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ORIGINAL ARTICLE

Intraoperative Vagus Nerve Stimulation Accelerates Postoperative Recovery in Rats

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Abstract

Introduction Postoperative ileus (POI) is a heavy burden for healthcare industries and reduces the postoperative quality of life. The aim of this study was to investigate the effects and mechanisms of the intraoperative vagus nerve stimulation (iVNS) on gastrointestinal motility in a rodent model of POI.

Methods For control group (control, $n = 8$), electrodes were placed on the chest wall for recording the electrocardiogram and on the stomach and small intestine for measuring gastric slow waves (GSWs) and small intestinal slow waves (SSWs). For sham group (sham, $n = 8$) and iVNS group (VNS, $n = 8$), after the same surgery as the control, intestinal manipulation (IM) was performed to induce POI. iVNS was performed during the surgery for the iVNS group. Small intestinal transit (SIT), gastric emptying (GE), postoperative pain, and plasma TNF-α were evaluated after operation.

Results IM delayed GE that was normalized by iVNS ($P < 0.05$). iVNS reduced plasma TNF-α increased by IM ($P = 0.04$). iVNS prevents the injury of ileum mucosa induced by IM ($P < 0.05$). iVNS reduced the postoperative pain ($P < 0.05$). iVNS prevented the IM-induced decrease in vagal activity (sham 0–30 min vs. 150–180 min, $P = 0.03$, VNS 0–30 min vs. 150–180 min, $P = 0.58$) and increase in sympathovagal balance (sham 0–30 min vs. 150–180 min, $P = 0.04$, VNS 0–30 min vs. 150–180 min, $P = 0.72$).

Conclusions iVNS accelerates postoperative recovery by improving GE, reducing postoperative pain, and preventing the injury of ileum mucosa mediated via the autonomic mechanisms.

Keywords Intraoperative vagus nerve stimulation · Postoperative recovery · Postoperative ileus · Motility · Pain · Inflammation

Introduction

The incidence of postoperative ileus (POI) after laparotomy has been reported to be 7.4 to 11.8%.[1–3] POI increases healthcare costs due to extended hospitalization time and has contributed to reduce the postoperative QOL after abdominal surgery. The prokinetic agents such as mosapride citrate are used in the event of POI.[4] However, the therapy for POI is limited. Because of these reasons, there is a need for exploration of treatments for gastrointestinal dysmotility due to POI.

There are no reports about the improvement of gastrointestinal motility in POI by subdiaphragmatic vagus nerve stimulation. However, vagal nerve is known to play an important role in gastrointestinal motility and vagal nerve stimulation was reported to improve gastrointestinal motility in rats.[5,6] Therefore, we hypothesized that intraoperative vagal nerve stimulation (iVNS) could improve gastrointestinal motility in POI.

In addition to gastrointestinal motility, postoperative pain and inflammation also contributed to symptoms of POI. However, it is unclear whether iVNS might exert multiple effects on postoperative dysmotility, pain, and inflammation. The analgesic agents such as the opioid have the adverse events such as gastrointestinal dysmotility. This dysmotility

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sometimes causes the POI. In perioperative period, the steroid has been used for anti-inflammation in the highly invasive surgery such as esophagectomy, hepatectomy, and cardiac surgery. However, the steroid has not been used in general abdominal surgery because of the lack of clinical benefits and the risk of the adverse events. Accordingly, new therapies are required to control pain and inflammation associated with POI.

The aim of this study was to investigate of the effect and mechanisms of iVNS on postoperative recovery by the evaluation of gastrointestinal motility, postoperative inflammation, and pain in a rodent model of POI induced by intestinal manipulation (IM).

Materials and Methods

Preparations of Animals

Adult male Sprague-Dawley rats (300–350 g, Charles River Labs, Wilmington, MA) were housed in wire-bottom cages in a temperature-controlled environment at 22 °C, 40% humidity, and a 12 h–12 h light-dark cycle. Rats had free access to water and solid food. All animal experiments were carried out in accordance with 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.

Surgical Procedure

Rats were randomly divided into control group (control, $n = 8$), sham treatment group (sham, $n = 8$), and iVNS group (VNS, $n = 8$) (Fig. 1). After an overnight fast, all rats were operated under anesthesia with the inhalation of 1.5–2.0% isoflurane (Forane; Abbott Laboratories, Abbott Park, IL). For the control, three abdominal electrodes were placed for the measurement of the electrocardiogram (ECG) as follows: two electrodes were implanted underneath the skin on the muscle layer across the heart with an interval of 1 cm. A reference electrode was placed in the same manner about 3–5 cm below the pair on the left costal margin. The abdomen was shaved and disinfected, and a midline incision was made. The length of incision was set as 7 cm. One pair of 28-gauge cardiac pacing wires (A&E Medical, Farmingdale, NJ) were implanted on the gastric antral serosa for the measurement of gastric slow wave (GSW). For the measurement of the small intestinal slow wave (SSW), same wires were sutured to the mid-jejunum along the small intestinal serosa with 5-0 polypropylene suture. For the sham and the VNS, after the same surgery as for the control, an IM was performed to induce ileus: the small intestine and the cecum were exteriorized for 5 min using cotton applicators rubbed in sterile saline; after that, the intestines were covered with gauze soaked in saline and the abdomen was left open for a total of 10 min and then placed back into the abdomen.[7] After the IM, all electrode-connecting wires were tunneled subcutaneously through the anterior abdominal wall and externalized at the back of the neck.[8] Abdominal muscles and skin were closed with 4-0 silk sutures. Following the surgery, each animal was administered with sterile saline (5 mL) and buprenorphine (0.1 mg/kg) subcutaneously to maintain hydration and for postoperative analgesia.

