Electroacupuncture Improves Rectal Distension-induced Delay in Solid Gastric Emptying in Dogs

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Short running head: Electroacupuncture on gastric emptying

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ABSTRACT

The aim of this study was to investigate the effects and mechanisms of electroacupuncture (EA) on rectal distension (RD)-induced delay in solid gastric emptying in dogs. Methods: Gastric emptying of solid was assessed in twelve dogs chronically implanted with a duodenal cannula by collecting samples at different time points from the cannula and measuring the dried weights of the samples. Bethanechol and atropine were used to qualitatively validate the method. In separate experiments, gastric emptying of solid was measured in a number of sessions: control, RD, RD + sham-EA, RD + EA of 6 mA, RD + EA of 3 mA and RD + EA + Naloxone. Results: 1) The method of gastric emptying by collecting and drying gastric chyme from the duodenal cannula was found to be accurate and reliable. Using the method, gastric emptying was found to be accelerated with Bethanechol (70.01 ± 8.10% vs. 82.61 ± 4.15%, P = 0.04) and delayed with atropine (4.31 ± 1.57%, P < 0.001 vs. control). 2) RD substantially and significantly delayed gastric emptying. EA but not sham-EA attenuated delayed gastric emptying induced by RD (48.79 ± 9.47% vs.74.28 ± 5.96%, P < 0.01). 3) The effect was more potent with EA of 6 mA than EA of 3 mA and blocked by Naloxone. Conclusions: EA is able to attenuate RD-induced delay in gastric emptying of solid and this ameliorating effect may be mediated via the opioid pathway. EA may have a therapeutic potential for treating delayed gastric emptying attributed to lower gut distension.

Key words: gastrointestinal motility; gastric emptying; acupuncture; rectal distension; gastroparesis; constipation.
INTRODUCTION

Patients with chronic, idiopathic, slow-transit constipation and constipation-dominant irritable bowel syndrome (IBS-C) frequently complain of upper abdominal symptoms such as bloating, upper abdominal discomfort or pain, and vomiting. These symptoms are related to the presence of reduced motor activities attributed to impaired gastric antral contractions and myoelectrical activity, and delayed gastric emptying as well as slow small bowel transit (1, 3, 14, 38). The abnormality of upper gastrointestinal motility may be related to fecal stasis in the rectum or colon, causing reflexive inhibition (5, 11, 20, 54). Rectal balloon distension (RD) mimicking fecal or gas stasis in the colorectal region is one of constipation models commonly used in the study of upper gastrointestinal motor activity. A number of studies have reported that RD impaired gastric myoelectrical activities, gastric tone and gastric accommodation, reduced antral contractions, delayed gastric emptying and inhibited small intestinal motility in dogs and humans (1, 2, 7, 11, 21, 39, 54).

Clinically, there are overlapping symptoms between functional dyspepsia (FD) and IBS-C (12, 44). Currently, there is a lack of effective medications for treating both upper and lower gastrointestinal symptoms. Acupuncture is one of the major treatment methods in traditional Chinese medicine and is performed by inserting the tips of needles on specific points (called acupoints) through the skin. Electroacupuncture (EA) is a modification of acupuncture that stimulates acupoints with electrical current instead of manual manipulations and appears to yield more consistent and reproducible results in both clinical and research settings (23, 26). During the last decade, a considerable number of studies have been performed to examine the efficacy of EA for the treatment of functional gastrointestinal disorders (32). Previous studies have reported that EA at ST36 accelerated gastric emptying in both dogs and humans (34, 49), and restored impaired gastric accommodation in vagotomized dogs (33). A recent study reported that EA improved RD-induced impairment of antral contractions (7). Mechanistically, a number of previous studies have shown the involvement of the opioid pathway with the prokinetic effect of EA on gastrointestinal contractions (7, 42). However, it is unknown whether EA is also able to improve or attenuate RD-induced delay in gastric emptying, a condition mimicking delayed gastric emptying attributed to colorectal stasis or gas in
constipation or IBS-C. Naloxone is a \( \mu \)-opioid receptor competitive antagonist in the central and enteric nervous systems and has been used as a blocker to investigate the possible involvement of the opioid pathway with EA (7).

