J Huazhong Univ Sci Technol［Med Sci］
DOI 10.1007/s11596-014-1351-8 34(5):775-781,2014

Clinical Study of Electro-acupuncture Treatment with Different Intensities for Functional Constipation Patients*

Fan XIONG (熊 繁), Ying WANG (王 颖), Shi-qi LI (黎诗祺), Man TIAN (田 曼), Cui-hong ZHENG (郑翠红), Guang-ying HUANG (黄光英)#
Institute of Integrative Traditional Chinese and Western Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

© Huazhong University of Science and Technology and Springer-Verlag Berlin Heidelberg 2014

Summary: Functional constipation (FC) is a common functional bowel disorder disease that affects life quality of a large number of people. This study aimed to explore the impact of different intensities of electro-acupuncture (EA) treatment for FC patients. Totally, 111 patients with FC meeting the Rome Ⅲ criteria were randomly assigned to different intensities of EA groups (low and high intensity of EA groups) and medicine-controlled (MC) group. In EA groups, patients were treated with EA at quchi (LI11) and shangjuxu (ST37) bilaterally for 4 weeks, 5 times/week in the first 2 weeks, and 3 times/week in the last 2 weeks. In MC group, 5 mg mosapride citrate was administered orally 3 times/day for 4 weeks. Spontaneous bowel movement frequency each day was recorded using a constipation diary. Self-rating anxiety scale (SAS) and self-rating depression scale (SDS) were used to assess the patients' psychological state. Cortisol (CORT), substance P (SP), and vasoactive intestinal polypeptide (VIP) were evaluated at baseline and at the end of 4 weeks after treatment. As compared with the baseline, there was statistically significant increase in stool frequency every week (P<0.01), but there was no statistically significant difference among the three groups. As compared with the baseline, after 4 weeks of EA therapy, the scores of SDS and serum levels of CORT were decreased significantly in low intensity of EA group (P<0.01), and the serum levels of SP and VIP were increased significantly (P<0.05); the scores of SAS and SDS and serum levels of CORT were decreased significantly in high intensity of EA group (P<0.05), and the serum levels of SP and VIP were increased significantly (P<0.05); the serum levels of CORT and VIP were increased significantly in MC group (P<0.05). As compared with MC group, after 4 weeks of treatment, the serum levels of SP were signifcicantly increased in low intensity of EA group (P<0.01). Low and high intensities of EA could increase the stool frequency, improve the FC patient's anxiety and depression, reduce the serum levels of CORT, and increase the serum levels of SP and VIP effectively. It is concluded that both low and high intensities of EA are effective for FC patients, but there is no significant difference between the low and high intensities of EA.

Key words: electro-acupuncture; current intensity; functional constipation; quchi (LI11); shangjuxu (ST37)

Constipation frequently occurs in the outpatients of gastroenterology division, 10%–15% of the general population in the US, UK and Canada suffer from constipation, the prevalence increases to 30%–40% among people aged >65 years, and women are affected up to three times more often than men[1]. Constipation is commonly defined as fewer than three bowel movements per week, straining, hard stools, incomplete evacuation, and sensation of anorectal obstruction. When constipation patients meet the ROME Ⅲ criteria, they are considered to have functional constipation (FC) once organic causes such as inflammatory, neoplastic, anatomical or neurological abnormalities and endocrine or metabolic disorders are ruled out[2]. Although the majority of patients do not have a clear etiology, many related factors have been identified: anxiety, depression, emotions, pressures, dietary, genetics, as well as therapy for comorbidities[3]. In addition, FC also brings about abdominal pain or discomfort, abdominal distension, headache, dizziness, nausea and poor appetite, which can interfere obviously with patient's quality of life[4].

Conventional therapeutic strategies have been proposed ranging from the use of various laxatives to stimulant cathartics, fibre and bulking agents[5]. Though various treatments are available for FC, most of patients still worry about their efficacy and safety. According to a recent meta-analysis, only a part of medicine, such as laxatives, polyethylene glycol solution, prucalopride, lubiprostone, and linaclotide, has been demonstrated to be effective in managing constipation[6]. The adverse effects of laxatives include cramp, abdominal distension, poor appetite and diarrhea; excessive laxative use has been

Fan XIONG, E-mail: fxiong369@163.com
#Corresponding author, E-mail: gyhuang@tjh. tjmu.edu.cn
*This project was supported by the National Basic Research Program of China (973 Program) (No. 2011CB505203).

linked to loss of colonic motility, leading to another rare occurrence, cathartic colon[1]. The goal of conventional treatment is to relieve constipation symptom, instead of solving the pathological abnormalities. Therefore, many patients are disappointed by current conventional treatments and seek help from alternative medicine.

