Physiological and Histopathological Effects of Electroporation Pulse on Stomach of Rats

Yuchi Zhang  
First Affiliated Hospital of Xi'an Jiaotong University

Xuan Han  
First Affiliated Hospital of Xi'an Jiaotong University

Zhuoqun Li  
First Affiliated Hospital of Xi'an Jiaotong University

Yu Zhang  
First Affiliated Hospital of Xi'an Jiaotong University

Lihong Liang  
First Affiliated Hospital of Xi'an Jiaotong University

Xiaoying Ma  
First Affiliated Hospital of Xi'an Jiaotong University

Haonan Liu  
First Affiliated Hospital of Xi'an Jiaotong University

Yihui Gao  
First Affiliated Hospital of Xi'an Jiaotong University

Qingshan Li  
First Affiliated Hospital of Xi'an Jiaotong University

Xue Chen  
First Affiliated Hospital of Xi'an Jiaotong University

Yi Lv  
First Affiliated Hospital of Xi'an Jiaotong University

Fenggang Ren  
Xí'ān Jiàotóng Dàxué  
renfenggang@xjtu.edu.cn

Research article

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Abstract

Background

Electroporation pulse (EP) is an emerging tissue ablation technique with widespread potential, including in the treatment of multiple cancer types. However, there is a lack of knowledge about its effect on the physiology and histopathology of stomachs. The aim of this study was to investigate biological effects of EP applied to stomachs of healthy rats on digestive function, serum marker levels, and gastric tissue structure.

Methods

Ninety male rats were divided into nine groups and examined up to 28 days post-treatment. A single burst of electroporation pulses (500 V, 99 pluses, 1 Hz, 100 μs) was delivered to the stomachs of the rats using a forceps electrode. Gastric emptying, small intestinal transit, and gastric secretion were measured to evaluate digestive function. Levels of serum markers were determined using ELISA. Haematoxylin–eosin, Masson trichrome, and immunofluorescence were performed for histopathological analysis.

Results

No significant effect on gastric emptying or secretion were found post-EP, whereas the small intestinal transit decreased at 4 h and rapidly recovered to normal on 1-day post-EP. Further, levels of serum markers such as TNF-α and IL-1β changed temporarily in the acute term but soon returned to normal within 28 days. Moreover, histopathological analysis revealed that that the cell death in ablation area occurred immediately post-EP, and the gastric wall scaffold in the ablation region remained intact post-EP.

Conclusions

This study demonstrates the safety and efficacy of EP on the physiology and histopathology of the stomach and lays a foundation for the wider use of this technique in future studies.

Background

A burst of microsecond pulsed electric field pulses with certain characteristics, which can achieve cell membrane irreversible electroporation (IRE), is called electroporation pulses (EP) [1]. Usually, the EP is a square wave with a pulse width on the order of tens to hundreds of microseconds, while the electric field intensity formed in the ablation area is approximately 700–1000 V/cm [2]. The cell transmembrane potential changes under the effect of EP, destroying the stability of the cell membrane and local environmental homoeostasis, ultimately leading to cell death. Therefore, EP has been recognised as an emerging tissue ablation technique and has been applied in the treatment of the pancreas, prostate, and liver cancer in clinical practise. Generally, this tissue ablation technique is also called the IRE [3, 4].

The duration of a single EP is ultra-short; hence, the heat generated during the IRE process can be quickly diffused or absorbed. Therefore, the cumulative temperature of the tissue is negligible when the IRE is achieved, while no thermal damage to the tissue is caused [5]. EP can inactivate malignant cells in situ rapidly while sparing tissue scaffolds such as the extracellular matrix, vascular wall, and nerve fibre as a result. Meanwhile, EP is not affected by the heat sink effect, so the blood vessel adjacent to the ablation area will not be a contraindication [6]. These
advantages of IRE prominently expanded the application of tissue ablation techniques, especially for heat-sensitive structures, which are usually out-of-bound areas for tissue ablation.

