Effect of Zusanli (ST 36) moxibustion on rat mesenteric microvascular system

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Abstract

OBJECTIVE: To investigate the effect of moxibustion on Zusanli (ST 36) on visceral-mesenteric vessels by observing circulation.

METHODS: Forty-five SD rats were randomly assigned to a moxibustion, electroacupuncture (EA), and blank group. In the moxibustion group, heat stimulation of moxibustion to the Zusanli (ST 36) area of normal rats was performed for 15 min. In the EA group, needles were inserted into the Zusanli (ST 36) and lateral point [0.5 cm lateral from Zusanli (ST 36)] for 15 min. The blank group was not given any treatment. We continuously monitored mesenteric microvascular changes with in vivo microscopic video.

RESULTS: Moxibustion and EA to Zusanli (ST 36) increase the diameter of mesenteric arterioles and venules ($P<0.05$). There were no obvious changes in the blank group. Fine arterial diameter peaked at 12 min in the moxibustion group, while it peaked at 15 min in the EA group.

CONCLUSION: The stimulation of moxibustion and acupuncture to Zusanli (ST 36) has immediate effects on expanding the microvasculature. This dilation may be the mechanism of the gastrointestinal effect of Zusanli (ST 36).

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Key words: Moxibustion; Electroacupuncture; Zusanli (ST 36); Splanchnic circulation

INTRODUCTION

Acupuncture and moxibustion affects the overall regulation of the nerve, vascular, endocrine, and immune network to achieve therapeutic effects, but its mechanism is complicated. However, vascular mechanisms likely play an important part. In our previous study, we found that moxibustion on Zusanli (ST 36) can cause temperature changes of the inner canthus, peri-oral area, nasal, forehead, supraclavicular fossa, mammary gland, and groin, where the blood is abundant. However, there was no obvious conduction of heat on the surface of body, suggesting that the numerous functions of Zusanli (ST 36) are related to improved circulation. Zusanli (ST 36) is one of the most important and widely studied acupoints on the human body, and is used in a wide range of disease treatment. Current research on Zusanli (ST 36) involves mostly acupuncture, and investigates central and peripheral effects. For example, research has shown that acupuncture on Zusanli (ST 36) can cause widespread activation of brain regions. The outer peripheral effects include inhibiting acute gastric mucosal injury, reducing inflammatory re-
sponse, preventing infection, reducing asthma attacks, suppressing lung ischemia-reperfusion injury, changing the levels of certain cytokines in rats, promoting colonic transmission function, and lowering blood pressure. There are also many studies investigating the effect of acupuncture on the stomach and intestine. For example, acupuncture can increase gastric blood flow perfusion, promote the recovery of bowel function after gastrointestinal surgery, and prevent and relieve nausea and hiccups in cancer patients after chemotherapy. Moreover, catgut implantation at acupoints has a reliable curative effect for constipation. This study investigates the effects of moxibustion at Zusanli (ST 36) on the gastrointestinal system. The effect of moxibustion at Zusanli (ST 36) on visceral-mesenteric vessels were immediately and visually observed. We aimed to further confirm moxibustion’s role in improving circulation of the internal organs, and possible neural regulation mechanisms.

MATERIALS AND METHODS

Animals and grouping
Forty-five healthy male three-month-old SD rats, weighing 240-270 g, were provided by the People’s Liberation Army General Hospital Medical Laboratory Animal Center (SCXK [Beijing]-2012-0001). Feeding was adapted to standard rodent animal feeding cage, at room temperature 20°C ± 2°C, with light for 12 h. As previously described, we randomized mice using Excel and the random function “Rand.” The rats were divided into moxibustion, electroacupuncture (EA), and control groups, with 15 rats in each. Differences in the number and weight of rats in each group were not statistically significant (P > 0.05). During the experiment, the disposal for animals conformed to the "Guiding Opinions on kind of experimental animals" published by the Republic of China Ministry of Science in 2006.