Intraoperative Vagus Nerve Stimulation

iVNS was performed at the dorsal subdiaphragmatic vagus nerve during the surgical procedure under anesthesia with an open abdomen. After the laparotomy, one pair of 28-gauge cardiac pacing wires (A&E Medical, Farmingdale, NJ) were implanted at the dorsal subdiaphragmatic vagus nerve for the VNS. The pair of the electrodes was connected to the universal pulse generator (Model DS8000, World Precision Instruments, Sarasota, FL, USA). iVNS was performed for 30 min during operation, using the following parameters: 5 Hz, 0.5 ms, 2.2 mA, 10 s on, 90 s off[9] (Fig. 2). This set of parameters was found to increase cardiac vagal activity assessed by the spectral analysis of the heart rate variability signal.[9]

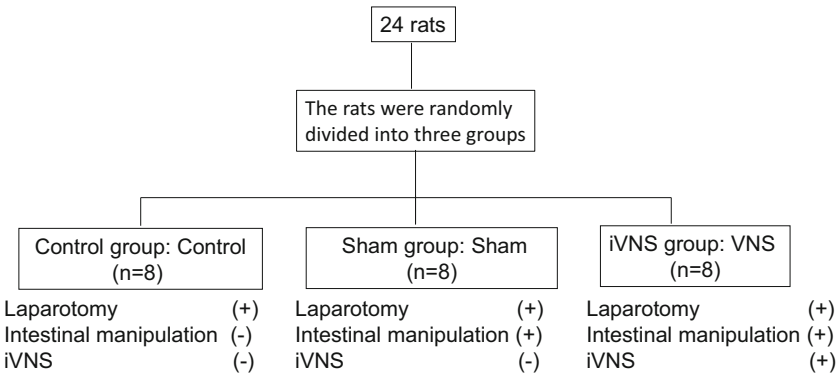

Fig. 1 The experimental protocol

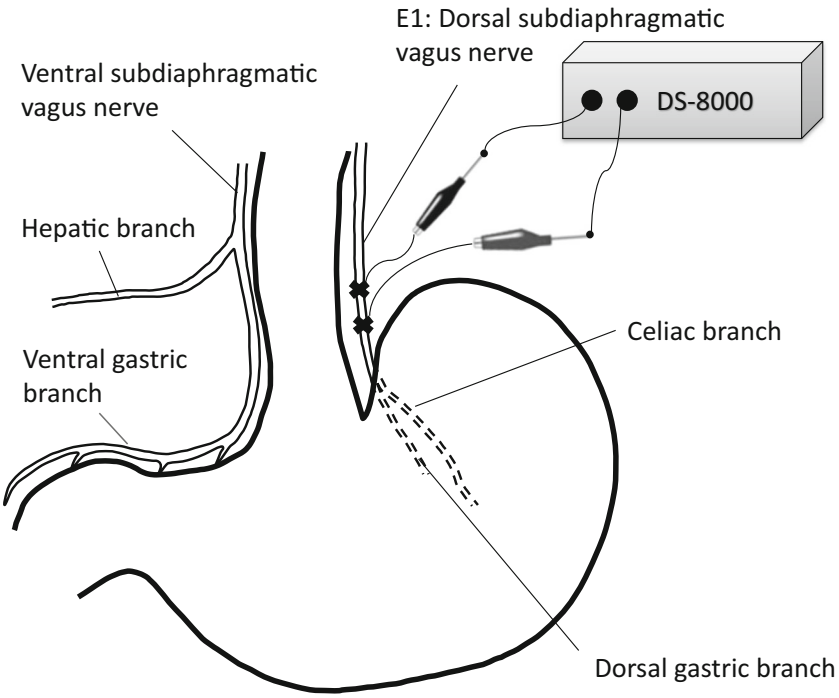

Fig. 2 Intraoperative vagus nerve stimulation (iVNS). iVNS was performed at the dorsal subdiaphragmatic vagus nerve. iVNS was performed for 30 min during operation, using the following parameters: 5 Hz, 0.5 ms, 2.2 mA, 10 s on, 90 s off. E1: the one pair of electrodes at dorsal vagal nerve for performing iVNS

Measurements

Immediately after the surgery, the rats were orally injected with a bolus of phenol red solution (1.5 ml) into the stomach using a feeding tube for the assessment of gastric emptying and intestinal transit. After that, the rats were placed in a Ballman's cage for 3 h. The SSW and GSW were recorded after the operation for 3 h (Fig. 3). During the slow wave recordings, the postoperative pain was evaluated and the ECG was also recorded (Fig. 4). Afterward, the rats were sacrificed with inhalation of 5% isoflurane and the sacrifice was confirmed by visual and physical inspection of the cessation of heart beat. Immediately after, the chest was cut open and the blood was drawn from the heart for the assessment of

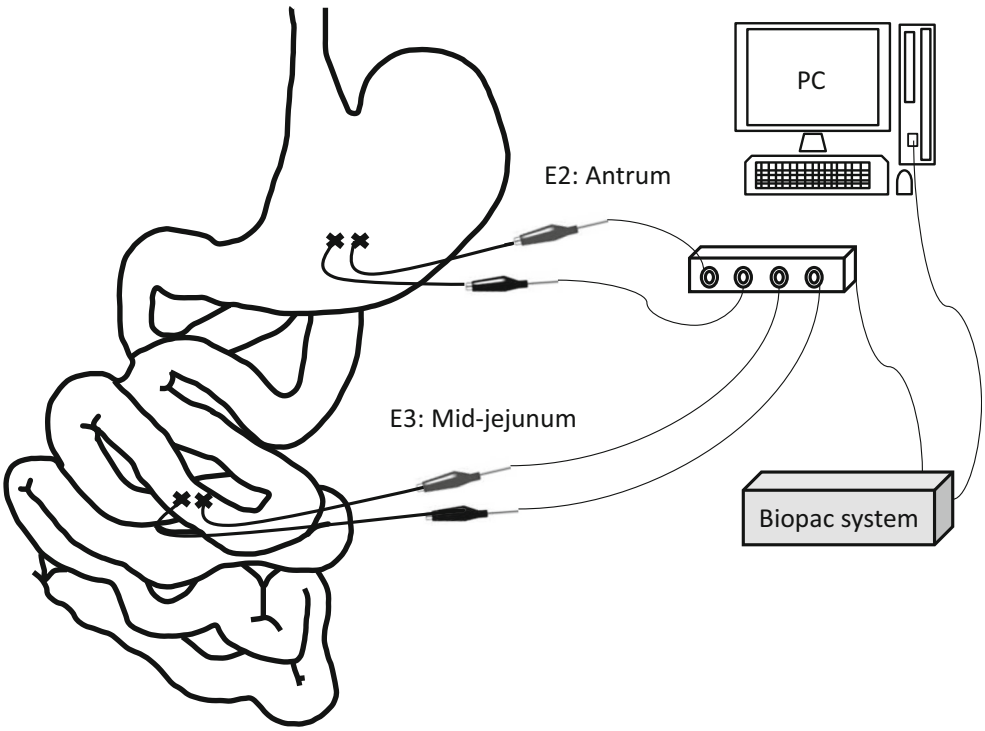

Fig. 3 Recording and analysis of gastric and small intestinal slow waves. One pair of electrodes was implanted on the gastric antral serosa for the measurement of gastric slow wave (GSW). For the measurement of the small intestinal slow wave (SSW), one pair of electrodes was sutured to the mid-jejunum along the small intestinal serosa. The GSW and SSW were recorded using a Biopac system. E2: the one pair of electrodes in the gastric antrum for measuring the GSW. E3: the one pair of electrodes in the mid-jejunum for measuring the SSW

