ELECTRICAL NEUROMODULATION AT ACUPUNCTURE ST36 NORMALIZES IMPAIRED COLONIC MOTILITY INDUCED BY RECTAL DISTENSION IN DOGS

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Short running title: Electroacupuncture on RD-induced impaired colonic motility

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ABSTRACT

Electroacupuncture (EA) has been shown to improve impaired gastric motility and slow waves in both humans and animals. However, its effects on colonic motility have rarely been investigated. The aim of this study was to investigate the effects and underlying mechanisms of EA on impaired colonic motility induced by rectal distension in dogs. Colon contractions and transit were measured in various sessions with and without EA in hound dogs chronically placed with a colonic cannula. Colonic contractile activity was assessed by motility index (MI). Autonomic functions were determined by the spectral analysis of the heart rate variability derived from the electrocardiogram. It was found: 1) Rectal distention (RD) suppressed colonic motility by 40.5% (10.8±0.9 with RD vs. 6.4±0.8 at baseline, P <0.002). EA at ST36 normalized colonic contractions suppressed by RD (12.9±2.8, P<0.002 vs. RD and P=0.1 vs. control). 2) Administration of atropine blocked the ameliorating effect of EA on colon motility. 3) RD also delayed colonic transit (65.0±2.0% with RD vs. 86.0±1.9% without RD, P<0.001) that was restored with EA (84.0±1.9%, P=0.178 vs. control). 4) EA increased vagal activity suppressed by RD (0.37±0.07 with RD+EA vs. 0.09±0.03 with RD without EA, P<0.001). In conclusion, RD inhibits colonic contractions and delays colonic transit in dogs; EA at ST36 restores the RD-induced impairment in both colonic contraction and transit, by enhancing vagal activity and mediated via the cholinergic pathway.
INTRODUCTION

The functional gastrointestinal disorders (FGIDs) are highly prevalent, accounting for 45–68% of gastrointestinal presentations to primary care facilities. It is estimated that 35 million individuals suffer from constipation in the USA (11). Irritable bowel syndrome (IBS) represent 29.8% (or 2.4–3.5 million) of all physician visits per annum in the USA (14). FGIDs cost healthcare industries hundreds of billion per year in USA (11, 4, 16). At least two of major FGIDs are related to dysfunction of colonic motility: chronic constipation and IBS. Colon dysmotility may cause symptoms, such as difficult defecation, diarrheal and bloating, and also some upper gastrointestinal symptoms such as upper abdominal discomfort or pain, and vomiting (50, 61).

Rectal distension (RD), attributed to fecal stasis in the colon-rectum region, is known to induce gastrointestinal dysmotility and certain gastrointestinal symptoms due to inhibitory reflexes (9, 25). In experimental settings, RD induced by intra-rectum balloon distention has been reported to impair gastric motility, such as gastric slow wave dysrhythmia, attenuated antral contractions and delayed gastric emptying, as well as colonic motility (7, 8, 66).

Main methods for the treatment of chronic constipation and constipation dominant IBS (C-IBS) include diet, pharmacotherapy and biofeedback training (10).
For diet treatment, fibers have long been used for treating functional bowel disease symptoms; the fibers are one of the safest and most important therapies for constipation (37). Increased dietary fiber intakes has been reported to reduce constipation rates and result in significant cost-saving (51). However, even when used carefully, fibers may exacerbate abdominal distension and constipation (15). Therefore some nuances are necessary regarding timing, dose and types, tailored to the need of individual patients. A meta-analysis on the effect of dietary fibers on constipation showed that dietary fiber intake could obviously increase stool frequency in patients with constipation, but without obvious improvement in stool consistency, laxative use or and painful defecation (64).

For pharmacotherapy, bulk forming and osmotic laxatives are used by patients with mild constipation, but more evidence needed to support the long-term use of them (24, 54). Biofeedback has been reported to be effective in treatment of constipation with paradoxical contractions of the rectum and anal sphincter (1, 48), not for constipation with slow transit. Some overlap symptoms of those diseases are resistant to those treatments because of the weak effects of some drugs or the long-term history of diseases and some of therapies are limited due to their side effects (11). So, safe and effective methods for treatment of those diseases are deeply needed.