Nowadays, acupuncture has become one of the most promising alternative medicine and gains increasing popularity in the world[7]. As compared to traditional acupuncture, electro-acupuncture (EA) has the advantage of increasing stimulation frequency and intensity objectively and quantifiably, so it has been broadly applied in various researches[8–11]. Though some studies have indicated that acupuncture was an effective treatment for FC[12, 13], how to strengthen the therapy effects of EA is still a problem. Only a few researchers explored the effects of different intensities of EA, which have been demonstrated to be an important factor in those studies[14, 15]. Meanwhile, some studies have shown that different intensities of transcutaneous electric nerve stimulation (TENS)[16] and EA[17] may exert significantly different degrees of analgesic effects on people. Whether there is a relationship between the intensity of EA and efficacy warrants further investigation. Therefore, the aim of our study is to explore the effects of different intensities of EA on improving spontaneous bowel movement frequency, psychology and gastrointestinal motility for FC patients.

1 MATERIAL AND METHODS

1.1 Subjects

This study was approved by the Clinical Trial Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. The trail was registered on http://clinicaltrials.gov (No. NCT01274793).

From May 2012 to June 2013, by the newspaper and network propaganda, the study of single center recruited 164 constipation patients during the 13 months. Patients were subjected to three-round selection. On the first round selection, the patients were selected according to the inclusion criteria and exclusion criteria[18], and the duration of FC was more than 6 months. On the second round selection, by recording the constipation card for 1–2 weeks, the patients meeting the FC diagnostic criteria were selected for further study. On the third round selection, routine physical examinations (routine tests of blood, urine and feces, hepatic and renal function, electrocardiogram) and special examinations (colonoscopy, or abdomen magnetic resonance) were performed to exclude any systemic diseases and gastrointestinal diseases. Written consent was obtained from each FC patient before entering the study formally.

1.2 EA and Study Design

The EA stimulation was an alternating wave with a frequency of 2–50 Hz by a Han's acupoint nerve stimulator (HANS-200E, China). The weakest EA stimulation that could be surely felt was identified as low intensity stimulation (LIS); the strongest EA stimulation that could be tolerated and was not noxious was defined as high intensity stimulation (HIS).

In a single-center clinical study, the selected FC patients were randomly assigned to the low intensity of EA group, high intensity of EA group, and medicine-controlled (MC) group. In the EA groups, the patients experienced EA therapy, but they were blinded to the current intensities. Patients were treated with EA at quchi (LI11) and shangjuxu (ST37) bilaterally for 4 weeks (5 times/week in the first 2 weeks, 3 times/week in the last 2 weeks). Each treatment lasted for 30 min, a total of 16 times for 4 weeks. In MC group, 5 mg mosapride citrate was administered orally 3 times/day for 4 weeks.

1.3 Acupunture Process

The disposable needles of Jianweishi brand were used (Shanghai Taicheng Technology Development Co. LTD., China). The size of acupuncture needles was 0.30 mm ×40 mm and 0.30 mm×50 mm; the size of auxiliary needle was 0.18 mm×13 mm. Acupuncture needle was used to deliver EA stimulation to acupoints (LI11 and ST37), and auxiliary needle was shallowly inserted in the proximal part nearby the acupoints. Both needles were used together only to ensure the acupoint receiving current stimulation, without any interference to any other acupoints. During the process of acupuncture, acupuncture needle was rotated manually for "deqi" sensation; auxiliary needle was only fixed without "deqi" sensation. All the procedure of each patient was operated by the same experienced and licensed acupuncturist.

1.4 Assessment of Outcomes

The assessment indexes consisted of spontaneous bowel movement frequency per week, levels of cortisol (CORT), substance P (SP) and vasoactive intestinal polypeptide (VIP). Self-rating anxiety scale (SAS) and self-rating depression scale (SDS) were used to assess patients' psychological state. Defecation frequency from the baseline to the end of 4 weeks' treatment, the scores of SAS and SDS and the serum levels of CORT, SP and VIP were evaluated at baseline and at the end of 4 weeks' treatment.

