Effect of acupuncture at different meridian acupoints on changes of related factors for rabbit gastric mucosal injury

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AIM: To explore the regularity of multi-meridians controlling a same viscus (MMCSV).

METHODS: The rabbit gastric ulcer model was established by ethanol intragastric instillation. Fifty-six rabbits were randomly divided into normal group, model group (MG), model plus acupuncture at Foot Yangming Meridian group (YMG), model plus acupuncture at Foot Taiyin Meridian group (TYG), model plus acupuncture at Foot Shaoyang Meridian group (SYG), model plus acupuncture at Foot Jueyin Meridian group (JYG), model plus acupuncture at Foot Taiyang Meridian group (TYMG), with eight rabbits in each group. Gastric mucosal nitric oxide (NO) and nitric oxide synthase (NOS) were assayed by the nitric acid reductase method, and prostaglandin E2 (PGE2) and epidermal growth factor (EGF) were measured by radioimmunoassay. The comprehensive effects were analyzed by weighing method.

RESULTS: Compared to MG, SYG, JYG and TYMG, the rabbits gastric mucosal injury index (GMII) reduced very significantly in YMG (P<0.01). Compared to MG, the GMII also reduced significantly in TYG (P<0.05). NO, NOS, PGE2 and EGF increased very significantly in YMG (P<0.01). The EGF in YMG also increased significantly than that in TYG. While compared to SYG, the NOS increased significantly in TYG (P<0.05). The PGE2 and EGF also increased very significantly in TYG than those in MG, JYG and TYMG (P<0.01). Compared to SYG, the NOS increased significantly in TYG (P<0.05). NOS was the highest in YMG (P<0.01), and was higher in TYG than in MG (P<0.01).

CONCLUSION: MMCSV is common. The Foot Yangming Meridian is most closely related to the stomach, followed by Foot Taiyin Meridian, Foot Shaoyang Meridian and Foot Jueyin Meridian. Foot Taiyang Meridian has no correlation with the stomach.

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Key words: Relationship between Meridian and Viscus; Gastric mucosal injury/acupuncture effects; MMCSV

INTRODUCTION

According to the meridian theory, the meridians in the body connections are sealed. Qi and blood flow recurrently, the meridians connect with each other directly or indirectly. Therefore, it is the underlying theory of multi-meridians controlling a same viscus (MMCSV).[1,2] Previous studies are focused on one meridian controlling one viscus[3-6], while MMCSV is seldom referred to. In general, NO, NOS, PGE2 and EGF are regarded as protective factors involved in repairing gastric mucosal injury. Based on our previous research on MMCSV,[7], in this study, we focused on the Foot Yangming Meridian (FYM) controlling the stomach. The Foot Shaoyang Meridian (FSY), Foot Jueyin Meridian (FJY), Foot Taiyin Meridian (FTY), as well as Foot Taiyang Meridian (FTYM) were selected to explore the regularity of MMCSV and meridian speciality for the Stomach.

MATERIALS AND METHODS

Animals

Fifty-six New Zealand 3-4 mo old pure rabbits, weighing 1.5-2.5 kg, were supplied by the Experimental Animal Center of Hunan College of Traditional Chinese Medicine (Permission number: 20030316).

Animal model and groups

The rabbit gastric ulcer model was established by ethanol intragastric instillation.[7] Fifty-six rabbits were divided into normal group (NG), model group (MG), model plus acupuncture at Foot Yangming Meridian group (YMG), model plus acupuncture at Foot Taiyin Meridian group (TYG), model plus acupuncture at Foot Shaoyang Meridian group (SYG), model plus acupuncture at Foot Jueyin Meridian group (JYG), model plus acupuncture at Foot Taiyang Meridian group (TYMG), with eight rabbits in each group.
Jueyin Meridian group (JYG), and model plus acupuncture at Foot Taiyang Meridian group (TYMG), with eight rabbits in each group. All the animals were fasted for 48 h before ulcer model-making. Rabbits in NG were given normal saline by intragastric instillation (2.35 mL/kg). Rabbits in other groups were given ethanol intragastrically (2.35 mL/kg). Twenty-four hours later, all animals were allowed free access to normal food. Except for rabbits in NG and MG groups, the animals received acupuncture therapy.