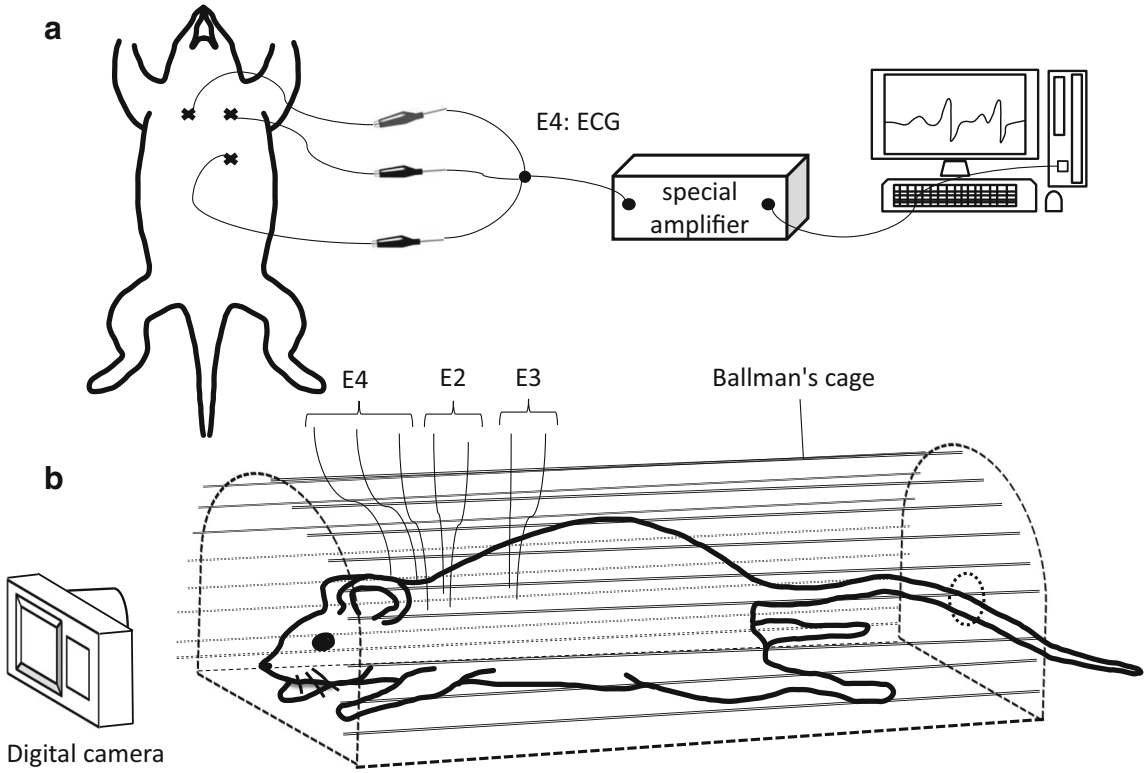

Fig. 4 Recording of ECG and analysis of the autonomic function and assessment of postoperative pain. The postoperative pain was evaluated, and the ECG was recorded for the heart rate variability. **a** The ECG signal was recorded using a special amplifier immediately after operation and 150 min after operation. The heart rate variability (HRV) signal was derived from the original ECG recording. E4: the one pair of electrodes underneath the skin in the chest for measuring ECG. **b** A digital camera was placed outside acrylic glass walls of Ballman's cage for clear head shots for the Rat Grimace Scale (RGS)

cytokines. The stomach and the small intestine were harvested; their contents were collected for the assessment of gastric emptying (GE) and small intestinal transit (SIT). Afterward, a segment (the most distal 5 mm of the ileum) of small intestine was fixed in 4% paraformaldehyde for histologic evaluation. Figure 5 shows the flowchart of the study.

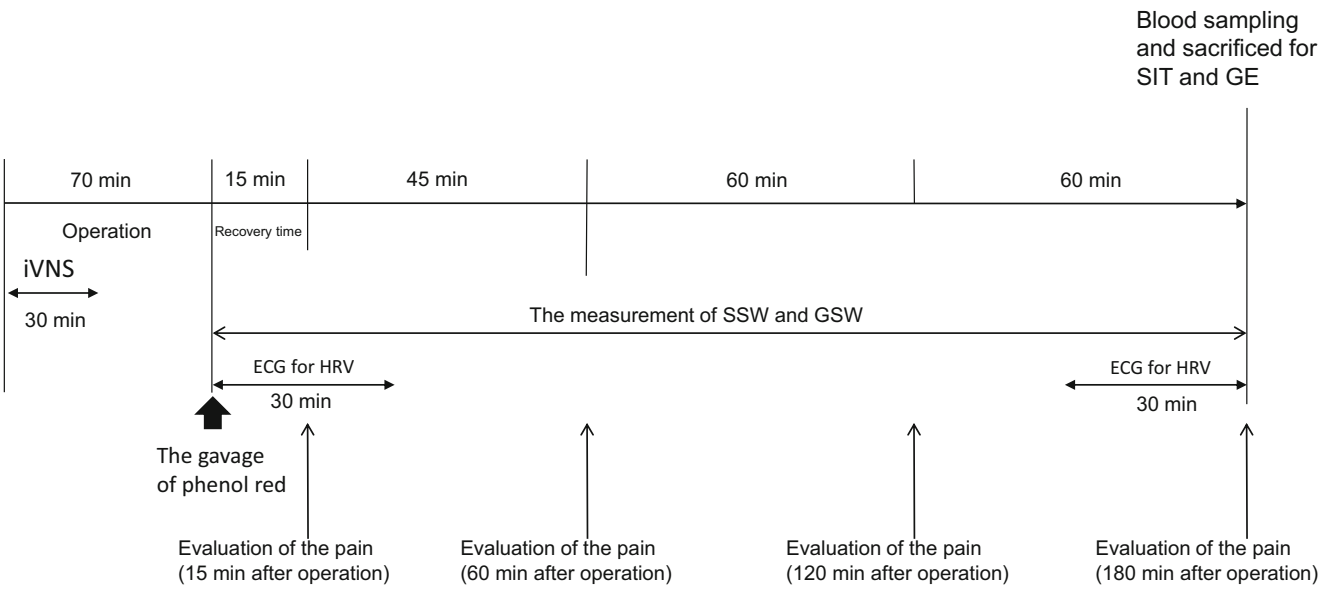

Fig. 5 The flowchart for whole experiment. iVNS intraoperative subdiaphragmatic vagus nerve stimulation, GE gastric emptying, SIT small intestinal transit, GSW gastric slow wave, ECG electrocardiogram, HRV heart rate variability

