Role of the nucleus tractus solitarii in the protection of pre-moxibustion on gastric mucosal lesions

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Abstract

Previous studies have shown that somatic sensation by acupuncture and visceral nociceptive stimulation can converge in the nucleus tractus solitarii where neurons integrate signals impacting on the function of organs. To explore the role of the nucleus tractus solitarii in the protective mechanism of pre-moxibustion on gastric mucosa, nucleus tractus solitarii were damaged in rats and pre-moxibustion treatment at the Zusanli (ST36) point followed. The gastric mucosa was then damaged by the anhydrous ethanol lavage method. Morphological observations, enzyme linked immunosorbent assays, and western immunoblot analyses showed that gastric mucosa surface lesion and the infiltration of inflammatory cells were significantly ameliorated after pre-moxibustion treatment. Furthermore, the gastric mucosal damage index and somatostatin level were reduced, and epidermal growth factor content in the gastric mucosa and heat-shock protein-70 expression were increased. These results were reversed by damage to the nucleus tractus solitarii. These findings suggest that moxibustion pretreatment at the Zusanli point is protective against acute gastric mucosa injury, and nucleus tractus solitarii damage inhibits these responses. Therefore, the nucleus tractus solitarii may be an important area for regulating the signal transduction of the protective effect of pre-moxibustion on gastric mucosa.

Key Words: nerve regeneration; traditional Chinese medicine; moxibustion; nucleus tractus solitarii; gastric mucosal lesion; heat shock protein-70; epidermal growth factor; somatostatin; NSFC grant; neural regeneration

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Introduction

According to the modern stress theory, moxibustion is a method to stimulate the body’s own potential and to adjust the internal resistance by stimulating relative acupoints. Stress can arouse an endogenous protective mechanism and thus, it belongs to defensive reactions[1]. Investigating the modulation of pre-moxibustion of the gastric mucosal lesion is a field of moxibustion prevention and treatment of disease that is currently receiving great attention[2-4]. With the continuous development of modern medicine, medical researchers are continuously exploring the treatment and drugs of the gastric mucosal lesion. However, drugs produce great damage to the gastric mucosa and human body[5], thus posing the question of whether a non-drug treatment for gastric mucosal lesion exists. Pre-moxibustion (“ni-jiu” in Chinese) is a method of moxibustion that increases healthy energy to prevent or reverse a disease before its onset[6-8]. Modern physicians have discussed the effect of pre-moxibustion on warming the spleen and stomach, and the harmonization of Yin and Yang to prevent spleen and stomach disease. Pre-moxibustion prevention and treatment has been widely applied for gastric mucosal lesions, however, its mechanism of action remains unclear.

Despite the increasing evidence of the protective effects of pre-moxibustion to the gastric mucosa[9-12], their potential mechanisms are unknown. The nucleus tractus solitarii is the lower central neutral core for the vagus nerve. Numerous neurotransmitters and neuromodulators distributed in the nucleus tractus solitarii play crucial roles for adjusting respiratory, cardiovascular, and digestive functions[13-14]. A variety of visceral mechanical and chemical signals, and nociceptive stimuli travel to the nucleus tractus solitarii via vagus nerve fibers. Therefore, the peripheral nerve is in close contact with the central nucleus tractus solitarius. Acupuncture stimulation on the body and the harmful stimulation to inner organs may converge at the nucleus tractus solitarii. Thus, inner organ functions may be ultimately influenced with a “comprehensive contribution”[15].

In addition, many gastrointestinal hormones and factors are involved in the damaging properties of acupuncture on gastric mucosa to stimulate a protective response. This response includes relevant protective factors, such as heat.
shock protein-70 and epidermal growth factor, which regulate gastric mucosa injury and induce protection[16-17]. Moxibustion may have protective effects on the stomach mucous membrane against stress gastric ulcer. The potential mechanism of moxibustion may be mediated by transforming growth factor-α, gastric mucosa cell proliferation, inhibition of apoptosis, and the expression of heat shock protein-70[16].

Previous studies have shown that warming moxibustion treatment suppresses the gastric mucosal lesion, and promotes the restoration of the damaged gastric mucosa by reducing the serum concentration of epidermal growth factor[17].

This present study aimed to analyze the protective effect of pre-moxibustion on the gastric mucosal lesion in rats with bilateral nucleus tractus solitarii damage. Epidermal growth factor and somatostatin content, and the expression of heat shock protein-70 were investigated in gastric mucosa tissue. These studies were performed to determine the effect of pre-moxibustion-mediated endogenous protection on internal organ damage and the information adjustment ability of the nucleus tractus solitarii.