Main equipment
Biological microscope (Olympus BH-2, Olympus Corporation, Tokyo, Japan) and Image-Pro image analysis software (Media Cybernetics, Silver Spring, MD, USA) were provided by Pathophysiology Department of the PLA General Hospital. Moxa stick (the 4th smokeless moxa) was produced by Ai Shang Moxa Supplies Company Limited (Suzhou, China), Huatuo electroacupuncture device (SDZ-V-type) by Suzhou Medical Supplies Company Limited (Suzhou, China), and sterile acupuncture needles (0.25 mm × 25 mm) by Guizhou Ande Medication Appliance., Ltd. (Guizhou, China). Surgical methods before the experiment, rats were fasted for 12 h, with normal water. Animal experiments were conducted according to the mesenteric microcirculation in vivo observation method. After using 20% urethane (0.7 mL/100 g) intramuscular anesthesia, rats were fixed on rat-fixing plates, and incised 1-2 cm along the ventral midline. With a small tweezers, some of the small intestine was gently raised, and the mesenteric vascular distribution areas where the blood vessels were tiny and less fatty were located. Then, the rats were flipped into a lateral position, fixed them on the mesenteric microcirculation observation box, and evenly separated the mesentery, avoiding overlap and stretch. The mesentery was placed on the microscope stage with a 37°C saline drip to maintain the temperature and humidity of the mesentery.

Treatment methods of each group
Common laboratory animal acupoints were located in reference to Experimental Acupuncture Science. Zusanli (ST 36) is posterolateral to the knees, about 5 mm below the fibular head. In the moxibustion group, hair at the point was removed to allow easy penetration of moxibustion heat. Suspended moxibustion was used with a No. 4 (4 mm) moxa stick 1.5 cm away from the point. In the EA group, needles were inserted 0.7 cm deep into the Zusanli (ST 36) and lateral point (0.5 cm lateral from ST 36) EA was connected with a frequency of 4-16 Hz and intensity to slight trembling. The blank group not given any treatment. Moxibustion and EA groups were treated for 15 min, and their videos were recorded 5 min before the treatment, and 15 min during and after the treatment. The blank group was recorded for 35 min.

Outcome measures
Capillary diameter: using Image-Pro image analysis software (Media Cybernetics, Silver Spring, MD, USA), the inside diameters of arterioles and venules were measured every 3 min. The measured capillaries were 30-120 μm in diameter, accompanied by fine arterio-venous tissue, and without cluttered surrounding blood vessels.

Statistical analysis
SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for data analysis. The paired t-test was used to compare the changes of each group before, during and after the treatments. Analysis of variance for repeated measures data was used for comparison among groups. If it satisfied the spherical requirements, namely P > 0.05, the direct analysis of variance was used, if not, ε correction was required. P < 0.05 was considered statistically significant.

RESULTS

Changes in fine arterial diameter during and after the treatment in each group
In the moxibustion and EA groups, there were significant differences in fine artery diameter during and af-
ter treatment compared with that before treatment (all \( P < 0.05 \)). Comparing different groups in the same period, it was found that the thin artery diameter value of the EA group was significantly higher than that of the control group (\( P < 0.05 \)). Moreover, thin artery diameter values of the moxibustion group 9, 12, and 15 min into treatment, and 3 and 12 min after treatment were significantly higher than those of the control group (all \( P < 0.05 \)). There were no significant differences (all \( P > 0.05 \)) between the two groups during other periods. Meanwhile, although changes of fine artery diameter in the EA group were greater than that of the moxibustion group, there were no significant differences (all \( P > 0.05 \)) between the two groups (Table 1).

