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Basic Study

Effects of different doses of ginger-partitioned moxibustion on trefoil factor 1, mucin 5AC and epidermal growth factor receptor in rats with spleen deficiency syndrome

不同灸量隔姜灸对脾虚证大鼠三叶因子1、粘蛋白5AC及表皮生长因子受体的影响

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Abstract

Objective: To observe the effects of different doses of ginger-partitioned moxibustion on serum trefoil factor 1 (TFF1) and mucin 5AC (MUC5AC) levels, as well as the expression of epidermal growth factor receptor (EGFR) in gastric mucosa of rats with spleen deficiency syndrome, therefore, to explore the possible mechanism and the dose-effect characteristics of ginger-partitioned moxibustion in spleen deficiency syndrome.

Methods: Seventy-five SPF grade Sprague-Dawley (SD) rats were randomly divided into a blank control group (group A), a model group (group B), a 3 moxa-cone ginger-partitioned moxibustion group (group C1), a 6 moxa-cone ginger-partitioned moxibustion group (group C2) and a 9 moxa-cone ginger-partitioned moxibustion group (group C3) using random number table method, 15 rats in each group. Except group A, rats in the other groups received intragastric administration of 4 ℃ 200% concentrated *Da Huang* (*Radix et Rhizoma Rhei*) to prepare spleen deficiency syndrome model. After successful modeling, rats in group B received no treatment; rats in group C1, C2 and C3 were treated with 3, 6 and 9 moxa-cone ginger-partitioned moxibustion at Zusanli (ST 36) and Zhongwan (CV 12) respectively for 8 continuous days. The general symptom score of rats was observed. The serum levels of TFF1 and MUC5AC were detected by enzyme-linked immunosorbent assay (ELISA). The expression of EGFR protein in gastric mucosa was detected by immunohistochemistry.

Results: After the treatment, compared with group A, the spleen deficiency symptom score was increased in group B, the levels of serum TFF1 and MUC5AC, the EGFR protein expression in gastric tissues of group C1, C2 and C3 were significantly increased (all $P<0.01$); compared with group B, the spleen deficiency scores were decreased in group C1, C2 and C3, and the serum levels of TFF1 and MUC5AC, as well as EGFR protein expression in gastric tissues were increased (all $P<0.01$). Compared with group C1, the spleen deficiency scores were decreased in group C2 and C3, the serum levels of TFF1 and MUC5AC, and the expression of EGFR protein in gastric tissues were increased (all $P<0.01$), however, there was no significant difference between group C2 and C3 (all $P>0.05$). The mechanism may be related to the increase of serum TFF1 and MUC5AC levels and activation of EGFR protein.

Conclusion: Ginger-partitioned moxibustion can improve the symptoms, as well as promote the proliferation and repair of gastric mucosa in rats with spleen deficiency. The therapeutic efficacy of 6 or 9 moxa-cone ginger-partitioned moxibustion is better than that of 3 moxa-cone ginger-partitioned moxibustion, while the efficacies are equivalent between 6 and 9 moxa-cone ginger-partitioned moxibustion groups.

Keywords: Moxibustion Therapy; Ginger-partitioned Moxibustion; Point, Zusanli (ST 36); Point, Zhongwan (CV 12); Research on Acupoints; Gastric Mucosal Damage; Spleen Deficiency Syndrome; Rats

【摘要】目的：观察不同灸量隔姜灸对脾虚证大鼠血清三叶因子1(TFF1)和粘蛋白5AC(MUC5AC)含量，以及胃黏膜表皮生长因子受体(EGFR)蛋白表达的影响，探讨隔姜灸治疗脾虚证的可能作用机制及量效特征。**方法：**将75只SPF级Sprague-Dawley(SD)大鼠按随机数字表法分为空白对照组(A组)、模型组(B组)、隔姜灸3壮组(C1组)、隔姜灸6壮组(C2组)和隔姜灸9壮组(C3组)，每组15只。除A组外，其余各组大鼠采用200%的大黄浓缩液4 ℃灌胃制作脾虚证