An extra vial of fasting blood was collected (at 8 to 9 a.m.) at baseline and at the end of 4 weeks' treatment. After centrifugation at 3000 r/min for 15 min, the serum was collected and stored in a –80°C refrigerator. The serum levels of CORT and SP were measured with ELISA kits (Enzo Life Sciences, Plymouth Meeting, USA) according to the manufacturer's instructions. The ELISA kits of CORT had a sensitivity of 27 pg/mL, range of 32–20 000 pg/mL, intra-assay CV<9%, and inter-assay CV<13%. The ELISA kits of SP had a sensitivity of 8.04 pg/mL, range of 9.76–10 000 pg/mL, intra-assay CV<8%, and inter-assay CV<8%. The serum level of VIP was detected by using the ELISA kits (USCN-LIFE, China) according to manufacturer's instructions. The ELISA kits of VIP had a sensitivity of 2.34 pg/mL, range of 6.17–500 pg/mL, intra-assay CV<10%, and inter-assay CV<12%.

If times of treatments was ≥8 and the time of medication was ≥2 weeks, the corresponding data would be deemed to be valid and included in the statistics; if the times of treatments was <8 or the time of medication＜2 weeks, the related data would be regarded as invalid and not included in the statistics.

During the whole study, the patients were asked not to make significant changes to their diet and not to continue their common medications. If the constipation symptoms became too severe to be tolerated, some laxatives were permitted, with documented.

1.5 Statistical Analysis

Quantitative data are expressed as $x \pm s$. Defecation

frequency before and after treatment within groups was compared by repeated measurement data analysis of variance; and the differences between two time points within groups were compared by Bonferroni method. The comparison of stool frequency among groups at each time point was carried out by multivariate analysis of variance. The comparison within group in SAS and SDS scores and serum levels before and after treatment was implemented by paired samples *t*-test, and the comparison of SAS and SDS scores and serum levels among groups before and after treatment was done by one way ANOVA analysis. SPSS17.0 statistical software was used for statistical analysis. $P<0.05$ was considered to be statistical significant.

2 RESULTS

2.1 Analysis of Patients' Information

Totally, 111 FC patients were randomly assigned to three treatment groups: low intensity of EA group (35 cases), high intensity of EA group (38 cases) and MC group (38 cases). The flow chart of the whole trial is shown in fig. 1. A total of seven patients (6.31%) withdrew during the whole course of the trial: two (5.71%) in low intensity of EA group, one (2.63%) in high intensity of EA group, and four (10.53%) in MC group. Among the seven withdrawn patients, four patients received ineffective treatment: one (2.86%) in low intensity of EA group, and three (7.89%) in MC group; the rest three patients were lost for other reasons: one patient in each group.

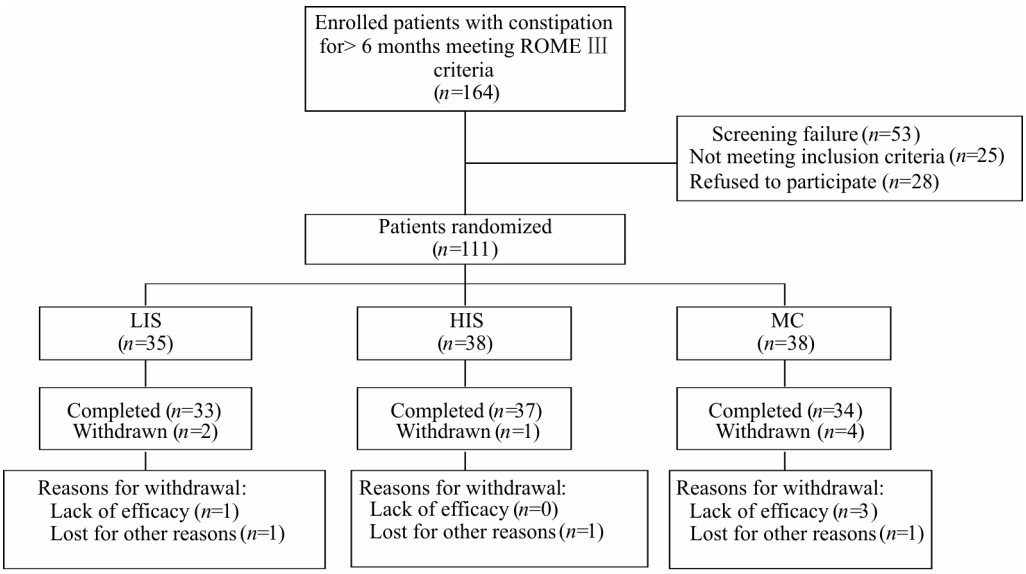

Fig. 1 The flow chart of the trail

2.2 Basic Data and Characteristics of Patients

In the baseline, there were no significant differences in terms of female ratio, age, height, weight, body mass index (BMI) and duration of constipation among the three groups (table 1).