Electroacupuncture (EA) at ST36 (Zusanli), a modified method Traditional Chinese Medicine, has been shown to be effective in the treatment of various functional gastrointestinal diseases (13, 29, 65). In our previous study, we found EA
at ST36 was able to accelerate gastric emptying and restore RD-induced impaired
gastric motility in dogs, possibly by enhancing vagal activity (8, 44), and enhance
intestinal contractions and glucagon-induced hypomotility in the fed state, and
accelerated intestinal transit via the opioid and cholinergic pathways in dogs (53).
However, to the best of our knowledge, little is known whether EA at ST36 is able to
improve impaired colon motility, such as slow transit and hypomotility of the colon.
Therefore, the aim of this study was to investigate effects and mechanisms of EA
at ST36 on RD-induced impairment in colonic contractions and transit in dogs.

MATERIALS AND METHODS

Animal Preparation

Five adult female hound dogs (3-4 yrs, 22-25 kg) were used in this experiment.
After an overnight fast, the dog was anesthetized with sodium thiopental at a dose of
5mg/kg, i.v. (Pentothal; Abbott Laboratories, North Chicago, IL., USA) and
maintained on IsoFlo (isoflurane 1.5%, inhalation anesthesia) (Abbott) in
oxygen-nitrous oxide (1:1) carrier gases delivered from a ventilator following
endotracheal intubation. Laparotomy was performed with the vital sign monitoring of
mucosal color, pulse rate and breathing rate. A colonic cannula was chronically and
surgically placed in the anterior of the ascending colon about 5 cm distal to the cecum,
as detailed in previous studies (7, 49). The cannula was used for the insertion of a
manometric catheter into the colon for the assessment of colon contractions and the
insertion of markers for the assessment of colon transit.

The female canine menstrual cycle usually lasts 1–3 wks and takes place only once
or twice a year. During the cycle, a small amount of bleeding may be noticed (8, 52).

To avoid possible effects of menstrual cycle on colon motility, none of the experiments was performed during the menstrual period. The Animal Care and Use Committee of the Veterans Affairs Medical Center (Oklahoma City, OK) approved the protocol of this study.

**Experimental Protocol**

The study was initiated after the dogs were completely recovered from the surgery (about 2 wks after the surgery). The experiment was composed of six sessions in the postprandial state on separate days in a randomized order described as follows (Fig. 1):

Session 1 (Control) served as a control without any intervention. The recordings were made for three 30min periods immediately after a standard test meal.

Session 2 (RD) was designed for studying the effect of RD on colonic contractions and transit. RD was performed at 33mmHg during the second 30-min period.

Session 3 (RD+EA) was for investigating the effect of EA at bilateral ST36 on the RD-induced suppression of colonic contractions and transit. RD and EA were performed simultaneously during the second 30min.

Session 4 (RD+sham-EA) was a sham stimulation session in which RD and sham-EA (needles were inserted at ST36 without electrical stimulation) were performed during the second 30-min period.

Sessions 5 and 6 were designed for determining the possible mechanism of EA involving the cholinergic pathway. In session 5, RD, EA at ST36 and intravenous infusion of atropine (0.05mg/kg, iv) (42) were given during the second 30-min period.
Session 6 was the same as session 5 except that no EA was performed. Colon contractions were measured during the entire experimental session immediately after the meal.

The procedure for each session was as follows: after an overnight fast, one bottle of enema liquid (Fleet enema saline; C.B. Fleet Co., Inc. Lynchburg, VA) was given to each dog at 9:00 AM one hour before the experiment. The experiment was performed in the laboratory with the animal standing on a table. During the study, the animal was minimally restrained: the dog was standing on the experimental table freely with its leash loosely attached to a fixed location at the same height to prevent the dog from jumping off the table accidentally (never happened in the study). In order to let the dogs accommodate to this experimental condition, they were trained to stand on the table for 3 hrs every day for one week before the initiation of the study (8, 66).

Each session was initiated immediately after a solid test meal consisting of 375g beef (Pedigree, Pedigree-Chopped Chicken). Colonic contractions, rectal tone and electrocardiogram were simultaneously recorded in all these sessions. Animal behaviors were closely monitored and recorded during the experiment.

