HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2017.07.017

Effect of retrograde colonic electrical stimulation on colonic transit and stress-induced visceral hypersensitivity in rats with irritable bowel syndrome

Xiao-Ri Qin[1,#], Yan Tan[2,#], Xiao-Ning Sun[1✉]

[1]*Department of Gastroenterology, Hainan Provincial People's Hospital, Haikou 570311, China*

[2]*Department of Gastroenterology, The Affiliated Hospital of Hainan Medical College, Haikou 570102, China*

ARTICLE INFO

Article history:
Received 6 Jun 2017
Received in revised form 16 Jul 2017
Accepted 26 Jul 2017
Available online 24 Aug 2017

Keywords:
Retrograde colonic electrical stimulation
Colonic transit
Irritable bowel syndrome
Visceral hypersensitivity

ABSTRACT

Objective: To evaluate the effects of retrograde colonic electrical stimulation (RCES) with trains of short pulses and RCES with long pulses on colonic transit in irritable bowel syndrome (IBS) rats and to investigate whether stress-induced visceral hypersensitivity could be alleviated by RCES so as to find a valuable new approach for IBS treatment.

Methods: A total of 48 male rats were randomly divided into model group and control group. Visceral hypersensitivity model was induced by a 6-day HIS protocol composed of two stressors, restraint stress for 40 min and forced swimming stress for 20 min. The extent of visceral hypersensitivity was quantified by electromyography and abdominal withdrawal reflex scores (AWRs) of colorectal distension (use a balloon) at different pressures. After the modeling, all rats were equipped with electrodes in descending colon for retrograde electrical stimulation and a PE tube for perfusing phenol red saline solution in the ileocecus. After recovering from surgery, RCES with long pulses, RCES with trains of short pulses, and sham RCES were performed in colonic serosa of rats for 40 min in six groups of 8 each, including three groups of visceral hypersensitivity rats and three groups of health rats. Colonic transit was assessed by calculating the output of phenol red from the anus every 10 min for 90 min. Finally, the extent of visceral hypersensitivity will be quantified again in model group.

Results: After the 6-day HIS protocol, the HIS rats displayed an increased sensitivity to colorectal distention, compared to control group at different distention pressures ($P < 0.01$). CRES with trains of short pulses and long pulses significantly attenuated the hypersensitive responses to colorectal distention in the HIS rats compared with sham RCES group ($P < 0.01$). The effects of RCES on rats colon transmission: In the IBS rats, the colonic emptying were (77.4 ± 3.4)%, (74.8 ± 2.4)% and (64.2 ± 1.6)% in the sham RCES group, long pulses group and trains of short pulses group at 90 min; In healthy rats, The colonic emptying was (65.2 ± 3.5)%, (63.5 ± 4.0)% and (54.0 ± 2.5)% in the sham RCES group, long pulses group and trains of short pulses group at 90 min.

Conclusion: RCES with long pulses and RCES with trains of short pulses can significantly alleviate stress-induced visceral hypersensitivity. RCES with trains of short pulses has an inhibitory effect of colonic transit, both in visceral hypersensitivity rats and healthy rats.

1. Introduction

Irritable bowel syndrome (IBS) is a common bowel disorder characterized by recurrent abdominal pain or discomfort associated with altered bowel habits in the absence of structural pathology [1]. Since IBS is diagnosed based on its symptoms and its pathophysiology is unclear, current treatments for IBS include constipating agents and muscle relaxants, but the efficacy of these drugs is limited [2]. It is difficult to treat chronic visceral pain, the cardinal feature of IBS, which decreases the quality of

First author: Xiao-Ri Qin, Department of Gastroenterology, Hainan General Hospital, Haikou 570311, China.
E-mail: jyxiaori@163.com.
First author: Yan Tan, Department of Gastroenterology, The Affiliated Hospital of Hainan Medical College, Haikou 570102, China.
✉Corresponding author: Xiao-Ning Sun, Department of Gastroenterology, Hainan Provincial People's Hospital, Haikou 570311, China.
E-mail: xnsun_0108@163.com
Peer review under responsibility of Hainan Medical University.
Foundation project: This work was supported by the Key Technology Project of Hainan Province (ZDXM20130071).
# These authors contributed equally to this study.