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大鼠模型。造模成功后，B组大鼠不予治疗；C1、C2和C3组大鼠分别接受3壮、6壮和9壮隔姜灸足三里和中脘治疗，连续治疗8 d。观察大鼠一般症状评分，采用酶联免疫吸附法(ELISA)检测血清中TFF1和MUC5AC含量；免疫组化法检测胃黏膜EGFR蛋白表达。**结果：**干预结束后，与A组比较，B组大鼠脾虚症状积分增高，C1、C2和C3组大鼠血清TFF1、MUC5AC含量及胃组织EGFR蛋白表达明显升高(均$P<0.01$)；与B组比较，C1、C2和C3组大鼠脾虚症状积分降低，血清TFF1、MUC5AC含量及胃组织EGFR蛋白表达升高(均$P<0.01$)；与C1组比较，C2和C3组大鼠脾虚症状积分降低，血清TFF1、MUC5AC含量及胃组织EGFR蛋白表达升高(均$P<0.01$)，但C2组与C3组差异无统计学意义(均$P>0.05$)。**结论：**隔姜灸能改善大鼠脾虚症状，促进脾虚证大鼠胃黏膜的增殖修复，其作用机制可能与提高血清TFF1和MUC5AC含量，激活EGFR蛋白的表达相关，且灸9壮和6壮的疗效优于灸3壮，但灸9壮和灸6壮的效果相当。

【关键词】灸法；隔姜灸；穴，足三里；穴，中脘；穴位研究；胃黏膜损伤；脾虚证；大鼠

【中图分类号】R2-03　　**【文献标志码】**A

Spleen deficiency syndrome is one of the common syndromes of traditional Chinese medicine. It is often seen in gastrointestinal mucosal diseases such as peptic ulcer and chronic gastritis. Studies have found that rats with spleen deficiency syndrome show damaged or shedding gastric mucosa, mucosal focal erosion, different degrees of reduction of gastric main cells, parietal cells and gastric glands, as well as the compensatory state of gastric parietal cell ultrastructure[1]. Therefore, some people think that spleen deficiency syndrome is the integration of multi-system and multi-functional abnormality based on the characteristics of digestive system morphology and dysfunction; gastrointestinal mucosal lesions and their corresponding dysfunction may be one of the pathological basis of spleen deficiency syndrome.

Studies have shown that trefoil factor 1 (TFF1) has the role in protection of gastric mucosa and repair of the injury[2-4]. On one hand, it plays a physiological function through the combination with Mucin 5ac (MUC5AC) ligand; on the other hand, it's involved in the reconstruction of gastric mucosal injury by inducing the epidermal growth factor receptor (EGFR) mediated signaling pathway. A large number of clinical studies have shown that acupuncture treatment has exact therapeutic efficacy on deficient cold of spleen and stomach, and the role of moxibustion therapy is more obvious[5]. Our previous study showed that ginger-partitioned moxibustion could not only improve the clinical symptoms of patients with spleen deficiency syndrome, but also had antioxidant effect[6]. Focused on TFF1, our current study explored the protective effect and the dose-effect characterization of ginger-partitioned moxibustion on the gastric mucosa in rats with spleen deficiency syndrome on the basis of our previous studies, therefore, to provide evidence for the clinical application of ginger-partitioned moxibustion in treatment of spleen deficiency syndrome.

1 Materials and Methods

1.1 Animals and groups

Seventy-five SPF grade Sprague-Dawley (SD) rats, weighing 200-250 g, were purchased from Hunan SJA Laboratory Animal Co., Ltd., China [license: SCXK (Hunan) 2011-0003]. Rats were randomly divided into a blank control group (group A), a model group (group B), a 3 moxa-cone ginger-partitioned moxibustion group (group C1), a 6 moxa-cone ginger-partitioned moxibustion group (group C2) and a 9 moxa-cone ginger-partitioned moxibustion group (group C3), 15 rats in each group by random number table method. Disposal of experimental rats followed the guidance of the *Guiding Opinions on the Treatment of Experimental Animals* issued by the Ministry of Science and Technology.

