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RAPID COMMUNICATION

Effect of electroacupunture on gastric mucosal intestinal trefoil factor gene expression of stress-induced gastric mucosal injury in rats

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Abstract

AIM: To investigate electroacupunture(EA) at the acupoints of Stomach Meridian of Foot-Yangming(SMFY), Gallbladder Meridian of Foot-Yangming(SMFY) on gastric mucosal intestinal trefoil factor (ITF) gene expression detection in stress-induced rats with gastric mucosal lesion, and to explore the regulatory mechanism and significance of EA-related gastric mucosal protective effect.

METHODS: Forty rats were randomly divided into 4 groups: Blank group, Model group, Model group+EA at acupoints of SMFY group("SMFY group"), and Model group+EA at acupoints of GMFY group(GMFY group). All rats (except blank group) were made model by water immersion and restraint stress (WRS). Then the gastric mucosa tissue in each rat was taken off after assessment of gastric mucosal lesion index(GUI), and the expression of ITF mRNA of the tissues was detected by reverse transcription-polymerase chain reaction(RT-PCR) method.

RESULTS: Compared with Model group(54.3 ± 1.34), the GUI value in SMFY group (31 ± 2.21) decreased significantly($P < 0.01$), so did that in GMFY group (39.8 ± 1.62, $P < 0.05$), meanwhile GUI value in SMFY group was significantly lower than in GMFY group($P < 0.01$). Compared with Model group (0.65 ± 0.01), EA had a tendency to improve the expression of gastric mucosal ITFmRNA gene: such tendency existed in GMFY group (0.66 ± 0.01) but with no signficant difference($P > 0.05$), in SMFY group(0.76 ± 0.01) with an extremely obvious difference ($P < 0.01$), furthermore the expression in SMFY group was significantly higher than in GMFY group ($P < 0.01$).

CONCLUSION: The gastric mucosal protective effect by EA at the acupoints of SMFY and GMFY was related to the expression variance of ITF, indicating certain meridian specificity exists. It could be one proof for the TCM theory "Relative particularity between SMFY and stomach".

Key words: EA; Relative particularity between Stomach Meridian of Foot-Yangming; Gastric mucosal damage; Stress; Intestinal trefoil factor; Gene expression

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INTRODUCTION

Acupuncture is one of the traditional Chinese medicine(TCM) therapeutic techniques that can be traced back at least 2500 years, gaining popularity in the West as an alternative and complementary therapeutic intervention[1-4]; while "Relative particularity between Foot Yangming meridian and stomach", is one of important TCM theories. Such theory was proved by our previous research results[5-7]: acupuncture at acupoints of Sibai (ST 2), Liangmen (ST 21), and Zusanli (ST36), could regulate gastric motion and gastric secretion. Recently, a group of new peptides have been discovered, called trefoil factor family (TFF) or trefoil peptides because of their uniquely distinctive cysteine-rich "three-leaf" secondary structure[8], which probably protects these peptides from the degradation by luminal acid and proteases within the gastrointestinal tract[9]. Intestinal trefoil factor (ITF) belongs to the growing family of trefoil peptides[10]. Most of the researches to date have revealed important roles for ITF in protection and repair against injury to the gastrointestinal mucosa[11-14].

To our knowledge, there existed still no evidence of ITF gene expression in stress-induced gastric mucosal lesion in rats by the treatment of acupuncture. In our present study, ITFmRNA was detected to probe gastric

mucosal protective mechanism of the factor, and to prove the classical TCM theory "Relative particularity between Stomach Meridian of Foot-Yangming(SMFY) and stomach".

MATERIALS AND METHODS

Reagents

Trizol reagent was obtained from Invitrogen Co. (USA). One tube RT-PCR kit was from Promega Co. (USA). Primers for rat ITF and GAPDH were designed by ourselves in accordance with gene sequence in GenBank, synthesized and purified by Gibco BRL Biological Engineering Co. All other reagents were analytically pure.