Typically, heat energy is not easy to control for thermal-basis tissue ablation, which may lead to serious side effects when applied to heat-sensitive structures [7]. On one hand, perforation or irreversible thermal damage may occur with a high intensity of heat energy. However, lower intensity may lead to residual tumour formation and decrease treatment efficacy [8]. Based on the non-thermal features of EP, several studies have found that EP is a potential ablation method for cavity organs such as the digestive tract. The safety and efficacy of IRE for the bile duct [9, 10], heart [11], and urinary tract [12] have been demonstrated in animal studies. In our previous study, the safety and efficacy of EP for gastric tissue ablation was investigated in an animal model [8]. However, there is a lack of basic research on the biological effects and outcomes of EP on the physiology and histopathology of the normal healthy stomach. Therefore, the aim of this study was to investigate the biological effects and outcomes of EP on changes in digestive function, serum marker levels, and gastric tissue structure in a rat model.

Methods

Animal care and ethics

Ninety male Sprague Dawley rats (180–280 g) were purchased and kept at the Experimental Animal Center of Xi’an Jiaotong University. The animals were maintained in standard day and night cycle (12 h light to 12 h dark) and environment temperature (25 ± 2 °C) with free access to food and water. All the animals were kept in a same condition. The study protocols were approved by the Institutional Animal Care and Use Committee of Xi’an Jiaotong University (No. XJTU2018-463).

Experimental protocol

The animals were randomly divided into eight groups according to their time of sacrifice: 0 h, 4 h, 1 d, 3 d, 7 d, 14 d, and 28 d post-EP and control groups. Random numbers were generated using a computer based random order generator. Among them, the 0 h group contained 6 rats for histopathological analysis only, while the other groups contained 12 rats for both physiological (n = 6) and histopathological analysis (n = 6).

All animals were fasted for 24 h before operation. Inhalation anaesthesia was maintained with isoflurane (RWD, Shenzhen, China). The oxygen flow rate was regulated to 0.6 L/min while the isoflurane flow rate was regulated to 2 L/min. The stomach was exposed with a 2–3 cm incision along the midline under the xiphoid. A forceps electrode was used to clamp the middle of the gland stomach near the cardia. After that, a single burst of EP (500 V, 99 pluses, 1 Hz, 100 µs) was delivered to the stomachs of rats in the experimental groups. As for the control groups, the same procedure was employed without delivering EP. After the operation, the activity status and feeding of animals were observed daily. The animals were sacrificed at each indicated time point by intraperitoneal injection of 3% sodium pentobarbital (800 mg/kg, Sigma Aldrich, St. Louis, MO, USA).

Digestive function measurement

Gastric emptying and small intestinal transit were measured by intragastric administration of the phenol red–methylcellulose (PR-HPMC) method as previously reported [13]. PR-HPMC food was prepared by dissolving 50 mg of phenolic red (Sangon Biotech, Shanghai, China) in 100 mL of 1.5% hydroxypropyl methyl cellulose water solution (Sangon Biotech). The stomach/small intestine specimens and gastric contents were harvested at 0.5 h
The supernatant of the gastric content was analysed with a microplate reader (Varioskan Flash, Thermo Fisher Scientific, Waltham, MA, USA) at 560 nm to determine the OD values. Then, the gastric emptying rate was calculated as percent emptying, which was calculated as follows: 1 – absorbance of the test sample/absorbance of the standard. The OD values of gastric contents from rats euthanized immediately after PR-HPMC gavage served as a standard. The furthest point of phenol red migration in the small intestine was verified, and the distance between this point and the initial intestinal segment was recorded as $D$. The overall length of small intestine specimens was determined as $L$. Thus, the small intestine transit was calculated as $D/L$.

Gastric secretion was evaluated using pyloric ligation. The stomach was ligated between the pylorus and duodenum, followed by fasting for 4 h. Then, the gastric specimens and contents were collected, and the volume of the gastric contents was measured as gastric juice (mL).