life for an important segment of the population worldwide. Transcutaneous electric nerve stimulation has been used extensively for treatment of various painful conditions and gastrointestinal diseases, including IBS, functional dyspepsia, constipation, and diarrhea [3,4]. In contrast, colonic electrical stimulation has lagged far behind cardiac pacing or electrical nerve stimulation [5]. Over the past decade, several stimulation patterns to modulate colon motility have been tested in animal and human models [6]. Shafik *et al.* found that colonic electrical stimulation succeeded in relieving the symptoms in nine patients with IBS [7]. Electrical stimulation including forward and retrograde electrical stimulation depends on the location of electrodes. Generally, electrodes implanted into the distal of gastrointestinal belong to retrograde electrical stimulation. Based on the differences of stimulation parameters, they can be divided into long pulse electrical stimulation, short pulse electrical stimulation and trains of pulses electrical stimulation. A few studies have demonstrated that retrograde gastric or intestinal electric stimulation (IES) is able to delay gastric emptying or intestinal transit [8], whereas forward IES is able to accelerate intestinal transit. However, little is known about potential roles of RCES, and maybe it is a new option for correct the disordered defecation and treatment of IBS. On the other hand research into RCES for chronic visceral pain is still in its infancy [9]. Thus, further investigations on RCES efficacy and its mechanisms are definitely merited. Clinical findings suggest that long-term stress, rather than short-term stress, exacerbates symptoms of IBS [10,11], and a rat model of visceral hypersensitivity induced by heterotypic intermittent stress (HIS) have been developed. These rats displayed no strong inflammation or injury in the colon, but a significantly higher visceromotor response to colorectal distention (CRD), compared with controls. Thus, the animal model can mimic the major characteristics of patients with IBS and thus it is suitable for study of the effect of RCES treatment. The aim of our study was to investigate whether RCES has therapeutic benefits on visceral hypersensitivity induced by HIS, and investigate the influence of RCES on colonic transmission.

2. Materials and methods

2.1. Animals

A total of 48 male Sprague-Dawley rats (Slack King of Laboratory Animals, Ltd., China) weighing 195–205 g, randomly divided into model group and control group. Then the two groups were divided each into three groups: sham RCES (no stimulation), RCES with long pulses, RCES with trains of short pulses, eight in each group. All rats were housed at a constant temperature and a humidity environment with free access to food and water. Before the experiments, all rats were starved overnight with free access to water. The Institutional Animal Care and Use Committee at the Research Center for Drug Safety Evaluation of Hainan Province approved the surgical and experimental protocol.

2.2. Establishment of rat model of IBS

2.2.1. Heterotypic intermittent stress protocol (HIS protocol)

Model rats were subjected to 6 consecutive days of a HIS protocol comprised of two stressors, which included restraint stress (RS) for 40 min and forced swimming stress (FSS) for 20 min, as described previously [10]. Stressors were applied between 8:00 am and 11:00 am. For RS, the rats were restrained in a clear plastic container (6 cm in diameter × 18 cm in length). The container had 2-cm diameter openings at each end for the rat to breathe normally. After 1-h break, for FSS, the rat was forced to swim for 20 min in a plastic bucket (35 cm high × 30 cm diameter) filed to a depth of 15 cm below the top with water at room temperature (approximately 22 °C). Control rats were brought to the laboratory without the stress protocol.

2.2.2. Electromyographic recordings

All rats were tested for EMG, after model rat termination of last stressor. An air sac urinary catheter (F6 BARD-FOLEY USA) was inserted 7 cm into the descending colon and rectum via the anus and held in place by taping the tubing to the tail. A pair of electrodes (Medtronic Inc., Minneapolls, USA) were implanted in the external oblique muscle and the other end of the wires connected with testing facilities [12]. Rats were put into fixation-machine (20 cm × 8 cm × 8 cm) (Beijing HeLiKeChuang Science and Technology Development Company, China) and allowed to adapt for 30 min. CRD was performed by rapidly inflating the air sac to constant pressure. Pressure was measured using a syringe injection of water [13], the balloon was inflated to various pressures (1.0 mL H_2O, 1.5 mL H_2O, 2.0 mL H_2O) for a 20 s stimulation period followed by a 2-min rest. EMG was recorded continuously during the experiment, the EMG signal was amplified, filtered at 500 Hz and digitized by BL-420E experimental system (ChengDu Technology Market, China). The area under the curve (AUC) for EMG activities during each 20 s of distention was calculated using an computer program [14]. The net value for each distension was calculated by subtracting the baseline value derived from the AUC for the 20 s pre-distention period. Each rat was tested for EMG twice for each distention pressure and the mean AUC of EMG calculated from the two groups was used for following statistical analysis.