A number of tests have been used for assessing gastric emptying of solid in humans. In the past few years, the most popular tests have been the scintigraphic method and the breath test (9, 10, 13, 36). These methods have also been used in animal studies (4, 9, 47). Scintigraphy is currently the gold standard for gastric emptying; however, it has limitations for use in animals since it requires the animal to be restrained during the test, which may induce stress and affect the gastric emptying rate (31). In addition, scintigraphy requires the use of a nuclear medicine facility with specialized and expensive equipment. The breath test for gastric emptying was used in mice using carbon isotope-enriched octanoic acid as a marker (9, 47) and does not require physical restraints, and may also be adopted to large animals (28, 41). In our lab, we have established a validated method for the assessment of liquid gastric emptying without the use of any expensive facilities or isotopes (34, 50). However, gastric emptying of solids is clinically more relevant and we were therefore motivated to establish a similar inexpensive and straightforward method for the assessment of solid gastric emptying in large animals.

We hypothesized that 1) solid gastric emptying can be assessed by directly collecting gastric chyme from an intestinal cannula; 2) EA is able to improve RD-induced delay in gastric emptying; and 3) the prokinetic effect of EA is reproducible, stimulation-intensity dependent and mediated via the opioid pathway. The aims of this study were 1) to validate a new method for the assessment of solid gastric emptying in dogs and 2) to investigate the effects and mechanisms of EA on RD-induced delayed gastric emptying in dogs using the new gastric emptying method.
MATERIALS AND METHODS

Animal model and surgical procedures

The study was performed in twelve healthy female hound dogs (22-26 kg). After an overnight fast, the dog was anesthetized with initial intravenous infusion of sodium thiopental (5 mg/kg, Abbott Laboratories, North Chicago, IL) 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. One cannula (see Fig.1) was placed in the duodenum, 20 cm beyond the pylorus, for the collection of gastric chyme used to assess gastric emptying of solid. The dog was transferred to a recovery cage for a few hours and then to the regular cage after receiving medications for postoperative pain control. The study was initiated after the dog was completely recovered from the surgery, usually 2 weeks after the surgery. The study was approved by the Animal Care and Use Committee of University of the Texas Medical Branch at Galveston, Texas.

Experimental protocols

Five experiments were performed in this study. The first experiment was designed to determine gastric emptying of solid and the second experiment was used to prove that the method of gastric emptying proposed in this study was able to assess alterations in gastric emptying (Bethanecol to accelerate and atropine to delay gastric emptying). Experiments 3-5 were designed to investigate the effects and mechanisms of EA on RD-induced delay in gastric emptying.

Experiment 1: Assessment of solid gastric emptying: This experiment was performed in 6 dogs. Each dog was studied in one session. The purpose of this session was to measure the total gastric emptying of solid. After an overnight fast, each dog was taken to the lab, slightly restrained and fed with 375g of solid dog food (Pedigree, Pedigree - Chopped Chicken). Immediately after the ingestion of the test meal, the duodenal cannula was opened for the collection of gastric chyme emptied from the stomach. The chyme was collected every 1 minute for the first 15 minutes and then every 15 min for the first hour and every 30 min for subsequent hours. The collection was continued for about 8 hours until the completion of gastric emptying (when nothing except secretion was collected from the cannula).
**Experiment 2: Effects of Bethanechol and atropine on solid gastric emptying:** This experiment was designed to qualitatively validate the method of gastric emptying and performed in the same 6 dogs used in Experiment 1. Each dog was studied in three sessions in a randomized order. One session served as a control. The procedure was the same as Experiment 1 except that the collection of gastric chyme from the duodenal cannula lasted for 3 hours. This was determined based on preliminary data from Experiment 1 in which more than 80% of gastric emptying was achieved within 3 hours. Bethanechol and Atropine were used in the other two sessions, respectively. The procedure of these sessions was the same as the control session except that Bethanechol (80 µg/kg, i.v) was injected 10 min before the test meal in one session and atropine (0.1 mg/kg, i.v.) was injected immediately before the test meal in the other session. These two doses were selected based on previous reports showing substantial alterations in gastrointestinal motility (27, 29, 30).

