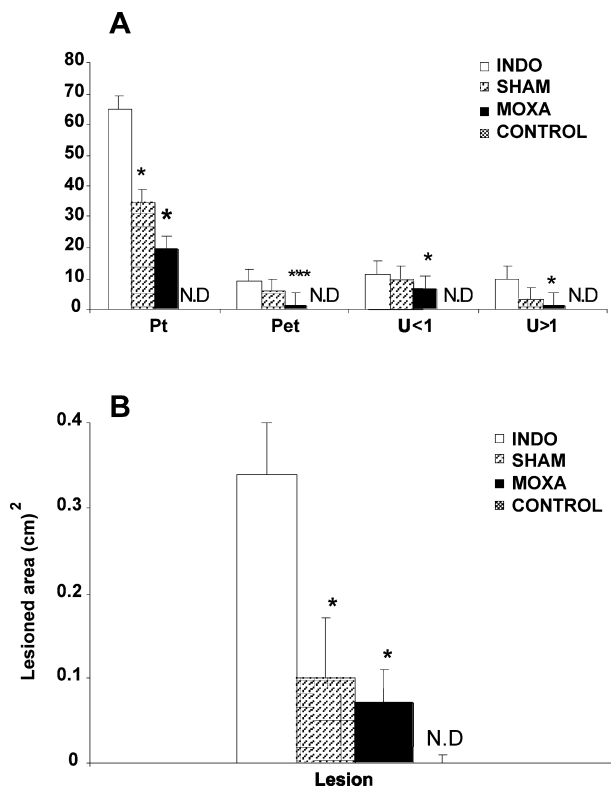


Fig 4. Comparative analysis of lesions in the gastric lining of rats subjected to treatment with indomethacin (indo) and later subjected to application of moxibustion at 50°C at acupuncture points (moxa) or nearby nonacupuncture points (sham). (A) Pt, lesion score; Pet, number of petechiae; U<1, number of ulcers smaller than 1 mm in diameter; U>1, number of ulcers greater than 1 mm in diameter. (B) Lesioned area measured in cm². Data on lesions expressed as mean \pm SE. * $P < 0.05$ vs indo; *** $P < 0.05$ vs sham; N.D., lesions not detectable (Fisher PLSD).

applied at anatomical areas not considered acupoints, i.e., the sham group (Figure 4). No macroscopic gastric lesions were observed in the control group (administered saline rather than indomethacin). The results for the moxa group differed significantly from those of the indo group on all measured parameters. Compared to the sham group, the moxa group had significantly fewer hemorrhagic spots (19%). The mean lesioned area of the stomachs was significantly smaller for both the sham group and the moxa group compared to the indo group; 77 and 88% reductions, respectively.

Effect of the Application of Different Materials to Heat the Acupoints

Figure 5 shows that the Moxa-*Artemisia* group showed effective prevention of gastric lesions at a significant level for all measured parameters compared to the indo group. Indeed the moxa-*Artemisia* group had a significantly smaller lesion area compared to the Moxa-charcoal

