Evaluation of the safety of irreversible electroporation on the stomach wall using a pig model

JIANNAN LI[1], JIANYING ZENG[2], JIBING CHEN[2], JIAN SHI[1], XIAOMEI LUO[2], GANG FANG[2], WEI CHAI[3], WENLONG ZHANG[4], TONGJUN LIU[1] and LIZHI NIU[2]

[1]Department of General Surgery, The Second Hospital of Jilin University, Changchun, Jilin 130041; [2]Department of Central Laboratory, Fuda Cancer Hospital, Jinan University School of Medicine, Guangzhou, Guangdong 510665; [3]Department of Gynecology and Obstetrics, The First Hospital of Jilin University, Changchun, Jilin 130021; [4]Department of Hematology and Oncology, China-Japan Union Hospital of Jilin University, Changchun, Jilin 130031, P.R. China

Received May 29, 2016; Accepted March 24, 2017

DOI: 10.3892/etm.2017.4559

Abstract. The aim of the present study was to evaluate the effects of irreversible electroporation (IRE) on the stomach wall following the direct application of IRE onto the organ surface. IRE ablation was performed in 8 Tibetan mini-pigs, which were randomly assigned into two groups based on their ablated areas: Group A, gastric cardia, fundus of stomach, gastric body and group B, lesser gastric curvature, greater gastric curvature, stomach pylorus. Two IRE needles were placed in the space between the stomach wall and the liver (not inserted into the stomach tissue), and three lesions were created in each pig. Serum aminotransferase and white blood cell (WBC) levels were measured. Gastroscopy and endoscopic ultrasonography were performed. From each group, 2 pigs were sacrificed on day 7 post-IRE; the remaining pigs were sacrificed on day 28 post-IRE. There were no signs of perforation on the stomach wall. Serum aminotransferase and WBC levels increased in both groups on day 1 post-IRE and decreased gradually thereafter. The gastroscopy procedure revealed oval ulcers on day 7 post-IRE and smaller ulcers on day 28 post-IRE. Transmural necrosis, inflammation and fibrosis were observed at 7 days post-IRE. Healing ulcers were observed at 28 days post-IRE. In conclusion, IRE ablation caused damage to the stomach wall; however, IRE did not induce any perforation.

Correspondence to: Professor Tongjun Liu, Department of General Surgery, The Second Hospital of Jilin University, 218 Ziqiang Street, Changchun, Jilin 130041, P.R. China
E-mail: tongjunliu@163.com

Professor Lizhi Niu, Department of Central Laboratory, Fuda Cancer Hospital, Jinan University School of Medicine, 2 Tangdexi Street, Guangzhou, Guangdong 510665, P.R. China
E-mail: fudaclab@gmail.com

Key words: irreversible electroporation, stomach, ablation

Introduction

Complete surgical resection is the most effective treatment for tumors; however, 60-70% patients are not candidates for this treatment (1), due to their tumor size, number and location, organ function and the presence of comorbidities. Due to the advantages of short treatment time, little injury and short recovery time, ablation technologies, such as radiation therapy and croablation, have increasingly attracted attention in the non-surgical treatment of tumors, including liver, spleen and prostate tumors (2). Although these traditional ablation methods can decrease the tumor burdens for patients, their side effects, such as causing damage to adjacent tissues (including vessels and nerves), limit their use and are a risk to health (2). Therefore, it is important to assess alternative treatment methods.

Irreversible electroporation (IRE) is an emerging ablation technology, which consists of the application of a high voltage field that generates nanopores in the membrane of target cells (3,4). As a result, cellular homeostasis is disrupted, leading to cell necrosis and apoptosis (5,6). Compared with other thermal ablation technologies, IRE does not rely on thermal energy, maintains connective tissue integrity and has little effects on vessels, nerves, bile and pancreatic ducts (1,7,8).

In 2012, the US Food Drug Administration agency approved the clinical application of IRE. Even though it has been successfully applied in the treatment of hepatic (9), pancreatic (10) and renal (11) carcinomas, IRE may cause injury to the stomach wall of certain patients, due to the close proximity of the organ to the liver and pancreas. The present study evaluated the effects of IRE on the stomach wall using a pig model.