2.2.3. Abdominal withdrawal reflex scores

Visceral hypersensitivity was also measured by grading behavioral response of rats to CRD. Behavioral response to CRD was measured by visual observation of AWR by a blinded observer, the AWRs criteria [15]. The experimenter, who assigned the AWR scores and performed the EMG analysis, was masked to the control or model group assignment, to the sham or RCES treatment. Each rat was tested twice for AWR score for each distention pressure and the mean was used for the following statistical analysis.

2.3. Surgical procedures

Under general anesthesia with chloral hydrate (4 mg/kg), the abdominal midline incision of the rats was performed. One fistula was made in the cecum, about 2 cm from the ileocecus. A polyethylene tube (PE-90) was inserted for perfusing phenol red solution. One pair of unipolar pacing wires (Medtronic Inc., Minneapolls, USA) were implanted into the seromuscular layer of the descending colon 4 cm above the anus. The two electrodes [12] in the pair were separated by approximately 1 cm. The other end of the pacing wires and polyethylene tube were tunneled to the back of the neck subcutaneously, through from the skin, fixed and numbered. Rats were allowed 1 week to recover from the surgery.

2.4. Experimental design

After recover from the surgery, model group and control group according to the type of stimulation were categorized into three groups at random: sham stimulation, RCES with long pulses, RCES with trains of short pulses, RCES were performed for 40 min simultaneously. After the electrical stimulation was completed, 1.5 mL of 5% phenol red (0.5 mg/mL) was given as a non-absorbable marker. From the polyethylene tube, then saline was continuously perfused for 90 min with a micro syringe pump (0.1 mL/min; TOP-5300, Terumo, Japan). The perfusion rate was chosen to ensure that saline in the solution had sufficient contact time with colon mucosa without leading to colon stasis, during the 90-min perfusion period. Colon contents were collected every 10 min. The phenol red in each of the samples were analyzed and calculated. After collection of the colon contents, the EMG and AWR scores were recorded again from model group. In the end, the rats were sacrificed after anesthetized.

2.5. RCES

Electrical stimulation was applied by BL-420E experimental system. The RCES with long pulses was performed using a frequency of 20 cpm, a pulse width of 200 ms, and amplitude of 10 mA (constant current output). The RCES with trains of short pulses was conducted using a train on-time of 2 s and off-time of 3 s, a pulse frequency of 40 Hz, a pulse width of 4 ms, and pulse amplitude of 10 mA.

2.6. Analysis and calculation of phenol red

The effluents from anus were added 100 mL of 0.1 mol/L NaOH and stored for 1 h at 20 °C. Totally 5 mL of supernatants were added 0.5 mL of TCA (20% w/v), followed by centrifugation for 20 min at 2 800 *g*. Then 3 mL of yielded supernatants were added 4 mL of NaOH (0.5 mol/L). The mixture was mixed thoroughly after each addition and then finally filtered. Phenol red in the filtrate was determined by measuring the absorption at a wavelength of 560 nm using a spectrophotometer [8] (Visible Spectrophotometer, 722s, INESA, ShangHai, China). The tested colonic transit time was expressed as the percentage of phenol red recovery. The percentage of phenol red recovery was calculated as the ratio between the recovered phenol red and the total amount of phenol red.

2.7. Statistical analysis

Statistical analysis was conducted using commercial software SPSS PASW Statistics (Chicago, United States). All data are presented as means ± SD. Two sample *t* test was applied to investigate the difference between the data, and two-way repeated-measures ANOVA was applied to assess the differences in phenol red recovery among the three groups. The level of significance was set at $P < 0.05$.