**Rectal Distension and Measurement of Rectal Tone**

A double-lumen catheter with a polyethylene balloon on its tip was inserted and positioned into the rectum with the proximal edge of the balloon 5 cm from the anal verge (34). The catheter was connected to an electronic barostat system (Distender Series IIR, Toronto, Canada) for rectal distention and the measurement of rectal tone. The pressure of rectal distention was set at 33 mmHg with which the animals
tolerated well without uncomfortable behaviors. The rectal tone was assessed by averaging the rectal volume during the last 10 min of RD; a higher volume was indicative of lower rectal tone; whereas a lower volume reflected higher rectal tone (34).

Electroacupuncture

Bilateral acupoints, ST36 were inserted with acupuncture needles for EA in this study. Sham-EA was performed by inserting needles at the ST36 without stimulation. A universal pulse generator (Model DS8000, World Precision Instruments, Sarasota, FL, USA) connected to the needles inserted into ST 36 was used to generate the electrical current for EA. The electrical stimuli consisted of pulse trains. The frequency of the pulse trains was 12 trains/min, the duration of each train was 2 s, and the pulse amplitude was 3-6mA according on animal tolerance and behaviors. The frequency and width of pulses in each train were 25 Hz and 0.5ms, respectively. The choice of this parameter set was based on our previous studies (8, 31, 45). EA at 25 Hz has been consistently and robustly shown to improve gastrointestinal motility. In a previous study, however, EA at 2Hz was found to be more potent in soliciting cardiovascular responses than frequencies higher 2Hz (68).

Recording and Analysis of Colonic Contraction

The colonic cannula was opened after the solid test meal and a manometric catheter was inserted into the colon through the cannula toward the direction of the rectum. An eight-channel water-perfused manometry system (Medkinetic Inc., Ningbo, China) was used to record colonic contractions during the entire period of each session. After
the recording, the colonic contractions were analyzed using a custom-made software (Medkinetic Inc. Ningbo, China). Motility index (MI) of the colonic contractions was defined as the mean area under the curve of each contraction (AUC).

**Recording and Analysis of Colonic Transit**

At the end of each study session, twenty polyvinyl ring markers (diameter of 5 mm and length of 2 mm) were placed into the ascending colon through the cannula. Colon transit was assessed by collecting and calculating the percentage of markers defecated at 24 hr, 48 hr and 72 hr after the insertion. During the subsequent 72 hrs, the animals were given regular food and water without any intervention.

**Recording and Analysis of Autonomic Function**

The autonomic function was assessed from the spectral analysis of the heart rate variability (HRV) signal derived from the ECG described previously (8). The ECG was recorded during each session (see Fig.2A). Custom-made software was used to detection R waves from the ECG signal, which resulted in R-R intervals (Fig.2B). An HRV signal was obtained by interpolating and uniformly sampling the R-R interval signal (Fig.2C). Finally, the power spectral analysis was performed on the HRV signal (Fig.2D). The power in the low-frequency (LF) band (0.04–0.15 Hz) represents mainly sympathetic activity and the power in the high-frequency (HF) band (0.15–0.50 Hz) represents vagal activity (8, 66).

**Statistical Analysis**

Statistical analyses were performed using SigmaStat17.0 (SPSS, Chicago, IL, USA). Data are reported as means ±SE. Analysis of variance (ANOVA) was applied
to investigate the difference among different sessions. Student’s t-test was used to investigate the difference between any two periods or two sessions. For multiple comparisons, the Tukey’s method was used and a P value of < 0.05 was considered statistically significant.

RESULTS

All dogs tolerated the procedures well. A pressure of 33mmHg of rectal distention maintained by the barostat system was the highest pressure the dogs could tolerate without adverse behaviors indicative of pain or discomfort.

Effects and Mechanisms of EA on Colonic Contraction

RD inhibited colonic contractions. The MI was 10.8 ± 0.9 at baseline, reduced substantially to 6.4 ± 0.8 during RD (P<0.002 vs. baseline) and 7.6 ± 0.9 (P<0.05 vs. baseline) during the 30 min after termination of RD.