1.2 Reagents and instruments

Moxa stick (Nanyang Wolong Chinese medicine moxa factory, China); ginger slices (2-3 mm in thickness, 1.8 cm in diameter); EGFR kit (Proteintech Group, Inc., USA); paraffin, neutral gum (Sigma, USA); hematoxylin, PBS buffer, citrate buffer, two-step kit (Wellbio Ltd., USA); DAB kit (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., China); conventional chemical reagents (Shanghai Sinopharm Biomedical Co., Ltd., China); shaker (Kylin-bell Instrument Manufacturing Co., Ltd., China); incubator (Beijing 61 Instrument Factory, China); microwave oven (Midea Group, China); slicers (Lycra, Germany); slicing machine (Zhejiang Jinhua City Yi Di Medical Equipment Co., Ltd., China); embedding machine (Changzhou Zhongwei Electronic Instrument Co., Ltd., China); ordinary refrigerator (Hefei Rongshida Sanyo Electric Co., Ltd., China); precision PH meter (Leici-Shanghai Instrument Technology Instrument Co., Ltd., China); microscope (Motic Mike Audi Industrial Group Co., Ltd., China); coverslips and slides (Haimen Yuantai Experimental Equipment Factory, China).

1.3 Modeling and model evaluation

Models were prepared according to the method developed by Peng Y and others[7]: except rats in group A, all rats in other four groups received intragastric administration of 4 ℃ and 200% concentrated *Da Huang* (*Radix et Rhizoma Rhei*) [10 mL/(kg·bw)], twice a day for 14 continuous days. The body weight, diet and water consumption, coat color, stools, mental status and behavior change were observed.

Evaluation criteria of spleen deficiency syndrome animal model were made according to the literature[7]. Main symptoms: diarrhea or prolapse of anus; eat less or anorexia. Secondary symptoms: ematiation, weight

loss; dispirited appearance, loose limbs, coat haggard; twisted and stacking together; easy fatigue. Spleen deficiency syndrome model was successful when two main symptoms and two secondary symptoms appeared simultaneously (Table 1).

1.4 Acupoints

Point positioning was conducted according to the method normally used for animal and personification control method based on *Experimental Acupuncture Science*[8]. The navel is the intersection of the lower 1/4 and upper 3/4 on the line between sternoclavicular symphysis and pubic symphysis.

Zusanli (ST 36): At back lateral of the knee joint, and about 5 mm below the capitulum fibulae.

Zhongwan (CV 12): At midpoint on the line between the umbilicus and xiphoid process, about 20 mm above the navel.

1.5 Treatment and sampling

Group A: Rats were not subject to modeling, with a 15 min bundling each day but without moxibustion for 8 continuous days.

Group B: Rats received a 15 min bundling each day without moxibustion for 8 continuous days after successfully modeling.

Group C1: Rat's hair was shaved (around 2.0 cm × 2.0 cm) at bilateral Zusanli (ST 36) and Zhongwan (CV 12) to expose local skin tissue, and the points were disinfected with ethanol treated cotton ball. Rats were fixed in a supine position with a self-made rat clip, and subjected to moxibustion treatment when they were still. Fixed the ginger slices (2-3 mm in thickness, 1.8 cm in diameter) with moxa cones on the acupoints, 3 cones daily (about 15 min) for constant 8 d.

Group C2: The treatment before moxibustion, materials and acupoints used for moxibustion were all same as in group C1. Ginger-partitioned moxibustion was performed for 6 cones (about 30 min) each day for 8 continuous days.

Group C3: The treatment before moxibustion, materials and acupoints used for moxibustion were all same as in group C1. Ginger-partitioned moxibustion was performed for 9 cones (about 45 min) each day for 8 continuous days.