Table 1 Patients' demographic data and baseline characteristics [$x\pm s$ **(%)**]

Parameters	LIS (*n*=35)	HIS (*n*=38)	MC (*n*=38)
Female [*n* (%)]	31 (88.57%)	34 (89.47%)	35 (92.11%)
Age (years)	30.29±12.95	30.49±15.89	30.77±12.41
Height (cm)	161.00±5.22	161.03±6.79	160.26±5.42
Weight (kg)	52.67±5.64	54.91±10.26	53.24±7.09
BMI	20.16±1.96	21.03±2.35	20.69±2.03
Duration of constipation (months)	98.00±87.06	109.49±117.12	92.91±79.02

2.3 Comparison of Average Spontaneous Bowel Movement Frequencies per Week

The results showed that time factor was statistically significant, and a significant improvement was observed in the average frequency/week from baseline to week 4 in all the three groups ($P<0.01$). There was no significant difference in the change of spontaneous bowel movement frequencies per week with time among the three groups.

By multivariate analysis of variance, there were significant differences in the spontaneous bowel movement frequencies per week before and after treatment (fig. 2).

2.4 Comparison of SAS and SDS Scores

Compared with the baseline, the SAS scores in high intensity of EA group was significantly decreased ($P<0.01$). As compared with MC group, the SAS scores in low and high intensity of EA groups were not significantly reduced.

Compared with the baseline, the SDS scores in low and high intensity of EA groups were significantly decreased ($P<0.01$ and $P<0.05$, respectively). As compared with MC group, the SDS scores in low and high intensity of EA groups were not significantly reduced (table 2).

Table 2 Comparison of SAS and SDS scores at the baseline and at the end of week 4 ($\bar{x}\pm s$)

Parameters	LIS (n=33)	HIS (n=37)	MC (n=34)
SAS scores			
Baseline	39.78±8.10	40.02±8.01	39. 83±10.35
Week 4	37.90±8.22	34.49±7.36*	40.17±13.01
SDS scores			
Baseline	43.31±9.67	41.08±8.91	42.93±11.85
Week 4	38.42±8.46△	35.91±8.41*	41.77±11.43

*P<0.01, △P<0.05 *vs.* the baseline

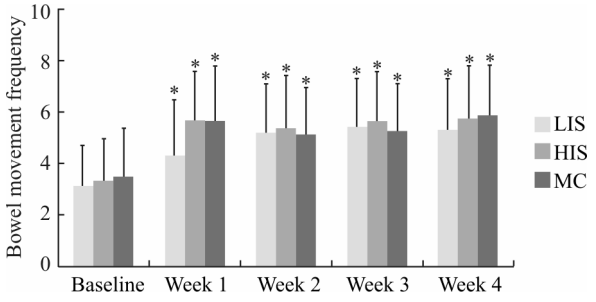

Fig. 2 Comparison of spontaneous bowel movement frequency
*P<0.01 *vs.* the baseline

2.5 Comparison of Serum Levels of CORT, SP, and VIP

2.5.1 Comparison of Serum Levels Within Groups As compared with the baseline, the serum levels of CORT were significantly decreased in low intensity of EA group and high intensity of EA group at the end of week 4 (P<0.01 and P<0.05, respectively), and those of CORT were significantly increased in MC group at the end of week 4 (P<0.01, fig. 3A).

As compared with the baseline, the serum levels of SP were significantly increased in low intensity of EA group and high intensity of EA group at the end of week 4 (P<0.05), and the serum levels of SP had a slight decline in MC group at the end of week 4 (fig. 3B).

As compared with the baseline, the serum levels of VIP were significantly increased in the three groups at the end of week 4 (P<0.01, P<0.05, and P<0.05, respectively) (fig. 3C).

2.5.2 Comparison of Serum Levels of CORT, SP and VIP Among Groups In the baseline, no significant differences were observed in each hormone among the three groups. At the end of week 4, as compared with MC group, the level of SP in low intensity of EA group was increased significantly (P<0.01, fig. 3B), while CORT and VIP in low intensity of EA group and high intensity of EA group had no significant changes.