Measurements of Small Intestinal Transit and Gastric Emptying

Phenol red (50 mg; Sigma, St. Louis, MO) was diluted in 100 ml aqueous methylcellulose (1.5%; Fisher Scientific, Fair Lawn, NJ) solution and used as the test substance. All rats were gavaged 1.5 ml phenol red solution immediately after operation. One hundred eighty minutes later, the rats were sacrificed for the measurement of SIT and GE. The entire stomach was carefully isolated, ligated just above the cardia and below the pylorus, and removed for GE. For the SIT, the entire small intestine except for the end of ileum (5 mm) was carefully harvested and divided into ten equal segments (Fig. 4). Each segment was individually homogenized using homogenizer with 100 ml of 0.1 N NaOH. The mixture was kept at room temperature for 1 h. Supernatant (5 ml) was added to 0.5 ml of TCA solution (20% wt/vol) to precipitate the proteins. After centrifugation (3000 rpm, 15 min), the supernatant was added to 4 ml of NaOH (0.5 N) to develop the maximum intensity of color. The solutions were read using a spectrophotometer (fixed wavelength of 560 nm). GE was calculated according to the following formula: C ingested − C recovered/C ingested. C ingested means the amount of phenol red ingested into the stomach. C recovered means the amount of phenol red recovered from the removed stomach. SIT was assessed using a parameter called geometric center (GC), which was calculated as follows: GC = sum of n × Pn for $n = 1, 2,...10$. Where "n" was the number of the intestinal segment and "Pn" was the percentage of phenol red recovered from the corresponding segment.[10,11]

Recording and Analysis of Gastric and Small Intestinal Slow Waves

The GSW and SSW were recorded using a Biopac system (EOG 100A; Biopac Systems, Santa Barbara, CA). The following parameters were derived from the recording using the fast Fourier transformation (FFT) spectral analysis method: dominant frequency (DF) and dominant power (dP) of the slow wave frequency.

Recording of Electrocardiogram and Analysis of the Autonomic Function

The ECG signal was recorded using a special amplifier (model 2283 Fti Universal Fetrode Amplifier, UFI, Morro Bay, CA, USA) with a recording range of 1.5 to 100 Hz for two 30-min periods immediately after operation and 150 min after operation. The heart rate variability (HRV) signal was derived from the original ECG recording by identifying R waves, interpolating R-R interval data at 100 Hz, and finally down-sampling the interpolated HRV data at 8 Hz using a previously validated software.[12] Spectral powers of the HRV at two frequency ranges were calculated[13] as follows: (1) a high-frequency (HF) band (0.8–4.0 Hz) reflecting vagal activity and (2) a low-frequency (LF) band (0.3–0.8 Hz) reflecting sympathetic and vagal activity. The ratio of LF/HF reflected sympthovagal balance.

Assessment of Plasma Inflammatory Cytokines

A blood sample was drawn at 180 min after the operation. The plasma was obtained by centrifuging the blood sample at 3000×*g* for 15 min at 4 °C. TNF-α 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 part of small intestine: the end of ileum (5 mm) was fixed in 4% paraformaldehyde for a histologic evaluation (Fig. 6). The paraformaldehyde-fixed intestine was embedded in paraffin and cut into 2-μm sections. Hematoxylin and eosin staining of the intestine was performed. The injury to the intestinal mucosa was scored using the modified histopathologic score by Cuzzocrea and co-workers[14] by the randomized and unlabeled specimen. A scale of 0–3 was used to assess intestinal damages: 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.

Assessment of Postoperative Pain

A digital camera (PowerShot SX400IS, Canon, Japan) was placed outside acrylic glass walls of Ballman's cage for clear head shots. Digital movies were taken for 5 min at 15 min, 60 min, 120 min, and 180 min after operation. The Rat Grimace Scale (RGS) was assigned by the evaluator who was blinded to this study. The scorer assigned a value of 0, 1, or 2 for each of the five RGS action units: orbital tightening, nose bulge, cheek bulge, ear position, and whisker change. The final RGS score was the average score across the five action units.[15]

Statistical Analysis

Statistical analysis of the obtained data was executed using Student's *t* test, one-way and two-way ANOVAs, and Tukey-Kramer test as multiple comparisons. Data are expressed as means ± standard deviation. Statistical significance was set at $P \leq 0.05$.

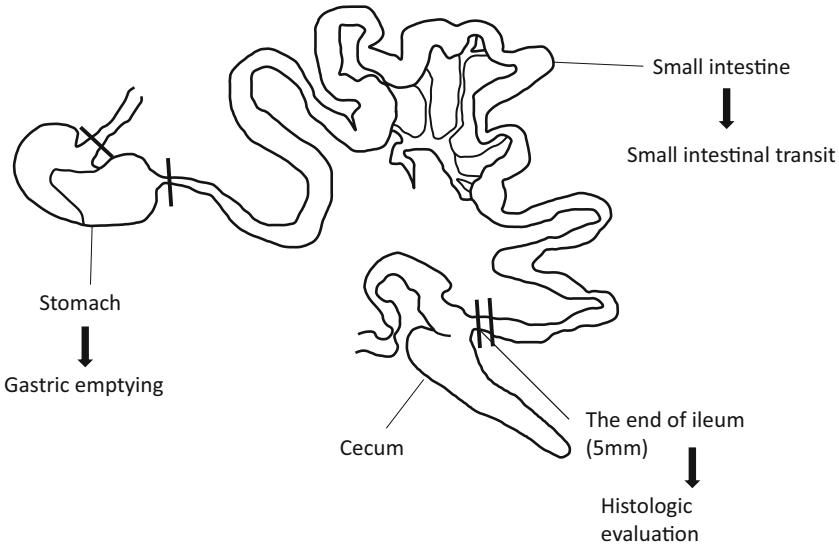

Fig. 6 Small intestinal transit, gastric emptying, and histologic evaluation of ileum. For the SIT, the entire small intestine except for the end of ileum (5 mm) for a histologic evaluation was carefully harvested and divided into ten equal segments. The entire stomach was carefully isolated, ligated just above the cardia and below the pylorus, and removed for GE. The end of ileum was fixed in 4% paraformaldehyde for a histologic evaluation

Results

Effects of Intraoperative Vagus Nerve Stimulation on Gastric Emptying and Small Intestinal Transit

IM significantly delayed GE and SIT; iVNS improved IM-induced delayed gastric emptying but not small intestinal transit. As shown in Fig. 7, IM dramatically and significantly delayed gastric emptying; iVNS normalized IM-induced delay in gastric emptying (Fig. 7a). However, iVNS was not able to significantly improve the delay in small intestinal transit induced by IM (Fig. 7b).