EA restored RD-induced inhibition on colonic contractions (Figs. 3 and 4). The MI during RD with EA at ST36 was 12.9 ± 2.8, which was significantly higher than that during RD without EA (P <0.002) and comparable to the baseline value before RD (P=0.5). However, this effect was not observed during sham-EA.

Atropine blocked the ameliorating effect of EA on colonic contractions. Administration of atropine did not alter MI (Fig.4, P=0.4, RD vs. RD+ atropine). However, at the presence of atropine, EA was not able to enhance colonic contractions (Fig.4, P=0.3, RD+ atropine vs. RD+ atropine+ EA).

Effects of EA on Colonic Transit

RD delayed colonic transit. The percentage of defecated markers at 24 hr was
significantly reduced from $86.0 \pm 1.9\%$ in the control session to $65.0 \pm 2.0\%$ in the RD session ($P<0.001$, Fig. 5), demonstrating a sustained effect of the 30-min RD on colon transit.

EA at ST36 restored the delayed colonic transit induced by RD. With EA at ST36 during RD, the amount of defecated markers increased up to $84.0\% \pm 1.9\%$ at 24 hr (Fig. 5, $P<0.01$, vs. RD without EA session; $P=0.29$, vs. control session).

**Effects of EA on Rectal Tone**

EA decreased rectal tone compared to the control session and sham-EA session. The intraballoons volume was higher during RD with EA than during RD without EA ($119.6 \pm 11.3$ ml with RD+EA vs. $90.9 \pm 9.7$ ml with RD without EA; $P<0.04$). The effect of EA on rectal balloon volume was blocked by atropine ($84.4 \pm 5.3$ mL with RD+ atropine+ EA, $P=0.3$ vs. RD only); atropine did not alter intraballoons volume (Fig. 6).

**Autonomic Mechanisms of EA**

RD with a pressure of 33mmHg decreased vagal activity (HF) from $0.31 \pm 0.03$ at baseline to $0.09 \pm 0.03$ ($P<0.05$) and increased sympathetic activity from $0.70 \pm 0.03$ at baseline to $0.91 \pm 0.03$ ($P<0.05$). EA normalized vagal activity that was decreased by RD ($P<0.01$, vs. RD; $P=0.5$, vs. baseline before RD (Fig. 7A). EA decreased sympathetic activity (Fig. 7B, RD+EA vs. RD, $P<0.01$). The effects of EA on the autonomic functions were blocked by atropine (Fig. 7).

**DISCUSSION**
This current study demonstrated that RD inhibited colonic contractions and transit in dogs; EA at ST36 restored the impaired colonic contraction and transit induced by RD by enhancing vagal activity and mediated via the cholinergic pathway. RD has been commonly used to establish a temporary model of impaired gastrointestinal motility in the research of gastrointestinal motility, such as function dyspepsia, constipation and constipation-dominant IBS. In previous studies, RD was reported to induce upper and lower gastrointestinal symptoms (2, 32), inhibit gastric tone in a volume-dependent manner and impair gastric accommodation (28), impair gastric myoelectrical activity which resulted in delaying gastric emptying and gastric dysrhythmia (38, 47), and inhibit small intestinal motility in humans and animals (25, 47). However, there is little information about the effects of RD on colonic contractions and colonic transit. This present study showed that RD at 33 mmHg significantly inhibited colonic motility, including reduced colonic contractions and delayed colonic transit in dogs. In this study, we performed RD after the meal, because of the following reasons: 1) the meal made the experimental condition consistent among different sessions. Without the test meal, the colon contractile activity in different sessions might vary substantially; 2) the meal enhanced colon motility and thus made it possible to observe a substantial inhibitory effect of rectal distention and 3) consistent inhibitory effects of RD on gastrointestinal motility have been observed after a meal in a number of our previous studies (8, 49, 66). For the treatment of gastrointestinal motility disorders, acupuncture and EA have been accepted by more and more patients in treatment of functional gastrointestinal
diseases because of their efficacy and acceptable safety profile (rare side effects) compared to pharmacotherapy (65). In our lab, EA at ST36 was found to accelerate gastric emptying and restore impaired gastric accommodation in dogs (44, 45, 66); EA at ST36 was able to improve upper and lower abdominal symptoms, and impaired gastric slow waves induced by RD mediated via the vagal pathway (32); EA at ST36 improved glucagon-induced hypomotility in the fed state and accelerated intestinal transit via the opioid and cholinergic pathways in dogs (53). In colon, EA at ST36 was reported to increase contractility of the distal colon via the cholinergic pathway in conscious rats (36) and increase colonic transit mediated via the sacral parasympathetic efferent pathway in rats (22). While these previous studies have shown the enhancement of vagal activity with EA, EA and direct vagal nerve stimulation are two totally different methods although they may have some similar effects and mechanisms (18, 41). EA is noninvasive and has excellent safety profile. Whereas direct vagal nerve stimulation is invasive and may have some unknown sides effects that may prevent its clinical applications; so basically, we still wanted to investigate the effect of EA on colon motility in this study.