**Experiment 3: Effects and mechanism of EA on RD-induced delay in gastric emptying:** This study was performed in 6 dogs; two of them were the same dogs from Experiment 1. The experiment consisted of four randomized sessions with an interval of 3-4 days: control, RD + Sham-EA, RD + EA and RD + EA + Naloxone sessions. RD with a volume of 120 ml was performed in the sham-EA, EA and Naloxone + EA sessions from 60 to 90 minutes after the test meal. The reasons we chose to use only 30 minutes of RD between 60 to 90 minutes were a) the animals would get irritated if RD was maintained too long and b) gastric emptying was fastest during 60-90 minutes after the test meal. EA was performed at bilateral ST36 (Zusanli) located at the proximal one-fifth of the craniolateral surface of the rear leg distal to the head of the tibia in a depression between the muscles of the cranial tibia and the long digital extensor. Sham-EA was performed during the same period with needle inserted at two non-acupoint locations that were not on any meridians on two thighs about 20 cm from the ST36 without electrical stimulation. The electrical current for EA was generated by a commercial electro-needling instrument (Electrotherapeutic Apparatus, model D-860; China). The same stimulation parameters that were used in a previous study showing an ameliorating effect of EA at ST36 on RD-induced antral hypomotility were used; they were composed of trains of pulses with train on-time of 2 s, off-time of 3 s, and pulse frequency of 25 Hz, width of 0.6 ms and amplitude of 6 mA (7).
On the study day, after the clearance of the rectum using enema liquid (Fleet enema; C.B. Fleet Lynchburg, VA), a rectal catheter with a balloon attached to its tip (the caudal pole of the balloon was about 6 cm from the anal verge) was inserted into the rectum and affixed before a test meal for gastric emptying. The balloon was inflated with 120 ml of air (7, 21) only during 60-90 minutes after the test meal that was identical to the meal in Experiment 1. In the control session, no catheter was inserted into the rectum and no RD or EA or sham-EA was performed. In the sham-EA or EA session, sham-EA or EA was performed during 15-90 minutes after the test meal. In the session with EA + Naloxone, Naloxone (1 mg/kg, iv) was injected 20 min before the EA was initiated. The dose of Naloxone was based on a previous study (7).

**Experiment 4: Reproducibility of the inhibitory effect of RD on gastric emptying:** Five dogs were used to test if the inhibitory effect of RD was reproducible. Three of the dogs were the same as in Experiment 3 and two of them were new. Each dog was studied in three sessions on separate days: control without RD, first RD with a volume of 120 ml (RD1), second RD (RD2) with a volume 120 ml 3 days after RD 1. The experimental protocol and the measurement of gastric emptying were the same as in Experiment 3.

**Experiment 5: Dose response of EA on RD-induced delayed gastric emptying:** This experiment was performed in the same 5 dogs used in Experiment 4 and composed of 3 randomized sessions (control with RD only, RD + EA at 3 mA and RD + EA at 6 mA). RD was performed during 60-90 minutes after the same test meal as in Experiment 1 in all three sessions. EA at ST 36 with parameters of 2 s on, 3 s off, 25 Hz, 0.6 ms and 6 mA or 3 mA was applied at the same time as RD was performed during 60-90 min in the other two sessions. However, the acupuncture needles were inserted into the acupoints at 45 min. Gastric emptying was measured every 15 minutes for the first 60 min, then every 10 min during RD (60-90 minutes), every 15 min during 90-120 minutes and every 30 minutes during 120 -180 minutes. The samples were more frequently collected during EA in order to find out the time for EA to be effective in accelerating gastric emptying. In addition, one more session was performed to investigate the effect of another form of sham-EA on RD-induced delay in gastric emptying. In this session, the sham-EA was performed by inserting needles at bilateral ST36 without electrical stimulation.
Measurements of gastric emptying

At the end of each experimental session, the collected chyme was centrifuged for 20 min at a speed of 3000 rpm using a table top centrifuge to separate liquids (composed of the liquid part of the can food and gastric secretions) and solids. After centrifuging, each sample was placed in a -20°C refrigerator for 4 hours and then kept for 30-45 min at room temperature to make it easier for removing the precipitate from the test tube. The entire precipitate of each collection was then taken out, placed in a paper plate and dried in air. The weight of each collection was measured daily until the weight became stable.