**Table 1 Changes in fine arterial diameter during and after treatment in each group (μm, \( \bar{x} \pm s \))**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Before treatment</th>
<th>3 min during treatment</th>
<th>6 min during treatment</th>
<th>9 min during treatment</th>
<th>12 min during treatment</th>
<th>15 min during treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>15</td>
<td>44±14</td>
<td>44±14</td>
<td>44±14</td>
<td>44±15</td>
<td>44±14</td>
<td>44±15</td>
</tr>
<tr>
<td>Moxibustion</td>
<td>15</td>
<td>48±9</td>
<td>52±10</td>
<td>53±9</td>
<td>54±9</td>
<td>56±9</td>
<td>55±9</td>
</tr>
<tr>
<td>EA</td>
<td>15</td>
<td>55±15</td>
<td>57±16</td>
<td>58±14</td>
<td>58±15</td>
<td>60±13</td>
<td>60±16</td>
</tr>
<tr>
<td>F value</td>
<td>-</td>
<td>2.528</td>
<td>3.711</td>
<td>4.329</td>
<td>4.639</td>
<td>6.512</td>
<td>5.362</td>
</tr>
<tr>
<td>P value</td>
<td>-</td>
<td>0.092</td>
<td>0.033</td>
<td>0.02</td>
<td>0.015</td>
<td>0.003</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Notes: moxibustion group: suspended moxibustion was used with a No. 4 (4 mm) moxa stick 1.5 cm away from the point; EA (electroacupuncture) group: needles were inserted 0.7 cm deep into the Zusanli (ST 36) and lateral point, with a frequency of 4-16 Hz and intensity of slight trembling; blank group did not receive intervention. EA: electroacupuncture.* \( P < 0.05 \), compared with prior treatment of the same group; result of Mauchly test of sphericity is 0.345, \( P > 0.05 \), which meets the spherical assumption; † \( P < 0.05 \), compared with the control group during the same period.

**Figure 1 Fine arterial diameter changes in each group during and after treatment (μm)**

Moxibustion group: suspended moxibustion was used with a No. 4 (4 mm) moxa stick 1.5 cm away from the point; EA (electroacupuncture) group: needles were inserted 0.7 cm deep into the Zusanli (ST 36) and lateral point, with a frequency of 4-16 Hz and intensity of slight trembling; blank group did not receive intervention. † represents the time of therapy discontinuation.

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**Time to peak of fine arterial diameter changes**

Fine arterial diameter peaked at 12 min in the moxibustion group, but 15 min in the EA group (Figure 1).

**Changes in fine vein diameter during and after treatment in each group**

In the moxibustion and EA groups, there were significant differences in fine vein diameter during and after treatment compared with that before treatment (\( P < 0.05 \)). Only the fine vein diameter of the moxibustion group was significantly higher than that of the control group (\( P < 0.05 \)), and in other periods during and after treatment, diameter changes of the moxibustion and EA groups were significantly higher than that of the control group (Table 2).

**Table 2 Changes in fine vein diameter during and after treatment in each group (μm, \( \bar{x} \pm s \))**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Before treatment</th>
<th>3 min during treatment</th>
<th>6 min during treatment</th>
<th>9 min during treatment</th>
<th>12 min during treatment</th>
<th>15 min during treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>15</td>
<td>44±14</td>
<td>48±14</td>
<td>53±14</td>
<td>58±14</td>
<td>58±14</td>
<td>58±14</td>
</tr>
<tr>
<td>Moxibustion</td>
<td>15</td>
<td>55±15</td>
<td>57±16</td>
<td>58±16</td>
<td>58±15</td>
<td>60±13</td>
<td>60±16</td>
</tr>
<tr>
<td>EA</td>
<td>15</td>
<td>55±15</td>
<td>57±16</td>
<td>58±16</td>
<td>58±15</td>
<td>60±13</td>
<td>60±16</td>
</tr>
<tr>
<td>F value</td>
<td>-</td>
<td>2.528</td>
<td>3.711</td>
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<td>4.639</td>
<td>6.512</td>
<td>5.362</td>
</tr>
<tr>
<td>P value</td>
<td>-</td>
<td>0.092</td>
<td>0.033</td>
<td>0.02</td>
<td>0.015</td>
<td>0.003</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Notes: moxibustion group: suspended moxibustion was used with a No. 4 (4 mm) moxa stick 1.5 cm away from the point; EA (electroacupuncture) group: needles were inserted 0.7 cm deep into the Zusanli (ST 36) and lateral point, with a frequency of 4-16 Hz and intensity of slight trembling; blank group did not receive intervention. EA: electroacupuncture. * \( P < 0.05 \), compared with prior treatment of the same group; result of Mauchly test of sphericity is 0.345, \( P > 0.05 \), which meets the spherical assumption; † \( P < 0.05 \), compared with the control group during the same period.
control group (all P<0.05). Although fine artery diameter changes of the moxibustion group were greater than that of the EA group, there were no significant differences (all P>0.05) between the two groups (Table 2).

