

## Effect of electro-acupuncture at Foot-Yangming Meridian on somatostatin and expression of somatostatin receptor genes in rabbits with gastric ulcer

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### Abstract

**AIM:** To discuss the protective effect of electroacupuncture at the Foot-Yangming Meridian on gastric mucosal lesion, somatostatin (SS) and the expression of SS receptor genes (SSR<sub>1</sub>mRNA) in rabbits with gastric ulcer and to further explore the relative specificity of meridians and viscera at gene expression level.

**METHODS:** Forty rabbits were randomly divided into control group (A), gastric ulcer model group (B), Foot-Yangming Meridian group (C), Foot-Shaoyang Meridian group (D) and Foot-Taiyang Meridian group (E). The gastric ulcer model was prepared by infusing alcohol into stomach. Groups C-E were treated with electroacupuncture at points along the above meridians using meridian stimulating instruments for 7 days respectively. By the end of treatment, the index of gastric ulcer was determined, the amount of epidermal growth factor (EGF) and somatostatin was measured by radioimmunoassay (RIA). SS-R<sub>1</sub>mRNA expression in gastric mucosa was determined by RT-PCR.

**RESULTS:** The value of EGF in model group was obviously lower ( $73.6 \pm 14.8$  vs  $91.3 \pm 14.9$  pg/mL,  $P < 0.01$ ) than that in control group. The index of gastric ulcer, content of SS and expression of SSR<sub>1</sub>mRNA in gastric mucosa were significantly higher than those in control group ( $24.88 \pm 6.29$  vs  $8.50 \pm 2.98$  scores,  $P < 0.01$ ;  $2978.6 \pm 587.6$  vs  $1852.4 \pm 361.7$  mIU/mL,  $P < 0.01$ ;  $2.56 \pm 0.25$  vs  $1.04 \pm 0.36$ ,  $P < 0.01$ ). The value of EGF in Foot-Yangming Meridian group was higher than that in model group ( $92.2 \pm 6.7$  vs  $73.6 \pm 14.8$  pg/mL,  $P < 0.01$ ). The index of gastric ulcer, content of SS and expression of SS-R<sub>1</sub>mRNA in gastric mucosa were significantly

lower than those in control group ( $10.88 \pm 3.23$  vs  $24.88 \pm 6.29$  scores,  $P < 0.01$ ;  $1800.2 \pm 488$  vs  $2978.6 \pm 587.6$  mIU/mL,  $P < 0.01$ ;  $1.07 \pm 0.08$  vs  $2.56 \pm 0.25$  mIU/mL,  $P < 0.01$ ). Compared to the model group, the content of SS and expression of SSR<sub>1</sub>mRNA in gastric mucosa in Foot-Shaoyang Meridian group decreased ( $2441.0 \pm 488$  vs  $2978.6 \pm 587.6$  mIU/mL,  $P < 0.05$ ;  $1.73 \pm 0.16$  vs  $2.56 \pm 0.25$  mIU/mL,  $P < 0.01$ ). But the above parameters in Foot-Taiyang Meridian group did not improve and were significantly different from those in Foot-Yangming Meridian group ( $P < 0.05$ ).

**CONCLUSION:** Electro-acupuncture at Foot-Yangming Meridian can protect gastric mucosa against injury. The mechanism may be related to the regulation of brain-gut peptides and the expression of SSR<sub>1</sub>mRNA.

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**Key words:** Foot-Yangming Meridian; Electro-acupuncture; Somatostatin; SSR<sub>1</sub>mRNA

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### INTRODUCTION

Studies have shown that needling the Foot-Yangming (stomach) Meridian can protect gastric mucosa against injury in rabbits<sup>[1-3]</sup>. This study was to further explore its mechanism and specificity in the experimental gastric ulcer model of rabbits and to compare its effect on the index of gastric mucosal lesion, brain-gut peptides and expression of SSR<sub>1</sub>mRNA by investigating the correlation between Foot-Yangming Meridian and stomach.

### MATERIALS AND METHODS

#### Main reagents and instruments

TRIZOL reagent and RT reagent kit as well as TagDNA polymerase were obtained from GZBOD BRL and Pro-

gmego Company respectively. RIA kits of EGF and SS were provided by Beijing SINO-UK Institute of Biological Technology. Hypothermia Eppendorf-flow-temperature centrifuge, Strategene Eagle Eye II figure recognition analytical system (made in USA), Xuanshou Meridian Unblocking Instrument (China Peace Economic Technic Counseling Corporation) were used in the study.