The total weight of all dried samples from Experiment 1 (complete emptying of the ingested food) in each dog was considered as 100% gastric emptying. The percentage of gastric emptying for each collection was calculated using the following formula:

\[
\% \text{ gastric emptying for each collection} = \frac{W_n}{W_t} \times 100
\]

Where, \( W_n \) was dried weight of the collection and \( W_t \) was total weight of all dried samples in that dog. That is, the percent of gastric emptying at a particular time in any experimental session was the dried weight of all samples collected up to that time point divided by the total dried weight of all samples collected in that particular dog in Experiment 1 (complete gastric emptying session) times 100.

To validate the method of gastric emptying, the same air-dry procedure was performed on 5 cans of the same dog food as follows: the content of each can was mixed with an appropriate amount of saline such that the total amount of the mixture was similar to the total amount of all collected samples in each individual dog. The mixture was then air dried in the same manner as the collected gastric chyme.

Statistical Analysis

All data are presented as mean ± SEM. One way ANOVA was used to investigate the difference in gastric emptying among three or more sessions. Paired student’s t-test was applied to investigate the difference in gastric emptying between any two of the sessions if ANOVA revealed a significant difference among the three or more sessions. A p value of < 0.05 was considered statistically significant.
RESULTS

Survival of the dogs post-operatively
All the dogs survived well after the surgery. During the experimental period, the only side effect from the surgery was mild infection around the cannula area in some dogs that was cleaned with povidone-iodine and treated with antibiotics whenever necessary. All dogs were kept for more than 6 months in healthy conditions and could be used for subsequent experiments after the completion of this study.

Validation of the Method of Gastric Emptying of Solid

According to Experiment 1, the mean time for complete gastric emptying was 435 ± 16 min. The weight of the collected sample dried in air became stable 5 days after the initiation of air drying. Accordingly, all samples collected in other experiments were air dried for 5 days. The mean total dried weight of all samples from each dog was 60.9 ± 3.1 g and the dried weight of one can of the original canine food that was not consumed by the dog was 67.8 ± 3.0 g, indicating a collection rate of 89.9%.

The complete gastric emptying curve is presented in Figure 2A. It can be seen that there was a lag-phase during the first 7 minutes when the percentage of gastric emptying was close to 0, followed by a “linear” phase of emptying from 8 minutes to 180 minutes after the meal during which more than 80% of gastric content was emptied from the stomach. The last 10% of gastric content was emptied slowly during a period of 3 hours or more (180 min to 520 min). Figure 2B showed the rate of gastric emptying (% per 15 min). The rate of gastric emptying gradually increased from 15 minutes, reached its peak at 90 minutes and then gradually decreased.

As expected, Bethanechol significantly accelerated gastric emptying of solid. As shown in Figure 3, Bethanechol increased gastric emptying at each time point during the entire 3 hours. The total gastric emptying after 3 hours was 70.0 ± 8.1% in the control session and it was significantly increased to 82.6 ± 4.2% with Bethanechol (P = 0.035). In contrast to Bethanechol, atropine substantially and significantly delayed gastric emptying. Gastric chyme did not appear at the duodenal cannula until 120 minutes after food ingestion in five out of the six dogs and until 90 minutes in the sixth dog. As shown in Figure 3, the percentage of gastric emptying with atropine was only 4.3 ± 1.6% at the end of the 3-hr test (P < 0.001, vs. control).
Reproducibility of the inhibitory effect of RD on gastric emptying
RD during 60 -90 min significantly delayed gastric emptying and the effect was reproducible. The gastric emptying was 50.1 ± 11.8% at 90 minutes in the control session, and reduced to 35.7 ± 6.9% with the first RD (P = 0.02, vs. control) and to 36.3 ± 3.7% with the second RD (P = 0.004. vs. control; P=0.3, vs. first RD). The RD-induced inhibitory effects were sustained until the end of the study in the both first and second RD sessions. These findings (similar values in gastric emptying between two RD sessions) demonstrated the reproducibility of RD on delayed gastric emptying.

