Comparative effect of electroacupuncture and moxibustion on the expression of substance P and vasoactive intestinal peptide in patients with irritable bowel syndrome

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Abstract

OBJECTIVE: To compare the impacts of electroacupuncture (EA) and moxibustion (Mox) on the primary gastrointestinal symptoms and the expressions of colonic mucosa-associated neuropeptide substance P (SP) and vasoactive intestinal peptide (VIP) in patients with either diarrhea-predominant or constipation-predominant irritable bowel syndrome (IBS-D and IBS-C, respectively).

METHODS: Eighty-five IBS patients were randomly allocated to the EA and Mox groups. Zusanli (ST 36) and Shangjuxu (ST 37) were selected as acupoints for electroacupuncture or warm moxibustion treatment once a day for 14 consecutive days. Before and after the treatment sessions, a Visual Analog Pain Scale and the Bristol Stool Form Scale were used to evaluate gastrointestinal symptoms. There were four dropout cases, leaving 81 participants (41 with IBS-D and 40 with IBS-C) who volunteered to undergo colonoscopy before and after the treatment sessions. During colonoscopy, sigmoid mucosa were collected to detect SP and VIP expression using immunohistochemistry assay.

RESULTS: Both EA and Mox treatments were effective at relieving abdominal pain in IBS-D and IBS-C patients. However, Mox was more effective at reducing diarrhea in IBS-D patients, whereas EA was more effective at improving constipation in IBS-C patients. EA and Mox treatments both down-regulated the abnormally increased SP and VIP expression in the colonic mucosa, with no significant difference shown between the two treatments.

CONCLUSION: Both EA and Mox treatments are effective at ameliorating gastrointestinal symptoms by reducing SP and VIP expression in the colonic mucosa of IBS patients.
INTRODUCTION

Irritable bowel syndrome (IBS) is the most common disorder presented to gastroenterologists, with a prevalence of up to 10%-20% in the UK and USA\(^4\,5\) and a prevalence of 5%-10% in most Asian countries.\(^6\,7\) The incidence of IBS increases with global economic growth. The most common IBS presenting symptoms are abdominal pain associated with altered bowel habits (diarrhea or constipation). Although the exact mechanism of IBS gastrointestinal symptoms has not been elucidated, visceral hypersensitivity and gastrointestinal motility disorders are the main pathological causes.\(^8\)

There is also evidence that IBS involves altered gut motility. Several brain-gut peptides, such as substance P (SP) and vasoactive intestinal peptide (VIP), are involved in the regulation of gastrointestinal motor and sensory functions. SP and VIP are important brain-gut peptides that are widely distributed in the central nervous system, gastrointestinal tract, and immune organs. These brain-gut peptides can affect gastrointestinal movements through nervous, endocrine, immune, and other means and may be involved in abdominal pain or discomfort, abnormal defecation, and other visceral hypersensitivity reactions.\(^9\,10\) Abnormal levels of SP and/or VIP have been demonstrated in patients with IBS with diarrhea (IBS-D) and IBS with constipation (IBS-C).\(^4\)

Sandler et al.\(^\text{11}\) revealed that abdominal pain was the symptom most likely to result in IBS patients seeking a medical consultation. The therapeutic options currently available are limited and their lack of efficacy is often disappointing. In recent years, the stimulation of certain acupuncture points or areas with electroacupuncture (EA) or moxibustion (Mox) has appealed to some scholars and a number of clinical\(^12\,13\) and experimental\(^14\,15\) studies have demonstrated its effectiveness, especially for curing abdominal pain or discomfort, abnormal defecation, and other gastrointestinal symptoms induced by visceral hypersensitivity. Our previous studies found that SP and SP receptors expression were greater in rats with IBS than in normal rats, indicating that SP and SP receptors are closely related to the development of IBS. EA at Zusanli (ST\(^37\)) and Shangjixu (ST\(^37\)) can decrease the number of mast cells and the expression of SP and SP receptors in the colon.\(^16\)

In this study, we investigated whether EA and/or Mox in IBS-D and IBS-C patients affected abdominal pain or discomfort, abnormal defecation and other gastrointestinal symptoms, the expression of SP in the colonic mucosa, and the expression of VIP. We compared differences in curative capacity between the two therapies in the hope of providing reliable evidence for the clinical treatment of IBS-D and IBS-C.

