



Ameliorating Effects of Electroacupuncture on Dysmotility, Inflammation, and Pain Mediated via the Autonomic Mechanism in a Rat Model of Postoperative Ileus

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Background/Aims

Postoperative ileus increases healthcare costs and reduces the postoperative quality of life (QOL). The aim of this study is to investigate effects and mechanisms of electroacupuncture (EA) at ST36 and PC6 on gastrointestinal motility in rat model of postoperative ileus.

Methods

Laparotomy was performed in 24 rats (control [n = 8], sham-EA [n = 8], and EA [n = 8]) for the implantation of electrodes in the stomach and mid-jejunum for recording of gastric and small intestinal slow waves. Electrodes were placed in the chest skin for electrocardiogram (ECG). Intestinal manipulation (IM) was performed in Sham-EA and EA rats after surgical procedures. Small intestinal transit (SIT), gastric emptying (GE), postoperative pain, and plasma TNF- α were evaluated in all rats.

Results

(1) Compared with sham-EA, EA accelerated both SIT ($P < 0.05$) and GE ($P < 0.05$) and improved regularity of small intestinal slow waves. (2) Compared with the control rats (no IM), IM suppressed vagal activity and increased sympathovagal ratio assessed by the spectral analysis of heart rate variability from ECG, which were significantly prevented by EA. (3) EA significantly reduced pain score at 120 minutes ($P < 0.05$, vs 15 minutes) after the surgery, which was not seen with sham-EA. (4) Plasma TNF- α was increased by IM ($P = 0.02$) but suppressed by EA ($P = 0.04$) but not sham-EA.

Conclusion

The postoperative ileus induced by IM, EA at ST36 and PC6 exerts a prokinetic effect on SIT and GE, a regulatory effect on small intestinal slow waves and an analgesic effect on postoperative pain possibly mediated via the autonomic-cytokine mechanisms.

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Key Words

Dysmotility; Electroacupuncture; Ileus; Inflammation; Pain

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Introduction

The incidence of postoperative ileus (POI) after laparotomy has been reported to be 7.4-11.8%.¹⁻³ POI increases healthcare costs due to extended hospitalization and has contributed to the worsening of the postoperative quality of life. Prokinetic agents, such as mosapride citrate, are commonly used in the event of POI.⁴ However, effective therapeutic options for POI are very limited, and there is a need for novel and effective treatments for gastrointestinal (GI) dysmotility due to POI.

Since ancient times, acupuncture has been used for the treatment of GI diseases and pain in East Asia. The prokinetic effect by acupuncture or electroacupuncture (EA) has been reported in animal⁵⁻¹⁰ and human^{11,12} studies. POI patients need to be treated not only for GI dysmotility but also for pain and inflammation. However, little is known whether acupuncture or EA has such multi-factorial effects on POI.

Treatment for pain and treatment for dysmotility are often contraindicated. Analgesic agents, such as opioids, impair GI motility, which aggregates POI. Prokinetics, on the other hand, may induce GI spasm, which worsens pain. In addition, inflammation could be a contributing factor of POI. It is not uncommon to use steroids to control inflammation in some highly invasive surgeries, such as esophagectomy, hepatectomy, and cardiac surgeries. However, steroids are not used in general abdominal surgery because of the lack of evidence of clinical benefits and possible adverse events. We hypothesized that EA with an appropriate method may have combined effects on postoperative GI dysmotility, pain, and inflammation via the improvement of autonomic functions.⁹⁻¹⁷

The aim of this study is therefore, to investigate possible multi-factorial effects of EA at ST36 and PC6 with appropriate parameters on postoperative dysmotility, pain and inflammation and

mechanisms involving autonomic functions in a rodent model of POI.

Materials and Methods

Preparations of Animals

Adult male rats (300-350 g, 8-10 weeks, Sprague-Dawley; Charles River Labs, Wilmington, MA, USA) were housed in regular cages in a temperature-controlled environment at 22°C, 40% humidity, and a 12 hour-12 hour light-dark cycle. The rats had free access to water and solid food. All animal experiments were conducted in accordance with the recommendations given by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committees of the VA Medical Center, Oklahoma City, OK, USA (IACUC No. 1405-002).

Surgical Procedure

Rats were randomly divided into a control group (control, n = 8), a sham-EA group (Sham-EA, n = 8), and EA group (EA, n = 8) (Fig. 1). After overnight fast, all rats were operated under anesthesia with the inhalation of 1.5-2.0% isoflurane (Forane; Abbott Laboratories, Abbott Park, IL, USA).

In the control, three electrodes (28-gauge cardiac pacing wires; A&E Medical, Farmingdale, NJ, USA) were placed for the measurement of the electrocardiogram (ECG) as follows: 2 electrodes underneath the skin on the muscle layer across the heart with an interval of 1 cm and a reference electrode about 3-5 cm below the pair on the left costal margin. Then, the abdomen was shaved and disinfected, and a midline incision was made. The length of the incision was set at approximately 7 cm. One pair of the same cardiac pacing wires was implanted on the gastric serosa in the antrum for

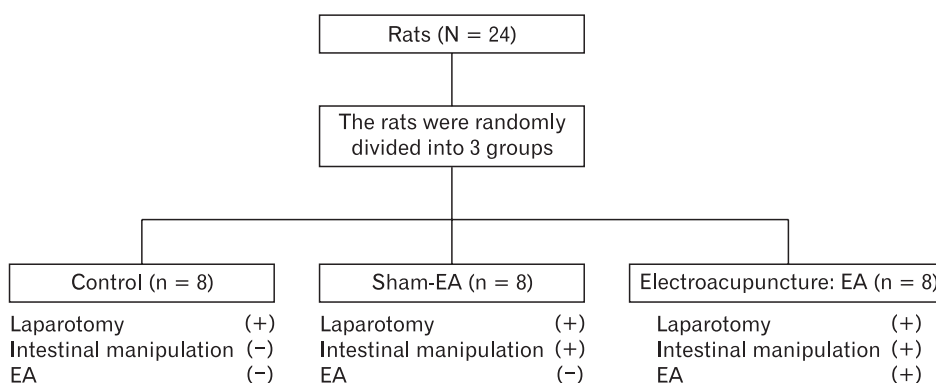


Figure 1. Animal groups and procedures. EA, electroacupuncture.

the measurement of the gastric slow wave (pace-making activity). For the measurement of the small intestinal slow wave, 2 identical wires were sutured on the serosa of the mid-jejunum.