Effects and mechanisms of EA on rectal distension-induced delayed gastric emptying
EA or sham-EA at the nonacupoints showed no effects on gastric emptying during the first hour after the test meal before the RD was applied. In Experiment 3, during the first 60 minutes after the meal, no RD was applied in any of the sessions whereas sham-EA or EA was applied during 15-60 minutes). From the data presented in Figure 4, we observed no significant differences in gastric emptying among the three sessions: no intervention (control), EA applied from 15-60 min or sham-EA applied from 15-60 min, suggesting that EA had no effects on gastric emptying under the normal condition without RD during the first hour after the meal. The ineffectiveness of EA during the first hour could be attributed to the fact that mixing and grinding are the major functions of the stomach during this period.

EA, but not sham-EA, attenuated RD-induced delay in gastric emptying. At 90 minutes, the gastric emptying in the sham-EA session was 25.1 ± 5.2% and significantly improved to 33.3 ± 5.3% with EA (P = 0.02). Similarly, EA improved the gastric emptying from 34.4 ± 7.8% with sham-EA to 48.3 ± 6.6% at 120 minutes (P = 0.03), from 43.7 ± 9.2% with sham-EA to 66.0 ± 6.7% at 150 minutes (P = 0.02) and from 48.8 ± 9.5% with sham-EA to 74.3 ± 6.0% at 180 minutes (P < 0.01). Actually, gastric emptying with RD and EA was not different from the control session (P>0.05 for any time points at 90 minutes or later), suggesting a complete normalization of RD-induced delay in gastric emptying with EA. On the other hand, the gastric emptying in the sham-EA session (RD + sham-EA) in Experiment 3 was not different from that in either of the two RD sessions in Experiment 4 (RD only) at any time point, demonstrating the ineffectiveness of sham-EA on gastric emptying.
In Exp. 5, it was found the sham-EA at ST36 without stimulation had no effect on RD-induced delay in gastric emptying during the entire 3-hour experimental period. At 90 minutes, gastric emptying was $35.7 \pm 6.9 \%$ in the RD session and $30.6 \pm 5.7\%$ with the sham-EA ($P = 0.6$); similar findings were noted at 120 minutes, 150 minutes and 180 minutes ($P > 0.5$).

Naloxone blocked the EA-induced excitatory effect on RD-induced delayed gastric emptying. From 90-180 minutes after the meal in Experiment 3, no significant changes were noted in gastric emptying between the sham-EA session and the EA plus Naloxone session (25.1 ± 5.2 % vs. 15.7 ± 4.7 at 90 min; 34.4 ± 7.8 % vs. 23.1 ± 6.7% at 120 min; 43.7 ± 9.2 % vs. 32.6 ± 10.5 at 150 min; 48.79 ± 9.47 % vs. 41.18 ± 14.16 % at 180 min, $P > 0.05$ for all paired data). These findings suggested the involvement of the opioid pathway with EA.

**Dose effects of EA on gastric emptying**

In Experiment 5, EA during 60 - 90 minutes at a stimulation output of both 3 mA and 6 mA significantly accelerated RD-induced delay in gastric emptying in comparison with control (RD only), starting at 90 min (see Figure 5). It can also be seen from this figure that gastric emptying was faster with EA of 6 mA and EA of 3 mA from 60 min to 150 min. The area under the curve of gastric emptying was significantly larger with EA of 6 mA than EA of 3 mA at 90 min (147.5 ± 23.2 %*min vs. 122.6 ± 24.4 %*min at 90 min, $P= 0.03$) and 120 min (222.2 ± 31.5 %*min vs. 187.8 ± 32.1 %*min, $P = 0.04$) and 150 min (302.8 ± 37.8 %*min vs. 267.0 ± 36.9 %*min, $P = 0.054$).
DISCUSSION

In the current study, we validated a simple method for the assessment of gastric emptying of solid in dogs without the use of any special equipment and reported a prokinetic effect of EA on RD-induced delay in gastric emptying with the involvement of the opioid pathway. The proposed gastric emptying method was straightforward: collecting and air-drying gastric chyme from a duodenal cannula; it was validated with Bethanechol and atropine showing expected acceleration and delay of gastric emptying, respectively. Gastric emptying of solid was found to be delayed with RD. EA at ST36 was able to attenuate the RD-induced delay in gastric emptying and this excitatory effect was blocked by Naloxone.