MATERIALS AND METHODS

Participants

This research was approved by the Chinese Clinical Trial Register Center (registration number: ChiCTR-TRC-11001349). All subjects were outpatients of the Department of Gastroenterology in Jinhua Hospital of Zhejiang University from October 2011 to September 2012. All patients gave informed consent and the trial was approved by the Ethics Committee of Yueyang Chinese and Western Medicine Integrated Hospital affiliated to Shanghai University of Traditional Chinese Medicine.

We adopted a simple randomized design using the SNOSE (sequentially numbered, opaque sealed envelopes) method. We used a random number table and the numbers generated were written on individual cards and enclosed sequentially in envelopes. Then, the order of each generated number was written on the envelopes and assigned to each eligible patient in order. The random number on the enclosed cards determined the group allocation for each patient; odd numbers denoted the EA group and even numbers denoted the Mox group. The same randomization method was applied separately to IBS-D and IBS-C patients.

We assessed the eligibility of the initial 85 IBS patients according to the Rome III diagnostic criteria.\(^17\) Participants with intestinal organic diseases; combined heart, liver, and kidney disease; mental illness; and pregnant or lactating women with diarrhea were excluded. Of the remaining patients, 43 patients aged 18 to 65 years with IBS-D were randomly allocated to the EA treatment group \((n = 21)\) or the Mox treatment group \((n = 22)\). Forty-two patients aged 18 to 65 years with IBS-C were randomly allocated to the EA treatment group \((n = 21)\) or the Mox treatment group \((n = 21)\). After signing an informed consent form that was approved by the ethics committee, all IBS-D and IBS-C patients provided information about their main digestive tract symptoms, including abdominal pain, abdominal distention, and diarrhea or constipation. 3 mm of sigmoid or ileocecal mucosa (selected according to the area in which the patient experienced abdominal pain or discomfort) was removed via painless colonoscopy before and after treatment. Sigmoid mucosa was collected from 10 healthy volunteers for comparison. The mucosa was placed in 10% formaldehyde solution for analysis. All healthy volunteers were examined for enucleated colonic polyps and intestinal organic diseases and had experienced no change in bowel habits or excrement character.

Treatment methods

As patients entered the clinic, they were randomly allo-
cated to a treatment group, as described above. For the EA group, the acupoints Zusanli (ST 36) and Shangjuxu (ST 37) were located using the national GB-12346-90 acupoint standard. After the skin was cleaned with a tincture of iodine and alcohol, sterile acupuncture needles (0.30 mm in diameter, 40 mm long, Hwatuo, Suzhou Medical Supplies Factory Co., Ltd., China) were inserted 20-25 mm into the skin. After twisting and obtaining Qi (soreness, numbness, distraction, and heaviness), each acupuncture needle was connected to the electrical leads of the HANS Acupoint Nerve Stimulator (Model LH 100A TENS, Nanjing Jisheng Medical Technology Co., Ltd., China) for 30 min, with a stimulation frequency of 2 Hz and a stimulation intensity of 3.0 mA. The treatment was applied once per day, six times per week, for 4 consecutive weeks.

For the Mox group, the acupoints Zusanli (ST 36) and Shangjuxu (ST 37) were selected, and patients were treated with warm moxibustion according to the following procedure. The lit moxa (2.5 cm in diameter, Hwatuo, Suzhou Medical Supplies Factory Co., Ltd., China) was placed 1-2 cm above the acupoints and the surface temperature of the acupoints was maintained at (46 ± 1) °C for 30 min. The treatment was applied once per day, six times per week, for 4 consecutive weeks.