Animals

Forty spraque-dawley rats weighing 180-250 g, male and female mixture, were used. They were housed three to four per cage at temperature 22 ℃ ± 2 ℃ with a 12/12 h light/dark (0:00 a.m. to 20:00 p.m.) cycle under controlled environment. Rats were fed standard laboratory chow, and water was given *ad libitum*. The rats were kept for 7 d in laboratory for habituation. This study was carried out according to the protocol approved by the Ethical Committee of Hunan University of Traditional Chinese Medicine, Changsha, China.

Animal groups and model

All rats were randomly divided into 4 groups(ten rats in each). The design of the experimental animal groups is shown in Table 1.

Table 1 Design of experimental animal groups

Group/code	Treatment
Group A: Blank group	Untreated rats as normal control group
Group B: Model group	having no EA or other treatment for 7 d, then WRS rat model was established.
Group C: SMFY group	After EA at points of SMFY for 7 d, WRS rat model was established
Group D: GMFY group	After EA at points of GMFY for 7 d, WRS rat model was established

Acupuncture method

Acupoints location was defined by reference of rat-acupoint-atlas[15] and analogy to human body. According to the induction stated above, three pairs of acupoints consisting of Sibai (ST 2), Liangmen (ST 21), Zusanli (ST36), in the Foot Yangming Meridian, were designed, which represent acupoints of different level(head, trunk, limb); thus 3 pairs of acupoints of the Foot Shaoyang Meridian in the same horizontal level were selected: Yangbai(GB 14), Riyue(GB 24), Yanglingquan(GB 34). Acupoint location: Sibai acupoint, at the depression of the infraorbital foramen; Liangmen acupoint, at intersection of the midline between anterior midline and midclavicular line and the middle horizontal line of omphalos and xiphoid.

Pairs of stainless-steel needles of 0.25 mm in diameter were inserted into the acupoints stated above of experimental rabbits(Groups C and D). The needles were connected to the output of an electronic pulse generator, a medical EA stimulator (Model G6805-1, made by Shanghai Medical Electro-apparatus Factory, China), which achieves intermittent-and-irregular wave(intermittent wave:4 Hz, irregular wave:20 Hz, intensity of 6-15 V, the depth of acupuncture of 0.5 cm, constant time of 20 min), while there was a light vibration in the rats' lower limbs.

Induction of gastric mucosal lesion index

Seven days after corresponding treatment, each rat was immobilized in a restraint cage and immersed for 10 h to the height of the xiphoid in a water bath kept at 20 ℃ ± 1 ℃, and then sacrificed under anesthesia with 10% urethane intraperitoneally (1 ml/100 g). The abdomen was opened, and the stomach was removed, opened along the great curvature and the gastric mucosa was carefully examined under a dissection microscope to determine gastric mucosal lesion index(GUI) by the score systems suggested by Guth[16]. Briefly, the stomach mucosa was examined with magnifier for the presence of erosions and scored as follows: (1) for small, round hemorrhagic erosion; (2) hemorrhagic erosions less than 1 mm in length; (3) hemorrhagic erosions between 1-2 mm in length; (4) hemorrhagic erosions between 2-3 mm in length; (5) hemorrhagic erosions longer than 4 mm in length. The score value was multiplied by 2 when the width of erosion was larger than 1 mm. The extent of the lesion (lesion index) is expressed as the sum of the length of these breaks per stomach.

RNA extraction

After the treatment stated above, each rat's gastric mucosal tissue was collected and put in freezing-and-storing tubes and kept in the nitrogen tank quickly. Five samples of each group were selected randomly for experiment. Expression of EGFR mRNA was evaluated with RT-PCR. Total RNA was isolated from gastric mucosal samples using a guanidinium isothiocyanate/phenol chloroform single step extraction kit from Stratagene(Gibco BRL, USA), precipitated in ethanol and resuspended in sterile RNAase-free water for storage at -80 ℃ until use. Total RNA was quantified spectrometrically at 260 nm, and the quality of isolated RNA was analysed on agarose gels under standard conditions.