Materials and methods

Animals. The present study was approved by the Animal Experimental Center of Southern Medical University (Guangzhou, China). All animals were under necessary and appropriate human care from professional staff. A total of 8 female Tibetan mini-pigs (weight, 25-30 kg; age, 4-6 months) were purchased from Southern Medical University and studied

under the supervision of the Division of Laboratory Animal Medicine at Southern Medical University. The housing conditions were: Temperature, 20-25˚C; relative humidity, 40-60%; light/dark cycle, 12 h. All animals had free access to food and water. As generating six lesions on the stomach wall may lead to overlapping of the lesions and cause extended damage to the stomach, the animals were randomly allocated into two groups, A and B, with four animals in each group. In group A, three lesions in three separate areas (Fig. 1A-C) of the stomach wall of the animals were generated, including the gastric cardia, fundus and body. In group B, three lesions were created in areas (Fig. 1D-F) of the lesser and greater gastric curvature and in the stomach pylorus of the pigs.

Anesthesia. All pigs were administered 20 mg/kg ketamine (Gutian Pharmaceutical Co., Ltd., Fujian, China) and 10 mg/kg promethazine (Suicheng Pharmaceutical Co., Ltd., Xinzheng, China) by intramuscular injection. General anesthesia was maintained with 2% isoflurane (Yapei Pharmaceutical Co., Ltd., Shanghai, China) per continuous inhalation and 0.1 mg/kg atropine (Jinyao Pharmaceutical Co., Ltd., Tianjin, China) via intramuscular injection. Respiratory support was administered via mechanical ventilation. Cisatracurium besylate (Hengrui Medicine Co., Ltd., Jiangsu, China) was administered intravenously at 60 μg/kg at the start of IRE and maintained by intravenous perfusion at 10 μg/kg/min during the procedure to reduce muscle contraction.

IRE procedure. Pigs were fasted for 24 h prior to IRE. Belladonna and aluminium capsules II (Jiangsu Hengrui Medicine Co., Ltd., Jiangsu, China) were administered to reduce gastric motility. Pigs were placed on a surgical table in a supine position, and the legs were fastened to ensure that the procedure was performed smoothly. The skin overlying the center of the abdomen was shaved, cleaned and sterilized prior to a 15-cm long midline laparotomy being performed. To simulate tumor ablation close to the stomach wall, electrode needles were placed in the space between the stomach wall and the liver (Fig. 2). Needles were not inserted into the stomach tissue, but sutures were used to fix the needles to the serosal surface of the stomach wall. The 16-g monopolar IRE probes (AngioDynamics, Queensbury, NY, USA) were attached to the stomach wall and secured with a spacer device; 10 IRE (NanoKnife; AngioDynamics) pulses at 1,500 V/cm were initially performed. There was an interruption in the IRE procedure in certain cases, due to high current, thereby leading to a reduced and adapted electric field. The final IRE electric field strength and other parameters were recorded (Table I). As the present study aimed to evaluate the most severe damage of IRE on the stomach wall, the above parameters were selected. All IRE pulses were delivered using an electrocardiographic synchronization instrument (AngioDynamics, Inc., Latham, NY, USA) to avoid cardiac arrhythmias. The skin incision was sutured at the end of the procedure. Pigs fully recovered within 2 h post-IRE and received daily intramuscular injections of 40 mg/kg body weight cephazolin (Qilu Pharmaceutical Co., Ltd., Jinan, China) for 1 week to reduce the risk of infection. To reduce pain, animals were administered intramuscular buprenorphine (0.01 mg/kg; Jin Lan Pharmaceutical Group Co., Ltd., Tianjin, China) and oral meloxicam (0.4 mg/kg; Boehringer Ingelheim Pharmaceutical Co., Ltd., Shanghai, China) for the first week.

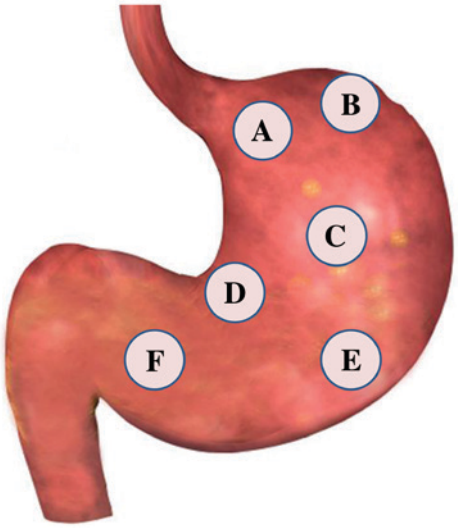

Figure 1. Irreversible electroporation ablation areas in the stomach wall. (A) Gastric cardia; (B) fundus of stomach; (C) gastric body; (D) lesser gastric curvature; (E) greater gastric curvature; and (F) stomach pylorus.