GASTRIC RECOVERY BY MOXIBUSTION

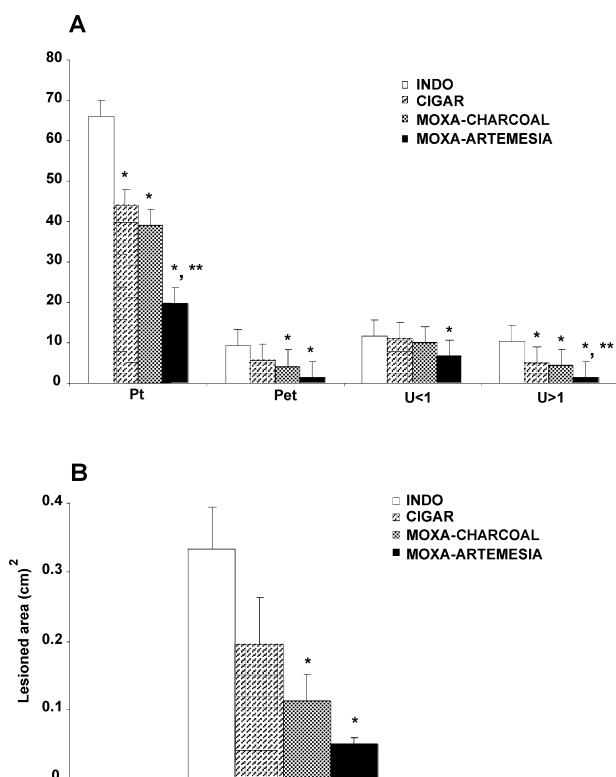


Fig 5. Comparative analysis of lesions in the gastric lining of rats subjected to treatment with indomethacin (indo) and later subjected to application of heat at 50°C by means of different kinds of materials (cigar, moxa-charcoal, moxa-Artemisia). (A) Pt, lesion score; Pet, number of petechiae; U<1, number of ulcers smaller than 1 mm in diameter; U>1, number of ulcers greater than 1 mm in diameter. (B) Lesioned area measured in cm². Data on lesions expressed as mean ± SE. **P* < 0.05 vs Indo group; ***P* < 0.05 vs cigar group (Fisher PLSD).

and cigar groups and differed significantly from the cigar group in both number of large ulcers and lesional score. The moxa-charcoal group showed effective diminution the indomethacin-induced lesions as measured by the number of petechiae and of large ulcers, the score, and the lesioned area. Finally, the cigar group showed effective reduction of lesions only in terms of large ulcers and overall score.

Comparative Effects of the Application of Moxibustion and a Water Pad

Here again the moxa group showed effective prevention of gastric lesions at a significant level for all measured parameters compared to the indo group (Figure 6). The moxa group also differed at a significant level from both the sham and the water pad groups in terms of petechiae, large ulcers, overall score, and lesioned area. Application of a water pad for a total of 5 min over the entire abdomen and hindlimbs was effective only in significantly reduc-

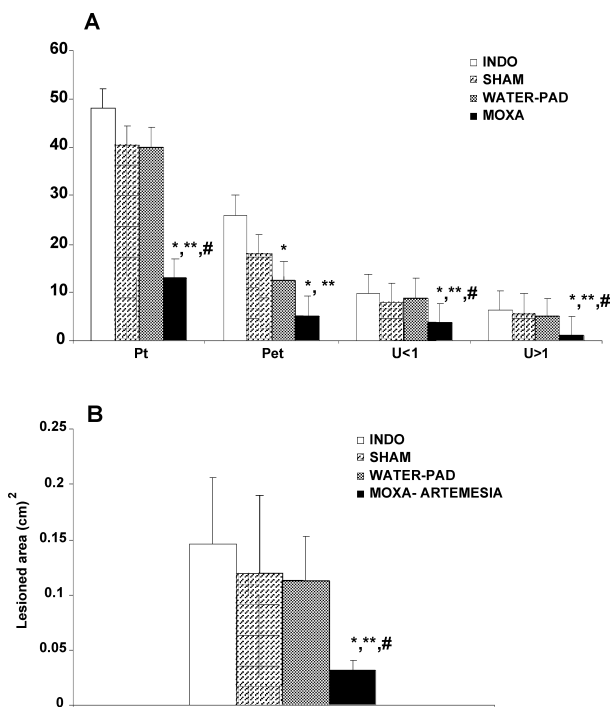


Fig 6. Comparative analysis of lesions in the gastric lining of rats subjected to treatment with indomethacin and later subjected to application of heat at 50°C by means of moxibustion in acupuncture points and nearby nonacupuncture points (sham) compared to a heating produced by a water pad. (A) Pt, lesion score; Pet, number of petechiae; U<1, number of ulcers smaller than 1 mm in diameter; U>1, number of ulcers greater than 1 mm in diameter. (B) Lesioned area measured in cm². Data on lesions expressed as mean ± SE. **P* < 0.05 vs indo group; ***P* < 0.05 vs sham group; #*P* < 0.05 vs water pad group (Fisher PLSD).

ing the number of petechiae compared to that in the indo group. Animals in the sham group did not differ from those in the indo group.