Results

Quantitative analysis of experimental animals
Forty healthy Sprague-Dawley rats were randomly assigned to four groups (10 rats per group): (1) control, (2) model (gastric mucosal lesion produced by the anhydrous ethanol lavage method), (3) pre-moxibustion (moxibustion pretreatment at the Zusanli (ST36) point) + model, and (4) pre-moxibustion + model + nucleus tractus solitarii damage.

Damage to the nucleus tractus solitarii
Hematoxylin-eosin staining showed damage of nucleus tractus solitarii in the pre-moxibustion + model + nucleus tractus solitarii damage group (Figure 1).

Comparison of the ulcer index between groups
Gastric mucosa in the model group was damaged (66.9 ± 15.1), indicating that the model was established successfully. The ulcer index in pre-moxibustion + model group (16.1 ± 8.1) and pre-moxibustion + model + nucleus tractus solitarii damage group (51.2 ± 17.2) was significantly (P < 0.05 and P < 0.01, respectively) decreased compared with the model group. These results indicated the protective effects of moxibustion on the gastric mucosa. The ulcer index in the pre-moxibustion + model + nucleus tractus solitarii damage group was significantly (P < 0.01) higher than that of the pre-moxibustion + model group. This finding demonstrated the pre-moxibustion adjustment effect form the damage of the nucleus tractus solitarii.

Effect of pre-moxibustion on the morphology of the gastric mucosa after damage to the nucleus tractus solitarii
In the control group, the gastric mucosa had an abundant blood capillary, a complete epithelial structure and ordered cell lines (Figure 2A). In the model group, gastric mucosal structures were destroyed, with massive glandular cell necrosis and disordered lines (Figure 2B). The nucleus and cytoplasm in this group were blurry by hyperemia and edema. In the pre-moxibustion + model group, partial abscission was observed on the gastric mucosa surface, with hyperemia in the cells, less infiltration of inflammatory cells, and gastric glands that were healed (Figure 2C). In the pre-moxibustion + model + nucleus tractus solitarii damage group, gastric mucosal surfaces were severely destroyed, with the infiltration of inflammatory cells, and hyperemia in the cells (Figure 2D).

Effect of pre-moxibustion on epidermal growth factor and somatostatin content in the gastric mucosa after nucleus tractus solitarii damage
Epidermal growth factor content in the pre-moxibustion + model group and pre-moxibustion + model + nucleus tractus solitarii damage group were significantly increased to varying degrees (Table 1). Somatostatin content was significantly (P < 0.01 for
pre-moxibustion + model group, and $P < 0.05$ for pre-moxibustion + model + nucleus tractus solitarii damage group) compared with the model group (Table 1). These results suggested that pre-moxibustion up-regulated and down-regulated the level of epidermal growth factor and somatostatin, respectively. Compared to the pre-moxibustion + model group, epidermal growth factor content was significantly ($P < 0.01$) lower and somatostatin content was higher in the pre-moxibustion + model + nucleus tractus solitarii damage group ($P < 0.01$), indicating that the regulatory effects were influenced by nucleus tractus solitarii damage.

**Effect of pre-moxibustion on the expression of heat shock protein-70 in the gastric mucosa after nucleus tractus solitarii damage**

Expression of heat shock protein-70 in the pre-moxibustion + model group and pre-moxibustion + model + nucleus tractus solitarii damage group was significantly ($P < 0.01$) increased compared with the model group. This result indicated that pre-moxibustion promoted the expression of heat shock protein-70 in the gastric mucosa. However, this effect may be influenced by nucleus tractus solitarii damage because heat shock protein-70 in this group was significantly ($P < 0.05$) lower than the pre-moxibustion + model group (Table 2, Figure 3). Therefore, the nucleus tractus solitarii may promote the expression of heat shock protein-70 in gastric mucosal tissue as a mechanism underlying the effects of pre-moxibustion.

**Discussion**

The use of moxibustion as physiological warm stimulation has a distinct advantage for the modulation of a sub-health status and the prevention of ulcer recurrence. Moxibustion can stimulate the human body to produce endogenous protective factors, activate the immune system, and treat gastrointestinal diseases\cite{18-21}. In the present study, results of the ulcer index show that pre-moxibustion at the Zusanli point is protective on gastric mucosal lesions, and that damage to the nucleus tractus solitarii may influence its protective effect.

Hydrochloric acid is one of the main factors in gastric juice that causes gastric mucosal lesions. Schwartz hypothesized in 1910 a “no acid, no ulcer” effect\cite{22}. 

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**Figure 2** Effect of pre-moxibustion on gastric mucosal tissue after damage to the nucleus tractus solitarii (hematoxylin-eosin staining, ×400).