**IBS gastrointestinal symptom scales**

A Visual Analog Pain Scale was used to assess IBS-D and IBS-C abdominal pain before and after treatment, which ranged from 0 = none to 10 = severe. The Bristol stool form scale was used to assess IBS-D and IBS-C feces before and after treatment. This is a descriptive, visual scale that comprises definitions and images of seven types of stool: Type 1: separate hard lumps, like nuts (hard to pass). Type 2: sausage-shaped, but lumpy. Type 3: like a sausage but with cracks on its surface. Type 4: like an Italian sausage or snake, smooth and soft. Type 5: soft blobs with clearcut edges (passed easily). Type 6: fluffy pieces with ragged edges, a mushy stool. Type 7: watery, no solid pieces, entirely liquid. And the scores ranged from 1 (Type 1) to 7 (Type 7). For IBS-D patients, the scores 4 = none, 5 = slight, 6 = moderate, and 7 = severe. However for IBS-C patients, the scores 4 = none, 3 = slight, 2 = moderate, and 1 = severe.

**Immunohistochemistry**

Immunohistochemical staining was used to detect SP and VIP expressions in colon tissue. A sample of sigmoid or ileocecal colon tissue fixed in 10% formalin was embedded in paraffin, cut into 4-μm serial sections and heated at 58 °C overnight. The sections were washed in PHOSPHATE BUFFERED SALINE (PBS) three times for 3 min. The sections were exposed to 0.01 M CITRATE BUFFER, pH 6.0, microwaved at 30% power for 20 min for heat fixation, and cooled to room temperature. The sections were washed three times with PBS for 3 min and exposed to 0.3% H2O2 for 20 min at room temperature to inhibit endogenous peroxidases. Following a final PBS wash (3 × 3 min), the samples were exposed to 20% normal goat serum and incubated for 30 min. Antibodies were added drop-wise (SP 1:50, VIP 1:200) and the sections were incubated at 37 °C for 2 h. The sections were washed with PBS three times for 3 min, incubated in HORSERADISH PEROXIDASE/RABBIT reagent at 37 °C for 30 min and PBS-washed three times for 3 min. The sections were then incubated in DIAMINOBENZIDINE chromogenic reagent for 8 to 12 min and dyed with hematoxylin lining and blue in the presence of hot water. After drying, the sections were wrapped with neutral gum. A semi-quantitative analysis of the staining was performed using the MIQAS medical image quantitative analysis system (Shanghai Qiuwei Biomedical Technology Company, Shanghai, China). Positive results were indicated by the presence of brown or tan particles in the stained colonic tissue cells. In each slice, three positive areas were counted and assessed for optical density (OD) in a high power field to calculate an immunohistochemical positive index (IHC index = positive area × OD / total area) for SP and VIP.

**Statistical analysis**

SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA) was used. For normally distributed variables, the paired-sample t test was used to test differences within the same group, and the two independent-samples t test was used to test differences between the groups before and after treatment. Non-parametric tests were used to analyze abnormally distributed variables; the Wilcoxon signed-rank test was used to analyze within-group differences and Wilcoxon rank sum test was used to compare the differences between two groups. A P value less than 0.05 was considered statistically significant.

**RESULTS**

**Final study sample**

Forty-two patients were randomly allocated to the EA group (21 with IBS-D and 21 with IBS-C), among which 38 patients completed all the treatment sessions and were included in data analysis. Two IBS-D patients withdrew halfway due to lack of therapeutic effect, one IBS-C patient failed to finish the treatment sessions, and one IBS-C patient withdrew for reasons unknown. Forty-three patients (22 with IBS-D and 21 with IBS-C) were randomly allocated to the Mox group, of which all received the allocated treatment and were included in data analysis (Figure 1).

**Comparison of age and duration of diarrhea-predominant and constipation-predominant IBS**

There were no significant differences in age (P =
Comparison of primary gastrointestinal symptoms in diarrhea-predominant and constipation-predominant IBS patients

Abdominal pain was assessed using the Visual Analog Pain Scale. Stool form was assessed using the Bristol Stool Form Scale.