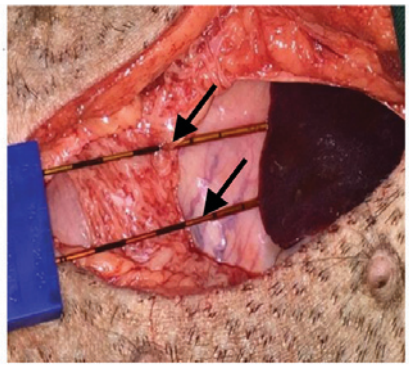

Figure 2. Representative image of the placement of irreversible electroporation electrodes (black arrows).

Serum aminotransferases and white blood cells. Blood samples were collected prior to IRE and on days 1, 3, 5 and 7 post-IRE. To monitor hepatic function and the presence of inflammation, the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and white blood cells (WBC) were assessed using an automatic biochemical analyzer, KHA-220 (Beijing Science and Technology Co., Ltd., Beijing, China).

Gastroscopy and endoscopic ultrasonography. Gastroscopy and endoscopic ultrasonography images were obtained on days 7 and 28 post-IRE. Gastroscopy and endoscopic ultrasonography were performed by two professional endoscopic doctors, who were blind to the aims of the study.

Gross pathology and histopathology. The present experiment was performed by a well-trained and professional pathologist with ~20 years experience. From each group, 2 pigs were sacrificed on day 7 post-IRE. The remaining 4 pigs were sacrificed on day 28 post-IRE. The ablated stomach wall

Table I. Irreversible electroporation applications.

Animal no.	Ablated areas	Electric field, V/cm	Feedback current, A
1	a	1,500	28
	b	1,400	26
	c	1,500	29
2	a	1,400	25
	b	1,300	23
	c	1,500	31
3	a	1,500	27
	b	1,400	26
	c	1,400	27
4	a	1,300	28
	b	1,300	24
	c	1,500	29
5	d	1,500	28
	e	1,500	30
	f	1,400	28
6	d	1,500	29
	e	1,400	25
	f	1,500	30
7	d	1,300	25
	e	1,300	23
	f	1,400	26
8	d	1,400	25
	e	1,400	27
	f	1,500	30

All animals had 2 cm electrode exposure and spacing and a 70 μsec pulse length for 90 pulses. Each animal underwent the procedure for 1 cycle.

tissue was grossly examined for any signs of perforation, edema, swelling and color changes. The mucosal and serosal surfaces of the ablated tissue and nearby normal tissue were imaged. The largest diameter of each lesion in the mucosal and serosal surface was measured with a ruler. Ablated tissue and surrounding normal tissue were serially sectioned at 5-mm intervals, fixed in 10% formalin for 24 h, and stained with hematoxylin and eosin and with Masson's trichome for histomorphologic and collagen proliferation analyzes, respectively. The photos were imaged using a NIKON LV150L microscope (Nikon Corporation, Tokyo, Japan), with magnification, x100.

Statistical analysis. Data were analyzed with SPSS version 22.0 (SPSS, IBM, Armonk, NY, USA). The size of the lesions was analyzed using Student's t-test. $P<0.05$ was determined to indicate a statistically significant difference.

Results

Imaging findings. Gastroscopy, which was performed on days 7 and 28 post-IRE, revealed differing degrees of gastric ulcers in the stomach wall of the pigs. Additionally, damage in the stomach wall gradually decreased. On day 7 post-IRE, tissue samples presented round/oval ulcers with whitish/yellowish fur and slight edema in the surrounding mucosa (Fig. 3A). Comparatively, on day 28 post-IRE, tissue samples had less pronounced edema, shallower and smaller ulcers, and smooth mucosal surfaces (Fig. 3B). The yellowish/whitish fur disappeared in the majority of the samples. In 2 animals of group A, 2 lesions in the gastric body did not present the visible healing changes (small ulcers and smooth mucosal surface) observed in the other animals on day 28 post-IRE. In these animals, the two lesions were shallow but not smaller in size.