The methodology of EA used in this project was based on a number of previous studies investigating the effects of EA on gastrointestinal motility. To the best of our knowledge, ST36 is the most commonly used acupoint for the treatment of gastrointestinal motility disorders, where the acupoint PC6 is best known for its anti-emetic effect (20). In most of previous studies investigating the effect of EA on gastrointestinal motility, both ST36 and PC6 were used simultaneously in order to
achieve the maximal effect (33, 40). In cardiovascular studies, PC6 was used in EA alone and shown to exhibit a strong autonomic action (57). In gastrointestinal motility studies, however, PC6 has rarely been used alone for EA. In a study investigating the effect of EA on gastric motility complications of systemic sclerosis, EA at PC6 alone was shown to reduce the percentage of normal myoelectric waves in the stomach (62). Accordingly, in this study we chose to use ST36 alone for EA. In clinical practice, as a precaution, some acupuncturists would place pairs of electrodes on one side of the body (unilateral) under assumption that electrical current would not go through the heart and thus avoid possible side effects on the heart. However, this has not been scientifically substantiated or proven. At a current of a few mA, no side effects have ever been reported on the heart with EA (55, 65). In almost all of our previous studies EA was performed bilaterally and therefore the same method was used in this study. Previously, we studied the effects of EA on RD-induced dysmotility of the stomach. In this study, we investigated the effect of EA on RD-induced hypomotility of the colon. In a study of gastric motility, we found that electroacupuncture at ST 36 could improve impaired gastric motility and slow waves induced by rectal distension in dogs (8) and restore impaired gastric accommodation in vagotomized dogs (44). Whereas the findings of this study suggested potential applications of EA for the treatment of slow-transit constipation and constipation-predominant IBS. In the present study, we found EA for 30 min could restore the impaired colonic contractions induced by RD in the postprandial state. The reason we just performed EA for 30 min was that in our previous study, we found the prokinetic effect of EA with a duration of
30 min was similar to that of EA with a duration of 75 min on delayed gastric emptying in dogs, suggesting that prolonged EA might not be necessary (66). For stimulation parameters, we used the parameters optimized in our previous studies: 25Hz, 2s on, 3s off, 0.5ms; EA at ST36 with these parameters has been reported to increase gastric motility in rats (21, 67), and accelerate gastric emptying and enhance antral contractions in dogs (8, 45, 66).

Concurrent with the acute effect on colon contractions, a brief EA at ST36 was found to exert a prolonged effect on colon transit in the present study. Under normal conditions without any intervention, 86% of inserted markers were defecated in 24 hr and all defecated in 48h. RD for 30 min decreased the colon transit 41% and this was normalized by EA at ST36 for 30 min. These findings suggest that both RD and EA have sustained and prolong effects on colon transit.

The utilization of the barostat device allowed us to perform RD at a fixed and constant pressure; meanwhile, it allowed us to assess rectal tone by measuring the intraballoon volume. Rectal tone has a close relationship with rectal pressure and relaxation of rectal muscle (27). The rectal pressure plays an important role in defecation; while rectal tone is important for rectal accommodation. In the stomach, EA at ST36 has been consistently reported to exert a prokinetic effect on gastric motility; meanwhile it was also shown to reduce gastric tone and improve gastric accommodation. This is critically important as certain prokinetics, such as erythromycin improves gastric motility but impairs gastric accommodation by increasing gastric tone; as a result these kinds of prokinetics are not effective in
treating overall symptoms resulting from gastric dysmotility. Similar to its effects on
gastric motility, EA at ST36 also showed an inhibitory effect on rectal tone while
enhancing colonic contractions and transit. This finding suggests that EA at ST36
may reduce symptoms related to rectal distention resulting from accumulation of
feces.