There were no significant differences in abdominal pain ($P = 0.885, P > 0.05$) or stool form ($P = 0.679, P > 0.05$) between IBS-D patients in the EA and Mox groups before treatment. There were no significant differences in abdominal pain ($P = 0.825, P > 0.05$) or stool form ($P = 0.815, P > 0.05$) between IBS-C patients in the EA and Mox groups before treatment (Table 2).

IBS-D and IBS-C patients in both the EA and Mox groups experienced a significant improvement in abdominal pain after treatment (all $P < 0.001$), with no difference between the two groups ($P = 0.361, P = 0.861, all P > 0.05$). In the IBS-D patients, the EA group experienced a significant improvement in stool features ($P = 0.009, P < 0.01$) after treatment, whereas the Mox group experienced a very significant improvement in stool features ($P < 0.001$), with the Mox group feeling significantly better than the EA group after treatment ($P < 0.001$). In the IBS-C patients, the EA group experienced a significant improvement in stool features ($P < 0.001$) after treatment, whereas the Mox group experienced no significant differences before and after treatment ($P = 0.065, P > 0.05$), with the EA group feeling significantly better than the Mox group after treatment ($P < 0.001$) (Table 2).

These findings suggest that both EA and Mox are effective at treating IBS-D and IBS-C patients with abdominal pain, which is the main symptom of digestive tract problems. However, Mox is better than EA at improving stool features in IBS-D patients, but EA is better than Mox at improving stool features in IBS-C patients.

Comparison of colonic mucosa SP expression in diarrhea-predominant and constipation-predominant IBS patients

SP in each group was mainly expressed in the basal portion of the colonic mucosa, lamina propria, and muscular plexus before treatment, with a strong brown reaction in staining and a scattered, knot-like, or clustered distribution (Figure 2).
Before treatment, SP expression in the colonic mucosa was significantly higher in IBS-D and IBS-C patients in both the EA and Mox groups compared with healthy volunteers (all $P < 0.001$). Furthermore, there were no significant differences in expression between the EA and Mox groups for either IBS-D or IBS-C patients ($P = 0.138$, $P = 0.940$, both $P > 0.05$) (Table 3). In comparison, after treatment, SP expression in the colonic mucosa was significantly reduced for IBS-D and IBS-C patients in both the EA and Mox groups (all $P < 0.001$).
groups (all \( P < 0.001 \)). There was no significant difference between the EA and Mox groups for IBS-D patients (\( P = 0.142 \), \( P > 0.05 \)), but there was a significant difference between the EA and Mox groups for IBS-C patients (\( P < 0.001 \)), indicating that EA was more effective at reducing over-expressed VIP in the colonic mucosa (Table 4).

**DISCUSSION**

IBS is a functional bowel disorder that is characterized by abdominal pain or discomfort and disturbed bowel habits. IBS pathogenesis involves many factors, such as abnormal gastrointestinal motility, visceral hypersensitivity, inflammation, and brain-gut axis and psychological abnormalities. Recommended treatments range from patient education, cognitive therapy, dietary modifications, and antispasmodics to psychological measures, including hypnotherapy, behavioral therapy, and newly developed neuropeptide receptor agonists and antagonists. The clinical applications of Western Medicine are hindered by adverse effects from long-term or repeated use of medications as well as huge economic costs. In recent years, clinical studies have demonstrated the curative effects of acupuncture and Mox for IBS. Under stress, various physiological and psychological stimuli can cause changes in gastrointestinal motility. These can be regulated by acupuncture and Mox, which can (individually and in combination) positively adjust physiological and psychological systems, thereby alleviating clinical symptoms. Acupuncture and Mox can also improve the visceral hypersensitivity that is characteristic of IBS.