Effect of the Application of Moxibustion in Wistar Rats Submitted to Desensitization of Afferent Sensory C Fibers by Capsaicin

Treatment of the animals with capsaicin tended to block the protection afforded by moxibustion against indomethacin-induced lesions. Capsaicin-treated rats did not differ from indomethacin-treated animals in terms of petechiae, small ulcers, and extent of lesioned area. In contrast, animals not injected with capsaicin (thus injected only with vehicle) but treated with moxibustion differed significantly from the indo group on all of the measured parameters. Capsaicin-treated animals still had significantly fewer large ulcers and a lower overall score compared to indo animals, indicating a partial effect of moxibustion (Figure 7).

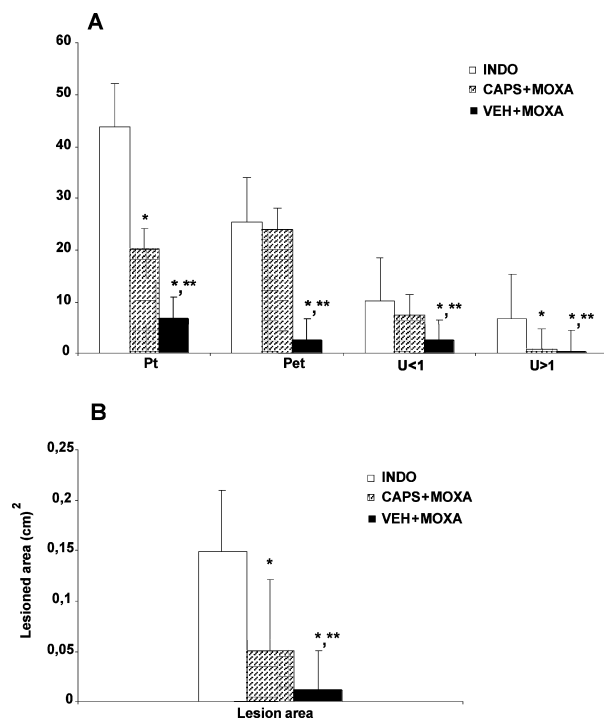


Fig 7. Comparative analysis of lesions in the gastric lining of rats subjected to treatment with indomethacin and capsaicin (CAPS) and later subjected to application of moxibustion (MOXA) at 50°C. (A) Pt, lesion score; Pet number of petechiae; U<1, number of ulcers smaller than 1 mm in diameter; U>1, number of ulcers greater than 1 mm in diameter. (B) Lesioned area measured in cm². Data on lesions expressed as mean ± SE. *P < 0.05 vs Indo group; **P < 0.05 vs vehicle (VEH) group (Fisher PLSD).

DISCUSSION

In the current study we investigated the effects of heat, applied under different conditions, in preventing gastric lesions induced by indomethacin in Wistar rats. The application of heat by means of the moxibustion of dry leaves of *Artemisia vulgaris* applied at acupoints Ren-12 (*Zhongwan*), St-25 (*Tianshu*), and St-36 (*Zusanli*) was significantly more effective in preventing gastric lesions, compared to a number of other procedures, including heating of nonacupoints with the same material as above, heating of the same acupoints with different materials (e.g., a regular tobacco cigar), and general abdominal heating with a warm water pad.