3. Results

3.1. HIS produced visceral hypersensitivity

To determine whether HIS induces visceral hypersensitivity, AWR scores to CRD were measured in model group (HIS group) and control group. The AWR scores were 1.2 ± 0.4, 2.0 ± 0.6 and 2.5 ± 0.7 for 1.0, 1.5 and 2.0 mL of balloon distention pressures in control group, respectively. After termination of the last stressor, the AWR scores (HIS group) were 2.1 ± 0.5, 2.9 ± 0.5 and 3.6 ± 0.5 for 1.0, 1.5 and 2.0 mL of balloon distention pressures, respectively (Figure 1A). There were significant effects of HIS on AWR scores for all pressures ($P < 0.01$, two way repeated measures ANOVA; $n = 24$ rats for each group). To further confirm the visceral hypersensitivity induced by HIS, EMG measurements were performed in the HIS group and control group. The AUC of EMG recordings was 34.7 ± 3.6, 43.5 ± 3.0 and 55.6 ± 3.7 for 1.0, 1.5 and 2.0 mL of balloon distention pressures in control group, respectively. After termination of the HIS protocol, the AUCs (model group) were 50.1 ± 2.6, 65.6 ± 4.4 and 82.7 ± 5.0 for 1.0, 1.5 and 2.0 mL of balloon distention pressures, respectively (Figure 1B). There were significant effects of HIS on EMG for all pressures ($P < 0.001$, two way repeated measures ANOVA; $n = 24$ rats for each group).

3.2. RCES treatment suppressed visceral hypersensitivity in HIS rats

To determine whether RCES suppressed visceral hypersensitivity, AWR scores and AUCs of EMG recordings after RCES treatment were compared with those after sham RCES treatment. To define the specificity of RCES-mediated analgesic effect in rats, for sham RCES group, implant electrodes but no electrical stimulation applied. AWR scores and EMG activities were recorded immediately after collected the colon contents. Both distention stress and RCES treatment affected AWRs ($n = 8$ rats for each group, two-way repeated measures ANOVA: under stress effect, $P < 0.01$; RCES treatment effect, $P < 0.01$). RCES treatment significantly decreased AWR scores in HIS rats. AWRs at 1.0 mL of balloon distention pressure: 2.0 ± 0.6 *vs* 1.4 ± 0.5 *vs* 1.6 ± 0.5; 1.5 mL of balloon distention pressure: 2.7 ± 0.5 *vs* 2.0 ± 0.8 *vs* 2.1 ± 0.7; 2.0 mL of balloon distention pressure: 3.6 ± 0.5 *vs* 2.7 ± 0.5 *vs* 2.9 ± 0.4 (sham RCES *vs* RCES with trains of short pulses *vs* RCES with long pulses). RCES treatment significantly decreased AWR scores in HIS rats ($P < 0.01$, two-way repeated measures ANOVA, Figure 2A). To further confirm the RCES effect on stressed rats, EMGs were performed after RCES or sham RCES treatment. Both distention pressure and RCES treatment affected AUCs of HIS rats significantly ($n = 8$ rats for each group, two-way repeated measures ANOVA: pressure effect, $P < 0.01$; RCES treatment effect, $P < 0.01$). HIS rats that received RCES treatment showed a significant decrease in their AUCs compared to rats that received sham RCES under 1.0, 1.5 and 2.0 mL of balloon distention pressures, EMG for, at 1 mL of balloon distention pressure: 51.2 ± 3.3 *vs* 40.0 ± 2.7 *vs* 43.6 ± 4.0; 1.5 mL of balloon distention pressure: 62.2 ± 4.4 *vs* 51.7 ± 4.4 *vs* 55.9 ± 4.1; 2 mL of balloon distention pressure: 78.7 ± 5.2 *vs* 64.2 ± 4.8 *vs* 67.0 ± 4.2 (sham RCES *vs* RCES with trains of short pulses *vs* RCES with long pulses) (Figure 2B). To exclude the non-specific effect of RCES treatment, RCES was applied at descending colon for 40 min significantly attenuated the hypersensitive responses to colorectal distention in HIS rats compared with sham RCES treatment.

3.3. Effects of RCES on colonic transit

As shown in Figure 3, RCES with trains of short pulses and not long pulses significantly reduced the percentage of recovered