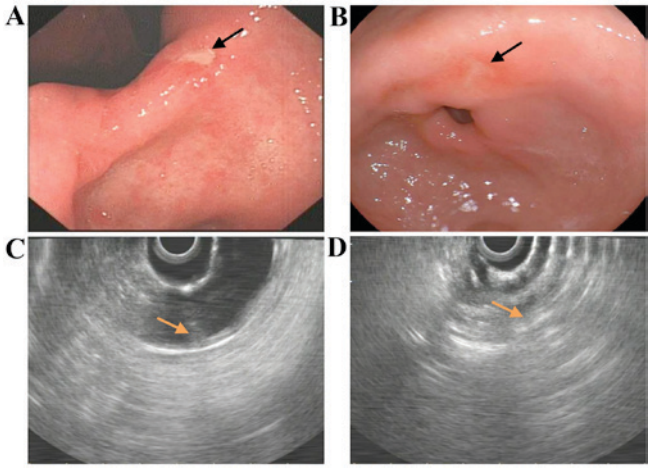

Figure 3. Gastroscopy and endoscopic ultrasonography on days 7 and 28 post-IRE. Gastroscopy indicated an (A) oval ulcer (black arrow) with whitish fur and surrounding congestion and edema on day 7 post-IRE; (B) this ulcer (black arrow) became shallower and smaller with less congestion and edema on day 28. Fusion of the stomach layers (yellow arrows) in the ablated areas on days (C) 7 and (D) 12 post-IRE, as indicated by endoscopic ultrasonography. IRE, irreversible electroporation.

It was challenging to completely eliminate air interferences during the endoscopic ultrasonography. On day 7 post-IRE, only fusion of stomach layers and edema in the ablated areas was observed (Fig. 3C). At day 28 post-IRE, there was no visible edema; however, it remained challenging to differentiate among the stomach wall layers (Fig. 3D).

Clinical course. In 3 pigs in group A and 2 pigs in group B, the body temperature increased (>40˚C) within 48 h after IRE. Administration of meloxicam (0.4 mg/kg; Boehringer Ingelheim Pharmaceutical Co., Ltd.) reduced body temperature to normal levels (38-39.5˚C). Notably, 3 pigs in group A and 2 pigs in group B presented with distended abdomens for 3 days. All pigs had reduced appetite and decreased fecal output for 5 days. Animal moaning and bruxism (teeth grinding), which are classic behavioral indicators of pain, were noted when the abdomen was pressed down firmly.

Changes in serum aminotransferase levels and white blood cell count. There were no significant differences in the levels of serum aminotransferase or WBC between groups A and B (data not shown). The results from group A and B were then pooled and are presented in Fig. 4. AST and WBC levels increased on day 1 post-IRE and gradually

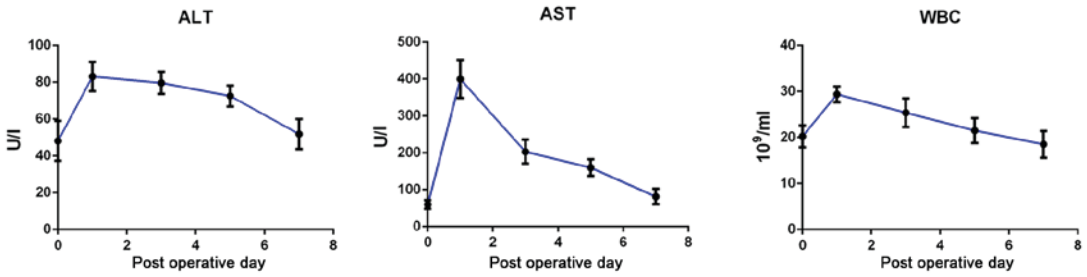

Figure 4. Changes in the levels of serum aminotransferase and WBCs. There were no significant differences between groups A and B (data not shown). Subsequently, data for groups A and B were pooled. ALT, AST, and WBC were measured prior to IRE and on days 1, 3, 5 and 7 post-IRE. ALT, alanine aminotransferase; AST, aspartate aminotransferase; WBC, white blood cells; IRE, irreversible electroporation.