For rats in the Sham-EA and EA, in addition to the same electrode placement, the following intestinal manipulation (IM) was performed: the small intestine and cecum were exteriorized and rubbed in sterile saline for 5 minutes using cotton applicators; after that, the intestine and cecum were covered with gauze soaked with saline and the abdomen was left open for an additional 10 minutes; the viscera were then placed back into the abdomen.¹⁸

In all rats, electrode-connecting wires were tunneled subcutaneously through the anterior abdominal wall and externalized at the back of the neck.¹⁰ Abdominal muscles and skin were closed with 4-0 silk sutures. Following surgery, each animal was subcutaneously administered with sterile saline (5 mL) and buprenorphine (0.1 mg/kg) for maintaining hydration and postoperative analgesia, respectively. The operation time was controlled 70 minutes for all rats (Fig. 1).

Experimental Protocol

Immediately after the surgery, a 1.5 mL methylcellulose solution mixed with phenol red was gavaged for measuring gastric emptying and small intestinal transit; the rats were then placed in a Ballman cage (transparent restrainer made of a clear acrylic wall and having thin metal bars). The gastric and small intestinal slow waves were recorded continuously for 3 hours, during which EA or sham-EA was performed in the EA or Sham-EA group. Postoperative pain was assessed using the Rat Grimace Scale (RGS).¹⁹ Three hours later, the rats were sacrificed under general anesthesia with 5% isoflurane inhalation, and their death was confirmed by opening their chest. Blood was drawn through the heart. The contents of the stomach and small intestine were obtained for the measurements of small intestinal transit and gastric emptying (see below). Finally, a part of the ileum was harvested and fixed in 4% paraformaldehyde for histologic evaluation. Figure 2 shows the experimental protocol and surgical procedures of this study.

Electroacupuncture and Sham-Electroacupuncture

EA was performed at bilateral ST36 (one channel) and bilateral PC6 (another channel) in the EA rats based on previous studies.⁹⁻¹⁵ ST36 is located 5 mm below the head of the fibula under the knee joint and 2 mm lateral to the anterior tubercle of the tibia. PC6 is located proximal to the accessory carpal pad of the forelimb, between the flexor carpi radialis and palmaris longus ligaments. Acupuncture needles (Seirin, Shizuoka, Japan), bended to form an L shape,

were inserted into each ST36 and PC6 at a depth of 3-5 mm and fixed by a 4-0 silk suture. The 2 pairs of needles were connected to a multi-channel universal pulse generator (Model DS8000; World Precision Instruments, Sarasota, FL, USA). EA at ST36 was designed to improve GI motility and the best parameters known to exert a prokinetic effect were: on time of 2 seconds, off time of 3 seconds, 25 Hz 0.5 milliseconds, and 4 mA.^{9,10,13,14,17} EA at PC6 was used to mainly provide an analgesic effect and the best stimulation parameters to reduce visceral pain were: on time of 0.1 seconds, off time of 0.4 seconds, 0.5 milliseconds, 100 Hz, and 1 mA.^{20,21} Sham-EA was performed in Sham-EA rats. The procedure was the same as EA without electrical stimulation.

The stimulation was initiated immediately after the surgery and the stimulation duration was chosen to be 60 minutes based on previous studies. In one study, a single 30-minute EA failed to suppress inflammation in a rodent model of POI,²² whereas in another study, a 90-minute EA was reported to improve tissue injury in hemorrhagic shock rats.²³ In this study we chose 60 minutes.

Assessment of Small Intestinal Transit and Gastric Emptying

Phenol red (50 mg; Sigma, St. Louis, MO, USA) was diluted in 100 mL aqueous methylcellulose (1.5%; Fisher Scientific, Fair Lawn, NJ, USA) solution, and 1.5 mL of the solution was ingested into the stomach by gavage immediately after the surgery. The rats were sacrificed 180 minutes later for the measurement of small intestinal transit and gastric emptying.

For measuring gastric emptying, the entire stomach was carefully isolated, ligated just above the cardia and below the pylorus, and removed. For measuring small intestinal transit, the entire small intestine was carefully harvested and divided into 10 equal segments. Each gut segment was individually homogenized using a homogenizer with 100 mL of 0.1 N NaOH. The mixture was kept at room temperature for 1 hour. Supernatant (5 mL) was added to 0.5 mL of TCA solution (20% wt/vol) to precipitate the proteins. After centrifugation (3000 rpm, 15 minutes), the supernatant was added to 4 mL of NaOH (0.5 N) to allow the development of maximum color intensity. The solutions were read using a spectrophotometer (fixed wavelength of 560 nm). Gastric emptying was calculated according to the following formula: $C_{\text{recovered}}/C_{\text{ingested}} - C_{\text{recovered}}/C_{\text{ingested}}$.¹⁰ C_{ingested} refers to the amount of phenol red ingested into the stomach. $C_{\text{recovered}}$ refers to the amount of phenol red recovered from the stomach.

Small intestinal transit was assessed using a parameter called geometric center, calculated as follows: $GC = \text{sum of } (n \times P_n) \text{ for}$