Mechanistically, we found that RD substantially decreased vagal activity by 72%
and significantly increased the sympathetic activity by 30%. The most common
explanation on possible mechanisms of RD on colonic motor motility is that RD
activates afferent mechanosensory fibers that project to the prevertebral ganglia
integrated with central synaptic input and then conveyed to the superior mesenteric
and celiac ganglia via intermesenteric nerves. The afferent input activates sympathetic
neurons in the prevertebral ganglia and inhibits motor activity in the colonic segment
proximal to the site of distension (19, 26).

EA at ST36 restored the RD-induced impaired colonic contraction and colonic
transit by normalizing the sympathovagal balance that was impaired by RD.
Location-specific effects of EA on autonomic function have been reported in the
literature. In this study, EA at ST36 enhanced vagal activity and reduced
sympathovagal balance (LF/HF). Whereas in a previous study (57), EA at PC6 was
shown to modulate gastric distention-induced reflex sympathoinhibition and vagal
excitation in rats. This opposite effect could be attributed to the use of a different
stimulation location (PC6 vs. ST36) and/or different experimental conditions
(sympathoinhibition and vagal activation vs. sympathtoactivation and vagal inhibition
In another study, however, clear location-specific effects were elucidated (21): in rats, EA at ST36 increased gastric motility and decreased the sympathovagal ratio, whereas EA at PC6 inhibited gastric motility and increased the sympathovagal ratio. Meanwhile, the normalizing effect of EA on colonic motility was found to be blocked by atropine as shown in Fig. 7. These findings suggest the involvement of the cholinergic pathway. However, there is a limitation in the use of atropine because atropine can cross the blood brain barrier and influence central cholinergic pathway and acetylcholine was reported to participate in central neural cardiovascular actions of EA (35).

While the autonomic mechanism involved in the ameliorating effect of EA on RD-induced colon hypomotility was implicated, the central action of EA was not investigated in this present study. However, a number of previous studies have shown EA has both spinal and supraspinal actions with respect to its effects on autonomic outflow. Longhurst et al reported that during and after EA stimulation, nociceptin, γ-aminobutyric acid (GABA), and opioids, including endorphins, and enkephalins, acting through μ- and δ-opioid receptors in the rostral ventrolateral medulla (rVLM), inhibit sympathetic outflow and the resulting cardiovascular sympathoexcitatory response (12, 58, 59). Furthermore, it was reported that low frequency EA could release endocannabinoids and activate presynaptic CB1 receptors to inhibit GABA release in the ventrolateral periaqueductal gray (vIPAG) resulting in disinhibiting vIPAG cells to modulate the activity of rVLM neurons to attenuate the sympathoexcitatory reflex responses (60).
Perspectives and Significance: The findings of the present study suggest the potential of EA for the treatment for patients with gastrointestinal symptoms related to weak colonic contraction and slow colonic transit, such as constipation, constipation-dominant IBS or other colorectal disorders. Clinical studies are warranted to explore such a therapeutic role of EA in the treatment of gastrointestinal symptoms related to impaired colonic motility.

In conclusion, EA at ST36 restores impaired colonic contractions and transit induced by RD by enhancing vagal activity mediated via the cholinergic pathway. EA may have a therapeutic potential for functional gastrointestinal disorders related to slow colonic transit.
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FIGURE CAPTIONS

Fig. 1 Experiment protocol. Colon contractions were measured during the entire study immediately after the meal.

Fig. 2 Calculation of sympathetic and vagal activities from the ECG. A: an ECG signal from a dog; B: a signal derived from the ECG showing peaks of R waves; C: the heart rate variability signal; D: power spectral analysis of the heart rate variability signal displaying LF (P1) (mainly sympathetic) and HF (P2, vagal activity).