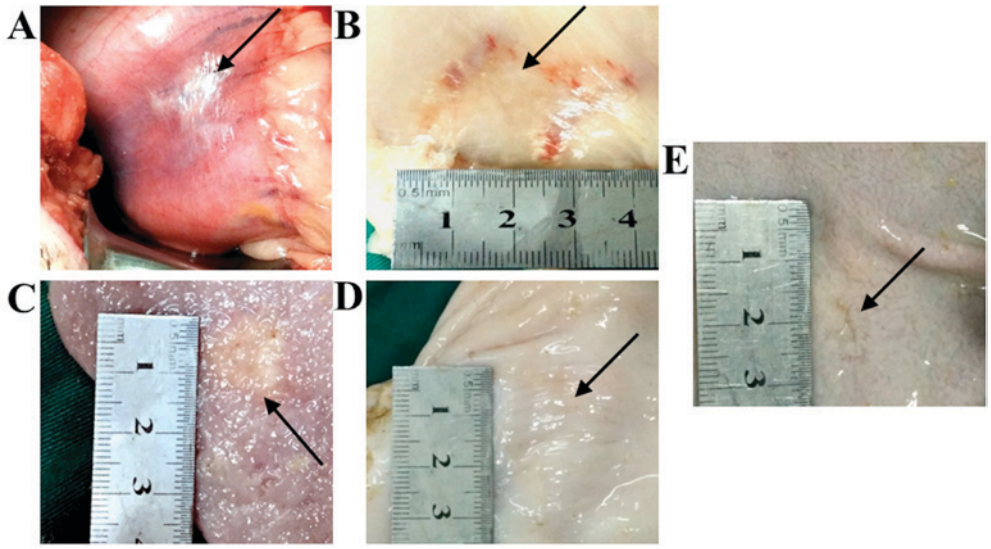

Figure 5. Representative images of IRE electrodes and stomach appearance post-IRE. (A) Stomach wall immediately following the ablation procedure, the black arrow indicates the ablated lesion. (B) Serosal surface of stomach wall on day 7 post-IRE. (C) Mucosal surface of stomach wall on day 7 post-IRE. (D) Serosal surface of stomach wall on day 28 post-IRE. (E) Mucosal surface of stomach wall on day 28 post-IRE. Black arrows in B and D indicate the lesions on the serosal surface; black arrows in C and E indicate the ulcers on the mucosal surface. IRE, irreversible electroporation.

decreased thereafter, reaching normal levels by day 7 post-IRE (Fig. 4). Changes in serum aminotransferase levels, which are indicative of hepatic damage, occurred for ~7 days post-IRE. The changes in WBC levels suggested that there may have been mild inflammation during the first week post-treatment.

Gross pathology. A whitish lesion with surrounding reddish areas was observed immediately following the ablation procedure (Fig. 5A). Demarcation between the whitish lesion and surrounding reddish parts was sharp. The appearance was similar for all ablation areas and there were no signs of perforation. At day 7 post-IRE, the lesions on the serosal surface of the stomach wall were observed to be a rectangular shape with dark red color where the electrodes had been attached (Fig. 5B). On the mucosal surface of the stomach wall, round/oval ulcers with or without whitish fur were observed (Fig. 5C). The demarcation between the lesion and nearby normal stomach tissue was sharp in all the pigs. However, at 28 days post-IRE, the lesions on the serosal surface were crumpled, markedly smaller in size and had the same color as that of nearby normal tissues (white or slightly reddish) with no signs of edema (Fig. 5D). Ulcers on the mucosal surface were shallower and smaller, and the color was similar to that of untreated tissue (Fig. 5E), with the exception of 2 lesions in the gastric body of 2 pigs in group A. The sizes of the lesions at 7 and 28 days post-IRE are presented in Table II. There was a significant difference in lesion size between the serosal and mucosal surfaces of the stomach wall.

Histopathology. At 7 days post-IRE, all layers of the stomach wall were damaged by IRE. Stomach wall damage was characterized by the presence of fibrosis and mild-to-moderate inflammation (plasma cells, lymphocytes, and eosinophils; Fig. 6A and B). The damage was present through the entire thickness of the stomach wall and the lesions were demarcated by sharp borders (Fig. 6C). The mucosa was obliterated by different degrees of inflammatory liquefactive necrosis, and the submucosa changes were characterized by hyperemia and edema (Fig. 6D). Muscle fibers in the muscular layer exhibited differing degrees of swelling and degeneration (Fig. 6E). The serosa of the stomach wall was directly exposed to IRE and was obliterated by inflammation, liquefactive necrosis and fibrous tissues (Fig. 6F). At 28 days post-IRE, the histopathology results revealed the presence of an almost normal

Table II. Size of stomach lesions on days 7 and 28 post-irreversible electroporation.