### Serum marker determination

Blood samples were collected before euthanasia. The serum levels of prostacyclin I2 (PG I$_2$), prostaglandin E2 (PG E$_2$) (Cusabio Biotechnology, Wuhan, China), nitric oxide (NO) (Jiancheng Bioengineering, Nanjing, China), ghrelin (USCN Kit, Wuhan, China), tumour necrosis factor alpha (TNF-α) (Thermo Fisher Scientific), interleukin 1β (IL-1β), interleukin-6 (IL-6), and interleukin-10 (IL-10) (Multi Sciences Biotech, Hangzhou, China) were determined using commercially available ELISA kits following the manufacturer’s instructions.

### Histopathological analysis

Stomach specimens were dissected along the greater curvature. After washing with phosphate buffer saline, specimens were fixed in paraformaldehyde, embedded in paraffin, and cut into 4 µm sections. Each sample was stained with haematoxylin–eosin (H&E) (Servicebio, Wuhan, China) and Masson trichrome (Servicebio, Wuhan, China) according to the manufacturer’s instructions. Moreover, terminal deoxynucleotidyl transferase-mediated nick end labelling (TUNEL) (Roche, Switzerland) and the expression of E-cadherin (1:200), β-Catenin (1:200), CD117 (1:2000), PGP9.5 (1:100), and proliferating cell nuclear antigen (PCNA) (1:100) (Servicebio) were detected by immunofluorescence following the manufacturer’s instructions. Histopathological analysis was performed by two experienced pathologists without aware of the group allocation.

### Statistical analysis

All statistical analyses were performed using GraphPad Prism 8.0 for Windows (GraphPad Software Inc., La Jolla, CA, USA). Quantitative variables were expressed as means ± SD and analysed by Student’s t-test or ANOVA. All statistical tests were bilateral, and the results were considered statistically significant at $P < 0.05$.

### Results

#### Digestive function

The changes in digestive function post-EP are summarised in Table 1 and Fig. 1. Compared with the control group, there was no significant difference among the groups in gastric emptying levels. The small intestine transit decreased from 67.71 ± 6.18% to 33.25 ± 10.49% ($P < 0.001$) at 4 h post-EP, while it rapidly recovered to 55.67 ± 13.58% at 1 d post-EP. As for gastric secretion, the volume was slightly decreased after EP, but without statistical difference.
Table 1
Changes in digestive function post-electroporation pulse

| Post-electroporation pulse time | Gastric emptying (%) | Small intestine transit (%) | Gastric acid secretion (mL) |
|-------------------------------|----------------------|-----------------------------|-----------------------------|
| Control                       | 79.82 ± 8.26         | 67.71 ± 6.18                | 5.29 ± 2.06                 |
| 4 h                           | 65.43 ± 13.59        | 33.25 ± 10.49               | 3.12 ± 1.70                 |
| 1 d                           | 60.62 ± 15.54        | 55.67 ± 13.58               | 2.72 ± 1.32                 |
| 3 d                           | 69.26 ± 9.99         | 59.12 ± 4.84                | 4.38 ± 1.34                 |
| 7 d                           | 66.75 ± 10.65        | 63.17 ± 10.93               | 3.00 ± 2.89                 |
| 14 d                          | 71.65 ± 6.32         | 54.85 ± 8.98                | 3.30 ± 0.90                 |
| 28 d                          | 59.13 ± 23.52        | 52.73 ± 14.53               | 2.96 ± 1.67                 |