**Acupuncture method**

Acupoints were selected based on the (Modern Acupunctureology)[8] and anthropomorphic method[6] as well as on our previous research results[5,4]. In the FYM, Neiting (ST 44), Jiexi (ST 41), Zusani (ST36), Liangqu (ST 34), Tianshu (ST 25) and Liangmen (ST 21) were selected for gastric ulcer therapy. The other acupoints of different meridians were almost in the same horizontal level to the FYM. In the FSY, Jixiai (GB 43), Qixu (GB 40), Yanglingquan (GB 34), Xiayangguan (GB 33), Dahei (GB 26) and Jingmen (GB 25) were selected. In the FYJ, the selected acupoints were Xingjian (LR 2), Zhongfeng (LR 4), Xiguan (LR 7), Yinbai (LR 9), Zhangmen (LR 13) and Qimen (LR 14). In the FTY, Dadu (SP 2), Shanque (SP 5), Yinlingquan (SP 9), Xuehai (SP 10), Dahen (SP 15) and Fu’ai (SP 16) were selected. In the FTYM, Tonggu (BL 66), Shenmai (BL 62), Chengji (BL 56), Fuxi (BL 38), Beixiadian and Beishangdian were selected.

Consecutive acupoint-stimulation in one meridian was applied by Xuanshou Meridian Dredging Apparatus (China Peace Economy Technology Consultation Company)[9]. Parameters for manipulation were as follows: single way running, six steps, at 0.5 s intervals; double direction narrow impulse and consecutive wave, 50 Hz, 0.5 ms wave width. The apparatus output was connected to the needle (diameter 0.25 mm, length 25 mm) which was inserted into the acupoints. Just mimicking human’s obeying meridian to propaganda sense, the excited order was from low leg to trunk, and the output strength was controlled within 2-3 intensity. Acupuncture stimulation was manipulated once a day, for seven days, 30 s each time. All the selected acupoints were in the left lateral body throughout the experiment.

**Determination of gastric mucosal injury index (GMII)**

After seven days, the animals were killed with their stomachs removed and opened along the greater curvature. The content in the stomach was washed with running water, mucus attached to gastric mucosa was removed by the brusher. Then the mucosa was extended in a flat plate. GMII was calculated by the Guth method[8] with a general magnifier.

**Determination of NO, NOS, PGE2 and EGF in gastric mucosa**

NO and NOS kit were supplied by Nanjing Jincheng Bioengineering Institute. EGF radioimmunoassay kit was provided by Beijing Huaying Radioimmunoassay Institute. [3H]PGE2 radioimmunoassay kit was obtained from Beijing Furui Bioengineering Co. Gastric mucosal NO and NOS were assayed by the nitric acid reductase method, and PGE2 and EGF were measured by radioimmunoassay. All assays were carried out according to the manufacturer’s instructions.

**Statistical analysis**

All the results were expressed as mean±SD. Comparison between groups was made using one-way analysis of variance (ANOVA). 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 index (Table1)**

There were some dots and strips of injury under the magnifier. The highest GMII was observed in MG and the lowest GMII in NG. There was a very significant difference between them (P<0.01), demonstrating that the ulcer model was successful. Compared to MG, GMII in YMG and TYG reduced significantly (P<0.05 or P<0.01), and the GMII in YMG was better than that in TYG. While the GMII in SYG, JYG and TYMG had no significant difference compared to that in MG (P>0.05). The GMII in YMG had a significant difference compared to that in SYG, JYG and TYMG (P<0.01). The GMII in TYMG also had a significant difference compared to that in TYGM (P<0.05).

**Table 1 Gastric mucosal injury index comparison (mean±SD)**

| Groups  | n  | GMII   |
|---------|----|--------|
| NG      | 8  | 8.50±2.98<sup>a</sup> |
| MG      | 8  | 24.88±6.29<sup>b</sup> |
| YMG     | 8  | 10.88±3.23<sup>c</sup> |
| SYG     | 8  | 19.38±3.66<sup>d</sup> |
| JYG     | 8  | 20.38±4.03<sup>e</sup> |
| TYG     | 8  | 14.62±3.20<sup>e</sup> |
| TYMG    | 8  | 24.13±1.64<sup>f</sup> |

<sup>a</sup>P<0.05, <sup>b</sup>P<0.01 vs MG; <sup>c</sup>P<0.05 vs MG; <sup>d</sup>P<0.01 vs YMG; <sup>e</sup>P<0.05, <sup>f</sup>P<0.01 vs TYG.

**NO and NOS changes in gastric mucosa (Table 2)**

Gastric mucosal NO and NOS in NG were both higher than those in MG (P<0.01). The highest NO content was found in YMG, than TYG, JYG and TYMG, while the lowest NO content was observed in SYG. The NO was higher in YMG than in MG, SYG, JYG and TYMG, and there was a very significant difference between YMG and MG, SYG, JYG, TYMG, respectively (P<0.01). However, no significant difference was found between YMG and TYG (P>0.05). The highest NOS content was observed in YMG, the lowest NOS content was found in TYMG. The NOS was higher in YMG and TYG than in
MG, SYG, JYG and TYMG ($P<0.05$ or $<0.01$). The NOS was also higher in SYG than in MG, and there was a very significant difference between them ($P<0.01$).