Effects of Intraoperative Vagus Nerve Stimulation on Gastric and Intestinal Slow Waves

Gastric slow waves showed a postoperative increase in frequency, and the increase was fastest in the control group and slowest in the VNS group (two-way ANOVA, $P < 0.01$, VNS vs. control; $P < 0.05$, VNS vs. sham). As shown in Fig. 7c, the DF of the GSWs was lower than that in the sham or control groups during 60–120 min and lower than the control group during 120–180 min. The DP of the GSWs was not affected by IM nor iVNS: no difference was noted in DP among the three groups at any time period.

Similar findings were noted with intestinal slow waves: iVNS reduced the DF of intestinal slow waves. As shown in Fig. 7d, the DF of the intestinal slow waves was lowest in the iVNS group during all three time periods. However, no difference was noted in the DP of intestinal slow waves among the three groups during any time period, suggesting that neither surgery nor iVNS alter the DP of intestinal slow waves.

Effects of Intraoperative Vagus Nerve Stimulation on Tissue Damages

IM induced tissue damages to the distal ileum, and iVNS normalized the IM-induced damages. As shown in Fig. 8, there were mild damages in the control group (Fig. 8a), severe damages in the sham group (Fig. 8b), and again mild damages in the iVNS group (Fig. 8c). The modified histopathologic score in the sham group showed one folder increase from the control group ($P = 0.02$); however, iVNS reduced this score to a value comparable to that in the control group ($P =$ NS vs. control; $P = 0.04$ vs. sham) (Fig. 8d).

Effects of Intraoperative Vagus Nerve Stimulation on Postoperative Pain

There was a progressive reduction in pain as assessed by the analysis of RGS during the three postprandial hours. iVNS substantially and significantly reduced pain caused by the surgery. As shown in Fig. 9a, a progressive reduction in the RGS was noted in all three groups during the postoperative hours. During the last hour (180 min), the RGS was significantly lower in the iVNS group compared with the other two groups (Fig. 9b). However, IM did not seem to induce extra pain: no difference in RGS was noted during any time period between the control (regular surgery) and the sham (regular surgery + IM).

Mechanisms Involving Inflammatory Cytokine

IM dramatically increased TNF-α, and iVNS normalized this IM-induced increase. As shown in Fig. 10a, the plasma level of TNF-α was increased by a few folder with IM ($P < 0.04$, sham vs. control). This increase was reduced with iVNS to a level comparable to the control ($P =$ NS vs. control; $P < 0.05$ vs. sham).