In a previous study in our laboratory, Tabosa and colleagues (25) showed that moxibustion on acupoints in the abdominal area produced effective gastric emptying in immobilized rats. In the current experiments we stimulated a similar set of acupoints with the same type of stimulus and thus should probably have generated a similar type of response as reported previously, i.e., more effective gastric emptying. A possible mechanism by which moxibus-

tion could generate a positive effect in preventing gastric ulcers induced by indomethacin is by means of promoting more effective gastric emptying. Conceivably such an effect could be achieved by either increased gastric motility or relaxation of the pylorus. Jiang and colleagues (27) showed in an experimental model that the motility of the internal anal sphincter could be modulated by somathermal stimulation in the popliteal fossa. Their data suggest that internal anal sphincter relaxation might be through an indirect effect, probably through stimulation of a heat-sensitive sensory and somatic reflex arc.

We have previously shown that acupuncture is effective in increasing and regulating the myoelectric activity of the small intestine (28). This effect was more intense in the proximal muscles of the duodenum. We therefore suggest that moxibustion may have caused both relaxation of the pylorus and more physiological contractions of the muscles in the stomach, speculating that the effect of moxibustion might be related to the modulation of peristalsis, consequently facilitating gastric emptying and thus diminishing the local gastric actions of indomethacin.

There is broad empirical knowledge on the positive effects of warming specific body areas to alleviate pain or discomfort. There is also some recent published evidence indicating that warming might be helpful in clinical practice. Kober and colleagues showed, in two different reports, that local abdominal active warming (with an electric heating blanket set to 42°C) is effective in alleviating pain resulting from acute cholelithiasis (29), and for pain, anxiety, and nausea caused by renal colic (30), in emergency care. Other studies showed positive effects of warming on cardiac pain (31), and also there seems to be some evidence for using warming in the treatment of menstrual discomfort in young women (32). These effects as well as those from our data might be explained by central nervous viscerosomatic convergence in the dorsal horn and supraspinal centers (33). One possible neural pathway for explaining the observed effects of moxibustion is related to the different nerves encountered in the stimulated areas, Ren-12 (*Zhongwan*) and ST-25 (*Tianshu*). Those areas are related to intercostal unisegmental nerves from T₈ to T₁₀ (3), corresponding to the same spinal segments where the sympathetic neurons responsible for the stomach and small intestine are located (34). Thus, heating afferents from the body wall converging with visceral high-threshold mechanical receptors to second- and third-order neurons may alter central viscerosensory and visceromechanical activity processing in the dorsal column, resulting in antinociceptive consequences and peristaltic regularization. However, conclusive data are lacking.

Additional evidence for that neural mechanism is based on embryonic development, which explains how the

GASTRIC RECOVERY BY MOXIBUSTION

visceral pain can be perceived as originating from a somatic area, a phenomenon known as “referred pain.” Actually, examples of such an influence, the somatovisceral reflex, have already been demonstrated (35) and comprise one of the proposed mechanisms of action of acupuncture (36–38). Therefore, it is reasonable to speculate that visceral function, particularly of the stomach in our experiment, is influenced by stimulation of the corresponding somatic area, thus preventing gastric ulcers induced by indomethacin.

We showed that treatment of the animals with capsaicin produced a significant reduction in the ability of moxibustion to prevent gastric lesions induced by indomethacin. Yet even in this case, animals pretreated with capsaicin and subjected to moxibustion stimulation had significantly fewer lesions than animals that were only administered indomethacin. Capsaicin is known to promote reversible lesions to unmyelinated, mostly C fibers, thus providing a valuable tool for dissecting the role of specific neural pathways in a given physiological response (39). The majority of capsaicin-sensitive afferent neurons have small-to medium-sized bodies connected to unmyelinated (C) or thinly myelinated (A δ) nerve fibers. However, not all unmyelinated afferent neurons conducting in the C and A δ fiber range are sensitive to capsaicin (40). The most consistent functional change associated with the neurotoxic effect of capsaicin is a long-term inhibition of chemoreception, polymodal nociceptors, and also some warmth receptors. Visceral afferent neurons sensitive to capsaicin have been found to respond to noxious or potentially noxious, as well as nonnoxious, physiological stimuli. Al-Swayeth and colleagues demonstrated that ablations of capsaicin-sensitive afferent neurons abolish gastric protection by honey (41), indicating a role of these neurons in the protective mechanism of honey. The same experiment also showed that sulcralfate was able to afford protection in the absence of these neurons, possibly through the activation of the prostaglandin system, which is shown to be unaffected following ablation of afferent sensory neurons (42). Similarly in our study the complete protective effect of moxibustion seems to be dependent on intact afferent sensory neurons.