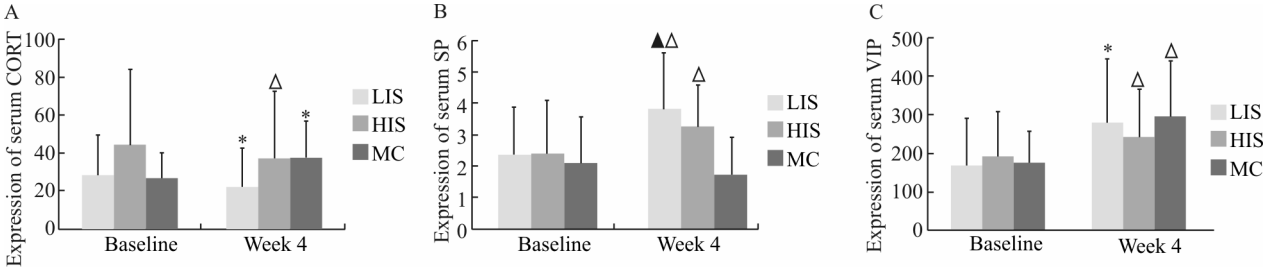

Fig. 3 Comparison of the serum levels of CORT (A), SP (B), and VIP (C)
*P <0.01, △P<0.05 *vs.* the baseline; ▲P<0.05 *vs.* MC group

3 DISCUSSION

In this study, the changes in the spontaneous bowel movement frequencies per week, SAS, SDS, stress hormones (CORT) and gastrointestinal hormones (SP and VIP) after 4 weeks of treatment were observed, and the efficacy of different current intensities of EA treatment for FC was assessed. Mosapride, a mixed type of 5-HT4 receptor agonist/5-HT3 receptor antagonist, can excite 5-HT4 receptor of gastrointestinal myenteric plexus, stimulate acetylcholine release, and enhance the gastrointestinal dynamics finally. The 5-HT4 receptors were distributed in the upper gastrointestinal tract[19, 20], so the mosapride citrate played an important role in gastric emptying and duodenum motility. Recently, 5-HT4 receptors were also found in the human colon and rectum[21, 22], thereby the 5-HT4 receptor agonist also could stimulate the lower gastrointestinal movement. Therefore, mosapride is used to strengthen the movement of colon and rectum, to relieve the constipation symptom. Moreover, as compared with the adverse reactions of traditional laxatives, such as abdominal distension, gastrointestinal stimulation, mosapride has less adverse reactions, and FC patients had better tolerance for it.

The reduced frequency of spontaneous bowel movement is the main symptom of FC, thus, it was used to evaluate the improvement of constipation symptoms. the intra-group comparison showed that as compared with the baseline, spontaneous bowel movement frequencies of different intensities of EA groups and MC group were significantly increased. There was no significant difference in therapy efficacy among the three groups. Therefore, SAS, SDS, CORT, SP and VIP were used to evaluate the differences of different intensities of EA treatment for FC.

In recent years, it has been shown that FC patients are more susceptible to anxiety and depression, as well as somatization, obsessive-compulsive tendencies and other neurotic personality characteristics[23]. The Symptom Checklist-90-R (SCL-90-R) Scales were used to

evaluate psychological status of patients with chronic constipation, and one-third of constipated patients got higher scores for somatization, anxiety and depression[24]. Some evidence demonstrated that under mental stress, the autonomic nervous system of rats would release the corticotropin-releasing hormone (CRH), which could induce colonic motor alterations[25]. The mental factors related to FC pathogenesis were still not very clear; psychological distress might be induced through inhibiting peripheral autonomic nerve innervating colon, or through influencing the brain cortex to affect the hypothalamus and autonomic nervous system, especially the parasympathetic nerve, and then constipation was caused[26]. Thus, the psychological factors could affect the movement of the gastrointestinal tract. In our results, the SAS and SDS scores were reduced after 4 weeks of EA therapy, while the sores were not decreased after 4 weeks of medicine therapy. Therefore, as compared with medicine therapy, EA therapy can effectively relieve anxiety and depression of FC patients.