Serum marker analysis

The changes observed in several serum markers after EP are summarised in Table 2 and Fig. 2. Serum TNF-α and IL-1β were higher in the post-EP group than in the control, but without statistically significant. IL-10 levels decreased from 22.30 ± 12.08 pg/mL to 8.47 ± 6.70 pg/mL (P < 0.05) at 4 h post-EP and further decreased to 4.71 ± 8.97 pg/mL (P < 0.01) on day 1 post-EP (P < 0.001), while it recovered to 21.57 ± 15.02 pg/mL at 3 d post-EP. The serum IL-6 level reached a peak (131.7 ± 104.2 pg/mL vs. 10.28 ± 29.23 pg/mL, P < 0.001) at 3 days post-EP, while there was no significant difference among other groups. The levels of serum PG I₂ and PG E₂ increased and reached a peak at 14 days (P < 0.001) The level of serum ghrelin decreased within 7 days post-EP and reached statistical significance (10136 ± 2484 pg/mL vs. 5268 ± 1781 pg/mL, P < 0.001) at day 7 and gradually recovered to normal within 14 days. NO was significantly increased to 1.543 ± 0.2082 µmol/L at 3 d post-EP, while it returned to normal within 14 days post-EP.
Table 2

| Post-electroporation pulse time | TNF-α (pg/mL) | IL-1β (pg/mL) | IL-10 (pg/mL) | IL-6 (pg/mL) | PG I2 (ng/mL) | PG E2 (pg/mL) | NO (µmol/L) | Ghrelin (pg/mL) |
|-------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|-------------|-----------------|
| Control                       | 0.35 ± 0.87   | 35.98 ± 48.16 | 22.30 ± 12.08 | 10.28 ± 29.23 | 0.51 ± 0.15   | 1.31 ± 0.46   | 1.01 ± 0.17   | 10136 ± 2484    |
| 4 h                           | 2.10 ± 2.50   | 94.17 ± 85.63 | 8.47 ± 6.70   | 3.25 ± 5.18   | 0.58 ± 0.21   | 1.65 ± 0.55   | 0.99 ± 0.15   | 11590 ± 2828    |
| 1 d                           | 1.92 ± 2.73   | 91.91 ± 77.72 | 4.71 ± 8.94   | 19.44 ± 37.99 | 0.62 ± 0.10   | 1.45 ± 0.38   | 0.98 ± 0.21   | 9184 ± 3001     |
| 3 d                           | 1.11 ± 2.06   | 63.81 ± 83.63 | 21.57 ± 15.02 | 131.70 ± 104.20 | 0.76 ± 0.50 | 1.49 ± 0.67   | 1.54 ± 0.21   | 7534 ± 965      |
| 7 d                           | 3.10 ± 4.05   | 76.68 ± 87.24 | 23.68 ± 12.01 | 11.23 ± 29.34 | 0.85 ± 0.50   | 1.72 ± 0.94   | 1.34 ± 0.26   | 5268 ± 1781     |
| 14 d                          | 3.02 ± 3.70   | 112.00 ± 114.60 | 13.88 ± 10.71 | 4.50 ± 9.64   | 1.44 ± 1.28   | 2.97 ± 2.04   | 1.00 ± 0.55   | 7589 ± 3155     |
| 28 d                          | 3.38 ± 3.31   | 38.39 ± 57.63 | 12.55 ± 10.98 | 8.04 ± 16.64  | 0.37 ± 0.11   | 1.01 ± 0.47   | 0.95 ± 0.28   | 7989 ± 3185     |

**Gross pathology**

Images of the gross pathology are shown in Fig. 3a–c. Clearly demarcated lesions with congestion were observed on the treated mucosa immediately post-EP. The shape and size of the lesions were broadly consistent with the electrode. On day 1 post-EP, the mucosa had sloughed off and the lesions turned into ulceration. The lesions started to recover from day 3 to day 7 post-EP. At day 28 post-EP, the mucosal face of the lesions was recovered and showed insignificant difference compared to the surrounding normal tissue. On the serosal face of the stomach, the ablation region turned darker after EP without bleeding or perforation. The serosal lesions became smaller in size and lighter in colour and completely repaired within 28 days post-EP.