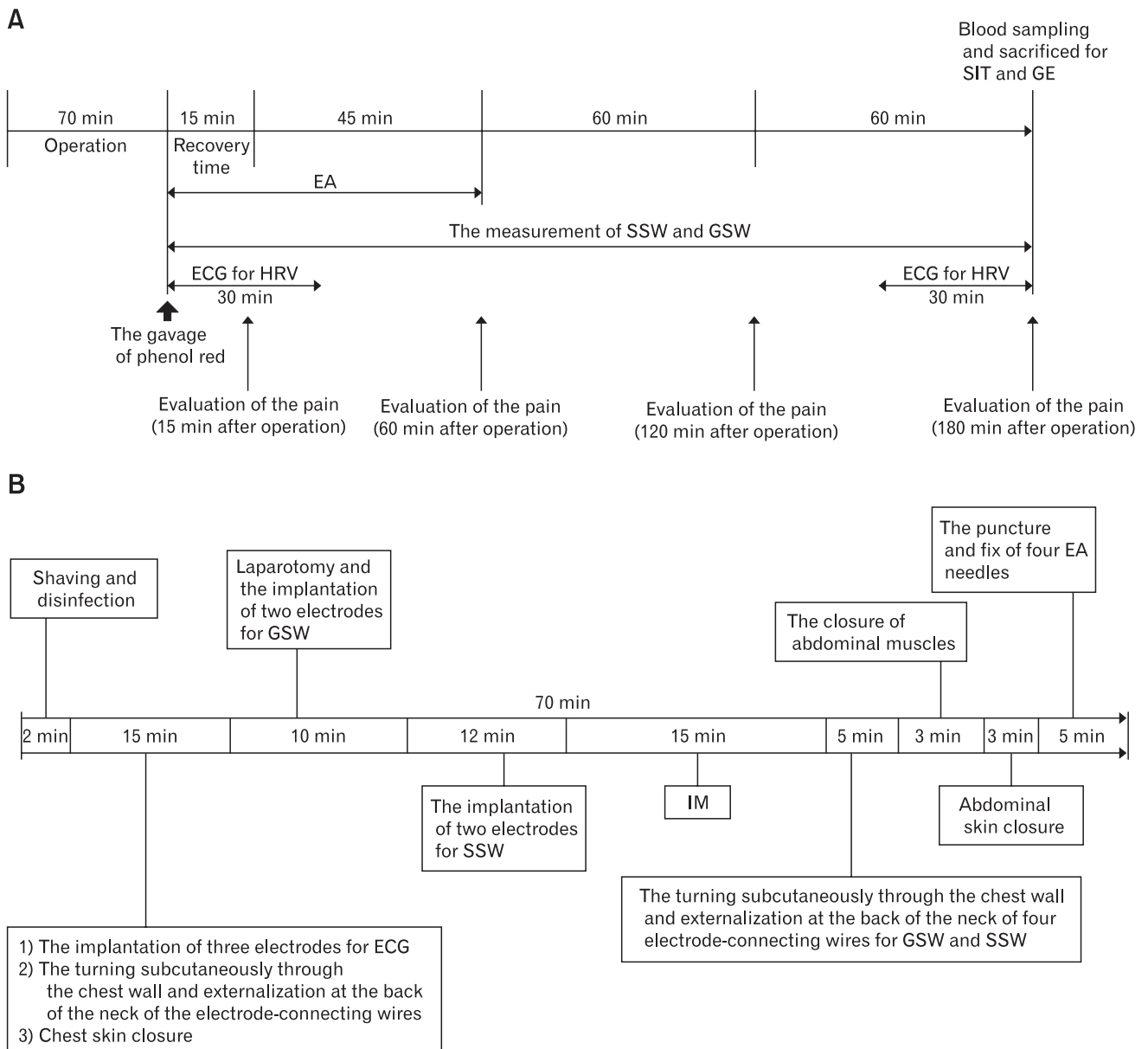


Figure 2. Experimental protocol (A) and surgical procedures (B). EA, electroacupuncture; SIT, small intestinal transit; GE, gastric emptying; SSW, small intestinal slow wave; GSW, gastric slow wave; IM, intestinal manipulation; ECG, electrocardiogram; HRV, heart rate variability.

$n = 1, 2, \dots, 10$.^{24,25} Where “n” is the number of an intestinal segment and P_n is the percentage of phenol red recovered from the segment.

Recording and Analysis of Slow Waves

The small intestinal and gastric slow waves were recorded using a Biopac system (EOG 100 A; Biopac Systems, Santa Barbara, CA, USA) as described previously.^{10,26,27} The following parameters were derived from the recording using the spectral analysis method^{10,26,28}: (1) dominant frequency (DF) in the power spectrum

of the signal; (2) dominant power (DP), defined as the power (dB) at the corresponding DF in the power spectrum; (3) the percentage power distribution of normal small intestinal slow waves (%S-normal), defined as the ratio of the power spectral density between the normal range of 38-42 cycles per minute (cpm) and the entire frequency range (30-49 cpm)²⁶; and (4) the percentage power distribution of normal gastric slow waves (%G-normal), defined as the ratio of the power spectral density between the normal range of 4-6 cpm and the entire frequency range (1-9 cpm).²⁶

Assessment of Autonomic Functions

The ECG was recorded using a special amplifier (model 2283 Fti Universal Fetrotode Amplifier; UFI, Morro Bay, CA, USA) with a recording range of 1.5-100 Hz for 180 minutes after the surgery. A heart rate variability (HRV) signal was derived from the ECG by identifying R waves, interpolating R-R interval data at 100 Hz, and finally down-sampling the interpolated data at 8 Hz suitable for analysis using a previously validated software.²⁹ The summations of 2 frequency ranges in the power spectrum of the HRV signal were calculated as follows³⁰: (1) a high-frequency band (HF; 0.8-4.0 Hz) reflecting purely vagal activity and (2) a low-frequency band (LF; 0.3-0.8 Hz) reflecting mainly sympathetic activity. The LF/HF ratio reflected the sympathovagal balance.

Assessment of Inflammatory Cytokine Tumor Necrosis Factor- α

Blood was drawn at 180 minutes after the surgery. Plasma was obtained by centrifuging the blood at $3000 \times g$ for 15 minutes at 4°C. The TNF- α level in the plasma was assessed using a commercial ELISA kit (Sigma, St. Louis, MO) according to the protocol provided by the manufacturer. The absorbance rate was read at 450 nm. The concentrations of the samples were calculated according to the standard curve. The plasma TNF- α level was expressed as pg/mL.

Histologic Evaluation

A 5-mm distal ileum (5 mm) fixed in 4% paraformaldehyde was used for histologic evaluation. The paraformaldehyde-fixed intestine was embedded in paraffin and cut into 2- μ m sections. He-

matoxylin and eosin staining of the intestine was performed. The injury to the intestinal mucosa was scored using the modified histopathologic score by Cuzzocrea et al³¹ by the randomized and unlabeled specimen. A scale of 0-3 was used to assess intestinal damage (0: normal, no damage; 1: mild, focal epithelial edema and necrosis; 2: moderate, diffuse swelling or necrosis of the villi; and 3: severe, diffuse necrosis of the villi with evidence of neutrophil infiltration in the submucosa or hemorrhage).

Evaluation of Postoperative Pain

A digital camera (PowerShot SX400IS; Canon, Japan) was placed outside the acrylic glass wall of the Ballman cage, and digital movies were taken for 5 minutes at 4 different time points after the surgery (Fig. 2). The RGS score was assessed by an evaluator in the lab who was blinded to the study. The RGS values were 0, 1, and 2 for each of the 5 RGS action units: orbital tightening, nose bulge, cheek bulge, ear position, and whisker change. The final RGS score was the average score of the 5 action units.³²

Statistical Methods

Statistical analysis of the data was performed using paired *t* test, one-way, repeated ANOVA, and the Tukey–Kramer test for multiple comparisons. Data are expressed as mean \pm standard deviation. Statistical significance was set at $P < 0.05$.