**Time to peak of fine vein diameter changes**

Fine vein diameter peaked at 12 min in the moxibustion group, but 15 min in the EA group (Figure 2).

**Image analysis**

The images of all groups before, during, and after treatment were captured by Image-Pro image analysis software. In the moxibustion (Figure 3) and EA group (Figure 4), the diameters of the fine vein and fine artery diameters increased during the treatment. Then diameters of the vessels decreased after treatment. However, in the blank group (Figure 5), there no obvious change.

**DISCUSSION**

Moxibustion is one of the primary means of treating the disease in Traditional Chinese Medicine. Ling Shu states that "if the needle is not suitable, moxibustion is appropriate," implying that moxibustion and acupuncture have similar effects for treatment and prevention. Zusanli (ST 36) has efficacy for gastrointestinal...

### Table 2 Changes in fine vein diameter during and after treatment in each group (μm, ±s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Before treatment</th>
<th>3 min during treatment</th>
<th>6 min during treatment</th>
<th>9 min during treatment</th>
<th>12 min during treatment</th>
<th>15 min during treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
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<td>67±19</td>
<td>67±20</td>
<td>67±20</td>
<td>67±19</td>
<td>67±20</td>
</tr>
<tr>
<td>Moxibustion</td>
<td>15</td>
<td>87±18b</td>
<td>92±19a</td>
<td>93±19b</td>
<td>94±20b</td>
<td>93±19a</td>
<td>93±19b</td>
</tr>
<tr>
<td>EA</td>
<td>15</td>
<td>80±18</td>
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<td>86±18b</td>
<td>86±18b</td>
<td>86±20a</td>
<td>88±18b</td>
</tr>
<tr>
<td>F value</td>
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<td>7.658</td>
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<tr>
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<td>0.002</td>
<td>0.001</td>
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<td>0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>3 min after treatment</th>
<th>6 min after treatment</th>
<th>9 min after treatment</th>
<th>12 min after treatment</th>
<th>15 min after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>15</td>
<td>67±20</td>
<td>67±20</td>
<td>67±20</td>
<td>67±19</td>
<td>67±20</td>
</tr>
<tr>
<td>Moxibustion</td>
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<td>95±19a</td>
<td>95±19a</td>
<td>95±19a</td>
<td>93±18a</td>
</tr>
<tr>
<td>EA</td>
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<td>87±19a</td>
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<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Notes: moxibustion group: suspended moxibustion was used with a No. 4 (4 mm) moxa stick 1.5 cm away from the point; EA (electroacupuncture) group: needles were inserted 0.7 cm deep into the Zusanli (ST 36) and lateral point, with a frequency of 4-16 Hz and intensity of slight trembling; blank group did not receive intervention. EA: electroacupuncture. *P<0.05, compared with prior treatment of the same group; result of Mauchly test of sphericity is 0.312, P>0.05, which meets the spherical assumption; **P<0.05, compared with the control group during the same period.

![Figure 2](image-url) Fine venous diameter changes of each group during and after treatment (μm)

Moxibustion group: suspended moxibustion was used with a No. 4 (4 mm) moxa stick 1.5 cm away from the point; EA (electroacupuncture) group: needles were inserted 0.7 cm deep into the Zusanli (ST 36) and lateral point, with a frequency of 4-16 Hz and intensity of slight trembling; blank group did not receive intervention. EA: electroacupuncture. *represents the time of therapy discontinuation.
In previous studies, we used moxibustion on body points and observed blood flow on the body surface with an infrared camera. We found that moxibustion on Zusanli (ST 36) could stimulate thermal image changes on specific parts of the body surface, particularly the inner canthus, perioral area, nasal, forehead, supraclavicular fossa, mammary gland, and groin, where the blood is abundant. Research has also shown that moxibustion stimulating Zusanli (ST 36) and Hegu (LI 4) both could cause a T-shaped hot temperature zone (blood distribution range) on the face. We aimed to investigate whether the moxibustion effects are only because of increased local blood flow. The results of this study showed the Zusanli (ST 36) moxibustion group had a larger mesenteric microvascular diameter during and 15 min after moxibustion than that before moxibustion. Compared with the EA group, the moxibustion group’s small artery change was less than that in the EA group, while the venule change was greater. We also found that moxibustion and EA at Zusanli (ST 36) caused the vascular diameter to change within 0-3 min. This effect is a reflection of the hypothesis that the body points “feel stimulated-by the central signal integration-react,”. When moxibustion and electrical acupuncture stimulate the rat, the peripheral receptors feel warm and electrical stimulation. The afferent nerves pass signals to the central nervous system. Through signal integration of the central somatosensory, applied to the respective blood vessels which perform as diameter increases. Its mechanism needs further study.