**PGE2 and EGF changes in gastric mucosa (Table 3)**

Gastric mucosal PGE2 and EGF were both higher in NG than in MG ($P<0.01$). The PGE2 was higher in YMG and TYG than that in NG and other acupuncture therapy groups ($P<0.01$). The highest EGF was found in YMG, compared to MG and other acupuncture therapy groups, and there were very significant differences between YMG and other groups respectively ($P<0.01$), demonstrating that acupuncture can raise gastric mucosal PGE2 and EGF content in YMG, while acupuncture could only raise gastric mucosal PGE2 content in TYG ulcer rabbits.

**Table 3 PGE2 and EGF changes in gastric mucosa (mean±SD, ng/L)**

| Groups | n | PGE2 (μg.mot/gprot) | EGF (μg/mgprot) |
|--------|---|---------------------|-----------------|
| NG     | 8 | 1.48±0.54$^{a}$     | 301.4±482.6$^{b}$|
| MG     | 8 | 0.45±0.20$^{a,f}$   | 762.1±156.3$^{c}$|
| YMG    | 8 | 1.38±0.27$^{a}$     | 193.1±612.6$^{c}$|
| SYG    | 8 | 0.45±0.19$^{a,f}$   | 137.0±319.4$^{c}$|
| JYG    | 8 | 0.76±0.25$^{a,f}$   | 987.2±365.1$^{c}$|
| TYG    | 8 | 1.25±0.68           | 1728.2±213.2$^{a}$|
| TYMG   | 8 | 0.57±0.24           | 750.9±254.3$^{c}$|

$^{a}P<0.05$; $^{b}P<0.01$ vs MG; $^{c}P<0.05$; $^{f}P<0.01$ vs TYG.

**Summarization of effects of acupuncture at different meridian acupoints (Table 4)**

To compare the protective effects of acupuncture at different meridian acupoints on the gastric mucosal injury, we ranked the intensity of effects. From the summarization in Table 4, we could see the comprehensive protection of acupuncture therapy against gastric mucosal injury of experimental rabbits was the best in YMG, followed by TYG, JYG or SYG, and TYMG.

**Table 4 Summary of effects of items after acupuncture therapy**

| Items | Order sequence | 1 | 2 | 3 | 4 | 5 |
|-------|---------------|---|---|---|---|---|
| GMII  | YMG           | TYG | SYG | JYG | TYG | TYMG |
| NO    | YMG           | TYG | JYG | TYG | TYMG | SYG |
| NOS   | YMG           | TYG | JYG | TYG | TYMG | SYG |
| EGF   | YMG           | SYG | JYG | TYG | TYMG | SYG |
| PGE2  | YMG           | TYG | SYG | JYG | TYG | SYG |

Order sequence refers to the effect intensity from strong to weak.

**Table 5 Comprehensive protective effects of different meridian acupuncture on gastric mucosa (mean±SD)**

| Groups | n  | WEV  |
|--------|----|------|
| NG     | 8  | 9.542±1.851.4$^{a}$ |
| MG     | 8  | 5.298±5.963.8$^{a,b}$ |
| YMG    | 8  | 9.653±1.064.7$^{a}$ |
| SYG    | 8  | 6.344±6.013.8$^{a,b}$ |
| JYG    | 8  | 5.660±7.491.9$^{a,b}$ |
| TYG    | 8  | 8.391±4.462.1$^{a,b}$ |
| TYMG   | 8  | 5.550±4.501.4$^{a,b}$ |

$^{a}P<0.01$ vs MG; $^{b}P<0.01$ vs SYG; $^{c}P<0.01$ vs TYG.

**DISCUSSION**

Excessive ethanol ingestion can result in gastritis characterized by mucosal edema, subepithelial hemorrhages, cellular exfoliation and inflammatory cell infiltration. Ethanol intragastric instillation breaks the gastric mucosal barrier and increases histamine release and pepsinogen output, thus leading to the damage in the gastric mucosa at least in part via hyperosmolarity. In the present study, after ethanol intragastric instillation, the highest GMII was found in MG, demonstrating that the model of gastric mucosal injury is reliable. It is known that neuronal modulating processes such as release of vasoactive mediators are crucial for the gastric mucosa to resist the continual onslaught of aggressive agents. Endothelial cells also release a highly labile humoral vasodilator method was used to analyze the differences.