Results

Small Intestinal Transit and Gastric Emptying

EA significantly improved both small intestinal transit and gas-

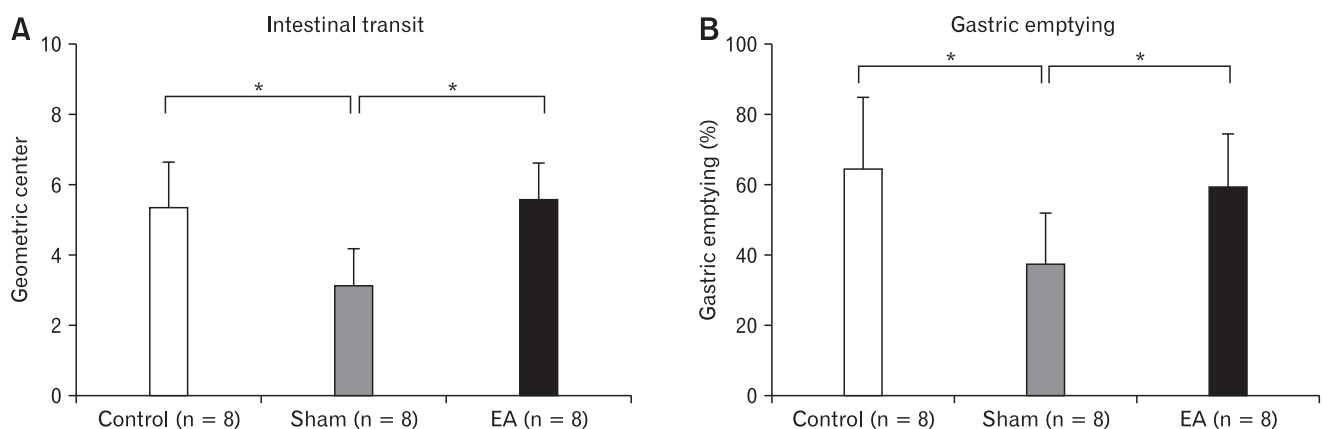


Figure 3. Small intestinal transit (A) and gastric emptying (B). Electroacupuncture (EA) normalized intestinal manipulation-induced delay in intestinal transit and gastric emptying. * $P < 0.05$ (Tukey–Kramer test).

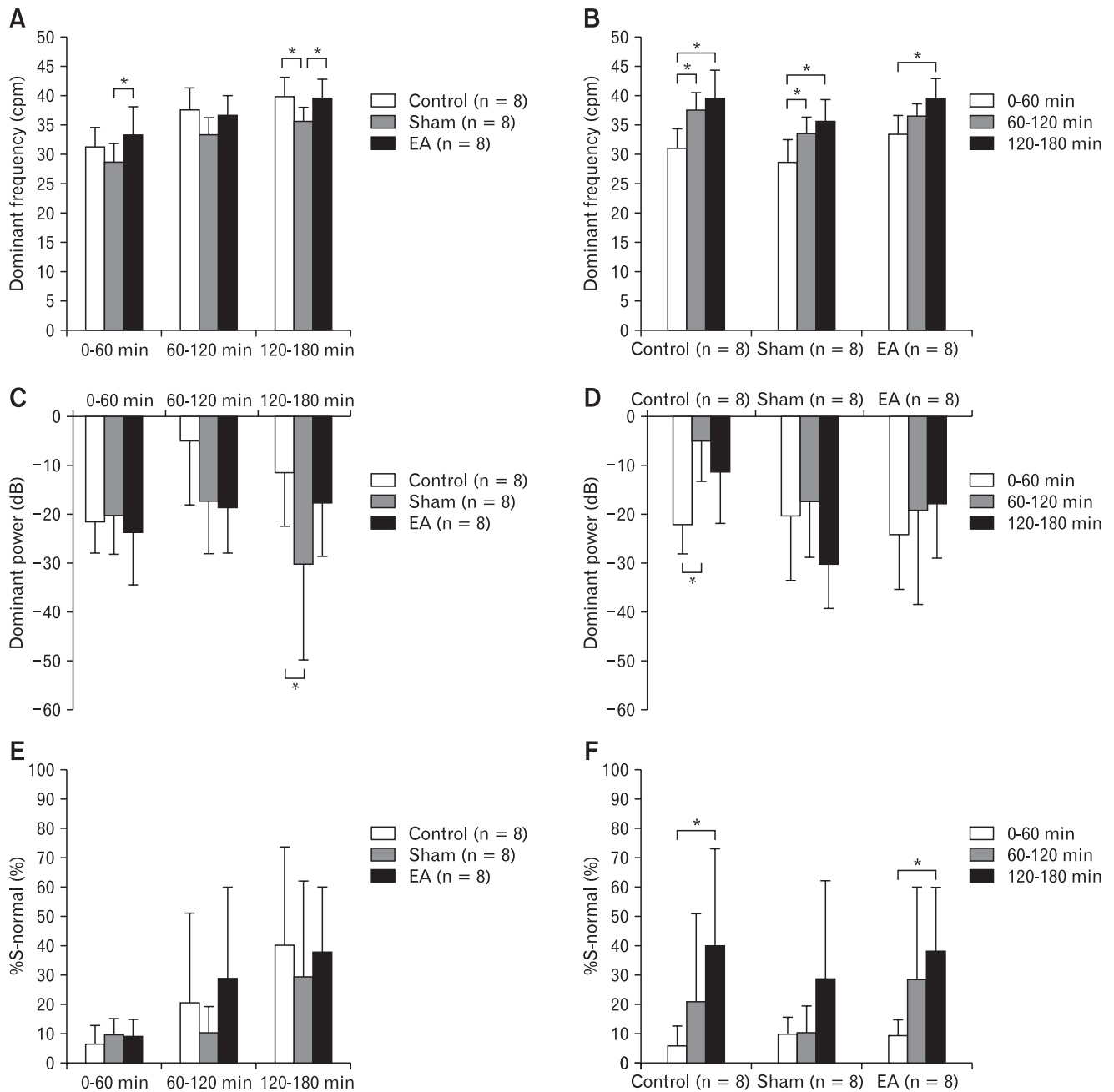


Figure 4. Effects of surgery, intestinal manipulation (IM), and electroacupuncture (EA) on intestinal slow waves. (A) Dominant frequency of slow waves in the 3 groups of rats during the 3 postoperative hours; it was reduced by IM but this reduction was normalized by EA during the third postoperative hour ($*P < 0.05$). (B) Dominant power of slow waves during the 3 postoperative hours in each group. A postoperative increase in dominant frequency was noted in all 3 groups ($*P < 0.05$). (C, D) Dominant power of intestinal slow waves in 3 groups of rats during different postoperative hours. No consistent effects were noted by surgery, IM, or EA. (E) %S-normal: the percentage of normal slow wave component distribution in the power spectrum in the 3 groups of rats during different postoperative hours. It was not altered by IM or EA. (F) %S-normal: the percentage of normal slow wave component distribution during the 3 postoperative hour in different groups of animals. A significant postoperative recovery was noted in the control and EA group but not sham-EA group, suggesting an IM-induced delay in postoperative recovery. $*P < 0.05$.