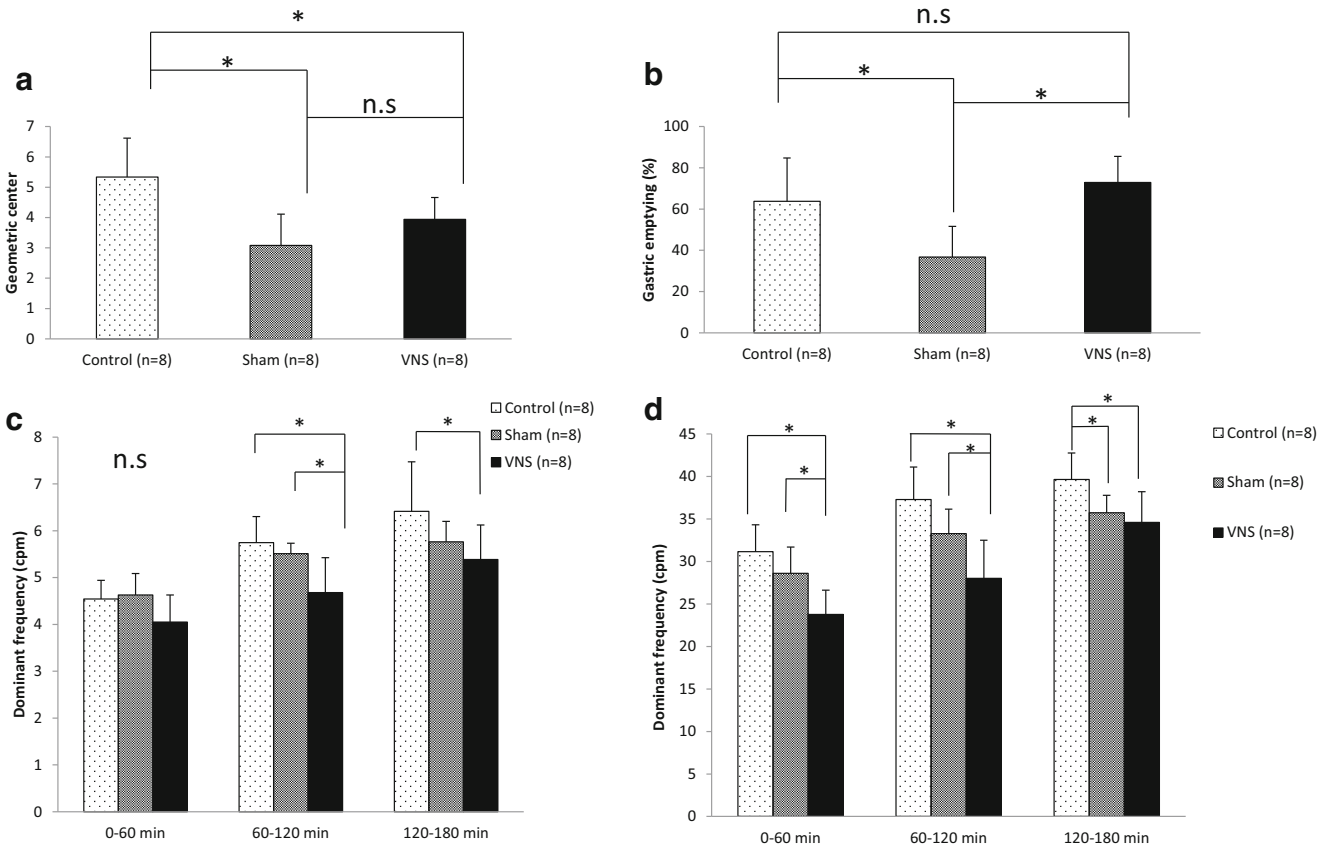

Fig. 7 Small intestinal transit, gastric emptying, gastric slow wave, and small intestinal slow wave. **a** Intestinal transit: intestinal manipulation significantly delayed small intestinal transit, and iVNS did not show significant difference between sham group and iVNS group. **b** Gastric emptying: intestinal manipulation delayed GE that was normalized by iVNS. **c** iVNS decreased gastric dominant frequency in postoperative time 60–120 min. **d** iVNS reduced the small intestinal dominant frequency in postoperative time 0–60 min. *$P \leqq 0.05$ (Tukey-Kramer test)

Mechanisms Involving Autonomic Functions

IM significantly and substantially increased the sympathovagal ratio and reduced the vagal activity; iVNS prevented these IM-induced alternations in the autonomic functions. As shown in Fig. 10b, c, in the sham group, there was a significant increase in LF/HF and a decrease in HF from the first 30 min to the last 30 min during the postoperative period (Fig. 10b, c). These postoperative changes were, however, not observed in the control group or the iVNS group, suggesting that the alterations were attributed to IM and iVNS exerted a preventive effect.

Discussion

In this study, we have demonstrated that intraoperative VNS exerted a prokinetic effect on gastric emptying, a preventive effect on tissue damages and an analgesic effect on postoperative pain. These effects seemed to be mediated via the autonomic function: iVNS prevented the IM-induced inhibition in vagal activity and increase in sympathovagal ratio. The iVNS induced suppression in TNF-α might also play a role.

Some researchers reported increased gastric emptying with vagal nerve stimulation.[4–6] However, there was no report on the effect of intraoperative VNS on gastric emptying in POI. In our study, we briefly stimulated the dorsal subdiaphragmatic vagus nerve during the surgery and normalized IM-induced delay in gastric emptying. In our study, iVNS decreased GSW frequency. Previously, Holle et al. reported that a prokinetic agent, erythromycin, increased gastric motility but decreased the frequency of GSW in a canine study.[16] It seemed the iVNS has similar prokinetic effects as erythromycin. The iVNS-induced improvement in gastric emptying might lead to improvement in postoperative symptoms, such as nausea and vomiting. There were no reports on the effect of VNS on intestinal motility. In our study, iVNS showed no effects on IM-induced delay in intestinal transit but reduced the frequency of intestinal slow waves. Further studies are needed to explore effects of VNS or iVNS on intestinal motility.

Jansen et al. reported that in children with refractory epilepsy, VNS induced a shift in sympathovagal balance towards sympathetic predominance and an improvement in autonomic

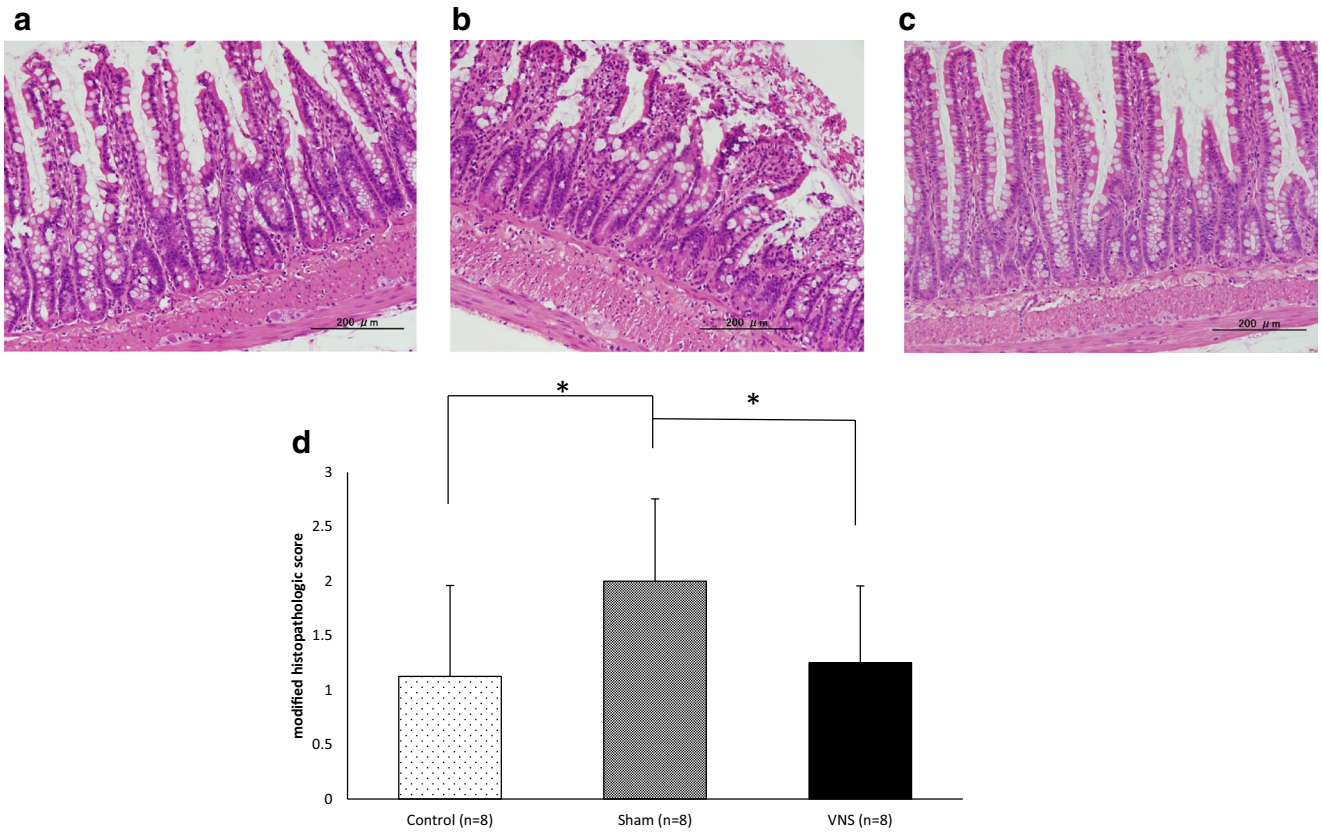

Fig. 8 Histologic analysis. **a** Control group. **b** Sham group. **c** iVNS group. **d** IM damaged the ileum mucosa. iVNS prevented the injury of ileum mucosa. *$P \leqq 0.05$ (paired t test). All images are taken at × 200 magnification with black bar = 5 μm