According to the importance of gastric mucosal protective factors including NO, NOS, PGE2 and EGF, the whole weight was set at 10, NO at 2.5, NOS at 1.25, EGF at 3.75 and PGE2 at 2.5. The comprehensive effect value (CEV) was made in the following process: NO, NOS, EGF and PGE2 content were multiplied with their own weight respectively, then added. ANOVA was used to compare the difference in the acupuncture therapy groups. The highest CEV was found in YMG followed by TYG, SYG, JYG, TYMG, and MG. The statistics showed that there was a very significant difference in CEV between YMG and other groups ($P<0.01$). In addition, CEV was also higher in TYG than in SYG, JYG, TYMG, and MG, respectively ($P<0.01$).
substance, now known as NO that mediates the vascular relaxation induced by vagal stimulation\textsuperscript{10}. NO could protect the gastric mucosa by increasing gastric mucosal blood flow\textsuperscript{11,12}, suggesting that gastroprotection can be induced by low level of central vagal stimulation and the consequent release of NO, and that NO plays a role also in ulcer healing by stimulating the formation of growth factors, such as epithelial proliferation and angiogenesis\textsuperscript{13}. NOS is a limited enzyme for NO synthesis. Studies have shown that chronic nitric oxide synthase inhibitor L-NAME enhances ulcerogenesis by decreasing mucosal resistance due to reduced mucosal blood perfusion\textsuperscript{14–16}. When the specific iNOS inhibitor L-N6-(1-iminoethyl) lysine (L-NIL) is given intravenously to rats, the blood flow increase in response to luminal acid is attenuated\textsuperscript{17}. Therefore, NOS is important for maintaining mucosal resistance through increasing the gastric mucosal blood flow. PGE2 is also involved in protecting gastric mucosa. PGE2 inhibits ethanol-induced apoptosis and increases cell viability in a dose-dependent manner in primary cultures of guinea pig gastric mucosal cells. PGE2 also inhibits hydrogen peroxide-induced apoptosis\textsuperscript{18}. EGF is also a key to the protection against mucosal injury. Since acute gastric lesions are induced by cold-restraint stress, the ulcer score is significantly reduced after intraperitoneal and intragastric administration of EGF solution\textsuperscript{19}. When EGF is added to primary monolayer cultures of guinea pig gastric mucous cells, the cytoprotection induced by EGF can be demonstrated. Mucin biosynthesis and PGE2 release are both significantly increased by EGF\textsuperscript{20,21}. EGF promotes gastric mucosal restitution by activating Na\textsuperscript+ /H\textsuperscript+ exchange of epithelial cells\textsuperscript{22}. In the present study, the comprehensive effects of different meridian acupuncture on gastric mucosal protective factors including NO, NOS, PGE2 and EGF, the relationship between meridian and viscera were revealed.

MMCSV is common in the theory of meridian and viscous correlation, manifested in two aspects. On the one hand, multiple meridians have different connections with the same viscus, and the function of meridians is complex. For example, there are five meridians connecting the stomach, namely Hand Taiyin Meridian (HTY), FTY, FJY, Hand Taiyang Meridian (HTY), FYM. On the other hand, stimulating different meridian acupoints could regulate the same viscus physical function, which is the underlying theory for treating diseases. For example, moxibustion at Neiguan (PC 6) of Hand Jueyin Meridian, Yangfu (GB 38) of FSY, as well as Shangqu (SP 5) of FTY, can be selected for stomachache therapy.

Though MMCSV is common, the meridians do have some speciality. It could be explained by the following two reasons. One is that since different meridian acupoints have a relative speciality, and meridian is composed of acupoints, it is no doubt that the meridian has some speciality. In addition, the meridian and viscous match theory also can explain the meridian’s speciality. Since FYM is the responsible meridian to stomach, acupuncture at FYM could regulate the stomach physical function and improve pathological conditions. According to viscous-viscus match theory, the stomach is matched with the spleen; since the FTY is responsible for the spleen, acupuncture at FTY also has good effects on the stomach function regulation. Gallbladder’s bile secretion from gallbladder could help the stomach to digest, while the secretion depends on the liver dredging function. Since the FSY is responsible for gallbladder, and FJY for the liver, acupuncture at FSY and FJY also has some effects on the stomach function regulation. In the present study, FYM had the best effect on stomach function regulation, followed by FTY, FSY and FJY, while the FTYM had no effect.

MMCSV is the unification for universality and specialty of meridian and viscus theory. In the present study, the protective effects of acupuncture at different meridian acupoints on gastric mucosa were various. For example, except for acupuncture at FTY, GMII decreased in all acupuncture therapy groups, while acupuncture at FYM, FTY and FSY, increased the NOS, suggesting that MMCSV is common. Acupuncture at FYM achieved the best intensity and width of protective effects on gastric mucosa. FJY had almost no effects on NO, NOS, PGE2, and EGF. FYTM has no significant effects on NO, NOS, PGE2, and EGF.

In summary, the FYM is most closely related to the stomach, followed by FTY, FSY, FJY, while FYTM has no correlation with the stomach.

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