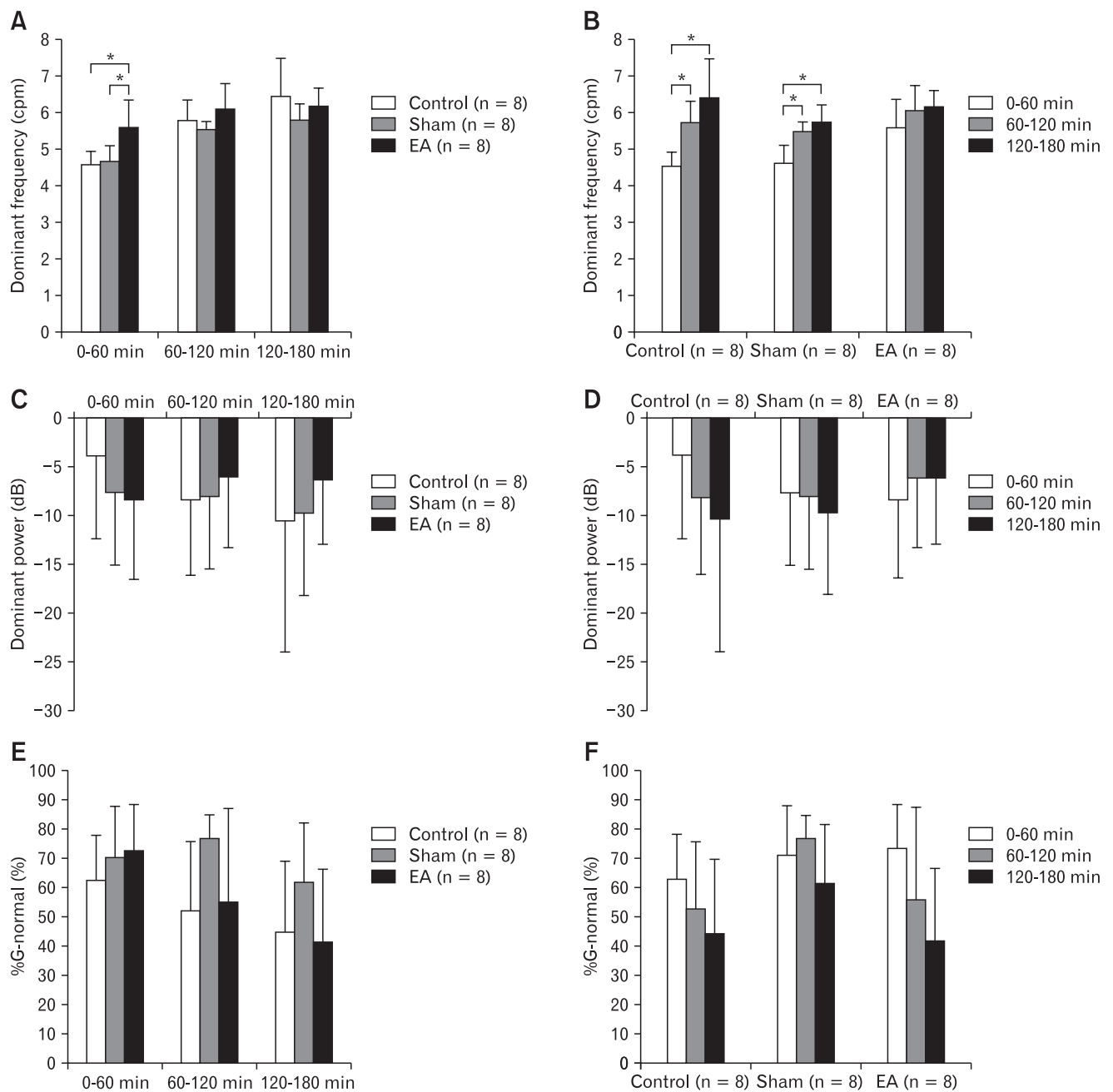


Figure 5. Effects of surgery, intestinal manipulation (IM), and electroacupuncture (EA) on gastric slow waves. (A) Dominant frequency of gastric slow waves in the 3 groups of rats during different postoperative hours. (B) Dominant frequency of gastric slow waves during hours in each of the 3 groups. (C, D) Dominant power of gastric slow waves in the 3 groups of rats during different postoperative hours. (E, F) %G-normal: the percentage of normal slow wave distributions in the power spectrum in the 3 groups of rats during different postoperative hours. **P* < 0.05 by Tukey–Kramer test.

tric emptying in rats with the IM. The IM delayed small intestinal transit, and EA normalized the IM-induced delay in small intestinal transit. The geometric center was 5.34 ± 1.28 in the control rats (no IM), 3.08 ± 1.03 in rats with IM and sham-EA (*P* < 0.01 vs

control) and 5.58 ± 0.98 in rats with IM and EA (*P* < 0.001, vs sham-sham EA) (*P* < 0.001, among the 3 groups, ANOVA) (Fig. 3A). Similarly, IM delayed gastric emptying that was normalized by EA (Fig. 3B).

Small Intestinal Slow Waves

In the small intestine, the DF of slow waves during the third hour after the surgery was reduced by IM and this decrease was prevented with EA (Fig. 4A, the third panel). The repeated ANOVA revealed that the DF during that period in the sham-EA group (with IM and sham-EA) was significantly lower than that in

the control ($P < 0.05$). The DF in the EA group was significantly higher than that in the sham-EA ($P < 0.05$) and comparable to the control without IM ($P = \text{NS}$). A postoperative increase in the DF of slow waves was noted in all three groups (Fig. 4B), suggesting a postoperative recovery of intestinal slow waves.

However, the DP of the slow waves was not consistently affected by either surgery (no significant postoperative changes; Fig.