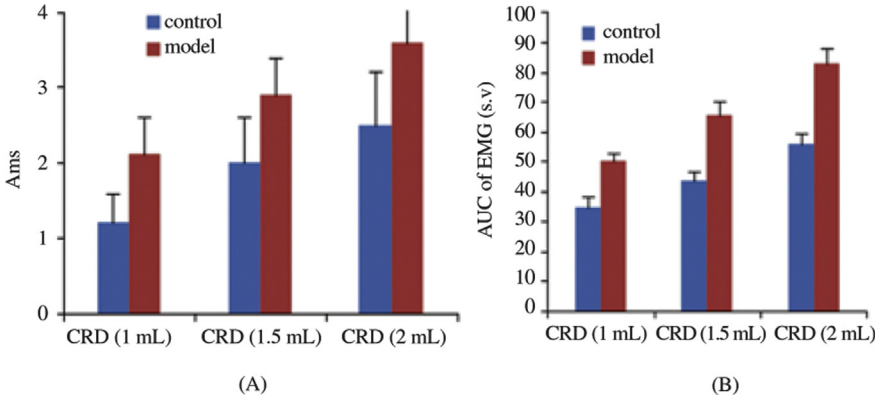

Figure 1. Effect of HIS on AWR scores and EMG.
(A) There was significant effect of HIS on AWR scores for all pressures ($P < 0.01$, two way repeated measures ANOVA; $n = 24$ rats for each group). (B) There was significant effect of HIS on EMG for all pressures ($P < 0.001$, two way repeated measures ANOVA; $n = 24$ rats for each group).

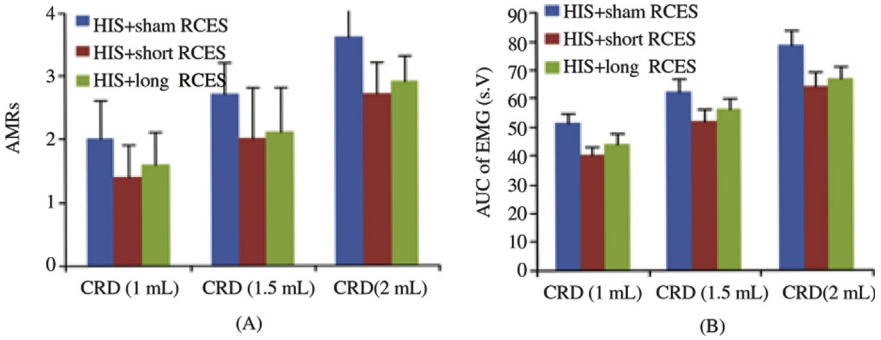

Figure 2. Effect of RCES treatment on abdominal withdrawal reflex (AWR) scores (A) and area under the curve (AUCs) (B).
(A) RCES treatment significantly decreased AWR scores in HIS rats ($P < 0.01$, two-way repeated measures ANOVA). (B) HIS rats that received RCES treatment showed a significant decrease in their AUCs compared to rats that received sham RCES under 1.0, 1.5 and 2.0 mL of balloon distention pressures.

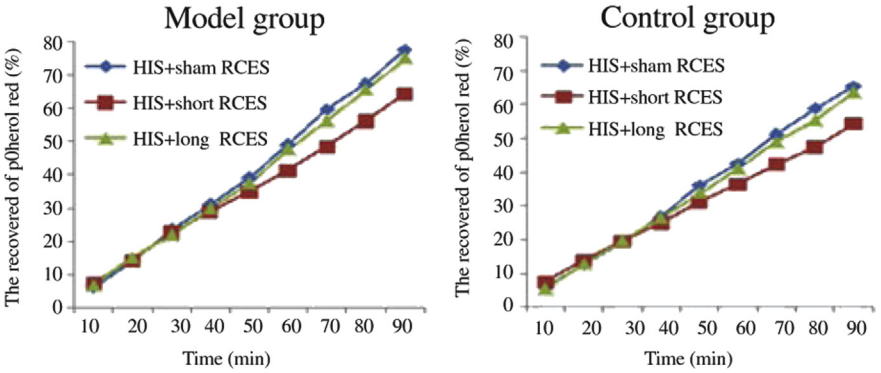

Figure 3. Colonic transit was assessed by calculating the output of phenol red from the anus every 10 min for 90 min.
RCES with trains of short pulses and not long pulses significantly reduced the percentage of recovered phenol red in model and control groups (ANOVA, $P < 0.001$).

phenol red (ANOVA, $P < 0.001$). In the model group rats, the percentage of recovered phenol red was (77.4 ± 3.4)% in the group of sham RCES and (74.8 ± 2.4)% in the group of RCES with long pulses ($P < 0.001$), (64.2 ± 1.6)% in the group of RCES with trains of short pulses ($P < 0.001$) at 90 min. And in control group rats, the percentage of recovered phenol red was (65.2 ± 3.5)% in the group of sham RCES, (63.5 ± 4.0) % in the group of RCES with long pulses ($P < 0.001$) and (54.0 ± 2.5)% in the group of RCES with trains of short pulses ($P < 0.001$).