Studies have found that stimulating Zusanli (ST 36) can cause gastrointestinal two-way adjustment, which is related to neural and humoral regulation. The main adjusting nervous systems are the central nervous system, autonomic nervous system, and the enteric nervous system. Li et al. studied the characteristics of peripheral afferent nerve discharges evoked by manual acupuncture and EA of Zusanli (ST 36) in rats. They found that acupuncture stimulation Zusanli (ST 36) can induce peripheral nerve domination in the region of discharge. Li et al. investigated the central nervous system, autonomic nervous system, and enteric nervous system neural mechanisms affecting gastrointestinal function. They found that gastrointestinal function was regulated by coordination of the internal and extern-
nal nervous system and humoral factors. Hu et al.\textsuperscript{21} found that EA at Zusanli (ST 36) alleviates intestinal ischemia-induced free radical injury in rats that had been scalded, and the mechanism might be related to the cholinergic anti-inflammatory pathway. Studies also found that acupuncture at Zusanli (ST 36) can improve neural regulation, promote gastrointestinal motility, and improve gastrointestinal function.\textsuperscript{22} Stimulating Zusanli (ST 36) can improve gastrointestinal function possibly \textit{via} regulating the central nervous system.\textsuperscript{23-27} In fact, the stimulation of other remote stomach channel points is associated with the central nervous system and can influence intestinal function.\textsuperscript{28-30} The central nervous system adjusts mesenteric microcirculation \textit{via} the sympathetic and parasympathetic nervous systems. For example, Song et al.\textsuperscript{31} studied the effects of capsaicin on induced action potential of inferior mesenteric nerves in rats. They found that capsaicin’s influence on the mesentery is affected by the sympathetic and parasympathetic nervous systems. Kong et al.\textsuperscript{32} showed that activation of the sympathetic nervous system plays an important role in regulating intestinal immunity. It is also thought that the stomach is mainly affected by the vagus nerve, and the small intestine is affected by sympathetic nerve activity.\textsuperscript{33} Therefore, the mesenteric microcirculation is chiefly regulated by the sympathetic and parasympathetic nervous systems after the signal is sent by the central nervous system and passes along the efferent nerve fiber to regular bowel function.\textsuperscript{34-35}

In summary, after moxibustion on Zusanli (ST 36) in normal rats for 15 min, the fine arteriovenous diameter was larger compared with that before treatment. Both moxibustion and EA could increase fine mesenteric artery and vein diameter, but changes in arterial diameter were slightly smaller, but small changes in venous diameter greater in the moxibustion group than that in the EA group. Moxibustion can produce effects on both the body surface and deep organs. Our observed changes in the fine mesenteric vascular diameter of microcirculation might be related to regulation of the central nervous system. Stimulating points on the rat might cause peripheral nerve structures around the point to generate afferent impulses and release acetylcholine, causing excitement of the nerve endings. This excitement spreads along the spinal cord to the central nervous system, where the information is integrated, and then the information spreads downward through the efferent system to sympathetic and parasympathetic mesentery. This signal could cause the release of adrenaline and noradrenaline to adjust intestinal vascular systolic and diastolic function, thus affecting the intestinal microcirculation. This may be an important mechanism of moxibustion on Zusanli (ST 36) that impacts the mesenteric microcirculation.

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