	Diameter of stomach lesions, cm		
Days post procedure	Serosal surface (n=12)	Mucosal surface (n=12)	P-value
7	3.13±0.46	1.78±0.31	<0.05
28	3.01±0.42	1.61±0.23	<0.05

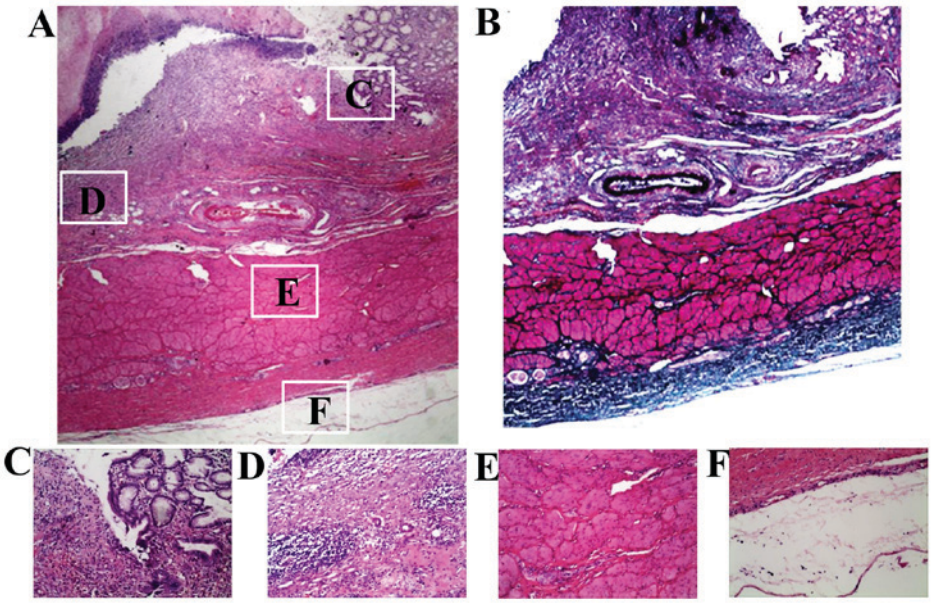

Figure 6. Histopathological changes on day 7 post-IRE. (A) Hematoxylin and eosin staining of the stomach wall in the ablated area (magnification, x20). (B) Masson's trichrome stain of the stomach wall in the ablated area (magnification, x20). (C) Ablated and nearby normal mucosa. (D) Submucosa with hyperemia and edema. (E) Muscular layer with swelled and degenerated muscle fibers. (F) Serosa with inflammation, liquefactive necrosis and fibrous tissues. (C-F) Magnification of x100 from the indicated areas in (A). IRE, irreversible electroporation.

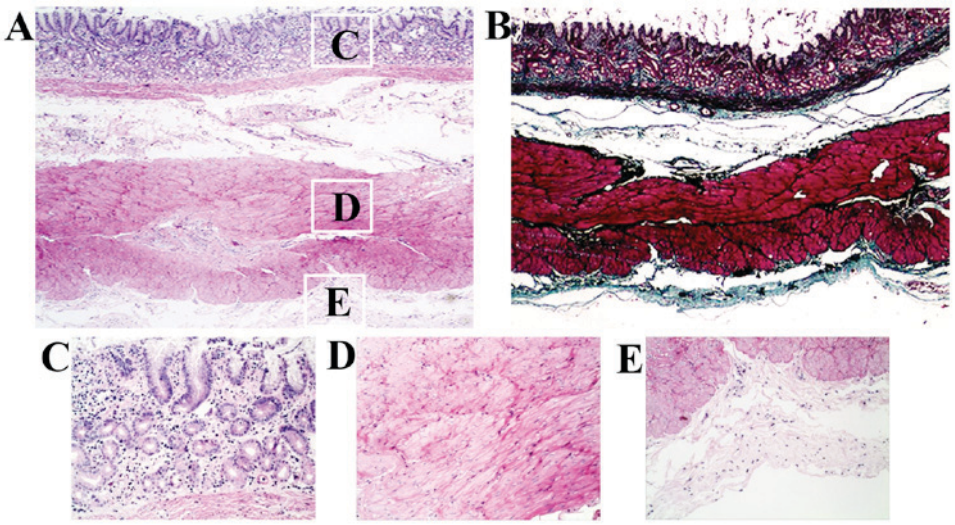

Figure 7. Histopathological changes on day 28 post-IRE. (A) Hematoxylin and eosin staining of the stomach wall in the ablated area (magnification, x20). (B) Masson's trichrome stain of the stomach wall in the ablated area (magnification, x20). (C) Regenerated mucosa. (D) muscular layer with normal myocytes. (E) Serosa with large amounts of fibrous tissues. (C-E) Magnification of x100 from the indicated areas in (A). IRE, irreversible electroporation.

stomach wall (Fig. 7A) and more fibrosis in the stomach layers (Fig. 7B). Small glands and short villus in the mucosa were also observed (Fig. 7C).