**Histopathology**

H&E staining is shown in Fig. 3c. The gastric wall showed a well-circumscribed ablation area immediately post-EP. Multiple erythrocytes, a decrease of nucleated cells, and epithelial cell necrosis can be seen in the ablation area. At 4 h post-EP, the mucosa layer showed oedema with hyperaemia and massive inflammatory cell infiltration. Even so, the muscular propria remained intact with local hyperaemia or haemorrhage. At 1 d post-EP, complete mucosal necrosis was observed, accompanied by diffusive inflammatory cells and red blood cells, indicating complete IRE on the full thickness of the gastric wall. The necrotic mucosa then fell off, resulting in ulceration on the gastric wall at 3 d post-EP. Meanwhile, the migration of the immature epithelium from the lateral normal tissue to the centre lesion was observed from 3 d post-EP, leading to a decrease in the lesion area until complete recovery took place within 14–28 days post-EP.
Masson's trichrome staining indicated that the gastric wall scaffold in the lesion remained intact after EP (Fig. 3d). In the 3-d sample, Masson trichrome staining showed that the muscularis mucosa, submucosa, and muscularis propria were replaced by collagen, suggesting that the muscular layer repair is mainly caused by fibrosis. Meanwhile, no significant change was observed in the thickness of the gastric wall within 14–28 days post-EP.

For TUNEL, a massive number of positive cells were observed in the mucosa and muscularis propria immediately post-EP (Fig. 4a–c). Viable epithelium decreased at 4 h post-EP, and the transition of TUNEL-positive cells from the mucous epithelium to the muscularis mucosa increased. Complete ablation of the mucosa and muscularis propria were observed within 12–24 h. The expression of E-cadherin and β-catenin in the ablated area was lower than that in normal tissue immediately and 4 h post-EP and rarely occurred in the 24 h samples, indicating that the epithelium intercellular junction was severely damaged by EP (Fig. 4d–e).

At the edge of the ablation region, PCNA staining showed an increase in the number of positive cells in the gastric fundus gland, suggesting that mucosal cells began the proliferation and repair process on day 3 post-EP (Fig. 5a–d). By day 7, PCNA showed increased expression in the fundus gland, suggesting active stem cell proliferation. Along with migration of the immature epithelium, mucosal regeneration and repair were completed by day 14 post-EP. Moreover, EP successfully induced a reduction of CD117 and PGP9.5 positive cells by day 1 in the muscularis propria, indicating the injury of Cajal cells and neurones by EP. At 14 d post-EP, no CD117- and PGP9.5-positive cells were observed in the regenerated muscularis propria (Fig. 5e–h).

**Discussion**

In this study, EP was performed on the stomachs of healthy rats to evaluate the effect of EP on digestive function, serum marker level, and gastric structure over 28 days. The safety and efficacy of EP for both physiology and histopathology have been confirmed in a rat model. The digestive function and serum markers changed temporarily in the acute term but soon returned to normal within 28 days. The gastric wall remained intact without bleeding or perforation after EP.

The stomach is a vital organ for digestion and secretion. Gastric emptying, small intestinal transit, and gastric secretion are evaluation indices of digestive function [14, 15]. After EP, no significant change was observed for gastric emptying or secretion. The small intestine transit decreased immediately at 4 h post-EP, whereas it recovered to normal at 1d later. This study confirmed that EP has limited impact on digestive functions in a rat model; thus, EP is safe for digestive function when used as an ablation method.

Serum markers were observed to be dynamic in the rat model. Post-EP, inflammation factors may be regulated by multiple factors. By and large, anti-inflammatory factors tended to be reduced in the acute phase and recover later, while proinflammatory factors show the opposite effect. Angiogenesis is an important component of gastric erosion and ulcer healing. Prostaglandins, iNOS, and ghrelin are important for gastric mucosal protection and play a role in angiogenesis [16]. Prostaglandin can protect the gastric mucosa by inhibiting acid secretion, promoting mucus generation, and increasing mucosal blood flow [17]. The prostaglandin level in the treatment group tended to rise and reached a peak at 14 days post-EP. In addition, the concentration of serum NO, which can improve microcirculation and reconstruction of mucosa, reached a peak at day 3, then returned to normal on day 14, which is consistent with the histopathology of mucosal regeneration. Ghrelin increased from day 7 to day 14 post-EP, during which the mucosa regenerated rapidly.
The thermal-basis ablation technique could generate coagulation necrosis instantly after application as well as a peripheral transition zone around the ablation region due to the temperature gradient [18]. All these changes can be determined by gross observation. However, a non-thermal ablated lesion was caused without evident coagulation necrosis on gross inspection [19, 20]. The cell viability, gross pathology, and histopathology show dynamic change post-EP, which varies in different tissues [21]. In general, the tissue damage process caused by EP ends within 24 h, then the repair procedure begins on days 3 to 7 post-EP and is completed within 28 days.