(A) In the control group, the gastric mucosal epithelium structure is intact. (B) In the model group, the gastric mucosal structure is damaged and the gastric mucosal lesion is evident. (C) In the pre-moxibustion + model group, gastric mucosal hyperemia in the cells is improved. (D) In the pre-moxibustion + model + nucleus tractus solitarii damage group, severe damage of the gastric mucosal surface damage is evident, and intracellular congestion is improved despite its visibility. Arrows represent damage to the gastric mucosal structure and necrotic gastric mucosal glandular cells.
Acid may determine the site of the ulcer in the gastric mucosal lesion, but not the insulin site\[26\]. Under continuous, excessive mental stress, a dysfunction in the neuroendocrine system often occurs in the human body, inducing abnormal changes of the gastrointestinal mucosa and ultimately causing a gastric mucosal lesion\[24-26\]. The gastric mucosa has a potential for ego protection, which can be activated as a result of the deterioration caused by damage, thus enhancing protective mechanisms and achieving reconstruction through homeostasis. Therefore, maximizing the potential of triggering a gastric mucosal protective mechanism is the key for the prevention and control of gastric mucosal lesions. Gastric mucosal lesion and repair is a dynamic process. The repair process plays a barrier role when external or internal stimuli are present. Mucosal protection may also increase. The regulatory mechanism is mainly mediated by nerve and body fluid. A variety of growth factors and cytokines are involved in the repair of gastric mucosal epithelial injury. Epidermal growth factor is resistant against the destruction of pepsin, trypsin and chymotrypsin\[32\]. Furthermore, this growth factor inhibits gastric acid and pepsin secretion, increases mucus generation in gastric mucosal cells, stimulates mucosal epithelial cell proliferation, increases mucosal DNA synthesis, prevents the formation of ulcers, and promotes gastric mucosa epithelial cell migration and proliferation\[27-28\]. Somatostatin inhibits the release and activity of gastrointestinal hormones, and inhibits mucosal nutrition factors (such as epidermal growth factor) to prevent excessive hyperplasia on the gastric mucosa\[29\].

Heat shock protein-70 serves as an important protective factor because it is related to stress reactions\[26-31\]. This protein has broad functions, such as self-stabilization, and a combination of immune, anti-apoptotic and -oxidative effects. Tsukimi et al.\[32\] have found that the expression of heat shock protein-70 is significantly increased in acetic acid-induced gastric mucosal injuries, particularly in the basal body of ulcers. Heat shock protein-70 was also slightly increased in the tissue around ulcers during healing. Omeprazole can strongly suppress gastric acid secretion and promote healing of ulcers. These effects were thought to be due to the enhanced expression of heat shock protein-70\[33\]. Therefore, enhanced expression of heat shock protein-70 can accelerate healing of an ulcer. A previous study has shown that heat shock protein-70 is highly related to cell stabilization via apoptosis\[33\]. The addition of meletin hinders the aggregation of heat shock protein-70 mRNA hence decreasing its expression, which may interfere with the repair of injured cells\[34-36\]. Therefore, we propose that heat shock protein-70 plays an important role in the fixation of the gastric mucosa.

Previous studies from our group have revealed the protective effects of pre-moxibustion on acute stress-related gastric injuries. These effects were based on a potential mechanism of up-regulating the expression of heat shock protein-70 by promoting the synthesis of transforming growth factor-alpha, triggering the proliferation of mucosal cells, and suppressing apoptosis\[9, 16\].

The experimental results of the present study demonstrated that in the gastric mucosa, pre-moxibustion at the Zusanli point increased and decreased epidermal growth factor and somatostatin levels, respectively, and up-regulated the expression of heat shock protein-70. These results provide evidence that pre-moxibustion has protective effects on gastric mucosal lesions.

In summary, moxibustion pretreatment at the Zusanli point is protective against acute gastric mucosal injury. The mechanism of action of this treatment may be associated with the induction of heat shock protein-70. The nucleus tractus solitarii may participate in the acceleration of pre-moxibustion on the expression of heat shock protein-70 in gastric mucosal tissue.

The nucleus tractus solitarii may be involved in the signaling pathway of the protective effect of pre-moxibustion on the gastric mucosa, thus playing an important role in signal gathering and integration. Therefore, the nucleus tractus solitarii is an important area for regulating central nervous information as a mechanism for signal transduction for the protective effects of pre-moxibustion.

Materials and Methods

Design

A randomized controlled animal experiment.