modulation by evaluation of HRV.[17] We also found that iVNS showed the amelioration of autonomic balance from analysis of HRV. In our study, IM increased sympathovagal balance. iVNS prevented the increase in sympathovagal balance. On the other hand, IM decreased the vagal activity. iVNS prevented the decrease in vagal activity. Therefore, iVNS suppressed the activity of the sympathetic nervous system, which was enhanced in the POI. iVNS was able to normalize the

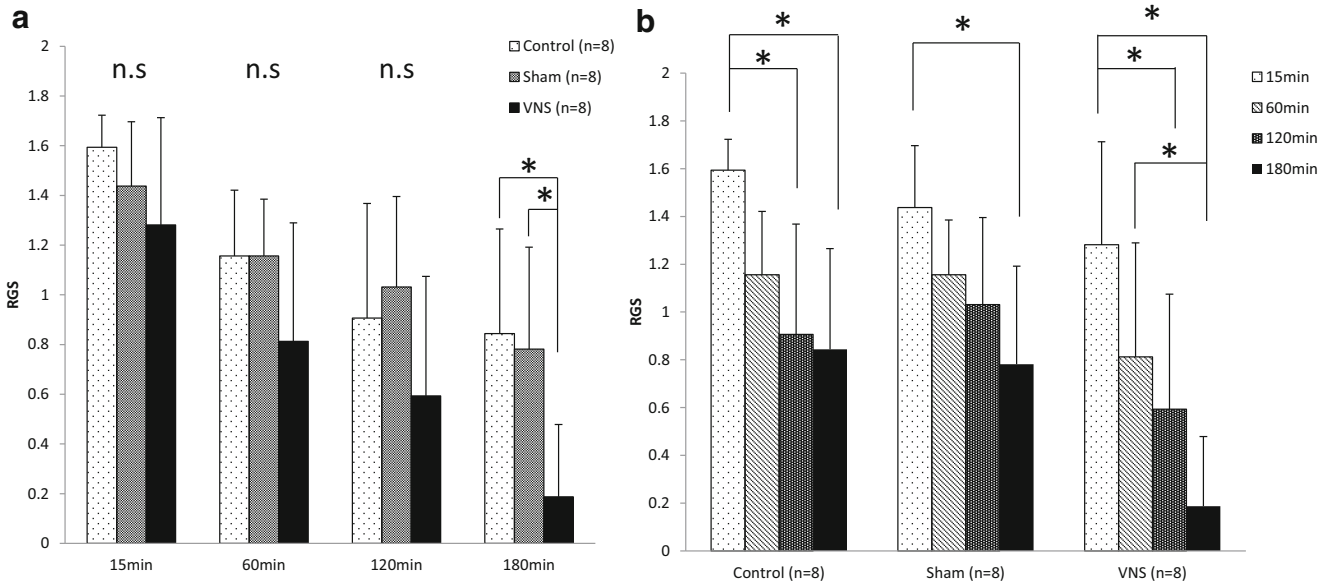

Fig. 9 The evaluation of postoperative pain by the Rat Grimace Scale (RGS). **a** After the operation 180 min, iVNS reduced postoperative pain. **b** iVNS accelerated postoperative pain relief. *$P \leqq 0.05$ (Tukey-Kramer test)