Autonomic dysfunction is common in IBS patients. IBS-D patients suffer from hyperactivity of the colon and rectum, manifesting an increased activity of the vagus (parasympathetic) nerve and decreased activity of the sympathetic nerve. SP and VIP, located in the enteric nervous system, are involved in the regulation of gastrointestinal motility and visceral hypersensitivity. SP has a strong stimulating effect on gastrointestinal smooth muscle. In combination with a variety of SP re-

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### Table 3 Comparison of SP and VIP expression before treatment between IBS-D and IBS-C patients and the normal control group (\( \bar{x} \pm s \))

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>SP</th>
<th>VIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBS-D EA</td>
<td>19</td>
<td>139±46</td>
<td>135±41</td>
</tr>
<tr>
<td>IBS-D Mox</td>
<td>22</td>
<td>162±49</td>
<td>139±45</td>
</tr>
<tr>
<td>IBS-C EA</td>
<td>19</td>
<td>132±29</td>
<td>152±39</td>
</tr>
<tr>
<td>IBS-C Mox</td>
<td>21</td>
<td>131±32</td>
<td>152±40</td>
</tr>
<tr>
<td>NC</td>
<td>10</td>
<td>61±8</td>
<td>63±9</td>
</tr>
</tbody>
</table>

Notes: EA and Mox groups received electroacupuncture or warm moxibustion treatment on Zusanli (ST 36) and Shangjuxu (ST 37) once for 14 consecutive days. SP: substance P; VIP: vasoactive intestinal peptide; IBS-D: irritable bowel syndrome with diarrhea; IBS-C: irritable bowel syndrome with constipation; EA: electroacupuncture group; Mox: moxibustion group. \( P < 0.001 \) compared with the normal control group.

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### Table 4 Comparison of SP and VIP expression before and after treatment in IBS-D and IBS-C patients (\( \bar{x} \pm s \))

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBS-D EA</td>
<td>19</td>
<td>139±46</td>
<td>100±37(^a)</td>
<td>135±41</td>
<td>100±23(^c)</td>
</tr>
<tr>
<td>IBS-D Mox</td>
<td>22</td>
<td>162±49</td>
<td>102±32(^c)</td>
<td>139±45</td>
<td>89±20(^c)</td>
</tr>
<tr>
<td>IBS-C EA</td>
<td>19</td>
<td>132±29</td>
<td>84±14(^d)</td>
<td>152±39</td>
<td>103±26(^c)</td>
</tr>
<tr>
<td>IBS-C Mox</td>
<td>21</td>
<td>131±32</td>
<td>100±25(^d)</td>
<td>152±40</td>
<td>140±32(^d)</td>
</tr>
</tbody>
</table>

Notes: EA and Mox groups received electroacupuncture or warm moxibustion treatment on Zusanli (ST 36) and Shangjuxu (ST 37) once for 14 consecutive days. SP: substance P; VIP: vasoactive intestinal peptide; IBS-D: irritable bowel syndrome with diarrhea; IBS-C: irritable bowel syndrome with constipation; EA: electroacupuncture group; Mox: moxibustion group. \( P < 0.001 \) compared with data before treatment in the same group; \( P < 0.05 \) compared with the IBS-C EA group after treatment; \( P < 0.001 \) compared with data before treatment in the same group; \( P < 0.001 \) compared with the IBS-C EA group after treatment.
Receptors in target cells, it causes high intestinal sensitivity (hyperalgesia) and intestinal motility disorders and induces IBS. Furthermore, SP can increase vascular permeability and promote the secretion of intestinal mucosal secretory cells. VIP, an inhibitory neurotransmitter, enacts an inhibitory regulation on gastrointestinal motility. It can promote vasodilation, relaxation of the gastrointestinal smooth muscles, reduction of visceral resistance, regulation of intestinal sodium metabolism, and the release of certain enzymes and hormones. Yao et al. found a significantly reduced abdominal contraction threshold in rats with visceral hypersensitivity and enhanced intestinal motility, which were related to SP and the significantly enhanced expression of its receptors (mainly neuropeptide type 1 receptors) in the intestinal mucosa and muscle layer. Valero et al. found that the combination of SP and the neuropeptide type 1 receptors directly causes small intestine smooth muscle contractions in rabbits, suggesting that SP may increase visceral sensitivity and accelerate gastrointestinal motility. Deng et al. found that small intravenous doses of SP in healthy rats can excite the subdiaphragmatic vagus nerve and enhance gastrointestinal motility, whereas large injection doses of SP enact an inhibitory effect on gastrointestinal motility, supporting the theory that SP affects gastrointestinal motility. Zhao et al. observed that VIP in the plasma of patients after abdominal surgery, and before the recovery of gastrointestinal function, was significantly higher than after the recovery of gastrointestinal function. They also found that VIP expression in the myenteric plexus in chronic constipation-predominant rats was higher than in healthy rats. Keller et al. reported that intravenous VIP injection in healthy individuals caused the secretion of intestinal sodium and increased water and diarrhea. They also found that VIP can both inhibit peristalsis and promote the secretion of water and electrolytes in the intestinal mucosa. Lan et al. demonstrated that both SP and VIP can promote intestinal mucosal mast cell degranulation and the release of histamine, leading to increased capillary permeability, glandular secretion, and other biological effects. In the present study, we found that SP and VIP in the colonic mucosa were significantly greater in both IBS-D and IBS-C patients than in healthy volunteers, indicating that these abnormally increased neurotransmitters may af-