Furthermore, we observed that moxibustion, in contrast to other kinds of heat, such as a water pad, a cigar, and moxibustion using charcoal, produced significantly greater changes in the ulcerogenic power of intragastric application of indomethacin. These findings suggest that the therapeutic effect was related not only to the acupoints but also to the material used to heat the points. The herb used to produce moxibustion, *Artemisia vulgaris*, was not a random choice. It has been used for a long time (1) and has its particular properties. The active

components in leaves of *Artemisia* such as cineol, thujone, adenine, and potassium chloride, have antipyretic, hemostatic and bactericidal effects (12, 13). Our findings offer experimental evidence supporting the use of moxibustion with dry leaves of *Artemisia vulgaris* rather than other kinds of heat stimulation when treating diseases of the gastric system. Moreover, our findings are relevant regarding the comparison of the effects of traditional moxibustion (leaves) with those of more expensive alternative forms (charcoal) and might suggest that electrical moxibustion is also not as effective. It must be stressed, however, that here we did not evaluate the effects of electrical moxibustion.

The temperature used is also an important parameter in reaching the ideal effect. Recently, local somatothermal stimulation, a maneuver similar to local heat therapy or even to moxibustion, has been reported to regulate the activity of the sphincter of Oddi in anesthetized cats, rabbits, and humans (43). The critical temperature evoking such visceral responses was about 41–43°C. In our study we observed that the ideal temperature was higher than that, with 60°C yielding more effective responses than 45°C. Possible reasons for this discrepancy are differences in the stimulation procedures. Thus, while in our experiments heating was performed for only a few minutes at most, in somatothermal stimulation it was applied for a longer time. Therefore it can be speculated that if we had stimulated the acupoints for longer periods at in the same temperature, we could have obtained better results at 45°C rather than 60°C. Nevertheless, it is well known that in the more traditional form of moxibustion, the ancient Chinese heated the acupoints until reaching a noxious stimulus, thus usually leaving a burn scar on the patient’s skin. In fact, this form of cutaneous stimulation generates a long-lasting consequence of the treatment. This procedure, however, was discontinued upon the introduction of acupuncture to the Western world—even though it is still employed in China, Japan, and Korea—given its obvious implications for limiting the acceptance of acupuncture by our culture.

In conclusion, our results show that moxibustion is effective in preventing acute gastric lesions induced by indomethacin. These effects of moxibustion are linked not merely to the thermal characteristics of the stimulation but also to the properties of the herb used for this procedure, *Artemisia vulgaris*, and depend on the integrity of neural pathways, mainly C fibers.

ACKNOWLEDGMENTS

This research was supported by FAPESP (99/06114-9), FAPESP–CEPID, and CNPq. A.O.F is a FAPESP and G.C.M.S and M.M.B are CNPq fellows.