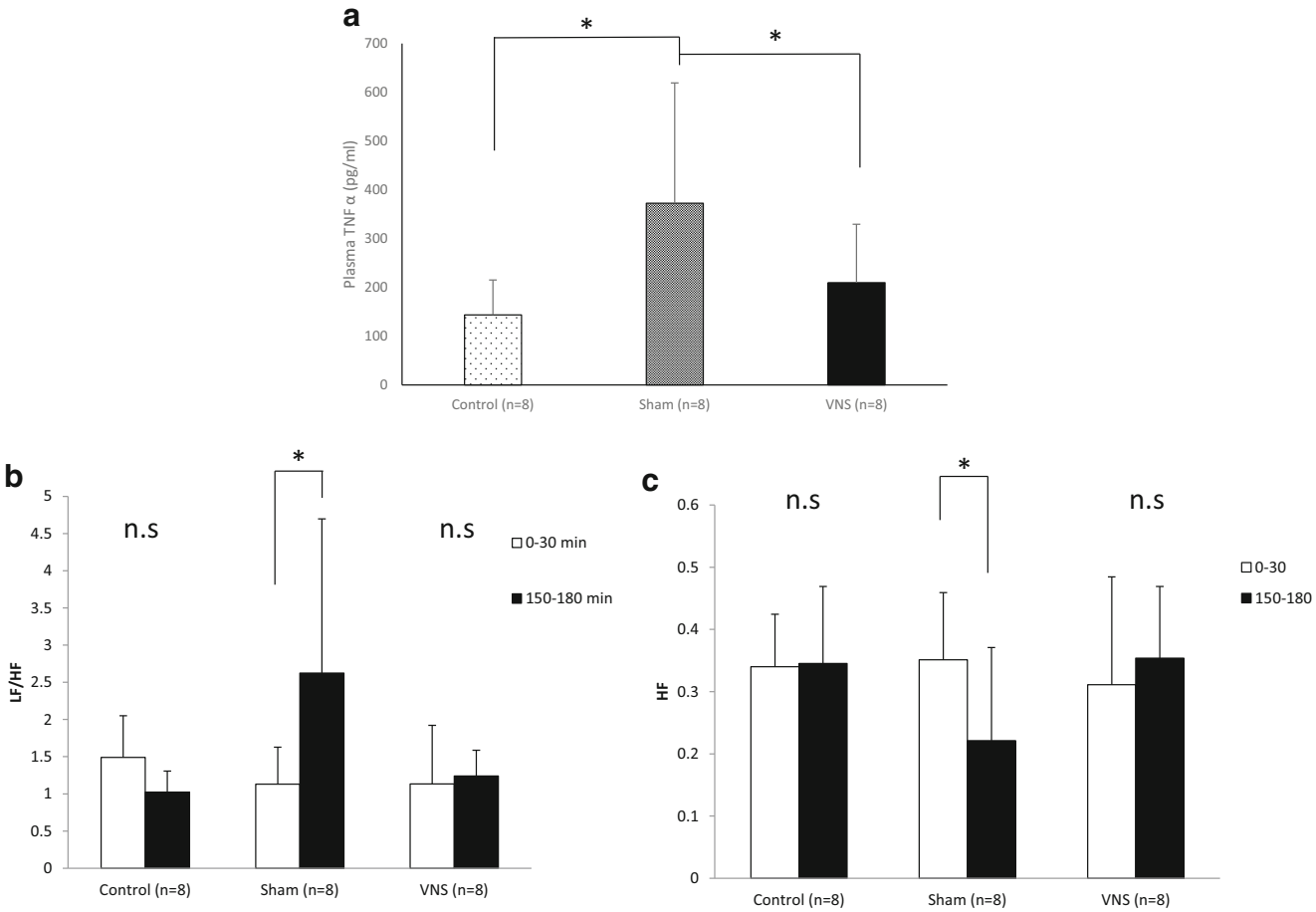

Fig. 10 Plasma TNF-α and heart rate variability. **a** Plasma TNF-α was increased by intestinal manipulation. iVNS decreased plasma TNF-α increased by intestinal manipulation. **b** Intestinal manipulation increased LF/HF: sympathovagal balance. iVNS prevented the increase of the sympathovagal balance. **c** Intestinal manipulation decreased HF: vagal activity. iVNS prevented the decrease of the vagal activity. *$P \leqq 0.05$ (paired t test)

activity of the parasympathetic nervous system, which is suppressed by the POI.

Low-intensity electrical vagal stimulation was reported to reduce visceral pain.[18] Zurowski et al. reported that analgesic effect of VNS was mediated in the peritonitis model of visceral pain .[19] In our study, iVNS significantly reduced postoperative pain. Therefore, iVNS has the possibility of postoperative pain relief. In our study, in all groups, RGS at postoperative time 180 min was significantly increased than RGS at postoperative time 15 min. Therefore, as time passed, it was found that the postoperative pain in all groups was improved. Furthermore, in the sham group, RGS at postoperative time 120 min was not significantly decreased than RGS at postoperative time 15 min. On the other hand, in control and VNS, RGS at postoperative time 120 min was significantly increased than RGS at postoperative time 15 min. Therefore, we thought that iVNS accelerated the pain relief. Recently, opioids are often used in reducing postoperative pain. However, opioids can cause nausea or vomiting by the stagnation of the stomach contents induced by disorders of the gastrointestinal tract peristalsis. iVNS in this study improved both gastric emptying and postoperative pain. Therefore, we thought from this finding that iVNS might be one of the new options for postoperative pain relief without impairing gastrointestinal motility. RGS in VNS was significantly lower than that in control. Therefore, we considered that iVNS reduced not only the visceral pain by POI but also the somatic pain by wound.

Some researchers reported an anti-inflammatory effect by the VNS.[20–24] There are no reports about anti-inflammatory effect of iVNS. It was considered that the comparison of plasma TNF-α in VNS and control was poor meaning from a clinical point of view. For that reason, at first, from the comparison between control and sham, we had confirmed the trend of plasma TNF-α by IM (POI). In our study, iVNS reduced plasma TNF-α in POI. We thought that if iVNS could control plasma TNF-α increased by surgical stress, there is a possibility that iVNS can prevent organ failure due to cytokine storm.

To the best of our knowledge, it was the first time that intraoperative VNS improved tissue damages induced by IM. Previously, Ay et al. reported that VNS reduced the extent of tissue injury in a model of middle cerebral artery

occlusion.[25] Lu et al. reported that VNS attenuated burn-induced histologic heart injury.[26] Some researchers reported that VNS improved the tissue damage induced by various disorders. In our study, the brief VNS during the surgery significantly improved tissue damages induced by intestinal manipulation. Improvement of tissue damages with iVNS is likely to contribute to the maintenance of postoperative functions of the small intestine.

We speculated autonomic-cytokine mechanisms involved in the ameliorating effects of iVNS on the postoperative recovery of gastric motility and pain. We believed that the invasion by the surgery and IM produced the increase of plasma inflammatory cytokine TNF-α. At the same time, these surgical and IM procedures also caused the imbalance of the autonomic nervous system: the enhancement of sympathetic nervous system and the suppression of the parasympathetic nervous system. Inflammatory cytokine promoted inflammatory cell infiltration. Inflammatory cell infiltration caused the tissue damage of the gastrointestinal tract. The tissue damage of the gastrointestinal tract caused gastrointestinal dysmotility. IM directly caused the damage to the tissue of gastrointestinal tract. The imbalance of the autonomic nervous system after surgery and IM caused gastrointestinal motility disorders and was also believed to contribute to the increase of pro-inflammatory cytokines. iVNS prevented the suppression of parasympathetic nervous system and the enhancement of sympathetic nervous system by invasion from surgery and IM. This amelioration of the autonomic nervous system by iVNS resulted in the improvement of gastric motility after surgery and IM. The prevention of the surgery and IM-induced suppression of parasympathetic nervous system by iVNS might reduce plasma TNF-α by anti-inflammatory effect of the parasympathetic nervous system. The iVNS-induced suppression of the sympathetic activity was believed to contribute to the relief of postoperative pain.

In conclusion, brief intraoperative VNS improved gastric emptying and postoperative pain in a rodent model of postoperative ileus, possibly mediated via the autonomic-inflammatory cytokine mechanisms. Further studies are warranted to explore the accelerative effects of intraoperative VNS on postoperative recovery.

Author Contributions Haruaki Murakami, Shiying Li, and Jieyun Yin, performed the research; Jiande DZ Chen, Robert Foreman, and Toshihiro Hirai designed the research study; Haruaki Murakami, Shiying Li, Robert Foreman, Jieyun Yin, and Jiande DZ Chen analyzed the data; Haruaki Murakami wrote the paper; and Haruaki Murakami, Shiying Li, Robert Foreman, Jieyun Yin, Toshihiro Hirai, and Jiande DZ Chen approved the final version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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