Ensuring the integrity of the gastric wall is important for evaluating the safety of EP. Phillips et al. studied the influence of IRE on the small intestine structure of rats and demonstrated that the small intestine can be ablated completely by IRE without obvious gastrointestinal side effects [22]. The epithelium starts repairing 3 days after surgery. In this study, EP was applied to the stomach, and the change in gastric structure within 24 h was evaluated. The demarcated lesions with congestion on both gastric mucosa and serosa were caused by EP, which may be related to the vascular lock-in effect of EP [6]. Histopathology showed immediate death of cells contacting the electrode after EP, and complete ablation of the mucous layer at 24 h. The 0 h and 4 h samples also revealed massive numbers of positive cells in the TUNEL assay, indicating that cell apoptosis started as early as 0 h and reached a peak at 24 h post-EP in the ablated area. The E-cadherin and β-catenin complex are vital for the tight junction between epithelial cells, which is crucial for the formation of the gastric mucosal barrier that protects the mucosa from gastric acid [23, 24]. This study revealed that the expression of E-cadherin and β-catenin decreased in mucosal epithelial cells immediately post-EP, suggesting a tight junction break and destruction of the mucosal barrier, which promotes corrosion and necrosis of the gastric mucosa.

There were several limitations to this study. First, normal gastric tissue differs from tumour tissue; hence, the efficacy of EP for tumour ablation has not been sufficiently elucidated. Second, since digestive function is closely related to nerve distribution, more research is needed in more treatment locations. Third, given the disparity between humans and rats, the practical treatment process cannot be fully evaluated in this study and requires further investigation in large animal models and/or humans.

Conclusions

This study demonstrated the safety and efficacy of EP on the physiology and histopathology of rat stomachs. Digestive function was slightly changed but soon returned to normal. The gastric wall remains intact, and the mucosa can be ablated using EP without perforation or bleeding. This study confirmed that EP is an attractive candidate for gastric tissue ablation and has laid the foundation for wider use of this technique in the future.

Abbreviations

IRE: Irreversible electroporation; EP: Electroporation pulse; TNF-α: tumour necrosis factor alpha; IL-1β: interleukin 1β; IL-10: interleukin-10; IL-6: interleukin-6; PG I₂: prostacyclin I₂; PG E₂: prostaglandin E₂; NO: nitric oxide.

Declarations

**Ethics approval and consent to participate**

The study protocols were approved by the Institutional Animal Care and Use Committee of Xi’an Jiaotong University (No. XJTU2018-463). All procedures performed in studies involving animals were in accordance with
the ethical standards of the institution or practice at which the studies were conducted.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

The study was designed by Fenggang Ren, Yi Lv; material preparation and animal experiment were performed by Yuchi Zhang, Xuan Han, Zhuoqun Li, Yu Zhang, Lihong Liang, Xiaoying Ma, Haonan Liu and Yihui Gao; data collection, analysis and interpretation were performed by Yuchi Zhang, Xuan Han, Qingshan Li and Xue Chen; the manuscript was drafted by Yuchi Zhang, Xuan Han and Zhuoqun Li; Fenggang Ren and Yi Lv revised the manuscript; all authors approved the final manuscript.