4. Discussion

In our study, RCES treatment significantly reduced AWR scores and suppressed EMG responses to colorectal distention in the HIS rats, indicating that RCES had an analgesic effect in this model. Although electric stimulation has been used clinically for alleviation of various types of pain [16,17], there is no enough scientific validation for the use of RCES in visceral pain. Two parameters of electric stimulation we choose significantly suppressed the visceral hypersensitivity. Since the parameter used for RCES treatment IBS rats, further experiments are needed to investigate the cause of different stimulation parameters produce the analgesic effect in this model.

The mechanisms that lead to chronic visceral hypersensitivity are unclear. However, several working models may be considered, including: nociceptive input from the colon that leads to hypersensitivity; increased intestinal permeability that induces a visceral nociceptive drive; and alterations in the expression of microRNAs

in gastrointestinal tissue that might be delivered via blood microvesicles to other target organs, such as the peripheral or central nervous system [18]. There are a lot of clinical researches on body surface electrical stimulation [19,20]. The endogenous opioid system is a well-established for explanation of electrical stimulation effects, and the involvement of non-opioid mechanisms in ES analgesia was confirmed by experiments in which administration of 5-HT or catecholamine or adrenoceptor antagonists or depletion of cellular monoamine content blocked the electrical stimulation-induced analgesic effect [21,22].

From cardiac pacemakers to colonic electrical stimulation, the basic principle is using different frequency current to normalizing the electric rhythm and treatment of organ dysfunction [6]. Colon dysmotilities generates abdominal pain and disordered defecation, which are symptoms accompanying IBS. In our research, the specific position of electrode and stimulation parameters are chosen for improvement in symptoms of IBS.

Electric stimulation at different locations leads to different results. Generally, electrodes implanted into the proximal of gastrointestinal belong to forward electrical stimulation, and it can trigger the myoelectrical propagation from proximal towards distal. Otherwise, they belong to retrograde electrical stimulation. Most previous studies with small intestinal electric stimulation have applied forward stimulation and have reported an acceleration of intestinal transit [23–26]. Retrograde electrical pacing increased small intestinal absorption of water, glucose and sodium, and decreased output of potassium in dogs with short bowel syndrome, without inducing any unacceptable symptoms. About the forward colonic electrical stimulation, Sallam *et al.* reported that implanted electrode into the seromuscular layer of the ascending colon in dog, with trains of short pulses has an excitatory effect in colonic transit [27]. A few studies have used in colon about retrograde electric stimulation, in order to achieve the goal of retrograde electrical pacing. We chose implanted the stimulate lead in the serosal of descending colon wall and focused on the RCES mediated effect in IBS rat models. The results showed that RCES at seromuscular layer of the descending colon significantly suppressed the visceral hypersensitivity to CRD and was able to delay colon transit.

Based on the differences of stimulation parameters can be divided into long pulse electrical stimulation (The pulse width greater than 10 ms), short pulse electrical stimulation (The pulse width less than 1 or 5 ms) and trains of pulses electrical stimulation (be composed of short pulse sequence) [28–30]. Different parameters of electric stimulation may have different effects on gastrointestinal functions. The long pulses has been used in the study of Shafi and Chen [7]. The parameters used for pacing comprised an amplitude of 6 mA, a pulse width of 150 ms and a frequency of 25% higher than that of the basal colonic waves. In 7/9 patients with IBS the improvement of symptoms after 6 months of daily pacing. Bertschi *et al.* with trains of short pulses was conducted using a pulse frequency of 120 Hz, a pulse width of 1 ms, and pulse amplitude of 7–15 mA [31]. Stimulation of the cecum of pig can induce contraction of the cecum and excretes of the colonic contents. Sevcencu *et al.* indicated that tens of ms long pulses depolarize muscle cells directly, while pulses shorter than 10 ms induce muscle contraction through the activation of cholinergic systems. Besides eliciting contraction, electrical stimulation of the colon wall can also initiate inhibitory responses [32]. The activation of NO-releasing resulted in relaxation of the muscles [33–35]. In our study, two parameters of electric stimulation, trains of short pulses and long pulses, were selected, and we found that the trains of short pulses delay colon transit, while long pulses had no significant effects on the output of colon. On the other hand, in order to avoid the damage of the colon, we choose single-site stimulation instead of multiple-site sequential stimulation. Vaucher *et al.* reported that single-site stimulation is a good means to promote transit in patients with constipation type of irritable bowel syndrome (IBS-C) and to attenuate symptoms related to its motor dysfunction [30].