Scintigraphy and the breath test are two methods commonly used to repetitively assess gastric emptying of solids in animals (4, 9, 47). However, special equipment is needed for both of these methods. In the current study, we established a canine model to assess solid gastric emptying without any special equipment and found that this method was reliable, accurate and reproducible. It is especially suited for the assessment of gastric emptying of solids with different interventions. The surgical procedure was feasible and safe. All the dogs survived well after the surgery and could be kept for a period of 6 months or longer. The operation did not cause any side effects except mild infections around the cannula that could be easily treated. The reliability of this gastric emptying method was demonstrated by the fact that 89% of the ingested solid food was collected from the cannula. The loss of 10% of the ingested food is believed to be reasonable and realistic and could be attributed to the following factors: 1) some of the gastric chyme might have passed through the duodenal cannula; 2) some of the food might have been absorbed; 3) some of the ingested solid might have been lost in the discarded liquid after centrifugation. However, these had little effects on the assessment of the percentage of gastric emptying, as all of these contributing factors would be proportional to the amount of gastric emptying. The reliability of the proposed method was also demonstrated by the pattern of gastric emptying observed in the study, which was similar to that reported in the literature. Gastric emptying of solid is known to exhibit three phases: a lag phase with no emptying, a “linear” phase during which the vast majority of gastric content is emptied from the stomach and a slow transit phase during which the small remaining gastric content is emptied from the stomach (51) as shown in Figure 2. The findings on gastric emptying in this canine study were comparable to those reported in healthy humans assessed by scintigraphy.
Further analysis of the gastric emptying data in this study indicated that the gastric emptying rate during the “linear” phase was actually not linear (Figure 2B); i.e., the rate of gastric emptying was not constant but increased almost linearly from 15 -90 min and decreased almost linearly from minute 90 -180 min with a peak value at 90 minutes. The reproducibility of the method was supported by the follow-up studies (Experiments 4 and 5) performed a few months after the first 3 experiments showing similar inhibitory effects of RD and accelerative effects of EA on gastric emptying.

To increase the accuracy of the method, gastric emptying was assessed by collecting and drying all gastric chyme samples. This is different from a previous canine study (45). In the previous study, only 1.5 ml was taken from each gastric emptying sample. According to our preliminary testing, the analysis of a small fixed amount of sample would lead to a large variation in final results because of the following reasons: 1) the sample of solid gastric chyme was not homogenous and therefore a small sample might not be an actual representation of the entire sample; 2) the amount of samples per 15 min varied from a few ml to up to 300 ml. Accordingly, a small error in the 1.5 ml sample taken from 300 ml would be amplified by 200, yielding inaccurate results. To further validate the method, we used Bethanechol and atropine to test changes in gastric emptying. Bethanechol is a cholinergic agonist used to activate the vagally mediated excitatory cholinergic pathway, and atropine has the opposite effect. Previous studies demonstrated that Bethanechol accelerated and atropine slowed gastric emptying (37, 40). Using these pharmacological agents, we confirmed the previous findings and showed significant changes in gastric emptying. The doses we chose for these agents were based on previous studies showing significant changes in gastrointestinal motility (29, 30).

The major aim of this study was to investigate whether EA was able to improve or attenuate RD-induced delay in gastric emptying since delayed gastric emptying is frequently reported in patients with slow transit constipation or constipation-dominant IBS. In the current study, we used RD, a constipation model commonly used in the study of upper gastrointestinal motor activity. In a previous study from our lab, RD induced upper gastrointestinal symptoms, reduced gastric tone and accommodation, and inhibited antral contractions in dogs (7, 21). The current study showed that RD delayed gastric emptying, which was in agreement with previous findings (2). The RD-induced delay in gastric emptying is believed to be attributed to the “rectogastric” reflex (5). Although a number of previous studies have investigated the
therapeutic potential of EA for gastrointestinal disorders (32) and reported ameliorating effects of EA on gastric slow waves and antral contractions impaired by RD in dogs, gastric emptying of liquid in dogs, vagotomy-induced impaired gastric accommodation in dogs and gastric emptying in patients with functional dyspepsia (7, 33, 34, 49), it was unknown whether EA would be able to improve RD-induced delay in gastric emptying. In this study, we found that EA was able to attenuate delayed gastric emptying attributed to rectal distension in dogs, suggesting a therapeutic potential of EA for treating delayed gastric emptying in patients with constipation or constipation dominant IBS. Although the effect of EA at ST36 on colonic motility was not investigated in the current study, a previous study by Iwa et al reported that EA at ST36 stimulated the parasympathetic pathway and accelerated colonic transit in rats (18). A few clinical studies have reported that EA or acupuncture increased bowel movement in constipated children (6, 24). The stimulatory effects of EA at ST36 on both gastric and colonic motility were anticipated to be beneficial for the overlapping symptoms of IBS-C and dyspepsia. Moreover, Xing et al reported that EA at ST36 and PC6 increased the threshold of rectal sensations induced by RD in patients with IBS, suggesting that EA was capable of reducing visceral perception in IBS patients (48).