The hypothalamus-pituitary-adrenal (HPA) axis can regulate the stress adaptation and has a close relationship with anxiety and depression. Enhanced HPA axis response to stress is prominent, and it regularly serves as potential regulatory factor to ameliorate health-damaging effects of psychosocial risk factors[27]. A study about increased HPA axis activation induced by stress showed that the impact of stress was correlated with the pathogenesis of metabolic syndrome[28]. These studies have demonstrated that one of the common reasons of HPA axis hyperactivity is the persistence of stress; HPA axis hyperactivity will pose a threat to body and mind of people, and affect the progression of diseases for the majority of patients. It has been manifested that CORT is released in response to various bodily stress in rodents, the CORT levels reflect acute adaptive responses, and the basal steady-state CORT levels are a predictor of stress susceptibility[29]. Increased stress was associated with a higher CORT awakening response and higher mean CORT levels of day and night[30]. The association existed between increased CORT reactivity to stress and the progression of coronary artery calcification[31]. The serum level of CORT was closely related with the psychological pressure in daily life, and it is also known as the stress hormone. Through the detection of the CORT levels in the serum, the anxiety and depression of patients can be revealed appropriately. Our results showed that after 4 weeks of treatment, the CORT levels in the serum were reduced significantly in EA groups, while the CORT levels in the serum were increased significantly in MC group. The findings in this study suggested that EA is more effective than medicine in alleviating the psychological pressure of FC patients.

As a neurotransmitter and a neuromodulator, SP plays an important role in regulating pain perception and emotional disorders, promoting gastrointestinal motility, protecting gastrointestinal mucosa, and improving electrolyte release of small intestine and large intestine[32–36]. It has been reported that as compared with normal subjects, the deficiency of SP immunoreactive nerve fibres is associated with intractable constipation[36, 37]. It has been found that, the decreased responses of SP peptidergic nerves play an important role in the impaired motility of the colon in patients with slow transit constipation (STC)[38], SP mediated diminished contractile responses of the colonic circular muscle in the STC patients, which is due to an increased release of inhibitory prostaglandins through activation of up-regulated NK1 receptors[39]. Moreover, it has been indicated that SP can promote gastrointestinal motility, and the low levels of SP will reduce the intestinal movement, and contribute to the chronic constipation[40]. In our trials, after 4 weeks of treatment, the serum levels of SP were increased significantly in EA groups, while the serum levels of SP were decreased significantly in MC group. Thus, EA has more advantages than mosapride citrate in improving gastrointestinal motility of FC patients.

VIP is a neuropeptide that functions as a neuromodulator and neurotransmitter. It helps smooth muscles relaxation along the gastrointestinal tract, increases water and electrolytes release from the pancreas and gut, inhibits gastric acid secretion and absorption, and maintains immune tolerance[41–43]. Some studies have reported that VIP neurons are decreased in colon of patients with severe chronic constipation[38, 40, 44]. Additionally, anxiety and depressive states of irritable bowel syndrome (IBS) patients lead to changes in the secretion of VIP and somatostatin (SS), and subsequent changes in gastrointestinal motility and function[45]. The VIP contents of plasma in stressed rat were obviously decreased, and the levels of VIP were elevated significantly after EA at Zusanli (ST36)[46]. This demonstrated that EA can effectively improve the stress state of rats and regulate the levels of VIP. Our results showed that as compared with the baseline, the serum levels of VIP in EA groups and MC group were significantly increased, and the serum levels of VIP were higher in EA groups than in MC group.

In conclusion, after treatment, there is no difference in spontaneous bowel movement frequency per week among the three groups. Through the changes of SAS, SDS scores and serum levels of CORT, our results show that EA can alleviate the FC patient's anxiety and depression, and affect the overall symptoms of FC patients. In addition, the changes of serum levels of SP and VIP suggest that EA can improve the gastrointestinal motility and has a better therapeutic effect for FC patients than mosapride. However, the differences between low intensity of EA group and high intensity of EA group are not significant.

Conflict of Interest Statement

The authors declare that there are no financial and personal relationships with other people or organizations that can inappropriately influence this work.

REFERENCES

1 Dennison C, Prasad M, Lloyd A, *et al*. The health-related quality of life and economic burden of constipation. Pharmacoeconomics, 2005,23(5):461-476

2 Longstreth GF, Thompson WG, Chey WD, *et al*. Functional bowel disorders. Gastroenterology, 2006,130(5): 1480-1491

3 Sun SX, Dibonaventura M, Purayidathil FW, *et al*. Impact of chronic constipation on health-related quality of life, work productivity, and healthcare resource use: an analysis of the national health and wellness survey. Dig Dis Sci,