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References

1. Davalos R V, Mir LM, Rubinsky B. Tissue ablation with irreversible electroporation. Ann Biomed Eng. 2005;33:223–31.
2. Kotnik T, Frey W, Sack M, Haberl Meglič S, Peterka M, Miklavčič D. Electroporation-based applications in biotechnology. Trends Biotechnol. 2015;33:480–8. doi:10.1016/j.tibtech.2015.06.002.
3. Weaver JC, Smith KC, Esser AT, Son RS, Gowrishankar TR. A brief overview of electroporation pulse strength-duration space: A region where additional intracellular effects are expected. Bioelectrochemistry. 2012;87:236–43. doi:10.1016/j.bioelechem.2012.02.007.
4. Rubinsky B, Onik G, Mikus P. Irreversible electroporation: A new ablation modality - Clinical implications. Technol Cancer Res Treat. 2007;6:37–48.
5. Ren F, Li Q, Gao X, Zhu K, Zhang J, Chen X, et al. Electrical and thermal analyses of catheter-based irreversible electroporation of digestive tract. Int J Hyperth. 2019;36:854–67. doi:10.1080/02656736.2019.1646928.
6. Geboers B, Scheffer HJ, Graybill PM, Ruarius AH, Nieuwenhuizen S, Puijk RS, et al. High-Voltage Electrical Pulses in Oncology: Irreversible Electroporation, Electrochemotherapy, Gene Electrotransfer, Electrofusion, and Electroimmunotherapy. Radiology. 2020;192190. doi:10.1148/radiol.2020192190.

7. Chu KF, Dupuy DE. Thermal ablation of tumours: Biological mechanisms and advances in therapy. Nat Rev Cancer. 2014;14:199–208. doi:10.1038/nrc3672.

8. Ren F, Li Q, Hu L, Yan X, Gao Z, Zhang J, et al. Safety and efficacy of magnetic anchoring electrode-assisted irreversible electroporation for gastric tissue ablation. Surg Endosc. 2020;34:580–9. doi:10.1007/s00464-019-06800-3.

9. Li Q, Ren F, Zhang Y, Chang P, Wang Y, Ma T, et al. Acute and subacute effects of irreversible electroporation on normal common bile ducts in a rabbit model. J Hepatobiliary Pancreat Sci. 2020;jhbp.807. doi:10.1002/jhbp.807.

10. Ueshima E, Schattner M, Mendelsohn R, Gerdes H, Monette S, Takaki H, et al. Transmural ablation of the normal porcine common bile duct with catheter-directed irreversible electroporation is feasible and does not affect duct patency. Gastrointest Endosc. 2018;87:300.e1-300.e6. doi:10.1016/j.gie.2017.05.004.

11. Wittkampf FHM, van Es R, Neven K. Electroporation and its Relevance for Cardiac Catheter Ablation. JACC Clin Electrophysiol. 2018;4:977–86.

12. Srimathveeravalli G, Cornelis F, Wimmer T, Monette S, Kimm SY, Maybody M, et al. Normal Porcine Ureter Retains Lumen Wall Integrity but Not Patency Following Catheter-Directed Irreversible Electroporation: Imaging and Histologic Assessment over 28 Days. J Vasc Interv Radiol. 2017;28:913-919.e1. doi:10.1016/j.jvir.2017.02.032.

13. Rtibi K, Selmi S, Saidani K, Grami D, Amri M, Sebai H, et al. Reverse Effect of Opuntia ficus-indica L. Juice and Seeds Aqueous Extract on Gastric Emptying and Small-Bowel Motility in Rat. J Food Sci. 2018;83:205–11.

14. Kong Y, Sun N-N, Dong A-Q, Yang G-T, Zhao H-Y. Study of the effects of nesfatin-1 on gastric function in obese rats. World J Gastroenterol. 2017;23:2940.

15. Shifrin Y, Fajardo AF, Belik J, Sobchak C, Pan J. Gastric and pyloric sphincter muscle function and the developmental-dependent regulation of gastric content emptying in the rat. Am J Physiol Liver Physiol. 2016;310:G1169–75.

16. Tarnawski AS, Ahluwalia A, Jones MK. Angiogenesis in gastric mucosa: An important component of gastric erosion and ulcer healing and its impairment in aging. J Gastroenterol Hepatol. 2014;29:112–23.