REFERENCES

1. Nghi VN, Tran VD: *Nguyen-Recours: Huangdi Neijing-Lingshu*. Marseille, NVN, Tome I, 1994
2. Nghi VN, Dong MV, Nguyen CR: *Semiologie et Therapeutique en Médecine Énergétique Orientale*, 2nd ed. Marseille, A. Robert, 1985
3. Yamamura Y: *Acupuntura tradicional: a arte de inserir*. São Paulo, Roca, 1993
4. Rogers PAM, Bossy J: Activation of the defense system of the body in animals and man by acupuncture and moxibustion. *Acupunct Res* 5:47–54, 1981
5. Yuan L: Keeping-fit moxibustion. *J Tradit Chin Med* 13(1):7–13, 1993
6. Ma Y, Wang C: Reports on illustrative cases of various diseases effectively treated with moxibustion. *J Tradit Chin Med* 16(2):121–124, 1996
7. Hu G, Chen H, Hou Y: A study on the clinical effect and immunological mechanism in the treatment of Hashimoto's thyroiditis by moxibustion. *J Tradit Chin Med* 13(1):14–18, 1993
8. Yamashita H, Ichiman Y, Takahashi M: Effects of moxibustion on the enhancement of serum antibody in rabbit against *Staphylococcus aureus*. *Am J Chin Med* 26(1):29–37, 1998
9. Okazaki M: Effects of single and multiple moxibustion on activity of platelet function, blood coagulation and fibrinolysis in mice. *Am J Chin Med* 36:67–74, 1990
10. Fang JQ, Aoki E, Seto A, Yu Y, Kasahara T, Hisamitsu T: Influence of moxibustion on collagen-induced arthritis in mice. *In Vivo* 12(4):421–426, 1998
11. Chiba A, Nakanishi H, Chichibu S: Effect of indirect moxibustion on mouse skin. *Am J Chin Med* 25(2):143–155, 1997
12. Ohnishi M: Inhibitory effects of moxa on production of superoxide. *J Jpn Soc Acupunct* 39:132, 1989
13. Kobayashi K: Organic components of moxa. *Am J Chin Med* 16:176–185, 1988
14. Lyons AS: *Medicine: An Illustrated History*. New York, Abrams, 1978
15. Guglietta A, Hervada R, Nardi RV: Computer-based quantitative morphometric analysis of the dynamic characteristics of indomethacin- and ethanol-induced gastric lesions in the rat. *J Pharmacol Meth* 24:73–78, 1990
16. Satoh H, Inada I, Hirata T, Yoshitaka M: Indomethacin produces gastric antral ulcers in the reefered rat. *Gastroenterology* 81:719–725, 1981
17. Takeuchi K, Ueshima K, Hironaka Y, Fuyioka, Matsumo J, Okabe S: Oxygen free radicals and lipid peroxidation in the pathogenesis of gastric mucosal lesions induced by indomethacin in rats. *Digestion* 49:175–184, 1991
18. Tramontana M, Renzi D, Calabro A: Influence of capsaicin-sensitive afferent on acetic acid-induced chronic gastric ulcers in rats. *Scand J Gastroenterol* 29:406–413, 1994
19. Jancsó-Gábor A, Szolcsányi J, Jancsó N: Irreversible impairment of thermoregulation induced by capsaicin and similar pungent substances in rats and guinea pigs. *J Physiol (London)* 206:495–507, 1970
20. Holzer P, Sametz W: Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons. *Gastroenterology* 91:975–981, 1986
21. Xu F, Chen R: Reciprocal actions of acupoints on gastrointestinal peristalsis during electroacupuncture in mice. *J Trad Chin Med* 19(2):141–144, 1999
22. Romita VV, Suk A, Henry JL: Parametric studies on eletro-acupuncture-like stimulation in a rat model: effects of intensity, frequency, and duration of stimulation on evoked antinociception. *Brain Res Bull* 42:289–296, 1997
23. Zhong-Xiang Z: Research advances in the electrical specificity of meridians and acupuncture points. *Am J Acupunct* 9:203–216, 1981
24. Wenbu X: *Tratado de Medicina Chinesa*. São Paulo, Roca, 1993