2011,56(9):2688-2695
4 Higgins PD, Johanson JF. Epidemiology of constipation in North America: a systematic review. Am J Gastroenterol, 2004,99(4):750-759
5 Riezzo G, Orlando A, D'Attoma B, *et al*. Randomised clinical trial: efficacy of Lactobacillus paracasei-enriched artichokes in the treatment of patients with functional constipation-a double-blind, controlled, crossover study. Aliment Pharmacol Ther, 2012,35(4):441-450
6 Ford AC, Suares NC. Effect of laxatives and pharmacological therapies in chronic idiopathic constipation: systematic review and meta-analysis. Gut, 2011,60(2):209-218
7 Dong Z, Sun Y, Lu P, *et al*. Electroacupuncture and lumbar transplant of GDNF-secreting fibroblasts synergistically attenuate hyperalgesia after sciatic nerve constriction. Am J Chin Med, 2013,41(3):459-472
8 Napadow V, Makris N, Liu J, *et al*. Effects of electroacupuncture versus manual acupuncture on the human brain as measured by fMRI. Hum Brain Mapp, 2005,24(3):193-205
9 Li M, Peng J, Song Y, *et al*. Electro-acupuncture combined with transcranial magnetic stimulation improves learning and memory function of rats with cerebral infarction by inhibiting neuron cell apoptosis. J Huazhong Univ Sci Technolog Med Sci, 2012,32(5):746-749
10 Zeng Q, Li M, Ouyang X, *et al*. Effect of electroacupuncture on reperfusion ventricular arrhythmia in rat. J Huazhong Univ Sci Technolog Med Sci, 2006,26(3):269-271
11 Zhao L, Shi J, Sun N, *et al*. Effect of electroacupuncture on TRPM7 mRNA expression after cerebral ischemia/reperfusion in rats via TrkA pathway. J Huazhong Univ Sci Technolog Med Sci, 2005,25(3):247-250
12 Ouyang H, Chen JD. Review article: therapeutic roles of acupuncture in functional gastrointestinal disorders. Aliment Pharmacol Ther, 2004,20(8):831-841
13 Broide E, Pintov S, Portnoy S, *et al*. Effectiveness of acupuncture for treatment of childhood constipation. Dig Dis Sci, 2001,46(6):1270-1275
14 Chesterton LS, Foster NE, Wright CC, *et al*. Effects of TENS frequency, intensity and stimulation site parameter manipulation on pressure pain thresholds in healthy human subjects. Pain, 2003,106(1-2):73-80
15 Bjordal JM, Johnson MI, Ljunggreen AE. Transcutaneous electrical nerve stimulation (TENS) can reduce postoperative analgesic consumption: a meta-analysis with assessment of optimal treatment parameters for postoperative pain. Eur J Pain, 2003,7(2):181-188
16 Olsén MF, Elden H, Janson ED, *et al*. A comparison of high-versus low-intensity, high-frequency transcutaneous electric nerve stimulation for painful postpartum uterine contractions. Acta Obstet Gynecol Scand, 2007,86(3):310-314
17 Barlas P, Ting SL, Chesterton LS, *et al*. Effects of intensity of electroacupuncture upon experimental pain in healthy human volunteers: A randomized, double-blind, placebo-controlled study. Pain, 2006,122(1-2):81-89
18 Li Y, Zheng H, Zeng F, *et al*. Use acupuncture to treat functional constipation: study protocol for a randomized controlled trial. Trials, 2012,13:104-111
19 Baxter GS, Craig DA, Clarke DE. 5-Hydroxytryptamine4 receptors mediate relaxation of the rat oesophageal tunica muscularis mucosae. Naunyn Schmiedebergs Arch Pharmacol, 1991,343(5):439-446
20 Mader R, Kocher T, Haier J, *et al*. Investigation of serotonin type 4 receptor expression in human and non-human primate gastrointestinal samples. Eur J Gastroenterol Hepatol, 2006,18(9):945-950
21 McLean PG, Coupar IM. Stimulation of cyclic AMP formation in the circular smooth muscle of human colon by activation of 5-HT4-like receptors. Br J Pharmacol, 1996,117(2):238-239
22 Sakurai-Yamashita Y, Yamashita K, Yoshimura M, *et al*. Differential localization of 5-hydroxytryptamine3 and 5-hydroxytryptamine4 receptors in the human rectum. Life Sci, 2000,66(1):31-34
23 Sun Y, Li XY, Yao LY, *et al*. Investigation of attention via the counting Stroop task in patients with functional constipation. Chin Med J, 2012,125(3):486-490
24 Merkel IS, Locher J, Burgio K, *et al*. Physiologic and psychologic characteristics of an elderly population with chronic constipation. Am J Gastroenterol, 1993,88(11):1854-1859
25 Gue M, Junien JL, Bueno L. Conditioned emotional response in rats enhances colonic motility through the central release of corticotropin-releasing factor. Gastroenterology, 1991,100(4):964-970
26 Chattat R, Bazzocchi G, Balloni M, *et al*. Illness behavior, affective disturbance and intestinal transit time in idiopathic constipation. J Psychosom Res, 1997,42(1):95-100
27 Brummett BH, Kuhn CM, Boyle SH, *et al*. Cortisol responses to emotional stress in men: association with a functional polymorphism in the 5HTR2C gene. Biol Psychol, 2012,89(1):94-98
28 Rosmond R. Role of stress in the pathogenesis of the metabolic syndrome. Psychoneuroendocrinology, 2005,30(1):1-10
29 Kim JG, Jung HS, Kim KJ, *et al*. Basal blood corticosterone level is correlated with susceptibility to chronic restraint stress in mice. Neurosci Lett, 2013,555:137-142
30 Kumari M, Badrick E, Sacker A, *et al*. Identifying patterns in cortisol secretion in an older population. Findings from the Whitehall II study. Psychoneuroendocrinology, 2010,35(7):1091-1099
31 Hamer M, Endrighi R, Venuraju SM, *et al*. Cortisol responses to mental stress and the progression of coronary artery calcification in healthy men and women. PLoS One, 2012,7(2):e31356
32 Gong S, Ding J, Chang Q. The expression of VIP and SP in the cochlea of spontaneously hypertensive rats and its implication. J Huazhong Univ Sci Technolog Med Sci, 2004,24(4):396-399
33 Datar P, Srivastava S, Coutinho E, *et al*. Substance P: structure, function, and therapeutics. Curr Top Med Chem, 2004,4(1):75-103
34 De Felipe C, Herrero JF, O'Brien JA, *et al*. Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. Nature, 1998,392(6674):394-397
35 Ebner K, Singewald N. The role of substance P in stress and anxiety responses. Amino Acids, 2006,31(3):251-272
36 Hutson JM, Chow CW, Hurley MR, *et al*. Deficiency of substance P-immunoreactive nerve fibres in children with intractable constipation: a form of intestinal neuronal dysplasia. J Paediatr Child Health, 1997,33(3):187-189
37 Hutson JM, Chow CW, Borg J. Intractable constipation with a decrease in substance P-immunoreactive fibres: is it a variant of intestinal neuronal dysplasia? J Pediatr Surg, 1996,31(4):580-583
38 Tomita R. Regulation of the peptidergic nerves (substance