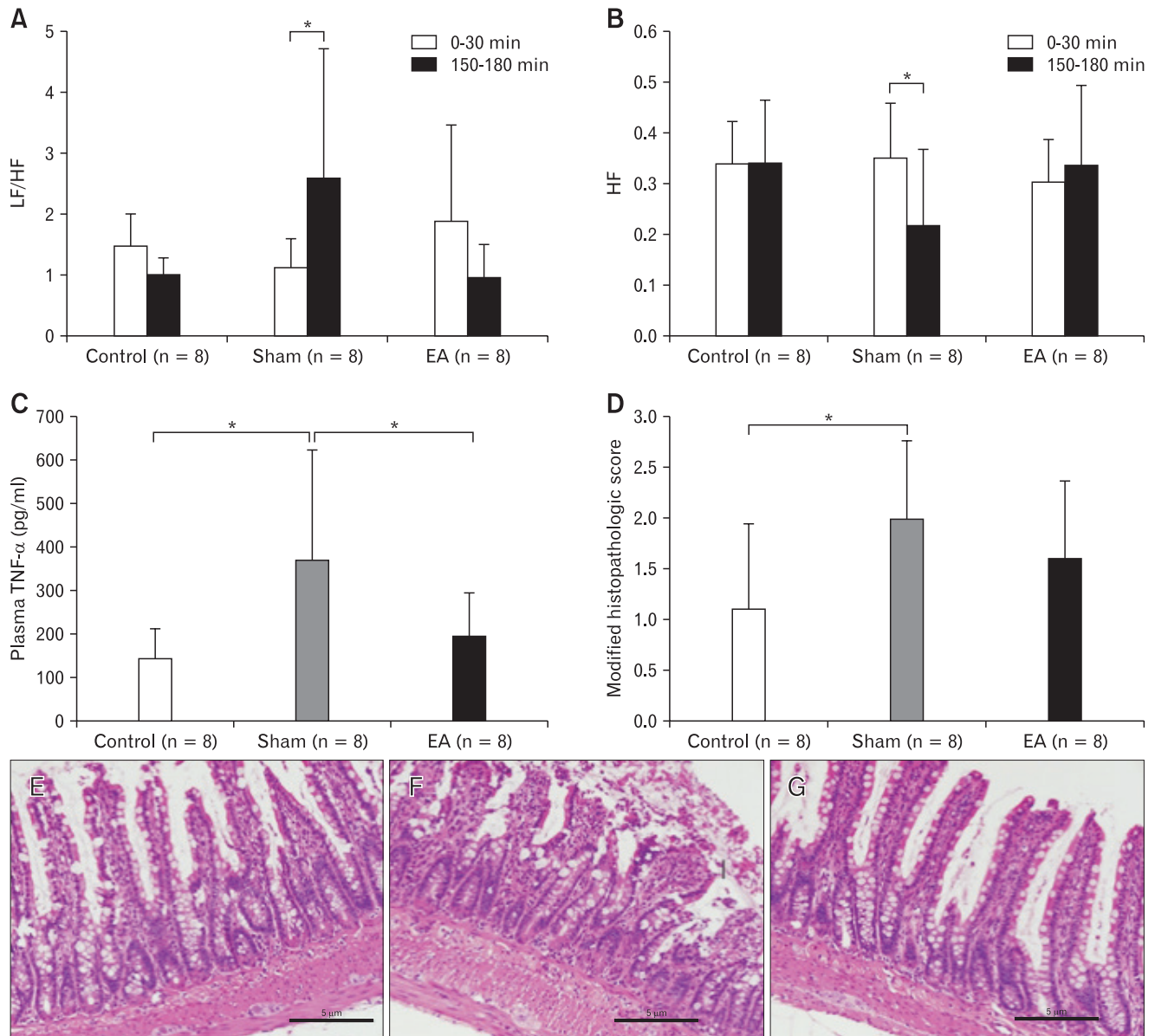


Figure 6. Effects of surgery, intestinal manipulation (IM), and electroacupuncture (EA) on autonomic functions, TNF- α , and intestinal tissue damages. (A, B) Sympathovagal balance (A) and vagal activity (B) in the 3 groups of animals. (C) Plasma TNF- α 3 hours after surgery in 3 groups of rats. (D) Histological score in 3 groups of rats. (E-G) Histologic evaluation of the distal ileum in a control rat (E), a sham-EA rat (F), and an EA rat (G). LF, low-frequency range; HF, high-frequency range. * $P < 0.05$ by Tukey-Kramer test. All images were taken at $\times 200$ magnification.

4D), IM (no significant difference between the sham-EA and control; Fig. 4C) or EA (no significant difference between EA and sham-EA; Fig. 4C).

The regularity (%S-normal: % of normal slow wave component in the entire power spectrum) of the intestinal slow waves was not altered by IM or EA; no difference was noted during any postoperative hour among the three groups of rats (Fig. 4E). However, it was affected by the surgical procedure and IM; as shown in Figure 4F, the %S-normal in the control rats (only surgery but no IM) was significantly increased from the 1st hour to the third hour after surgery, suggesting a postoperative recovery. This postoperative recovery was not noted in the sham-EA group, demonstrating a delayed recovery due to IM but was observed in the EA group, suggesting an accelerative effect of EA on post-IM recovery.

Gastric Slow Waves

The frequency of gastric slow wave was reduced in the first hour due to surgery; this reduction was not worsened by IM but prevented by EA. As shown in Figure 5A, during the first hour after surgery, the DF of gastric slow waves was significantly lower in the control and sham-EA groups than the EA group, and there was no difference between the control (no IM) and sham-EA (IM); this difference was, however, not seen during the second and third hour after surgery. Similarly it can also be seen from Figure 5B, in the control and sham-EA rats, the DF in the second and third hour was significantly higher than that in the first hour, suggesting

a postoperative recovery. In the EA group, however, the DF in the first hour was not different from that during the second or third hour, demonstrating that EA prevented the surgery-induced decrease in DF as seen in the control and sham-EA groups.

Dominant power of the gastric slow waves or %G-normal was however, not altered by surgery, IM or EA, as shown in Figure 5C and 5D and Figure 5E and 5F, respectively.

Autonomic Functions

IM increased the sympathovagal balance and decreased the vagal activity during the 3 postoperative hours, and EA treatment prevented such postoperative changes in the autonomic functions. As shown in Figure 6A and 6B, there was a significant increase from minutes 0-30 to minutes to 150-180 in LF/HF and a significant decrease in HF in the rats treated with IM and sham-EA, demonstrating a postoperative increase in sympathetic dominance; these changes were not noted in the control rats, suggesting that the effect was attributed to IM or the EA rats, demonstrating that EA prevented the IM-induced changes in autonomic functions.