Figure 3 Comparison of VIP expression within IBS-D and IBS-C patients before and after EA or Mox treatment.

EA and Mox groups received electro-acupuncture or warm moxibustion treatment on Zusanli (ST 36) and Shangjuxu (ST 37) for once a day for 14 consecutive days. A-I: VIP expressions in colonic mucosa were dyed by immunohistochemical staining method (×200). A-B: VIP expression of IBS-D patients before and after EA treatment; C-D: VIP expression of IBS-D patients before and after Mox treatment; E-F: VIP expression of IBS-C patients before and after EA treatment; G-H: VIP expression of IBS-D patients before and after Mox treatment; I: VIP expression of normal control. IBS-D: irritable bowel syndrome with diarrhea; IBS-C: irritable bowel syndrome with constipation; EA: electro-acupuncture group; Mox: moxibustion group; VIP: vasoactive intestine peptide.
fect intestinal motility and mucosal secretion and increase visceral hypersensitivity through nervous, endocrine, or immune means. These effects can cause abdominal pain or discomfort, diarrhea, constipation, and other gastrointestinal symptoms in IBS-D and IBS-C patients. These results indicate that SP and VIP in the colonic mucosa are involved in the visceral hyperactivity and intestinal disorder mechanisms of IBS. Our research group previously found that EA on Zusanli (ST 36) and Shangjuxu (ST 37) in IBS model rats can significantly inhibit SP in the colonic mucosa and VIP expression.11,20 In the present study, Zusanli (ST 36) and Shangjuxu (ST 37) were selected to determine whether there were any curative differences between EA and Mox in IBS-D and IBS-C patients. Both IBS-D and IBS-C patients demonstrated significantly reduced SP in the colonic mucosa and reduced VIP expression compared with before the treatment, and their abdominal pain symptoms were significantly alleviated. However, we found that Mox was significantly better than EA at improving thin or watery stools in IBS-D patients, whereas EA was significantly better than Mox at improving unsmooth or dry stools, reducing SP in the colonic mucosa, and reducing the expression of VIP in IBS-C patients.

In conclusion, this study suggests that either EA or Mox are effective clinical treatments for IBS patients suffering from abdominal pain. However, we recommend that IBS-D patients should choose Mox, and IBS-C patients choose EA, for optimal curative effects. Furthermore, we lost four IBS patients who received EA treatment throughout the whole study. Of which two IBS-D patients withdraw halfway due to lack of therapeutic effect, and the optimal choice of EA and MOX when treated IBS patients with different master gastrointestinal disorders (diarrhea or constipation) maybe the main reason. We will improve the research design in the future.

REFERENCES