Reverse transcription reaction

Total RNA (10 μL, about 0.5 μg/sample) was reverse transcribed (RT) using oligo(dT)18 primers 1 μL (30 pmol/L), 5×RT buffer 4 μL(Promega Co.), dNTPs(10 mmol/L) 1 μL, RNasin(20 MU/μL, Promega Co. Madison, America) 0.5 μL, M-MULV reverse transcriptase (200 MU/μL, Promega Co., Madison, America) 1 μL, and DEPC water 2.5 μL in a 20 μL reverse transcription reaction system, and such system was performed at 42 ℃ for 30~60 min, then cooled and centrifuged for several seconds so that target mRNA of total RNA sample was transcribed into target cDNA.

Polymerase chain reaction (PCR)

An aliquot of the same RT product from each sample (1/20

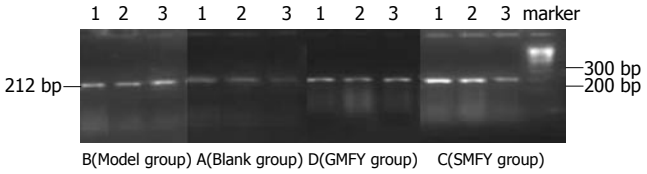

Figure 1 Electrophoresis of ITFmRNA RT-PCR product in gastric mucosal tissue (1, 2 and 3 are randomly selected from each group).

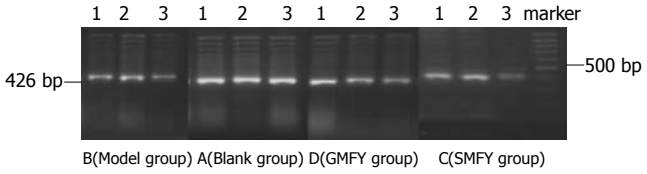

Figure 2 Electrophoresis of GAPDH mRNA RT-PCR product in gastric mucosal tissue (1, 2, and 3 are randomly selected from each group).

of the total volume) was used in the PCR amplification reactions for ITF and GAPDH. The PCR reaction system compound contained 4 μL cDNA, 10×PCR buffer(Promega Co., Madison, America)5 μL, dNTPS (10 mmol/L) 1 μL, oligonucleotide primers sense/ antisense (10 mmol/L) 1 μL (the related primer sequence as stated below), Taqase(5 MU/μL Promega Co.) 1 μL, ddH_2O 32 μL in a total volume of 50 μL. Reaction mixtures were incubated for predenaturation at 94 ℃ for 2 min, followed by 35 cycles for ITF (denaturation at 94 ℃ for 30 s, annealing at 58 ℃ for 1 min, and extension at 72 ℃ for 1 min) and 35 cycles for GAPDH (denaturation at 94 ℃ for 30 s, annealing at 58 ℃ for 30 s, and extension at 72 ℃ for 30 s), and a final extension at 72 ℃ for 5 min.

PCR primer design

By use of the relatively quantitative method to measure ITF-mRNA, rat GAPDH was selected as internal control substance. The primer sequences and sizes of amplification products are as follows: ITF sense, 5' -ACAACCCTGCTGCTGGTCCT -3'; antisense, 5' -TCTGTCTCTTGCAGAGGTTTG-3' (212 base pairs); GAPDH sense, 5'-TGCTGAGTATGTCGTGGAGTC-3'; antisense: 5'- AAGGCCATGCCAGTGAGCTTC-3' (426 base pairs).

RT-PCR product electrophoresis

Five microliter PCR products were analyzed on 10 g/L agarose gel containing ethidiumbromide with TBE buffer at 80 V for 40 min and photographed under UV illumination. The band intensities were quantified by densitometry. ITF and GAPDH PCR products were respectively 212 base pairs(Figure 1), and 426 base pairs(Figure 2). ITF and GAPDH were determined by computer-assisted densitometric scanning. Signals were quantified by density analysis of the digital images using Eagle Eye Ⅱ image software (Stratagene Co.) and ITF/ GAPDH quotient indicated the relative expression of ITF.

