Effect of moxibustion treatment on cell apoptosis and expressions of heat shock protein and second mitochondrial activator of caspase in acute gastric mucosal lesion of rats

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Abstract

OBJECTIVE: To investigate the effect of moxibustion-acupoint treatment with acupoints of Zusanli (ST 36) and Zhongwan (RN 12) on cell apoptosis and the expressions of heat shock protein (HSP) 60, HSP70 and second mitochondrial activator of caspase (Smac) in rat models of acute gastric mucosal lesion (AGML), and explore the mechanisms underlying protection of gastric mucosal lesion.

METHODS: Twenty-four Sprague Dawley rats were divided into 3 groups, blank controlled group (group A), controlled-point group (group B) and acupoint group (group C), 8 for each. After 8-day moxibustion treatment in group B and C, gastric lavage of anhydrous ethanol was used to created AGML in all three groups. The Guth method was employed to measure the ulcer index (UI) of gastric mucosal lesion and immunohistochemistry used to measure apoptosis with apoptosis index (AI) and examine the expressions of HSP60, HSP70 and Smac.

RESULTS: Compared with group A, the expressions of UI, AI, Smac and HSP60 were markedly elevated in group B (P<0.05 or P<0.01). However the expression of HSP70 showed no obvious change (P>0.05); the expressions of UI, HSP60 and HSP70 were markedly elevated in group C (P<0.01) while those of AI and Smac became obviously suppressed (P<0.01). Compared with group B, the expressions of UI, AI and Smac decreased significantly in group C (P<0.01) while those of HSP60 and HSP70 increased markedly (P<0.01), and the expressions of HSP60 and HSP70 were considerably up-regulated (P<0.01).

CONCLUSION: The moxibustion treatment could alleviate the gastric mucosal lesion caused by anhydrous ethanol, induce the over-expressions of HSP60 and HSP70, and down-regulate the expression of Smac, which could suppress cell apoptosis.

Key words: Moxibustion; Apoptosis; Caspase; Gastric mucosal lesion; Heat shock protein

INTRODUCTION

Occurring under multiple critical circumstances, acute gastric mucosal lesion (AGML) refers to acute stress-induced lesion of human body. It is characterized by erosion, ulceration and hemorrhage of gastric mucosa. Due to its major clinical manifestation of upper gastrointestinal tract bleeding, it is also called acute hemorrhagic erosive gastritis. In recent years, the morbidity rate of AGML is on the rise year by year. In light of its
clinical importance, it is a pressing issue for medical profession to seek an effective intervention for AGML. The protective roles of acupuncture and moxibustion mainly were realized through regulating secretion of gastric acid, strengthening barrier of gastric wall, improving blood flow of gastric mucosa, increasing release of cytokines and enhancing antagonism of radical group lesions etc. Also it was capable of promoting the restoration and healing of ulcers." The present study tried to explore the protective role of moxibustion for AGML and its relation with heat shock protein (HSP) 60, HSP70 and Smac in order to decipher the mechanism that underpinned moxibustion-treatment’s activation of the endogenous pathway for the protection of gastric mucosa. The ultimate objective was to provide fundamental rationale for clinical prevention and treatment of gastric mucosal diseases.

MATERIALS AND METHODS

Animals
Twenty-four SPF grade Sprague Dawley rats, weighted at 180-220 g, were purchased from Shanghai Xi-pu-Bikel Laboratory Animal Ltd.

Equipment
Moxa sticks, with a brand name of Natural Huatuo Nanying, were purchased from Nanyang City Wolong TCM Moxa Wool Factory (model: mg-454, specification: 18 mm × 200 mm). Microtome (USA Type 820 Equipment) was specially designed to confine moxa heat-covered area to 0.2 cm only in diameter. Every acupoint as well as its controlled one was treated for 30 min daily for 8 consecutive days.

Modeling and specimen collection
After 8-day moxibustion treatment, all rats fasted for 24 h and then gastric lavage was performed with 95% anhydrous ethanol (0.6 mL/100 g). 20 h after gastric lavage was performed, gastric specimens were collected.