Rats in each group were fasted for 24 h and deprived of water for 6 h at the end of treatment. Blood samples were collected after anesthesia using 10% urethane (10 mL/kg·bw). After the belly was opened, stomachus pyloricus and gastric cardia were ligated with hemostatic forceps, then the esophagus and duodenum were cut off to separate the whole stomach. The stomach was opened along the arcus major ventriculi, and the stomach residue was rinsed with iced saline. On the super-clean bench, 1.0 cm × 0.5 cm size of tissue was sampled from the gastric mucosa appearing obvious injury, rinsed with saline, and fixed with 4% paraformaldehyde at 4 ℃ for 24 h. The 5 μm thick slices were used for enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry assay.

1.6 Observation items

1.6.1 General symptom observation

The stools, mental state and behavior changes were observed; the body weight, food intake and pulling tail resistance duration were recorded; and the score was quantified according to the syndromes of animal models with spleen deficiency syndrome[7] and the total points were calculated before the experiment, on the 14th and the 22th experimental day respectively.

With the two symptoms of loose stools and less diet, and the total score ≥4 points indicated the successful spleen deficiency modeling. The score ≤5 points was mild degree, 6 to 10 points was moderate degree, >10 points was severe degree.

Table 1. Quantitative score table for symptoms of animal model with spleen deficiency syndrome

Symptom	0 point (asymptomatic)	1 point (mild)	2 points (moderate)	3 points (severe)
Loose stools	No, forming	Semi-forming, slightly loose, positive -1 min pull tail for defecation test	Loose stools, positive -1 min pull tail for defecation test	Loose stools, perianal foul, strong positive -1 min pulling tail for defecation test
Poor appetite	No	Reduced appetite ≥10%, <20%	Reduced appetite ≥20%, <40%	Unwilling eating all the day
Body weight growth rate	≥10%	≥7%, <10%	≥4%, <7%	<4%
Fatigue	No fatigue, fast reaction, pulling tail resistance duration ≥60 s[1)]	Slightly tired limbs, faster reaction, pulling tail resistance duration ≥30 s, <60 s[1)]	Less movement, fatigue, pulling tail resistance duration ≥5 s, <30 s[1)]	Powerless, getting together, pulling tail resistance duration <5 s[1)]
Fur	Glossy fur	Slightly glorious, less luster	Fluffy and not glorious	Withered and dull

Note: 1) Rats were suspended by caught the tail with hand. The time started to count was when the rat stopped struggle and used as pulling tail resistance duration, which could reflect the degree of fatigue in rats

1.6.2 Serum TFF1 and MUC5AC

Serum TFF1 and MUC5AC were determined by ELISA.

Coated antibody: the specific antibody globulin was diluted with a coating buffer to the optimal concentration, and added into wells by 0.3 mL/well; kept at 4 ℃ overnight or 37 ℃ water bath for 3 h, then stored in refrigerator. Wash: removed the coating buffer and washed the wells with washing buffer for 5 min × 3 times. 0.2 mL of the TFF1 or MUC5AC containing buffer was respectively added to each well and incubated at 37 °C for 1-2 h. Wash: removed the buffer and washed the wells with washing buffer for 5 min × 3 times. Added 0.2 mL of the enzyme labeling specific antibody solution diluted with dilution buffer and incubated at 37 °C for 1-2 h. Wash: removed the buffer and washed the wells with washing buffer for 5 min × 3 times. Added 0.2 mL of substrate solution to each well, incubated at room temperature for 30 min (for the blank control, 0.4 mL substrate and 0.1 mL terminator). Adding terminator: added 0.05 mL 2 mol/L H_2SO_4 or 2 mol/L citric acid per well. Observation of the results: visual observation or measured the integral optical density (IOD) value with a standard colorimeter at 492 nm [substrate: o-phenylenediamine (OPD)].

1.6.3 EGFR protein

The EGFR protein was determined by immune-histochemistry.