P and vasoactive intestinal peptide) in the colon of women patients with slow transit constipation: an *in vitro* study. Hepatogastroenterology, 2008,55(82-83):500-507

39 Liu L, Shang F, Morgan MJ, *et al*. Cyclooxygenase-dependent alterations in substance P-mediated contractility and tachykinin NK1 receptor expression in the colonic circular muscle of patients with slow transit constipation. J Pharmacol Exp Ther, 2009,329(1):282-289

40 King SK, Sutcliffe JR, Ong SY, *et al*. Substance P and vasoactive intestinal peptide are reduced in right transverse colon in pediatric slow-transit constipation. Neurogastroenterol Motil, 2010,22(8):883-892

41 Said SI, Mutt V. Polypeptide with broad biological activity: isolation from small intestine. Science, 1970,169(3951): 1217-1218

42 Said SI, Mutt V. Potent peripheral and splanchnic vasodilator peptide from normal gut. Nature, 1970,225(5235): 863-864

43 Qiao Y, Fang JG, Xiao J, *et al*. Effect of baicalein on the expression of VIP in extravillous cytotrophoblasts infected with human cytomegalovirus *in vitro*. J Huazhong Univ Sci Technolog Med Sci, 2013,33(3):406-411

44 Cortesini C, Cianchi F, Infantino A, *et al*. Nitric oxide synthase and VIP distribution in enteric nervous system in idiopathic chronic constipation. Dig Dis Sci, 1995, 40(11):2450-2455

45 Han B. Correlation between gastrointestinal hormones and anxiety-depressive states in irritable bowel syndrome. Ther Med, 2013,6(3):715-720

46 Shen GM, Zhou MQ, Xu GS, *et al*. Role of vasoactive intestinal peptide and nitric oxide in the modulation of electroacupucture on gastric motility in stressed rats. World J Gastroenterol, 2006,12(38):6156-6160

(Received Jan. 16, 2014; revised Aug. 13, 2014)