### Preparation of experimental gastric ulcer

Gastric ulcer model was induced by ethanol<sup>[4]</sup>. In brief, the animals were perfused with 100% ethanol (2.35 mL/kg) into the stomach after fasted for 48 h. Twenty-four hours after the perfusion, the animals were allowed to have normal foods. The model group received normal saline (2.35 mL/kg) through an esophageal cannula.

### Grouping and disposing

Forty New Zealand white rabbits of either sex, weighing 1500-2500 g were supplied by the Medical Animal Center of Hunan College of Traditional Chinese Medicine. The animals were randomly divided into 5 groups: control group (A), gastric ulcer model group (B), Foot-Yangming (stomach) Meridian group (C), Foot-Shaoyang (gallbladder) Meridian group (D) and Foot-Taiyang (bladder) Meridian group (E). NS was perfused into stomach of the control group. Dehydrated alcohol was administered intragastrically (i.g.) using a metal tube in B-E groups. C-E groups were treated with electro-acupuncture at points along the meridians of stomach, gallbladder and bladder for 7 d respectively. All the animals were killed at the end of the treatment. Gastric mucous membrane was removed for the detection of the indexes.

### Location of acupoints and method of electro-acupuncture

Acupoints were selected on the basis of *Experiment Acupuncture Science*<sup>[5]</sup> and the method was chosen according to the human body<sup>[6]</sup>. For Foot Yangming (stomach) Meridian, the acupoints of "Neiting", "Jiexi", "Zusanli", "Liangqiu", "Tianshu" and "Liangmen" were chosen as stimulating points. According to the above stimulating points, acupoints in other meridians were selected at the same level of Foot Yangming Meridian in corresponding regions. For example, the acupoints of "Jiexi", "qiuxu", "Yanglingquan", "Qiyangguan", "Daimo", "Jingmen" were selected for Shaoyang Meridian while the acupoints of "Zutonggu", "Shenmo", "Chengjin", "Fuxi", "Beishangdian", "Beixiadian" were selected for Taiyang Meridian.

The method of stepwise stimulation along the meridians was used as previously described<sup>[7]</sup>. Then the instrument entered the state of making program and the single way of running was turned at the running speed of 0.5 second. The stimulation parameters included two-direction narrow pulse and continuous wave with a frequency of 50Hz and a width wave of 0.5ms. The output claps of stimulating instrument were clapped with 6 inserted needles at acupoints. Stimulating excitation was turned from low limber to body. The output intensity of instrument was controlled between "2-3" sections. Acupuncture was performed for 30 min a day for 7 d.

### Index of gastric ulcer measurement

The abdomen was opened with the stomach removed 7 d

after acupuncture. The stomach was then cut from pylorus to cardia. The index of gastric ulcer was calculated as previously described<sup>[8]</sup>.

### Determination of EGF and SS

Samples of gastric mucosa were homogenized and disposed with the test kit according to the manufacturer's instructions. EGF and SS were determined by radioimmunoassay.

### Determination of SS-R<sub>1</sub>mRNA

The expression of SS-R<sub>1</sub>mRNA was analyzed RT-PCR. A small piece of gastric mucosa tissue was cut with a sterilized operating knife and mounted on a mortar stuffed with liquid nitrogen. After the tissue was ground into powders, the total RNA of was extracted by one step method with Trizol reagent kit. The rRNA bands of 28S, 18S and 5S were observed by electrophoresis. The proportion of A260/A280 of the total RNA in all samples was between 1.7 and 1.95. mRNA from 2 µg total RNA in samples was reversed to cDNA at 65°C using oligo (dT)<sub>18</sub> as primer for 5 min. Then, 20 µL RT reaction system comprised of 20 uRNA enzyme inhibitor (offered by Promega corporation), 0.5 mmol/L dNTP, 10u AMV RT enzyme as well as 5xRT buffer. PCR was performed with the follow primers. The sense primer of SS-R<sub>1</sub> was 5'-CAAGAC-GACGCCACCGTGAGCCA-3', antisense primer was 5'-GGGGTTGGCACAGCTGTTG-3'. The sense primer of cyclophilin(cyc) was 5'-CCATCGTGTCATCAT-CAAGGACTTCAT-3' and antisense primer was 5'-TTGCCATCCAGCCAGGAGGTCT-3'. The 50 µL PCR reaction system comprised of 5 µL 10×PCR reaction buffer, 1.5 mmol/L MgCl<sub>2</sub>, 200 umol/L dNTP, 5 µL cDNA template, 0.1 mmol/L specific primers, and 3u Taq DNA polymerase covered by paraffin oil. The PCR conditions for SS-R<sub>1</sub> and cyc cDNA were as follows: predenaturation at 94°C for 2 min; denaturation at 94°C for 30s, annealing at 60°C for 30s, and an extention at 72°C for 30s for 26 cycles; then a final extention at 72°C for 5 min. Ten µL product of PCR was put in 1.5% sepharose to go on electrophoresis in ranks in different groups. The products of SS-R<sub>1</sub> and cyc were 66 bp and 216 bp, respectively. The products of electrophoresis were stained with ethidium bromide and photographed under ultraviolet lamp. The electrophoresis band was scanned by figure recognition analytical system. The proportion of SS-R<sub>1</sub>/cyc was calculated as the relative expression level of SS-R<sub>1</sub>mRNA.