To confirm the true effect of EA at specific acupoints, various methods of sham-EA may be applied: 1) the use of same acupoints without stimulation. This provides an indication between stimulation and no-stimulation; 2) the use of non-acupoints without stimulation. This emphasizes the importance of both acupoints and electrical stimulation; 3) the use of same stimulation at non-acupoints. This distinguishes between acupoints and non-acupoints; 4) the use of same stimulation at other acupoints. This distinguishes between two different acupoints. In this study, sham-EA was performed by inserting needles at either ST36 or non-acupoints without stimulation. Neither form of the sham-EA methods showed any effect on the RD-induced delay in gastric emptying. These findings suggest that both the acupoint, ST36 and the electrical stimulation are needed to improve the RD-induced delay in gastric emptying and the mere insertion of needles at acupoints without stimulation did not show any sustained effects, as previously reported (55). In human studies, electrical stimulation is often needed for sham-EA since the subject feels electrical stimulation. In one previous clinical study, we found that electrical stimulation at ST36 was more effective than electrical stimulation at non-acupoints in improving functional dyspepsia symptoms and vagal activity (25).
Typically, there are a number of issues that need to be addressed with the application of EA, such as selection of stimulation parameters and duration of EA. Some of these issues were addressed in this study. In clinical practice, EA is commonly applied for 30 min. In our initial experiment (Experiment 3), EA was performed for 75 min: 45 min before RD and 30 min during RD. We initially speculated that a period of EA immediately before RD might improve the outcome of EA. To test this, in Experiment 5, EA was only applied for 30 min during RD. Interestingly, the prokinetic effect of EA with a duration of 30 min was similar to that of EA with a duration of 75 min, suggesting that prolonged EA is not necessary. Among stimulation parameters, two most important ones are stimulation intensity (pulse amplitude) and stimulation frequency. For stimulation intensity, we compared EA of 6 mA with that of 3 mA and found that EA of 6 mA was slightly more potent than EA of 3 mA. Regarding stimulation frequency, we were aware of several studies in which the effects of different stimulation frequencies were investigated. In the literature, there are discussions of the use of EA with low or high frequency. Low frequency EA (2 - 5 Hz) was reported to be more effective in suppressing abdominal cardiovascular reflexes than high frequency stimulation (200 Hz) and to increase the release of opioid peptides in the central nerve system (46, 55). In the treatment of pain, the release of central opioid peptides was reported to be frequency-dependent. More Met-enkphalin was released with EA at 2 Hz, whereas, more dynorphin was released with EA at 100 Hz (8, 16). In GI motility studies, the commonly used stimulation frequency for EA ranged 10 Hz to 25 Hz: EA at 10 Hz/25 Hz was found to increase gastric motility in rats (17, 52, 53) and EA at 25 Hz accelerated liquid gastric emptying and enhanced antral contractions in dogs (7, 34). In this study, EA was performed at 25 Hz and the findings were consistent with previous studies. None of the previous studies investigated the effect of EA at low frequency, such as 2-4 Hz, on GI motility. However, strong cardiovascular effects have been consistently reported with EA at low frequencies (46, 55). It is therefore necessary to study the effects of EA at lower frequencies on GI motility. If EA is to be used for treating patients with delayed gastric emptying induced by rectal stasis, a dose response study should be performed with different stimulation frequencies.