Fig. 3 Typical tracings of colonic contractions. A: colonic contraction was decreased during rectal distension (RD) and recovered after RD. B and C: electroacupuncture (EA) at ST36 normalized the impaired colonic motility induced by RD, but the sham stimulation did not show such an effect. D: After administration of atropine, the effect of EA on RD-induced impaired colonic motility was inhibited, suggesting that the effect of EA was mediated via the cholinergic pathway. E: Atropine plus RD significantly decreased the colonic contraction.

Fig. 4 Colonic motility index at baseline and during different interventions RD significantly decreased colonic motility index compared to baseline (*P <0.002 vs. Baseline) and EA but not sham-EA normalized RD-induced impairment of colonic motility ("P <0.002 vs. RD without EA). Atropine blocked the effect of EA on colonic motility.

Fig. 5 Colon transit assessed by the defecated markers in 24 hrs. RD significantly decreased colonic transit compared to the baseline condition without any intervention
EA but not sham-EA normalized impaired colonic transit induced by RD (#: \( P<0.01 \) vs. RD or RD+ Sham-EA; \( P=0.796 \) vs. baseline). The effect of EA on RD-induced impairment of colonic transit was blocked by atropine: no difference between the RD+Atropine session and RD+Atropine+EA session.

**Fig. 6** Rectal volume under various conditions. The intraballoon volume was significantly increased with EA (\( ^*: P<0.04 \), RD+EA vs. RD) but not sham-EA. The relaxing effect of EA was blocked by atropine (\( P>0.05 \), RD+Atropine+EA vs. RD or RD+Atropine).

**Fig. 7** Effects of RD and EA on vagal and sympathetic activities. A: RD significantly decreased vagal activity (HF) by 71% \( (P<0.05) \). EA but not sham-EA normalized RD-induced impairment of vagal activity and the effect was blocked by atropine. B: RD significantly increased sympathetic activity (LF) by 30% \( (P<0.05) \). EA normalized RD-induced increase in sympathetic activity and the effect was blocked by atropine.
Figure 1

**Session 1: Control**

Meal

| Time (min) | 0 | 30 | 60 | 90 |

**Session 2: RD**

Meal | RD | Stop RD

| Time (min) | 0 | 30 | 60 | 90 |

**Session 3: RD+EA**

Meal | RD+EA | Stop RD+EA

| Time (min) | 0 | 30 | 60 | 90 |

**Session 4: RD+ Sham-EA**

Meal | RD+ sham-EA | Stop RD+ sham-EA

| Time (min) | 0 | 30 | 60 | 90 |

**Session 5: RD+ atropine+ EA**

Meal | RD+ atropine+ EA | Stop RD+ atropine+ EA

| Time (min) | 0 | 30 | 60 | 90 |

**Session 6: RD+ atropine**

Meal | RD+ atropine | Stop RD+ atropine

| Time (min) | 0 | 30 | 60 | 90 |
Fig. 3

A

Meal  RD  Deflate

30 mmHg

B

Meal  RD + Sham-EA  Deflate

30 mmHg

C

Meal  RD + EA  Stop RD + EA

30 mmHg

D

Meal  At, RD + EA  Stop RD + EA

30 mmHg

E

Meal  At, RD  Stop RD

30 mmHg

Time (min)
Fig. 4
Fig. 5
Fig. 6
Fig. 7

A

![Graph showing HF vagal activity with bars for Baseline and Treatment conditions for different groups: RD, RD+Sham-EA, RD+EA, RD+Atropine, and RD+Atropine+EA.](image)

- RD: P=0.04
- RD+Sham-EA: P<0.001
- RD+EA: P=0.455
- RD+Atropine: P=0.003
- RD+Atropine+EA: P=0.018

B

![Graph showing LF sympathetic activity with bars for Baseline and Treatment conditions for different groups: RD, RD+Sham-EA, RD+EA, RD+Atropine, and RD+Atropine+EA.](image)

- RD: P=0.041
- RD+Sham-EA: P=0.002
- RD+EA: P=0.439
- RD+Atropine: P=0.014
- RD+Atropine+EA: P=0.03