Cytokine Tumor Necrosis Factor- α

IM increased the plasma TNF- α level, which was prevented by EA. As shown in Figure 6C, the plasma TNF- α level at 3 hours after the surgery was elevated by 159% in the sham-EA rats (with IM), compared with the control rats ($P = 0.020$). The EA treatment significantly reduced the plasma TNF- α level to a level com-

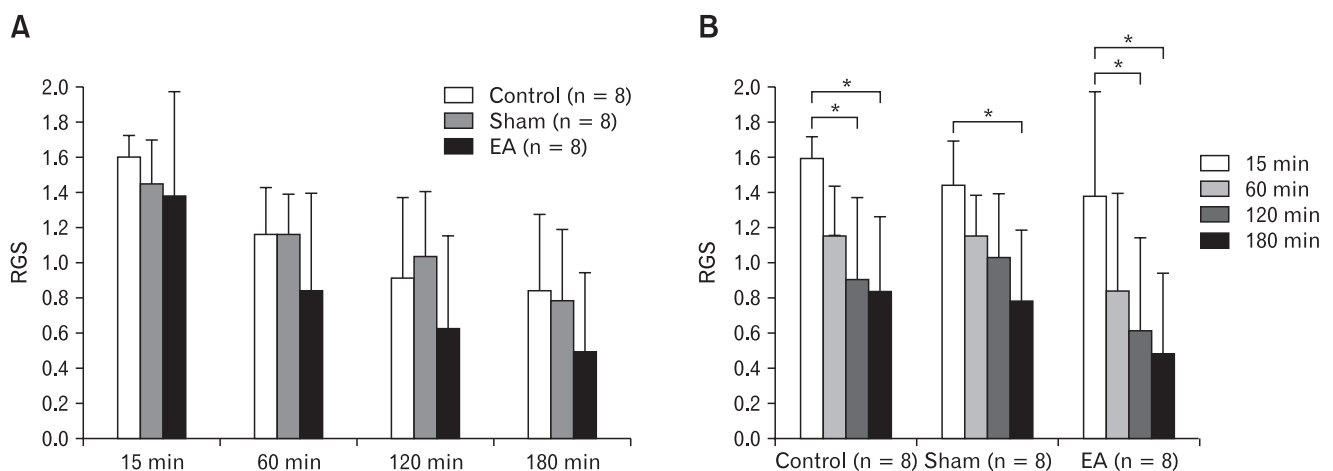


Figure 7. Effects of surgery, intestinal manipulation (IM), and electroacupuncture (EA) on postoperative pain. (A) The Rat Grimace Scale (RGS) pain score in the 3 groups of rats during different postoperative hours. No difference was noted among the groups. (B) The RGS pain score during different postoperative hours in each group of rats. A significant reduction in the pain score was noted only during the third hour in the sham-EA group, suggesting an IM-induced delay in the recovery of pain. * $P < 0.05$ (Tukey–Kramer test).

parable with that of the control rats (Fig. 6C).

Histologic Evaluation of the Ileum

IM increased inflammation in the distal ileum, which was prevented by the EA treatment. As shown in Figure 6D, the histopathologic score was significantly higher in the rats treated with sham-EA and IM than that in the control rats ($P = 0.045$); however, this difference was not noted between the EA and control groups ($P = 0.170$), suggesting a preventive effect of EA on the IM-induced increase in tissue damages.

Postoperative Pain

EA accelerated the recovery of postoperative pain. Although no difference was noted among the three groups in the pain score during any hour after surgery (Fig. 7A), the postoperative recovery of the pain was accelerated with EA. As shown in Figure 7B, the RGS pain score in the control group was significantly reduced during the second and third hours after surgery, compared with the first 15 minutes after surgery, and this was the same in the EA group. However, in the sham-EA, the RGS score was significantly reduced only during the third postoperative hour, suggesting a delayed recovery of pain due to IM.

Discussion

In the present study, we have demonstrated the comprehensive effects of EA at ST36 and PC6 on IM-induced delay in gastric emptying and small intestinal transit, postoperative pain and inflammation, as well as the mechanism involving autonomic functions. Compared with the surgical procedures of placing various electrodes, the special manipulation of the small intestine delayed gastric emptying and small intestinal transit, delayed postoperative pain recovery, and increased tissue damages and inflammatory cytokine, TNF- α . Autonomically, IM reduced vagal activity and increased the sympathovagal balance. EA with previously approved methodology accelerated gastric emptying and small intestinal transit, accelerated postoperative pain recovery, and prevented postoperative inflammation and the postoperative increase of TNF- α . Mechanistically, EA prevented the IM-induced increase in sympathovagal balance and decrease in vagal activity.

The methodology used in this study was slightly different from those published in the literature. In addition to the IM to induce POI, we also implanted electrodes at the serosa of the stomach and small intestine, and underneath the skin in the chest. The total surgical time was 70 minutes. These factors might have contributed

some discrepancies between the findings of the present study and those in previous studies. For example, moderate tissue damages were noted in this study (not typically reported in other POI studies) and much delayed gastric emptying was noted even without IM (about 64% at 3 hours).

A few previous studies have reported prokinetic effects of EA on small intestinal dysmotility in various disorders or conditions, such as acceleration of small intestinal transit delayed by glucagon and improvement of rectal distention-induced impairment in small intestinal transit in dogs^{13,14} and improvement of intestinal transit in STZ-induced diabetic rats.¹⁰ Fang et al²² reported improvement of gastric emptying and intestinal transit including the contents of the stomach, small bowel cecum, and colon in POI by EA. However, there was no report on the improvement of dysmotility in small intestinal transit and gastric emptying in POI combined with the analysis of the small intestinal slow wave and gastric slow wave.

In the present study, EA improved small intestinal transit impaired by IM. We speculated that the improvement of small intestinal transit was due to the improvement of the small intestinal slow wave. Compared with the gastric slow wave, the small intestinal slow wave has been relatively less intensively studied. In the present study, we carefully assessed a number of major parameters of the small intestinal slow wave. It was found that abdominal surgery (without IM) caused a decrease in intestinal slow wave frequency (reflected as a postoperative increase in frequency) and rhythmicity, and IM impaired the recovery of the slow wave frequency and rhythmicity. The proposed method of EA prevented these IM-induced delays in slow wave recovery.

Although the manipulation was in the small intestine, the IM also delayed gastric emptying, and EA was able to improve the IM-induced delay in gastric emptying. Similar findings have been reported in previous studies.⁵⁻¹⁰ Unlike in the small intestine, the IM-induced delay in gastric emptying was unrelated to the gastric slow wave. This was because IM did not alter gastric DF, DP, or rhythmicity in comparison with the control rats (same abdominal surgery without IM). Interestingly, however, EA had a preventive effect on the surgery-induced decrease in gastric DF during the first postoperative hour.