### Statistical analysis

All data were presented as mean ± SD and analyzed by the software of SPSS10.0. Statistically significant differences were calculated by analysis of variance (ANOVA). If the mean square deviation was regular, it was analyzed by LSD test. Otherwise it was analyzed by Dunnett T3 test.

## RESULTS

### Effect of electro-acupuncture on index of gastric ulcer

The highest gastric ulcer index was observed in the model group (24.88 ± 6.29,  $P < 0.01$ ). After electro-acupuncture, the gastric ulcer index in Stomach Meridian group was significantly lower than that in the other groups ( $P < 0.01$ ),

Table 1 Index of gastric ulcer in different groups (mean  $\pm$  SD)

Group	n	Index of gastric ulcer
Group A	8	8.50 $\pm$ 2.98 <sup>b</sup>
Group B	8	24.88 $\pm$ 6.29 <sup>d</sup>
Group C	8	10.88 $\pm$ 3.23 <sup>b</sup>
Group D	8	19.38 $\pm$ 3.66 <sup>d</sup>
Group E	8	24.13 $\pm$ 1.64 <sup>d</sup>

<sup>b</sup> $P$ <0.01 vs group B; <sup>d</sup> $P$ <0.01 vs group C.

Table 2 EGF and SS in gastric mucosa in different groups (mean  $\pm$  SD)

Group	n	EGF(pg/mL)	SS(mIU/mL)
Group A	8	91.3 $\pm$ 14.9 <sup>b</sup>	1852.4 $\pm$ 361.7 <sup>b</sup>
Group B	8	73.6 $\pm$ 14.8 <sup>d</sup>	2978.6 $\pm$ 587.6 <sup>d</sup>
Group C	8	92.2 $\pm$ 6.7 <sup>b</sup>	1800.2 $\pm$ 488.1 <sup>b</sup>
Group D	8	74.9 $\pm$ 9.0 <sup>d</sup>	2441.0 $\pm$ 488.1 <sup>a,c</sup>
Group E	8	65.4 $\pm$ 12.8 <sup>d</sup>	2592.7 $\pm$ 426.8 <sup>d</sup>

<sup>a</sup> $P$ <0.05, <sup>b</sup> $P$ <0.01 vs group B; <sup>c</sup> $P$ <0.05, <sup>d</sup> $P$ <0.01 vs group C.

Table 3 SS-R1mRNA expression in different groups of rabbits with gastric ulcer (mean  $\pm$  SD)

Group	n	SSR1/cyc
Group A	8	1.04 $\pm$ 0.36 <sup>b</sup>
Group B	8	2.56 $\pm$ 0.25 <sup>d</sup>
Group C	8	1.07 $\pm$ 0.08 <sup>b</sup>
Group D	8	1.73 $\pm$ 0.16 <sup>b,c</sup>
Group E	8	2.39 $\pm$ 0.39 <sup>d</sup>

<sup>a</sup> $P$ <0.05, <sup>b</sup> $P$ <0.01 vs group B; <sup>c</sup> $P$ <0.05, <sup>d</sup> $P$ <0.01 vs group C.

indicating that electro-acupuncture at Stomach Meridian could significantly decrease the gastric ulcer index in rabbits with experimental gastric ulcer (Table 1).

### Effect of electro-acupuncture on content of EGF and SS

The content of EGF in the control group was significantly higher than that in the other groups ( $P$ <0.01) except for the control group. However, the content of SS in the Stomach Meridian group was significantly lower than that in the other groups except for the control group ( $P$ <0.05), indicating that electro-acupuncture at Foot-Yangming Meridian could increase the content of EGF in ulcer rabbits and decrease the content of SS (Table 2).

### Effect of electro-acupuncture on SS-R1mRNA expression in gastric mucosa

The expression of SS-R1mRNA in model group was obviously lower than that in control group and there was a significant difference ( $P$ <0.01). The expression of SS-R1mRNA in gastric mucosa after acupuncture treatment was Foot-Yangming Meridian group>Foot-Shaoyang Meridian group>Foot-Taiyang Meridian group. There was a significance difference ( $P$ <0.05), suggesting that

acupuncture at Stomach Meridian had a distinct effect on inhibiting expression of SS-R1mRNA of experimental gastric ulcer (Table 3).