Outcome measurement
Ulcer index: the GUTH method was employed to calculate ulcer index (UI). It was the total length (in millimeter) of all lesions in a stomach. Ulcer length ≤1 mm (including erosion foci) was scored 1; 1 mm< ulcer length ≤2 mm scored 2; 2 mm <ulcer length ≤3 mm scored 3; 3 mm <ulcer length ≤4 mm scored 4; >4 mm scored 5; ulcer width >2 mm, score was doubled.

Preparation of specimens: the pyloric region was ligated with sutures, and 4% paraformaldehyde 5 mL was injected intra-gastrically. After injection, gastric cardia was ligated. At both ends of two sutures, esophagus and duodenum were removed from the stomach. 10 min later, dissection was performed along the greater gastric curvature. After rinsed thoroughly with normal saline, qualitative filter paper was used to blot-dry normal saline. Then tissue pieces of 0.5 cm ×0.5 cm dimension were collected from gastric antrum and immobilized for 24 h and then gastric lavage was performed with 95%

Reagents
HSP70, HSP60, cell apoptosis and Smac immunohis-tochemical assay reagent kit (Wuhan Boster Inc. China) ; SABC immunohistochemical staining reagent kit, brownish yellow DAB colorant, 0.1 mol/L PBS, TBS buffer solution (Wuhan Boster Inc. China), 20% urethane, 4% paraformaldehyde and other reagents all were analytic grade.

Animal grouping
24 rats were divided randomly into 3 groups: blank controlled group (group A), controlled-point group (group B) and acupoint group (group C), 8 for each.

Localization of acupoints and controlled points
Acupoints of Zusanli (ST 36) and Zhongwan (RN 12) were pinpointed following the methods described in Experimental Acupuncture & Moxibustion, a textbook for National Higher Institutions of Traditional Chinese Medicine in the New Century; their controlled points were 0.5 mm away from them.

Moxibustion treatment
Group C and B were treated with lit moxa at acupoints and controlled points respectively while group A was left alone and nothing done on it. In a supine position, the rats of group B and C were immobilized on the boards. Hair was clipped around treated acupoints and their controlled points. The lit moxa sticks were fixed on an exclusively-made device to make sure that the lit ends were 3 cm apart above those points. The device was specially designed to confine moxa heat-covered area to 0.2 cm only in diameter. Every acupoint as well as its controlled one was treated for 30 min daily for 8 consecutive days.

Detections of HSP60, HSP70 and Smac: the S-P immunohistochemical method was employed for the purpose. The procedure was as follows: 1) 3% H2O2 was added in drops. Humid kit was used at room temperature for 20 min. Redundant fluid was discarded without rinsing; 4) The 1:125 diluted polyclonal rabbit antibody was added in drops. Humid kit was used at room temperature for 20 min. Redundant fluid was discarded without rinsing; 4) The 1:125 diluted polyclonal rabbit antibody was added in drops. Humid kit was used at room temperature for 2 h; 5) Biotinylated goat-anti-rabbit immunoglobulin G was added in drops. Humid kit was used at room temperature for 30 min; 6) Horseradish peroxidase-labeled streptavidin solution was added in drops. Humid kit was used at room temperature for 30 min. Except for steps 1 and 3, all other steps were followed by rinsing with 0.1 M PBS thrice.
for 3 min; 7) DAB coloration method was applied at room temperature. And coloration time was controlled under microscope. Rinsing with distilled water was performed; 8) The slides were slightly re-stained with hematoxylin. After dehydration, hyalination and blocking, the slides were viewed under microscope. PBS was used to replace primary antibody for negative control. Protein positivity was judged by the appearance of brown-yellow granules in cytoplasm or nucleus. The slides were observed under light microscope with the magnification of 10 × 40. For each slide, 5 random viewing fields were selected to count the number of positive cells. Detection of cell apoptosis: immunohistochemical method was applied. The procedure was basically the same as that of HSP60. PBS was used to replace primary antibody for negative control. Positivity was judged by the appearance of brown-yellow granules in nucleus. The slides were observed under light microscope with the magnification of 10×40. For each slide, 5 random viewing fields were selected to count the number of positive cells.

Data analysis
Data were processed with SPSS 14 and expressed as mean ± standard deviation (χ±SD). One-way ANOVA was employed for quantitative data. The LSD method was used for those with homogeneity of variance and Tamhane’s T2 for those with heterogeneity of variance.