The studies performed show that trains of short pulses RCES can delay colon transit and have a significant analgesic effect on the visceral hyperalgesia. It may become a valuable new approach in treating diarrhea irritable bowel syndrome. However, it should also be noted that the current study had certain limitations. Most of the stimulation patterns have been developed in animal models and further experiments are necessary to investigate whether they are applicable to humans. In addition, improvement of the stimulation methods is necessary. It can be achieved by better understanding the mechanisms activated by electrical stimulation of the colon wall.

Conflicts of interest statement

The authors declare that there is no conflict of interests.

Acknowledgment

This work was supported by the Key Technology Project of Hainan Province (ZDXM20130071).

References

[1] Cremonini F, Talley NJ. Irritable bowel syndrome: epidemiology, natural history, health care seeking and emerging risk factors. *Gastroenterol Clin North Am* 2005; **34**(2): l89-204.
[2] Liu HR, Yang Y, Wu HG. Clinical study on acupuncture in treating diarrhea-predominant irritable bowel syndrome. *J Acupunct Tuina Sci* 2008; **6**: 360-362.
[3] Takahashi T. Acupuncture for functional gastrointestinal disorders. *J Gastroenterol* 2006; **41**: 408-417.
[4] Schneider A, Streitberger K, Joos S. Acupuncture treatment in gastrointestinal diseases: a systematic review. *World J Gastroenterol* 2007; **13**: 3417-3424.
[5] Yin J, Chen JD. Implantable gastric electrical stimulation: ready for prime time? *Gastroenterology* 2008; **134**: 665-667.
[6] Sevcencu C. Electrical stimulation – an evolving concept in the treatment of colonic motor dysfunctions. *Neurogastroenterol Motil* 2006; **18**: 960-970.
[7] Shafik A, EI-Sibai O, Shafik AA, Ahmed I. Colonic pacing in the treatment of patients with irritable bowel syndrome: technique and results. *Front Biosci* 2003; **8**: b1-5.
[8] Sun Y, Chen JD. Intestinal electric stimulation decreases fat absorption in rats: therapeutic potential for obesity. *Obes Res* 2004; **12**(8): 1235-1242.
[9] Reynolds JA, Bland JM, MacPherson H. Acupuncture for irritable bowel syndrome an exploratory randomised controlled trial. *Acupunct Med* 2008; **26**: 8-16.
[10] Winston JH, Xu GY, Sarna SK. Adrenergic stimulation mediates visceral hypersensitivity to colorectal distension following heterotypic chronic stress. *Gastroenterology* 2010; **138**: 294-304.
[11] Zhou YY, Wanner NJ, Xiao Y. Electroacupuncture alleviates stress-induced visceral hypersensitivity through an opioid system in rats. *World J Gastroenterol* 2012; **18**(48): 7201-7211.