The slices were heated at 60 ℃ for 45 min; dewaxed to water: in xylene for 10 min × 2 times, then in 100%, 95%, 85% and 75% ethanol for 5 min, and washed with distilled water for 5 min; repair of the antigen by heating: immersed the slices in 0.01 mol/L citrate buffer (pH 6.0) and heated to boiling by electric furnace or microwave oven and switched off the power, repeated twice with an interval of 5-10 min; washed with 0.01 mol/L PBS (pH 7.2-7.6) for 3 min × 3 times after cooling. Added 3% H_2O_2, incubated at room temperature for 10 min to inactivate endogenous enzyme, and washed 3 min × 3 times with PBS. Incubation with primary antibody: added appropriate dose of diluted EGFR primary antibody, incubated at 4 ℃ overnight, rinsed 5 min × 3 times with PBS; incubation with secondary antibody: added 50-100 μL anti-rabbit IgG antibody-HRP polymer, incubated at 37 ℃ for 30 min, washed with PBS for 5 min × 3 times.

DAB color: added 50-100 μL pre-prepared DAB working solution, incubated at room temperature for 1-5 min, and controlled the reaction time under microscope, and washed with distilled water. Counterstained with hematoxylin for 5-10 min × 2 times and rinsed with distilled water, blued with PBS; dehydrated with 60%, 70%, 80%, 90% and 100% alcohol, 5 min for each concentration. Exposed to xylene for 10 min × 2 times, and sealed with neutral gum. Observed under microscope, and analyzed using Image-Pro-Plus image analysis software.

1.7 Statistical analysis

The SPSS 21.0 statistical software was used for data processing and analysis. Normally distributed measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). One-way ANOVA was used for comparison among groups. The least significant difference (LSD) and Student-Newman-Keuls (SNK) *Q*-test were used for data with homogeneous variance; Tamhane's T2 or Dunnett's T3 was used for data with heterogeneous variance; paired *t*-test was used for self-comparison before and after intervention. Rank-sum test was used if the data did not fit the normal distribution. $P<0.05$ indicated that the difference was statistically significant.

2 Results

2.1 Comparison of syndrome scores in rats with spleen deficiency syndrome among groups

After modeling, in addition to rats in group A, all the other rats showed a slow increase of body weight, decreased appetite and activity, curled up, with loose stool and dull hair; the syndrome scores of spleen deficiency in group B, C1, C2 and C3 were higher than those before modeling, as well as in group A ($P<0.01$), suggesting that the model was successful. After treatment with ginger-partitioned moxibustion, the symptoms of spleen deficiency were not obvious in group C1, C2 and C3, and the syndrome scores of spleen deficiency were lower than those of group B (all $P<0.01$).

Compared with group C1, the syndrome scores of spleen deficiency were lower in rats of group C2 and C3 ($P<0.01$), but there was no significant difference in the syndrome score of spleen deficiency between group C2 and C3 ($P>0.05$),(Table 2).

Table 2. Comparison of syndrome scores of spleen deficiency among groups ($\bar{x} \pm s$, point)

Group	*n*	Before modeling	After modeling	After treatment
A	15	0.91±0.48	1.11±0.64	1.15±0.10
B	15	1.08±0.47	6.18±0.67[1)2)]	6.11±0.73[1)2)]
C1	15	1.03±0.35	5.97±0.45[1)2)]	2.45±0.37[3)]
C2	15	0.96±0.31	6.05±0.52[1)2)]	1.62±0.45[3)4)]
C3	15	1.02±0.41	6.13±0.57[1)2)]	1.47±0.53[3)4)]

Note: Compared with the same group before modeling, 1) $P<0.01$; compared with group A, 2) $P<0.01$; compared with group B, 3) $P<0.01$; compared with group C1, 4) $P<0.01$

2.2 Comparison of serum TFF1 and MUC5AC levels

After the intervention, the levels of serum TFF1 and MUC5AC in the other groups were significantly higher than those in group A ($P<0.01$). Compared with the model group, the levels of serum TFF1 and MUC5AC in

group C1, C2 and C3 were increased after treatment with ginger-partitioned moxibustion (P<0.01). Compared with group C1, the levels of serum TFF1 and MUC5AC in the group C2 and C3 were increased (P<0.01), while the difference was not statistically significant between group C2 and C3 (P>0.05). This indicated that there was no significant difference in the regulation of serum TFF1 and MUC5AC levels between the 6 and 9 moxa-cone ginger-partitioned moxibustion groups (Table 3).