17. Sgambato D, Capuano A, Giuseppa Sullo M, Miranda A, Federico A, Romano M. Gut-Brain Axis in Gastric Mucosal Damage and Protection. Curr Pharmaceutic. 2016;14:959–66.

18. Cornelis FH, Durack JC, Kimm SY, Wimmer T, Coleman JA, Solomon SB, et al. A Comparative Study of Ablation Boundary Sharpness After Percutaneous Radiofrequency, Cryo-, Microwave, and Irreversible Electroporation Ablation in Normal Swine Liver and Kidneys. Cardiovasc Intervent Radiol. 2017;40:1600–8. doi:10.1007/s00270-017-1692-3.

19. Lyu C, Lopez-Ichikawa M, Rubinsky B, Chang TT. Normal and fibrotic liver parenchyma respond differently to irreversible electroporation. Hpb. 2019;1:10. doi:10.1016/j.hpb.2019.01.019.

20. Lee JM, Choi HS, Kim ES, Keum B, Seo YS, Jeen YT, et al. Characterization of irreversible electroporation on the stomach: A feasibility study in rats. Sci Rep. 2019;9:9094. doi:10.1038/s41598-019-45659-1.

21. Mercadal B, Beitel-White N, Aycock KN, Castellvi Q, Davalos R V., Ivorra A. Dynamics of Cell Death After Conventional IRE and H-FIRE Treatments. Ann Biomed Eng. 2020. doi:10.1007/s10439-020-02462-8.
22. Phillips MA, Narayan R, Padath T, Rubinsky B. Irreversible electroporation on the small intestine. Br J Cancer. 2012;106:490–5. doi:10.1038/bjc.2011.582.

23. Čemažar M. Effects of Electroporation of Mammalian Cells on Cytoskeleton and Intercellular Connections. In: Handbook of Electroporation. Cham: Springer International Publishing; 2017. p. 307–21. doi:10.1007/978-3-319-32886-7_18.

24. Van Roy F, Berx G. The cell-cell adhesion molecule E-cadherin. Cell Mol Life Sci. 2008;65:3756–88.

Figures

**Figure 1**

Changes in the digestive function of rats following electroporation pulse treatment. (a) Gastric emptying; (b) small intestine propulsion rate; (c) gastric acid secretion. Compared with control group; ***, P < 0.001; n = 6.

**Figure 2**

Changes in serum marker factor levels within 28 days following electroporation pulse application. (a) TNF-α; (b) IL-1β; (c) IL-10; (d) IL-6; (e) prostacyclin I2; (f) prostaglandin E2; (g) ghrelin; (h) NO. Compared with control group; *, P < 0.05; **, P < 0.01; ***, P < 0.001; n = 12.
Figure 3

Gross appearance and histopathology of the rat gastric wall post-treatment with electroporation pulses. (a) mucosal surface; (b) serosal surface; (c) H&E staining (4×); (d) Masson trichrome staining (4×).
Figure 4

Histopathology analysis of TUNEL (40×), E-cadherin, and β-catenin (20×) in gastric mucosa post-electroporation pulse (EP), as determined by immunofluorescent. (a) TUNEL immediately post-EP; (b) TUNEL at 4 h post-EP; (c) TUNEL at 24 h post-EP; (d) E-cadherin and β-catenin levels in control group; (e) E-cadherin and β-catenin immediately post-EP; (f) E-cadherin and β-catenin at 4 h post-EP.

Figure 5
Histopathology analysis of PCNA in gastric mucosa and PGP9.5 and CD117 in gastric serosa following application of electroporation pulses, as determined by immunofluorescent (40×). (a) PCNA at 1 d. (b) PCNA at 3 d. (c) PCNA at 7 d. (d) PCNA at 14 d. E. PGP9.5 and CD117 levels in the control group. (f) PGP9.5 and CD117 at 4 h. (g) PGP9.5 and CD117 at 1 d. (h) PGP9.5 and CD117 at 14 d.

**Supplementary Files**

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