Discussion

IRE is an emerging technology that consists of the application

of a high voltage electric field across target cells to generate nanopores on the cell membranes, thereby contributing to cell necrosis and apoptosis (12,13). IRE, which selectively targets cell membranes and preserves connective tissues, induces rapid tissue regeneration (7,14). IRE has been widely used in the treatment of hepatic and pancreatic tumors; however, there is little evidence on the effects of IRE on the stomach wall.

In a study by Schoellnast *et al* (15), computed tomography (CT)-guided IRE adjacent to the rectum wall was performed in 5 pigs without a water-filled endorectal coil (group A) and in 6 pigs with the coil (group B) to avoid displacement of the rectum wall. A 16-g bipolar probe was inserted adjacent and tangential to the rectum wall and adjacent to the internal obturator muscle. The results revealed that transmural necrosis was present in all group B animals, while necrosis was limited to the external layer of the muscularis propria in the group A animals (15). Schoellnast *et al* (15) therefore, concluded that IRE ablation of the rectum may have harmful effects when the rectum wall is adjacent to the IRE-probe. In the present study, the IRE probes were placed in the space between the stomach wall and the liver, fixed in placed by sutures. As a result, no tissues were present between the IRE probes and the stomach wall; therefore, IRE ablation directly affected the stomach. As the electrode tips cannot be exposed to air while ablating, the liver was slightly pressed down to ensure the successful completion of the IRE procedure. This step may limit stomach contraction during ablation, which may contribute to more injury (15). In a study by Srimathveeravalli *et al* (16), 2 endorectal IRE ablations were performed. The first ablation procedure (1,050-1,125 V) was performed on the mucosal and submucosal layers but not on the muscularis propria. The second procedure (2,100 V) consisted of a transmural ablation of the rectal wall. In the current study, the stomach wall was ablated using an electric field >2,100 V, which likely induced cell death in the stomach layers. Transmural necrosis was observed in the lesions of the stomach wall, without any evidence of perforations. The stomach wall was structurally intact, characterized by the presence of fiber and regenerated mucosa.

The conditions of the present study, including IRE probes placement and ablation parameter settings, contributed to marked damage to the stomach wall. However, when ablating tumors adjacent to the stomach, the IRE probes cannot affect the stomach wall directly and the IRE settings may be modified to limit stomach injury. Therefore, the damage to the stomach wall must be lighter when ablating tumors adjacent to the stomach.

Compared with other thermal ablation technologies, IRE, which primarily targets the cellular membrane, preserves the basic structure of tissue (17,18). In the present study, the mucosa layer of the stomach presented with marked inflammatory liquefactive necrosis. The stomach wall structure was preserved, which allowed collagen, elastic fiber and regenerated stomach tissue to replace the damaged cells. Edema and yellowish/whitish furs disappeared by 28 days post-IRE. At day 28 post-IRE, the presence of regenerated mucosa was indicative of healing. In the present study, there were no evident complications associated with IRE treatment in the pigs.

In 2 pigs of group A, 2 gastric body lesions did not heal by day 28 post-IRE. This was attributed to the convenient ablation area of the gastric body, which is just beneath the exposed hepatic tissue. IRE probes were fixed tightly to the stomach by sutures, and movement of the liver or of the IRE probes was less possible, which may contribute to more severe effects of IRE on the gastric body. However, in other ablation lesions, due to the inconvenient ablation area, it was difficult to avoid the electrode dislocation during the whole procedure and this may lighten the IRE effects on the stomach wall.

The current study had a number of limitations. Firstly, the three lesions may increase stomach wall damage and decrease the healing process, although normal tissues were observed between the lesions in this study. Secondly, there was an interruption caused by the high IRE current, and the voltage required adjusting to complete the ablation procedure, which may have affected the extent of the stomach wall damage. Thirdly, IRE electrodes were placed on the stomach wall, not inserted into the stomach tissue; any probe movement during ablation was able to affect the procedure. Also, the IRE effects on the anterior stomach wall were evaluated, but not on the posterior stomach wall. Finally, while there were no perforations in the stomach wall, gastrointestinal function was not assessed in these pigs post-IRE.

In conclusion, the current study demonstrated that IRE does cause injury to the stomach if it is applied directly on the organ surface. However, no perforation was observed in the pigs used in the present study. If translating the results of the current study into clinical use, IRE of hepatic or pancreatic tumors, which are adjacent to the stomach wall cannot lead to stomach perforation. However, further studies that evaluate different IRE settings and assess gastric function post-IRE are required.