Table 3. Comparison of serum TFF1 and MUC5AC levels among groups ($\bar{x}$ ±s, ng/mL)

Group	n	TFF1	MUC5AC
A	15	3.58±0.43	127.75±18.92
B	15	4.29±0.51[1)]	160.43±21.54[1)]
C1	15	5.53±0.47[1)2)]	229.51±27.37[1)2)]
C2	15	7.68±0.62[1)2)3)]	277.86±32.41[1)2)3)]
C3	15	7.92±0.58[1)2)3)]	291.64±40.25[1)2)3)]

Note: Compared with group A, 1) P<0.01; compared with group B, 2) P<0.01; compared with group C1, 3) P<0.01

2.3 Comparison of EGFR protein expression in gastric mucosa

At the end of the intervention, the EGFR protein expression in gastric mucosa of the other groups was significantly higher than that of group A (all P<0.01); compared with group B, the EGFR protein expression in rat gastric mucosa of group C1, C2 and C3 was increased (P<0.01).

EGFR protein expression in gastric mucosa of group C2 and C3 was higher than that in group C1 (P<0.01), but there was no significant difference between group C2 and C3 (P>0.05), indicating no significant difference in regulation of EGFR protein expression in gastric mucosa between 6 and 9 moxa-cone ginger-partitioned moxibustion (Table 4).

Table 4. Comparison of EGFR protein expression in gastric mucosa of rats among groups ($\bar{x}$ ±s)

Group	n	EGFR protein (IOD)
A	15	48.25±7.32
B	15	62.48±10.69[1)]
C1	15	74.56±13.15[1)2)]
C2	15	88.53±12.78[1)2)3)]
C3	15	93.37±15.36[1)2)3)]

Note: Compared with group A, 1) P<0.01; compared with group B, 2) P<0.01; compared with group C1, 3) P<0.01

3 Discussion

Gastric mucosal injury is the main pathological factor leading to acute and chronic gastritis, gastric ulcer and other diseases, mainly reflected by the increased gastric mucosal attack factors and decreased gastric mucosal self-protection/defense, while the gastric mucosal injury is closely related to both the pathological process and syndrom of spleen deficiency[9]. With the establishment of the diagnostic standard of spleen deficiency syndrome, various animal models of spleen deficiency were successfully replicated. At home and abroad, many institutes have confirmed that feeding rhubarb decoction to animals is the most stable method to establish of the spleen deficiency model[10], after study and exploring of the functional changes, physiological and biochemical changes, as well as the immunocompromice.

Chinese medicine indicates that 'a strong spleen helps to defend against invasion of pathogen', suggesting that the spleen and stomach are closely related to the defensive function, meanwhile, spleen and stomach are intrinsically linked with the digestive system, especially the local defensive function of the gastric mucosa.

Therefore, it should focus on the spleen and stomach to correct the pathological basis of spleen and stomach deficiency, and improve the gastric mucosal self-protection/defensive function during the treatment.

Moxibustion is one of the external therapies in traditional Chinese medicine. The warm stimulation by moxibustion can produce a good warm and steady effect, thus playing a role in disease prevention and treatment[11]. Ginger-partitioned moxibustion is the comprehensive treatment combining pungent-warm and moving nature of ginger with dispelling cold to circulate blood and warm to dredge the meridian of moxibustion. Ginger and moxibustion produce synergies to complement each other. Experiments have confirmed that ginger partitioned moxibustion could significantly improve the general symptoms of experimental spleen deficiency rats, and correct the indiscriminate plasma β-endorphin (β-EP), motilin (MTL) and somatostatin (SS) levels in rats with spleen deficiency, which was better than conventional mild moxibustion[10]. Moxibustion must reach a certain dose to produce the best therapeutic effect. And the moxibustion time is an important factor affecting the dose of moxibustion, therefore, to explore the effect of different dose of moxibustion in ginger-partitioned moxibustion is essential to improve the clinical efficacy.