[12] Larsson M, Arvidsson S, Ekman C. A model for chronic quantitative studies of colorectal sensitivity using balloon distension in conscious mice – effects of opioid receptor agonists. *Neurogastroenterol Motil* 2003; **15**(4): 371-381.
[13] Xu GY, Shenoy M, Winston JH, Mittal S, Pasricha PJ. P2X receptor-mediated visceral hyperalgesia in a rat model of chronic visceral hypersensitivity. *Gut* 2008; **57**: 1230-1237.
[14] Winston J, Shenoy M, Medley D, Naniwadekar A, Pasricha PJ. The vanilloid receptor initiates and maintains colonic hypersensitivity induced by neonatal colon irritation in rats. *Gastroenterology* 2007; **132**: 615-627.
[15] Ma XP, Tan LY, Yang Y. Effect of electro-acupuncture on substance P, its receptor and corticotropin-releasing hormone in rats with irritable bowel syndrome. *World J Gastroenterol* 2009; **15**(41): 5211-5217.
[16] Sun S, Cao H, Han M, Li TT, Zhao ZQ, Zhang YQ. Evidence for suppression of electroacupuncture on spinal glial activation and behavioral hypersensitivity in a rat model of monoarthritis. *Brain Res Bull* 2008; **75**: 83-93.
[17] Kim HN, Park JH, Kim SK, Sun B, Koo S, Choi SM, et al. Electroacupuncture potentiates the antiallodynic effect of intrathecal neostigmine in a rat model of neuropathic pain. *J Physiol Sci* 2008; **58**: 357-360.
[18] Zhou Q, Verne GN. New insights into visceral hypersensitivity–clinical implications in IBS. *Nat Rev Gastroenterol Hepatol* 2011; **8**(6): 349-355.
[19] Lin JG, Chen WL. Acupuncture analgesia: a review of its mechanisms of actions. *Am J Chin Med* 2008; **36**: 635-645.
[20] Zhao ZQ. Neural mechanism underlying acupuncture analgesia. *Prog Neurobiol* 2008; **85**: 355-375.
[21] Koo ST, Lim KS, Chung K, Ju H, Chung JM. Electroacupuncture-induced analgesia in a rat model of ankle sprain pain is mediated by spinal alpha-adrenoceptors. *Pain* 2008; **135**: 11-19.
[22] Kim JH, Kim HY, Chung K, Chung JM. Electroacupuncture reduces the evoked responses of the spinal dorsal horn neurons in ankle-sprained rats. *J Neurophysiol* 2011; **105**: 2050-2057.
[23] Yin J, Chen JD. Mechanisms and potential applications of intestinal electrical stimulation. *Dig Dis Sci* 2010; **55**(5): 1208-1220.
[24] Sun Y, Chen JD. Intestinal electric stimulation accelerates whole gut transit and promotes fat excrement in conscious rats. *Int J Obes (Lond)* 2009; **33**(8): 817-823.
[25] Sallam HS, Chen JD. Colon electrical stimulation: potential use for treatment of obesity. *Obesity* 2011; **19**: 1761-1767.
[26] Yan Y, Xiang XL, Qian W, Xu JY, Hou XH. Changes of neuronal activities after gut electrical stimulation with different parameters and locations in lateral hypothalamus area of obese rats. *J Huazhong Univ Sci Technol Med Sci* 2014; **34**: 510-515.
[27] Sallam HS, Chen JD. Colonic electrical stimulation: potential use for treatment of delayed colonic transit. *Colorectal Dis* 2013; **15**: 244-249.
[28] Sanmiguel CP, Casillas S, Senagore A, Mintchev MP, Soffer EE. Neural gastrointestinal electrical stimulation enhances colonic motility in a chronic canine model of delayed colonic transit. *Neurogastroenterol Motil* 2006; **18**: 647-653.
[29] Sevcencu C, Rijkhoff NJ, Gregersen H, Sinkjaer T. Propulsive activity induced by sequential electrical stimulation in the descending colon of the pig. *Neurogastroenterol Motil* 2005; **17**: 376-387.
[30] Vaucher J, Cerantola Y, Gie O. Electrical colonic stimulation reduces mean transit time in a porcine model. *Neurogastroenterol Motil* 2010; **22**: 88-92.
[31] Bertschi M, Schlageter V, Vesin JM, Aellen S, Peloponissios N, D'Ambrogio A, et al. Direct electrical stimulation using a battery-operated device for induction and modulation of colonic contractions in pigs. *Ann Biomed Eng* 2010; **38**: 2398-2405.
[32] Sevcencu C, Rijkhoff NJM, Sinkjaer T. Muscular vs. neural activation in propulsion induced by electrical stimulation in the descending colon of rats. *Neuromodulation* 2005; **8**: 131-140.
[33] Shafi A, El-Sibai O. Rectal pacing: pacing parameters required for rectal evacuation of normal and constipated subjects. *J Surg Res* 2000; **88**: 181-185.
[34] Liu S, Chen JD. Colonic electrical stimulation regulates colonic transit via the nitrergic pathway in rats. *Dig Dis Sci* 2006; **51**: 502-505.
[35] Chiba T, Bharucha AE, Thomforde GM, Kost LJ, Phillips SF. Model of rapid gastrointestinal transit in dogs: effects of muscarinic antagonists and a nitric oxide synthase inhibitor. *Neurogastroenterol Motil* 2002; **14**: 535-541.