Autonomic and opioid mechanisms are believed to be involved in the prokinetic effect of EA on RD-induced delay in gastric emptying. Although not investigated in the current study, the vagal mechanism has been consistently reported to be involved in the excitatory effect of
EA at ST36 on gastric motility. Anatomically, ST36 is overlying the deep peroneal nerve, EA at ST36 activates somatic afferents and further activates the nuclei in the brain. It is believed that the somatic stimulation induced by EA is conveyed to the nucleus tractus solitariu (NTS) (43). Using c-Fos staining in the rodent brain, Takahashi et al reported site-specific effects of EA on gastric motility. They found that EA at ST36 enhanced gastric motility by stimulating the dorsal motor nucleus of the vagus (DMV) through the caudal NTS whereas EA at ST25 (abdomen) inhibited gastric motility by stimulating the rostral centrolateral medulla through the medio-caudal NTS (19). Therefore EA at ST36 stimulates gastric motility via the somatosensory-NTS-DMV-parasympathetic efferent pathway (43). In addition, a previous study investigating the effect of EA at ST36 on gastric emptying under normal physiological conditions, a significant increase in vagal efferent activity was noted with EA at ST36 (34). A similar increase in vagal activity was also observed in dogs when EA at ST36 was applied to improve RD-induced antral hypomotility (7). The exact involvement of the opioid pathway was not investigated in the present study. It is possible that EA may activate central opioids that inhibit the sympathetic outflow, resulting in vagal dominance (15, 22).

**Perspectives and Significance:**

Delayed gastric emptying is common in functional motility disorders; however, there is a lack of effective treatment options for delayed gastric emptying. In the current study, delayed gastric emptying was established with rectal distention, which is commonly seen in patients with constipation. While electroacupuncture has been reported to improve gastric motility, not much has been shown on its effects on solid gastric emptying. The findings of the present study suggest that EA at ST36 with appropriate parameters is effective in treating delayed gastric emptying associated with rectal distention. It is likely that the similar method of EA may also be effective in improving delayed gastric emptying due to other causes. Further clinical studies are warranted to investigate the therapeutic potential of EA on delayed gastric emptying.

In conclusion, gastric emptying of solid in dogs can be accurately assessed without the use of any special equipment. Electroacupuncture is able to attenuate RD-induced delay in gastric emptying of solid and that this ameliorating effect may be mediated via the opioid and autonomic pathways. EA may have a therapeutic potential for treating delayed gastric emptying attributed to lower gut distension.
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REFERENCES


Figure Legends

Figure 1: Intestinal cannula. The left panel shows the exterior view of the cannula. The metal screw is used to close the cannula opening when it is not in use. The middle panel shows the inside view of the cannula. The oval part of the cannula is within the lumen of the intestine. The diameter of the opening of the cannula is 12 mm. The right picture shown the side view of the cannula. The bottom part is inserted into the intestine and the top part is externalized in the abdomen. The length of the cannula is adjusted according to the thickness of the intestinal and abdominal walls.

Figure 2: Complete gastric emptying of solid in dogs. A: Percent of gastric retention during 450 min after a test meal. B: Gastric emptying rate per 15 min during the entire 450 min.

Figure 3: Effects of Bethanechol and atropine on gastric emptying of solid. Bethanechol significantly accelerated gastric emptying at all time points in comparison with the control session (P < 0.05). In contrast, atropine substantially and significantly delayed gastric emptying at all time points in comparison with the control session (P < 0.05).

Figure 4: Effects and opioid mechanism of EA on delayed gastric emptying induced by RD. RD from minute 60 to minute 90 after the meal significantly delayed gastric emptying from minute 90 to 180 after the meal (P < 0.03, vs. control without RD). EA (15-90 min) significantly improved RD-induced delayed gastric emptying (P < 0.03 vs. sham-EA with RD); this excitatory effect was blocked by Naloxone (P > 0.05), suggesting involvement of the opioid pathway.

Figure 5: Effects of EA (60-90 min) with different amplitude on RD-induced (60-90 min) delayed gastric emptying. EA at both 3mA and 6mA significantly accelerated gastric emptying (P < 0.03 vs. control). There was a trend that 6mA was more potent than 3mA from 90-120 min.
*P < 0.03 vs. Control

Gastric Emptying (%)

EA/sham EA

Minutes

Control

EA 3mA

EA 6mA

RD