TFF1 is a class of small molecules secreted by the gastrointestinal tract, and its specific site can be bound by the carbohydrate chain of the mucin to form a stable gel complex and stabilize the gastrointestinal slime layer, thus to play an important role in protection and repair of the gastrointestinal tract[12]. Ren JL, *et al*[13] pointed out that TFF1 expression was higher in human peptic ulcer and drug-induced rabbit gastric ulcer, suggesting that it plays an important role in gastric mucosal

protection and epithelial reconstruction. Li TL, *et al*[14] found that moxibustion treatment could heal the gastric ulcer caused by spleen deficiency by increase of the TFF1 expression level and repair of the damaged gastric mucosa. MUC5AC is a family of mucin, and secreted by epithelial cells. The main role of MUC5AC is to maintain lubrication of the cavity organs (such as the gastrointestinal tract, respiratory lumen). MUC5AC plays an important role during the repair of the damaged gastrointestinal mucosal barrier, and is also an important biological marker for predicting the development, progression and prognosis of gastric cancer[15]. EGFR has a small amount of expression in normal gastric mucosa epithelial cells, intrinsic membrane cells and mucosal muscle cells. Of which, the distribution is the most in the gastric mucosal epithelial cells, playing a very important role during the repair of gastric mucosal injury. EGFR expression is increased during the gastric mucosal injury[16]. Liu XL, *et al*[17-18] showed that MUC5AC and EGFR protein expressions were significantly decreased, when the self-defense ability of the gastric mucosal was decreased in rats with spleen deficiency. *Bu Zhong Yi Qi* decoction can improve the rat spleen syndrome and stimulate the mechanism of gastric mucosal reconstruction, thus to achieve gastric mucosal repair, by increasing the expression of MUC5AC and EGFR proteins.

The results of this study showed that the serum TFF1 and MUC5AC levels, as well as the expression of the EGFR protein in the gastric mucosa of the model group were increased in the successful rat model of spleen deficiency, which indicated that the gastric mucosa had some self-repair function when it was damaged by initiation of cell proliferation. It's similar to the findings of Li TL, *et al*[14], while it's different from that of Liu XL, *et al*[17-18]. The reason may be related to the duration of intragastric administration of concentrated *Da Huang* (*Radix et Rhizoma Rhei*), which was 4 d less in the study conducted by Liu XL, *et al*[17] than our current study, and may not reach enough stimuli to induce gastric mucosal self-repair function. In addition, this may also be related to the quality of herbs, constitution of the experimental animals, the physical existence of a certain relationship, which are to be further validated in the future experiments. The levels of serum TFF1 and MUC5AC, as well as the EGFR protein in the gastric mucosa were further increased in rats of each group after ginger-partitioned moxibustion at Zusanli (ST 36) and Zhongwan (CV 12), therefore, we inferred that ginger-partitioned moxibustion promoted the proliferation and repair of gastric mucosal injury in rats with spleen deficiency syndrome, by up-regulation of TFF1 expression and binding with the specific mucin MUC5AC, as well as co-activation of EGFR.

At the same time, the results showed that the levels of TFF1 and MUC5AC, as well as the expression of EGFR protein in gastric tissue were higher in the 6 and 9 moxa-cone ginger-partitioned moxibustion groups versus the 3 moxa-cone ginger-partitioned moxibustion group, suggesting that in a certain range, the proliferation and repair of gastric mucosa will be enhanced with the dose increase of moxibustion. However, the effect was similar in 6 and 9 moxa-cone ginger-partitioned moxibustion groups, indicating that it is not that the greater the amount of moxibustion, the stronger the effect.

In summary, ginger-partitioned moxibustion can promote the proliferation and repair of gastric mucosa in rats with spleen deficiency syndrome. The mechanism may be related to the increase of serum TFF1 and MUC5AC levels, as well as activation of EGFR protein expression. Within a certain range, the proliferation and repair of gastric mucosa will be enhanced with the dose increase of moxibustion.

Conflict of Interest

The authors declared that there was no potential conflict of interest in this article.

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Statement of Human and Animal Rights

The treatment of animals conformed to the ethical criteria in this experiment.

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