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**Table 1** Effect of pre-moxibustion on the level (μg/L) of epidermal growth factor and somatostatin in the gastric mucosa after damage to the nucleus tractus solitarii

<table>
<thead>
<tr>
<th>Group</th>
<th>Epidermal growth factor</th>
<th>Somatostatin</th>
</tr>
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<tbody>
<tr>
<td>Normal control</td>
<td>2,579.3±308.2</td>
<td>207.8±24.4</td>
</tr>
<tr>
<td>Model</td>
<td>2,923.7±251.2</td>
<td>231.8±22.4</td>
</tr>
<tr>
<td>Pre-moxibustion + model</td>
<td>4,037.1±300.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>165.4±11.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pre-moxibustion + model + nucleus tractus solitarii damage</td>
<td>3,176.2±242.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>195.6±21.3&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a</sup>P<0.05, <sup>b</sup>P<0.01, vs. model group; <sup>c</sup>P<0.05, vs. pre-moxibustion + model group; <sup>d</sup>P<0.05, <sup>e</sup>P<0.01, vs. normal control group. Data are expressed as mean ± SD (n = 10) and analyzed by one-way analysis of variance followed by the least significant difference test.

**Table 2** Effects of pre-moxibustion on the expression of heat shock protein-70 in the gastric mucosa after damage to the nucleus tractus solitarii

<table>
<thead>
<tr>
<th>Group</th>
<th>Heat shock protein-70</th>
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<tbody>
<tr>
<td>Normal control</td>
<td>0.52±0.05</td>
</tr>
<tr>
<td>Model</td>
<td>0.54±0.03</td>
</tr>
<tr>
<td>Pre-moxibustion + model</td>
<td>0.74±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pre-moxibustion + model + nucleus tractus solitarii damage</td>
<td>0.64±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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</table>

<sup>a</sup>P<0.01, vs. model group; <sup>b</sup>P<0.05, vs. pre-moxibustion + model group; <sup>c</sup>P<0.05, vs. model group. Data are expressed as mean ± SD (n = 6) and analyzed by one-way analysis of various and the least significant difference test. Values represent the expression of heat shock protein-70/β-actin.
Time and setting
This experiment was conducted in the Key Laboratory of Biological Information Analysis for Acupuncture and Moxibustion, Hunan University of Chinese Medicine, China from April to September, 2012.

Materials
A total of 40 pathogen-free healthy Sprague-Dawley rats (20 females and 20 males, 3–4 months of age, 220–250 g) were provided by the Animal Scientific Center of Hunan University of Chinese Medicine, China from April to September, 2012.

Methods
Nucleus tractus solitarii damage
Rats were anesthetized with 20% urethane (0.6 mL/100 g, intraperitoneal injection) 24 hours after fasting, and then secured on an animal operating table in the prone position. According to the Rat Brain Stereotaxic Coordinates, under sterile conditions, a bone window (1 mm²) was cut through the cranium at 13–14 mm lateral to bregma and 1 mm left or right using a dental drill and straight blade. An insulated electrode (diameter 0.35 mm, tip exposure 0.5 mm) was inserted into the animal’s brain (coordinate: anterior posterior 13.2 mm, 0.9 mm lateral to bregma, 7.7–7.8 mm below the dorsal surface). The bilateral solitary tract nuclei were damaged by anode direct current electricity using the BL-410 Biology Signal System (Taimeng Electronics Co., Ltd., Chengdu, Sichuan Province, China). The anode was connected to the electrode, and the cathode was connected to the operative incision. After surgery, hemostatic sutures were made, and penicillin (160,000 U/d) was intramuscularly injected for 7 days.

Pre-maxibustion
The pre-maxibustion + model group and pre-maxibustion + model + nucleus tractus solitarii damage group were secured on a board 1 week after the operation. After fur removal, an insulated fabric (Jinjiang Yaoxi Reflective Material Co., Ltd., Quanzhou, Fujian Province, China; hole 3 mm) was pasted onto the Zusanli (ST36) point at both sides, approximately 5 mm under the capitulum fibulae. Moxa rolls (Hanyi-Moxi Limited Liability Company, Nanyang, China) were placed 0.5 cm above the skin, and were lit for moxibustion treatment at the Zusanli (ST36) for approximately 30 minutes. Skin temperature was maintained at 42°C by an electronic thermometer (WYX8039, Tonglixing Technology Co., Ltd., Shenzhen, Guangdong Province, China). This treatment was carried out once a day for 8 days.

The gastric mucosal lesion model
Gastric mucosal lesions were established by the dehydrated alcohol lavage method. In brief, approximately 24 hours after pre-maxibustion, rats were given absolute alcohol (99%, 0.6 mL/100 g; Sinopharm Group Co., Ltd., Shanghai, China) using a stomach tube to produce gastric mucosal lesions. Rats in the control group were given physiological saline (0.6 mL/100 g).