## DISCUSSION

Cytoprotection is referred to the fact that some substances can protect epithelial cells of the digestive tract against injury. Studies showed that acupuncture confers quite good protective effect on gastric mucosal lesion<sup>[1,2]</sup>. The protective mechanism may be as follows. Acupuncture can increase the level of PGE<sub>2</sub> in gastric mucosa<sup>[3]</sup>, inhibit release of GAS, influence metabolism of oxygen free radicals<sup>[9]</sup>, increase gastric mucosal NO and NOS<sup>[10]</sup>, regulate expression of Bcl-2 and Fas and depress gastric mucosal epithelial cell apoptosis<sup>[11]</sup>. Acupuncture at Stomach Meridian can decrease gastric mucosal ulcer index, thus protecting gastric mucosa against injury.

Epidermal growth factor is secreted by salivary gland, duodenum Brunner gland and pancreas<sup>[12]</sup>, which can resist the destructive effect of gastric and pancreatic protease and chymotrypsin, inhibit secretion of gastric acid and gastric protease, promote proliferation of gastric epithelial cells and synthesis of DNA in gastric mucosa, prevent formation of ulcer<sup>[13-15]</sup>. Studies showed that epidermal growth factor can accelerate healing process<sup>[16-18]</sup>. Zhao *et al.*<sup>[19]</sup> showed that electro-acupuncture at "Zusanli" can increase EGF in upper alimentary canal and depress gastric acid secretion. Jin *et al.*<sup>[20]</sup> reported that EGF remains normal in stress rats and electro-acupuncture can relieve the gastric ulcer index, suggesting that EGF plays an important role in protecting gastric mucosa from injury during acupuncture. In our study, acupuncture at Stomach Meridian could increase EGF in gastric mucosa of experimental gastric ulcer rabbits, suggesting that acupuncture at Stomach Meridian can promote the healing of gastric mucosal lesion by regulating EGF.

SS is the main inhibitory hormone in digestive tract and can inhibit release and activity of the gastrointestinal hormones as well as gastric acid secretion and participates in protecting gastric mucosa<sup>[21-23]</sup>. Somatostatin receptors exist in gastrointestinal tract and central nervous system. Among the 5 subtypes of SS-R, SS-R<sub>1</sub> is highly expressed in gastric mucosa and can inhibit secretion of gastrin, histamine and gastric acid and plays an important role in adjusting gastric acid secretion. SS can also inhibit upper gastrointestinal hemorrhage and epithelial hyperplasia<sup>[24-26]</sup>. There are reports on the change of SS in digestive ulcer<sup>[27-29]</sup>. Lin *et al.*<sup>[30]</sup> found that stomach perfusion with alcohol can result in gastric mucosal lesion and increase SS, but after electro-acupuncture at Foot-Yangming Meridian, SS in gastric antrum and medulla is decreased. Wang *et al.*<sup>[31]</sup> showed that under psychological stress, gastric mucosal lesion of rats is associated with level of SS, but after electro-acupuncture at Zusanli, the degree of gastric mucosal lesion is reduced and SS in blood plasma tends to decrease. Chen *et al.*<sup>[32]</sup> showed that Codonopsis pilosula, one of the Chinese herbs, can increase SS in stomach and duodenum mucosa, thus promoting healing of alimentary tract ulcer, suggesting that the level of SS and expression

of SS-R1mRNA are increased in rabbits with experimental gastric ulcer due to the process of compensation feedback.

Konturek *et al*<sup>[33]</sup> found that SS may play a role in inhibiting epithelial proliferation and mucosal healing in stomach. However, Pfeiffer *et al*<sup>[34]</sup> found that epithelial growth factor receptors and SS receptors can increase the healing process of experimental gastric ulcer. Our study showed that the level of SS and expression of SS-R1mRNA in Foot-Yangming Meridian group were significantly lower than those in profler group, suggesting that the effect of SS on cell proliferation is weakened during the process of ulcer healing<sup>[35]</sup>.

The protecting effect of acupuncture at Foot-Sanyang Meridian on gastric mucosal lesion indicates that the action of acupuncture at Foot-Yangming Meridian is the strongest, suggesting that Foot-Yangming Meridian is closely related with stomach. This result accords with traditional meridian theory and clinical practice of acupuncture and has confirmed once again the relative specificity between Foot -Yangming Meridian and stomach.

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