RESULTS

UI of acute gastric mucosal lesion in rats
As shown in Table 1, after gastric lavage was performed, the means of UI of group B and C were all significantly higher than that of group A (P<0.01). It indicated that anhydrous ethanol resulted in gastric mucosal lesion and the modeling was successful. And the mean of UI of group C was markedly lower than that of group B (P<0.01). It suggested that the moxibustion treatment of Zusanli (ST 36) and Zhongwan (RN 12) acupoints could alleviate the gastric mucous lesion caused by anhydrous ethanol.

Expressions of AI and Smac in rats with acute gastric mucosal lesion
As shown in Table 2, the expressions of AI and Smac in group B were markedly higher than those of group A (P<0.05 or P<0.01). It suggested that gastric lavage of anhydrous ethanol caused gastric mucosal lesion, increased the cell apoptosis of gastric mucosa and promoted the up-regulated expression of Smac. As compared with two other groups, the expressions of AI and Smac were markedly down-regulated in group C (P<0.01). It suggested that the moxibustion treatment at acupoints of Zusanli (ST 36) and Zhongwan (RN 12) could suppress the cell apoptosis of gastric mucosal lesion and down-regulate the expression of Smac.

DISCUSSION

There are multiple precipitating factors contributing to gastric mucosal lesion. As important source of ulcerogenic factors, some dietary components, such as ethanol, cigarette and bacteria, have the potentials to stimulate and damage gastric mucosa. As a kind of highly conservative stress-induced protein, HSP might be ex-
pressed at a level of ≥15% under such stress conditions as high temperature, hypoxia, viral/bacterial infections and metal intoxication. Under the condition of AGML, according to the principle of adaption, epithelial cells of gastric mucosa could make active adjustment and promote HSP synthesis. From the viewpoint of Moriyama et al, HSP70 played an important role in mucus turnover. Tsukimi et al in their study revealed that HSP70 had a higher expression in reconstructed new cells and it was probably associated with the rapid turnover of ulcerated gastric mucosa. Serving as a molecular companion, HSP60 could also participate in cell apoptosis. Through the treatment of thermal shock, Zhang et al induced the over-expressions of HSP60 and HSP70 that alleviated the condition of AGML caused by severe burns in rats. In previous study by Lin et al, the moxibustion treatment at acupoints of Zusanli (ST 36) and Liangmen (ST 21) could promote the restoration of AGML under the stress of water immersion and boost resistance of gastric mucosa. After moxibustion treatment at acupoints of Zusanli (ST 36) and Liangmen in rats, there was an up-regulated expression of HSP70. This suggested that the treatment at acupoints of Zusanli (ST 36) and Liangmen (ST 21) could induce a high expression of HSP70 in gastric mucosa. After gastric lavage was performed with anhydrous ethanol, the gastric mucous expression of HSP60 in group B was markedly higher than that in group C. It suggested that, under the acute external stimulation, the stress expression of HSP60 was elevated in gastric mucosa. However, high expressions of HSP70 and HSP60 were induced in gastric mucosa of group C. It suggested that the moxibustion treatment at acupoints of Zhongwan (RN 12) and Zusanli (ST 36) could induce high expression of HSP70 and HSP60 in gastric mucosa of rats with AGML.

Smac, discovered in 2000, also known as direct IAP binding protein with low PI, is a kind of pro-apoptotic protein released by mitochondria. It participated in the binding protein with low PI, is a kind of pro-apoptotic Smac, discovered in 2000, also known as direct IAP AGML. HSP70 and HSP60 in gastric mucosa of rats with Zusanli (ST 36) could induce high expression of Smac. As demonstrated by the data of the study, the levels of cell apoptosis and Smac in group B were markedly higher than those in group C (P<0.01). It indicted that the expression of Smac responded to the degree of gastric mucosa ulcer in group B. And the levels of cell apoptosis and Smac in group C declined markedly. It suggested that moxibustion treatment could suppress cell apoptosis and the expression of Smac. Based on that moxibustion-acupoint treatment could induce the expressions of HSP60 and HSP70, suppressed cell apoptosis, and down-regulated the expression of Smac, identified was the mechanism underlying the promoted restoration of AGML, which might provide clinical rationale for its prevention and intervention.

REFERENCES