Table 2 Effect of EA on GUI and ITF-mRNA expression in gastric mucosal tissue (mean ± SD)

G	GUI score (n = 10)	ITF mRNA/GAPDH mRNA (n = 5)
A(Blank group)	0.3±0.48	0.45±0.01
B(Model group)	54.3±1.34[b]	0.65±0.01[b]
C(SMFY group)	31±2.21[bd]	0.76±0.01[bd]
D(GMFY group)	39.8±1.62[bcf]	0.66±0.01[bf]

[b]$P<0.01$ *vs* blank group, [c]$P<0.05$ *vs* Group B, [d]$P<0.01$ *vs* Group B, [f]$P<0.01$ *vs* Group C.

Experiments were performed in triplicate.

Statistical analysis

The data were expressed as mean±SD of 10 rats per group. Comparison between groups was assessed using one-way analysis of variance (ANOVA) on ranks. Differences were considered statistically significant if the P value was less than 0.05. Software SPSS 10.0 was used in all statistical tests.

RESULTS

Gastric mucosal injury condition and GUI

There were some dots and strips injury detected by the magnifier(10×). The GUI in Model group was highest, while in Blank group lowest, with significant difference between them ($P<0.01$). It demonstrated the ulcer model was successful. Compared with Model group, GUI in SMFY group and GMFY group reduced significantly ($P<0.05$ or $P<0.01$), and the GUI in SMFY group was lower than that in GMFY group($P<0.01$, Table 2).

Effect of ITF-mRNA expression on gastric mucosal tissue

The expression of ITF mRNA using RT-PCR was detected in the intact gastric mucosa of Blank group as a weak signal but it was well-defined among other groups: Model, SMFY, and GMFY groups($P<0.01$). Compared with Model group, EA at acupoints of SMFY group could upregulate significantly the expression of ITF-mRNA expression in gastric mucosal tissue($P<0.01$), while there did not exist difference of expression between GMFY group and model group($P>0.05$), but obvious difference between SMFY group and GMFY group was found ($P<0.01$, Table 2).

DISCUSSION

According to classical TCM theory, SMFY, running from head, via chest and abdomen, along anterior lateral lower limb to foot, is a crucial meridian for its good bidirectional modulation of digestive diseases. Previously, we studied the effect of single acupoint of SMFY on gastric function as well as the whole SMFY's functional mechanism. Acupuncture at acupoints of head and face, trunk, or lower limb (such as acupoint "Sibai", "Tianshu", "Liangmen", "Zusanli", "Shangjuxu"), could produce certain ameliorative effect through the following mechanisms: augmentation of gastric antrum anrea, reinforcement of

pressure power of gastric pyloric sphincter, promotion or inhibition of related gastrointestinal peptide secretion[17-19]. All of these have provided experimental evidence for the theory "Relative particularity between SMFY and stomach".

The cytoprotective functions in protecting gastrointestinal tract against ongoing damage may be accomplished in several ways, and evidences for participation in both the early phase of epithelial repair known as restitution (marked by increased cell migration but no proliferation), and in the subsequent, protracted phase of glandular renewal (marked by proliferation, differentiation and migration) have been published[20-22]. ITF is one of the most recently described members of the trefoil peptide family, a peptide of 59 amino acids, expressed normally by mucus secretory cells of the small and large intestine[9,23]. It is secreted onto the luminal surface of the gastrointestinal tract, and may act in conjunction with the mucin glycoprotein products of goblet cells to promote reestablishment of mucosal integrity after injury through mechanisms distinct from those that may act at the basolateral pole of the epithelium[11,24].

This study assessed for the first time ITF expression by RT-PCR analyses in rat gastric mucosa after exposure to water immersion and restrained stress. It showed that expression of ITF in gastric mucosa was enhanced shortly after the stress, leading us to hypothesize that this process might be mediated by ITF. Meanwhile, it was proved that EA had a tendency to improve the expression of gastric mucosal ITFmRNA gene, and such expression of SMFY group was strongly higher than model group and GMFY group, indicating that the expression discrepancy of ITFmRNA may be the underlying mechanism of different effect of EA at acupoints of SMFY and that of GMFY, thus it could be one proof for the TCM theory "Relative particularity between SMFY and stomach".

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