The postoperative analgesic effects of acupuncture or EA at ST36 have been reported in both animal³³⁻³⁵ and human studies.^{36,37} Feng et al³³ reported that EA at ST36 and SP6 for 30 minutes showed an analgesic effect on post-laparotomy pain in rats assessed by the Von Frey filaments test. Teixeira et al³⁴ reported that 100-Hz EA could reduce mechanical nociception in a rodent model of incisional pain. To the best of our knowledge, there had been no

reports investigating the analgesic effect of EA on postoperative pain associated with POI. In clinical practice, analgesics (mostly, opioids) are commonly used. However, opioids are known to inhibit GI motility.^{38,39} In the present study, the stimulation parameters for EA at PC6 were specifically designed to reduce pain. These parameters have been shown to reduce gastric hypersensitivity²⁰ and ameliorate visceral pain in a rodent model of functional dyspepsia.²¹ From Figure 7, it is clear that abdominal surgery induced pain, but the pain gradually subsided because there was a significant decrease in the RGS score: pain score starting at 2 hours after the surgery in the control rats. IM, however, delayed this pain recovery by 1 hour; the decrease in the pain score was significant only at 3 hours after the surgery. The EA treatment, however, prevented such a delay in pain recovery. The decrease in the pain score in the EA rats was significant at 2 hours after the surgery, the same as in the control rats without IM. These findings demonstrated an accelerative effect of EA on pain recovery induced by IM. Although not significant, the overall pain score was lower in the EA-treated rats than in the control rats, suggesting an analgesic effect on postoperative pain in general. These findings suggest that EA may be a novel option for postoperative pain relief. In the present study, we evaluated postoperative pain using the RGS scoring method proposed by Chi et al⁴⁰ We felt that this method was useful because it did not induce pain and could be used to evaluate spontaneous pain associated with laparotomy and POI. The analgesic effect observed in this study seemed to be contracting with the prokinetic effect of EA as it is generally believed that opioids inhibit GI motility. However, previous studies have also shown excitatory effects of opioids on GI motility, particularly in the case of hypotensive motility. In one study, μ and δ , but not κ opioid agonist was shown to induce small intestinal contractions in dogs.⁴¹ In another study, morphine was shown to inhibit gastric antral contractions but stimulate small intestinal contractions in dogs mediated via the cholinergic mechanisms; further the prokinetic effect was partially blocked by naloxone.⁴² Similar findings were also reported in another study: the blockage of μ receptor with naloxone enhanced contractions of the small intestine in dogs.⁴³ In the present study, the major impairment in motility was in the small intestine and the major action of EA was also in the small intestine. Therefore, these studies seem to support the combined prokinetic and analgesic effects of EA observed in this present study.

The noninvasive assessment of autonomic functions using spectral analysis of HRV has been well-established and used in various animal research and clinical studies.^{10,44,45} Yin et al¹⁰ reported that EA decreased LF/HF and increased HF in a rodent model

of diabetes. Sakai et al⁴⁵ reported that EA at the right trapezius in humans decreased LF/HF. In the present study, IM increased the sympathovagal balance and EA prevented this increase. Furthermore, IM decreased the vagal activity and EA prevented such a decrease. It is well known that the enhancement of the vagal activity improves GI motility, whereas the activation of sympathetic activity suppresses GI motility. Accordingly, we believe that the improvement in GI motility (gastric emptying, small intestinal transit, gastric slow wave, and small intestinal slow wave) can be attributed to the improvement in these autonomic functions.

The anti-inflammatory effects of acupuncture and EA at ST36 have also been reported in both animals^{23,46-48} and humans.^{15,49} However, there is a lack of studies investigating the effect of EA on inflammatory cytokines associated with POI. To do so, we chose one major inflammation cytokine, TNF- α . Compared with the control rats, IM significantly increased the level of plasma TNF- α . This IM-induced increase was completely blocked with EA. These findings suggest an anti-inflammatory effect of EA in POI. We speculate that if EA can control the increase in plasma TNF- α due to surgical stress, there is a possibility that EA can prevent organ failure due to cytokine storm. While the exact anti-inflammatory mechanism of EA was not investigated in this study, we speculated a cholinergic anti-inflammation pathway as reported in a previous study in a rodent model of intestinal inflammation⁵⁰: EA enhanced vagal activity and release of acetylcholine in the gut; acetylcholine inhibited the release of TNF- α via the $\alpha 7$ nicotinic acetylcholine receptor in the macrophage of the gut.⁵¹

Some studies have shown that EA improves the gut injury occurring after intestinal ischemia and reperfusion in hemorrhagic shock rats.^{47,48} In the present study, we found a significant increase in the histopathologic score in the distal ileum with IM. Although EA was not able to significantly block this increase, there was a trend toward improvement with EA in the ileum mucosa injury. The modified histopathologic score in sham-EA group was statistically elevated compared to the control group. On the other hand, the score in the EA group did not differ statistically from that in the control group, suggesting that EA normalized the modified histopathologic score. EA seemed to prevent the IM-induced significant increase in the histopathologic score (control vs EA: $P = 0.170$).

There was a report showing that 30-minute EA did not suppress inflammatory cytokines in a rodent model of POI.²² In the previous study, 30-minute EA at ST36 at 5 Hz did not alter the expression of IL-1 β and TNF- α mRNA expression in the intestinal muscularis (small bowel) 6, 12, and 24 hours after the surgery. Whereas EA at the present study suppressed plasma TNF- α .

The discrepancy could be attributed to the followings: (1) The different time in the assessment of TNF- α . In the previous study, TNF- α mRNA expression was assessed 6, 12, and 24 hours after the surgery, whereas in the present study, plasma TNF- α was assessed 3 hours after the surgery. It was previously reported that TNF- α was elevated during the first few hours after surgery and almost recovered to baseline at 6 hours after surgery.⁵² (2) Different stimulation methodologies used in the 2 studies. EA was performed for 60 minutes in the present study but only for 30 minutes in the previous study. It might be possible that a longer stimulation was needed to exert an anti-inflammatory effect. In addition, stimulation frequencies were also different: 5 Hz vs 25 Hz. (3) Methods in the assessment of inflammation were also different. We thought that the POI was accompanied by a systemic inflammation. For this reason, the inflammatory cytokine was measured from the blood. In the previous study, the effect of EA on TNF- α was assessed from the mRNA expression. In addition, we used a modified histopathologic score to investigate tissue injury; it could evaluate several factors such as edema, necrosis, neutrophil infiltration, and hemorrhage.

In this study, a rodent model of POI was established by intraoperative IM and EA at ST36 and PC6 with special parameters was found effective in improving GI motility, suggesting a therapeutic potential of EA with appropriate methodologies for POI. However, this pre-clinical study was limited in sample size due to the interest of animal welfare. Large-sample clinical studies are warranted to prove the clinical role of appropriate EA for post-surgical recovery and POI.

In conclusion, in a rodent model of POI induced by IM, EA at ST36 and PC6 exerts a prokinetic effect on gastric emptying and small intestinal transit, a regulatory effect on small intestinal slow waves, and an analgesic effect on postoperative pain possibly mediated via the autonomic-cytokine mechanisms.

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Author contributions: Haruaki Murakami, Shiyong Li, and Jieyun Yin performed the research; Jiande D Z Chen, Robert Foreman, and Toshihiro Hirai designed the research study; Haruaki Murakami, Shiyong Li, Robert Foreman, Jieyun Yin, and Jiande D Z Chen analyzed the data; and Haruaki Murakami wrote the paper.

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