Acknowledgements

The current study was supported by International Science Foundation of Guangzhou Fuda Cancer Hospital (grant no. Y2015-ZD-001). The authors would also like to thank Elixigen Corp. (Huntington Beach, CA, USA) for helping in proofreading and editing the English of final manuscript.

References

1. Cannon R, Ellis S, Hayes D, Narayanan G and Martin RC II: Safety and early efficacy of irreversible electroporation for hepatic tumors in proximity to vital structures. J Surg Oncol 107: 544-549, 2013.
2. Ahmed M and Goldberg SN: Basic science research in thermal ablation. Surg Oncol Clin N Am 20: 237-258, 2011.
3. Lee EW, Wong D, Prikhodko SV, Perez A, Tran C, Loh CT and Kee ST: Electron microscopic demonstration and evaluation of irreversible electroporation-induced nanopores on hepatocyte membranes. J Vasc Interv Radiol 23: 107-113, 2012.
4. Novickij V, Grainys A, Novickij J and Markovskaja S: Irreversible magnetoporation of micro-organisms in high pulsed magnetic fields. IET Nanobiotechnol 8: 157-162, 2014.
5. Scheffer H, Nielsen K, van Tilborg A, Vieveen JM, van den Tol MP and Meijerink MR: Irreversible electroporation: A new form of image-guided tumour ablation. Ned Tijdschr Geneeskd 158: A7176, 2014 (In Dutch).
6. Al-Sakere B, André F, Bernat C, Connault E, Opolon P, Davalos RV, Rubinsky B and Mir LM: Tumor ablation with irreversible electroporation. PLoS One 2: e1135, 2007.

7. Lee EW, Chen C, Prieto VE, Dry SM, Loh CT and Kee ST: Advanced hepatic ablation technique for creating complete cell death: Irreversible electroporation. Radiology 255: 426-433, 2010.
8. Schoellnast H, Monette S, Ezell PC, Deodhar A, Maybody M, Erinjeri JP, Stubblefield MD, Single GW Jr, Hamilton WC Jr and Solomon SB: Acute and subacute effects of irreversible electroporation on nerves: Experimental study in a pig model. Radiology 260: 421-427, 2011.
9. Narayanan G, Froud T, Lo K, Barbery KJ, Perez-Rojas E and Yrizarry J: Pain analysis in patients with hepatocellular carcinoma: Irreversible electroporation versus radiofrequency ablation-initial observations. Cardiovasc Intervent Radiol 36: 176-182, 2013.
10. Martin RC II, McFarland K, Ellis S and Velanovich V: Irreversible electroporation in locally advanced pancreatic cancer: Potential improved overall survival. Ann Surg Oncol 20 (Suppl 3): S443-S449, 2013.
11. Pech M, Janitzky A, Wendler JJ, Strang C, Blaschke S, Dudeck O, Ricke J and Liehr UB: Irreversible electroporation of renal cell carcinoma: A first-in-man phase I clinical study. Cardiovasc Intervent Radiol 34: 132-138, 2011.
12. Maor E, Ivorra A, Leor J and Rubinsky B: The effect of irreversible electroporation on blood vessels. Technol Cancer Res Treat 6: 307-312, 2007.
13. Edd JF, Horowitz L, Davalos RV, Mir LM and Rubinsky B: In vivo results of a new focal tissue ablation technique: Irreversible electroporation. IEEE Trans Biomed Eng 53: 1409-1415, 2006.
14. Onik G, Mikus P and Rubinsky B: Irreversible electroporation: Implications for prostate ablation. Technol Cancer Res Treat 6: 295-300, 2007.
15. Schoellnast H, Monette S, Ezell PC, Single G, Maybody M, Weiser MR, Fong Y and Solomon SB: Irreversible electroporation adjacent to the rectum: Evaluation of pathological effects in a pig model. Cardiovasc Intervent Radiol 36: 213-220, 2013.
16. Srimathveeravalli G, Wimmer T, Monette S, Gutta NB, Ezell PC, Maybody M, Weiser MR and Solomon SB: Evaluation of an endorectal electrode for performing focused irreversible electroporation ablations in the Swine rectum. J Vasc Interv Radiol 24: 1249-1256, 2013.
17. Deipolyi AR, Golberg A, Yarmush ML, Arellano RS and Oklu R: Irreversible electroporation: Evolution of a laboratory technique in interventional oncology. Diagn Interv Radiol 20: 147-154, 2014.
18. Rubinsky B, Onik G and Mikus P: Irreversible electroporation: A new ablation modality-clinical implications. Technol Cancer Res Treat 6: 37-48, 2007.