Detection of ulcer index
24 hours after model establishment, stomachs were dissected (under anesthesia; 20% urethane) from the pylorus to the cardia along the greater gastric curvature. Stomachs were then gently cleaned with physiological saline, flattened on filter paper, and observed under a microscope (10 x magnification). According to the method of Guth and colleagues, the ulcer index was calculated 24 hours after the last pre-maxibustion treatment.

Morphology of gastric mucosa under light microscopy
Pathological analysis on rat brain tissues was performed 24 hours after damage to the nucleus tractus solitarii, moxibustion, and model establishment. The lesioned gastric mucosal tissues (1.0 cm x 0.5 cm) were washed with normal saline (0°C) and stored in 10% formaldehyde solution for 24–48 hours. Tissues were then embedded in paraffin wax, dehydrated by gradient ethanol, hyalinized by xylene, and re-embedded in paraffin wax. Sections were cut (4-μm thickness) for hematoxylin-eosin staining. Damage to the nucleus tractus solitarii was observed under the light microscope (Olympus, Tokyo, Japan) using the Motic TeK3.1 Graph System.

Measurement of epidermal growth factor and somatostatin content in gastric mucosa via the enzyme linked immunosorbent assay
A small piece of gastric mucosal tissue was sheared, weighed, then added with physiological saline (1.5 mL/400 mg) and homogenized (40 times). Specimens were centrifuged (1,238 x g, 4°C, for 10 minutes), and then remaining tissue was stored at −20°C. Prior to use, samples were equilibrated at room temperature for 20 minutes. Each standard well contained 50 μL standard solution. Each sample well con-
Conflicts of interest: design of the study. Yang Z and Peng Y provided the collection of samples who supplied hematoxylin-eosin staining technology for tissues organization.

Acknowledgments: We would like to thank Professor Zhigao D, Peng L and Chang XR designed the study advance on biophysical characteristics of acute stomach pain. Zhonghua Shiyong Zhongxiyi Zazhi. 2010;26(7):1382-1386.

References


Statistical analysis

Data are expressed as mean ± SD and were analyzed using one-way analysis of variance followed by the least significant difference test. SPSS 16.0 software (SPSS, Chicago, IL, USA) was used for data analysis. Significance was reached at values of P < 0.05 or P < 0.01.

Acknowledgments: We would like to thank Professor Zhigao D, who supplied hematexylin-eosin staining technology for tissues and assays performed by Changsha Well Biotech Company.

Author contributions: Peng L and Chang XR designed the study, performed the majority of experiments and wrote the manuscript. Liu M also performed the majority of experiments. Yan J and Yi SX provided analytical tools and were also involved in the design of the study. Yang Z and Peng Y provided the collection of all the material, in addition to providing financial support for this work. All authors approved the final version of the manuscript.

Conflicts of interest: None declared.

Peer review: This study investigated the protective effect of pre-moxibustion via neural pathways when the nuclear tractus solitarii was damaged. This work provides an in-depth analysis on moxibustion modulation of gastric mucosal lesions and its protective mechanism.

Western immunoblot analysis for heat shock protein-70 in gastric mucosa

Gastric mucosa tissue samples were solubilized in radio- immunoprecipitation assay buffer (Pierce, Woburn, MA, USA) for 30 minutes on ice. The lysate was then centrifuged (1,238 × g, 4°C, for 10 minutes), and the protein concentration was determined according to the manufacturer’s instructions (Wellbio). Equal volumes of reducing sodium dodecyl sulfate loading buffer (× 5) were added and boiled at 98°C for 5 minutes. Total protein samples (10 μg protein/lane) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on 10% polyacrylamide gels. The separated proteins were transferred to polyvinylidene fluoride membranes. Membranes were blocked with 5% skim milk and 5% bovine serum albumin in PBS solution containing 0.05% Tween-20 (Sigma-Aldrich, Beijing, China) for 1 hour at room temperature. Membranes were incubated with rabbit anti-heat shock protein-70 polyclonal antibody (0.2 μg/mL; Proteintech Group, Chicago, IL, USA) at 4°C overnight, then incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:3,000; Proteintech Group) for 1 hour at room temperature. Bands were detected via enhanced chemiluminescence (Thermo, Shanghai, China) for 3 minutes, and developed on X-ray film. A Chemidoc EQ system with Quantity One software (Bio-Rad, Alfred Nobel Drive Hercules, CA, USA) was used to determine the absorbance of protein bands.

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