25. Tabosa AMF, Yamamura Y, Forno ER, Mello LEAM: A comparative study of the effects of electroacupuncture and moxibustion in the gastrointestinal motility of the rat. *Dig Dis Sci* 49(4):602–610, 2004
26. Arroyo MT, Lanás A, Sáinz R: Prevention and healing of experimental indomethacin-induced gastric lesions: effects of ebrotidine, omeprazole and ranitidine. *Eur J Gastroenterol Hepatol* 12(3):313–318, 2000
27. Jiang JK, Chiu JH, Lin JK: Local somatothermal stimulation inhibits motility of the internal anal sphincter through nitrergic neural release of nitric oxide. *Dis Colon Rectum* 43(3):381–388, 2000
28. Tabosa A, Yamamura Y, Forno ER, Mello LEAM: Effect of the acupoints St 36 (*Zuzanli*) and SP 6 (*Sanyinjiao*) on intestinal myoelectric activity of Wistar rats. *Br J Med Biol Res* 35:731–739, 2002
29. Kober A, Scheck T, Tschabitscher F, Wiltshning S, Katzenschlager S, Madei W: The influence of local active warming on pain relief of patients with cholelithiasis during rescue transport. *Anesth Analg* 96:1447–1452, 2003
30. Kober A, Dobrovits M, Djavan B, Marberger M, Barker R, Scheck T: Local active warming: an effective treatment for pain, anxiety and nausea caused by renal colic. *J Urol* 170:741–744, 2003
31. Hartman CH: Response of anginal pain to hand warming: a clinical note. *Biofeedback Self Regul* 4:355–357, 1979
32. Campbell MA, McGrath PJ: Non-pharmacological strategies used by adolescents for the management of menstrual discomfort. *Clin J Pain* 15:313–320, 1999
33. Al-Chaer E, Traub R: Biological basis of visceral pain: recent developments. *Pain* 96:221–225, 2002
34. Kimmy MB, Silverstein FE: *Diseases of the gastrointestinal tract*. In: *The Management of Pain*. Bonica JJ, Loeser JD, Chapman CR, Fordyce WE (eds). Philadelphia, Lea & Febiger, 1990, pp 1186–1213
35. Sato A, Sato Y, Sugimoto H, Terui N: Reflex changes in the urinary bladder after mechanical and thermal stimulation of the skin at various segment level in cats. *Neuroscience* 2:111–117, 1977
36. Guimarães CM, Pinge, MCM, Yamamura Y, Mello LEAM: Effects of acupuncture on behavioral cardiovascular and hormonal responses in restraint-stressed Wistar rats. *Br J Med Biol Res* 30:1445–1450, 1997
37. Omura Y: Patho-physiology of acupuncture treatment: effects of acupuncture on cardiovascular and nervous system. *Acupunct Electro-Ther Rev Int* 1:51–141, 1975
38. Sato A, Sato Y, Suzuki A, Uchida S: Neural mechanisms of the reflex inhibition and excitation of gastric motility elicited by acupuncture-like stimulation in anesthetized rats. *Neurosci Res* 18:53–62, 1993
39. Szolcsányi J, Anton F, Reeh PW, Handwerker HO: Selective excitation by capsaicin of mechano-heat sensitive nociceptors in the rat skin. *Brain Res* 446:262–268, 1988

GASTRIC RECOVERY BY MOXIBUSTION

40. Lawson SN, Harper AA, Garson JA, Anderton BH: A monoclonal antibody against neurofilament protein specifically labels a subpopulation of rat sensory neurons. *J Comp Neurol* 228:262–272, 1984
41. Al-Swayeh OA, Mobarok ATM: Effect of ablation of capsaicin-sensitive neurons on gastric protection by honey and sulcralfate. *Hep Gastrol* 45:297–302, 1988
42. Holzer P, Sametz W: Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons. *Gastroenterology* 91:975–981, 1986
43. Chiu JH, Lui WY, Chen YL, Hong CY: Local somatothermal stimulation inhibits sphincter of Oddi motility in cats, rabbits and humans through the neural release of nitric oxide. *Life Sci* 63:413–428, 1998

Queries to Author

A1: Au: "in the abdominal cavity" as meant? If not, pls, correct sentence.

A2: Au: "Placed" as meant? clarify?