Surgical Endoscopy (2019) 33:658–662
https://doi.org/10.1007/s00464-018-6425-4

NEW TECHNOLOGY

EUS-guided irreversible electroporation using endoscopic needle-electrode in porcine pancreas

Jae Min Lee[1] · Hyuk Soon Choi[1] · Hoon Jai Chun[1] · Eun Sun Kim[1] · Bora Keum[1] · Yeon Seok Seo[1] · Yoon Tae Jeen[1] · Hong Sik Lee[1] · Soon Ho Um[1] · Chang Duck Kim[1] · Hong Bae Kim[2]

Received: 9 April 2018 / Accepted: 5 September 2018 / Published online: 29 October 2018

Abstract

Background Endoscopic irreversible electroporation (IRE) can be performed using a flexible, thin, needle-shaped electrode for an endoscopic ultrasound (EUS)-guided procedure. This study aimed to evaluate the feasibility and efficacy of performing EUS-guided IRE with endoscopic needle-electrode in porcine pancreas.
Methods Experimental endoscopic IRE on the pancreas were performed by EUS-guided approach in three pigs and compared with surgical approach in three pigs. The animals were killed after 24 h and their pancreases collected.
Results IRE ablation using endoscopic needle-electrode was successful technically in EUS-guided approaches for the pancreas. Immediately following IRE, the ablated pancreatic tissue showed no gross change except focal hemorrhage. H&E staining presented a well-demarcated ablation site measuring 1.0–1.5 cm in diameter in the pancreas. TUNEL immunohistochemistry showed diffuse cell death along the puncture site 24 h after IRE. No complication was observed in pigs after endoscopic IRE ablation.
Conclusion EUS-guided IRE ablation was feasible and effective for pancreas using the newly developed device.

Keywords Irreversible electroporation · Pancreas · Endoscopy · EUS · Animal

Pancreatic cancer is one of the most aggressive cancers, and only 15–20% of the patients affected could be treated by radical surgery. Despite the improvement in survival rates after combination chemotherapy, it still has a poor prognosis and survival rates of 9–11 months [1–3]. Therefore, additional ablation therapies could be considered for the treatment of pancreatic tumors for clinical or research purposes. Among the ablation therapies, irreversible electroporation (IRE) was known as a novel non-thermal ablation therapy that disrupts cellular homeostasis and induces apoptosis of the parenchyma without damaging surrounding vessels [4–6]. In the previous studies, IRE ablation can be a feasible therapeutic modality for treatment of advanced pancreatic cancer [7]. Combination therapy using IRE ablation and systemic chemotherapy showed a favorable response in patients with metastatic pancreatic cancer [8]. However, current IRE ablation therapy for pancreatic tumors has been usually performed intraoperatively and required laparotomy. General anesthesia and surgical approach would be a heavy burden for the patients.

Endoscopic ultrasound (EUS)-guided thermal ablation of the pancreas was first described in 1999 [9]. Recently, minimally invasive procedures have been suggested as a therapeutic option for the treatment of pancreatic tumor [10]. The development of linear EUS makes it possible to perform various endoscopic treatments for pancreatic diseases. EUS-guided procedure allows an endoscopic treatment of pancreatic cancer under real-time imaging without laparotomy [11]. We developed a novel electrode for endoscopic IRE procedure. In this study, we investigated the feasibility, efficacy, and safety of EUS-guided IRE with endoscopic needle-electrode on porcine pancreas.

Jae Min Lee and Hyuk Soon Choi have contributed equally to this work.

✉ Hoon Jai Chun
drchunhj@chol.com

1 Division of Gastroenterology and Hepatology, Department of Internal Medicine, Korea University College of Medicine, Seoul, Republic of Korea

2 Department of Biosystems & Biomaterials Science and Engineering, Seoul National University, Seoul, Republic of Korea

Materials and methods

Animals

The study was performed on Yorkshire pigs (Orient Laboratory Animal Company, Seongnam, Korea) that weighed 35 ± 5 kg. All animals were housed for acclimation for 7 days in the animal facility. They were kept in temperature (22–24 °C)- and humidity (55%)-controlled rooms under 12-h/12-h light/dark cycles. The animals were fasted the night before the procedure. The procedure was performed under general anesthesia using midazolam 0.5 ml/kg and ketamine 25 mg/kg administered intravenously. This study was approved by the Institutional Animal Care & Use Committee, Korea University (IACUC number: KOREA-2016-0062).

Device

The procedure was performed using the newly developed endoscopic needle-electrode (patent pending). The endoscopic electrode consisted of a needle-shaped tip, two electrodes arranged at 1 cm intervals on the fore end and long flexible body that can be inserted through the channel of the endoscope (Fig. 1). ECM 830 square wave electroporation system (BTX Genetronics, San Diego, CA) was used for IRE ablations of the pancreas. EUS-guided IRE was performed using ProSound Alpha 10 ultrasound system (Hitachi-Aloka, Tokyo, Japan).

Procedure

This study employed two approaches for IRE ablation, namely, the surgical approach and EUS-guided endoscopic approach. In the surgical approach, a midline laparotomy was performed after induction of general anesthesia. The pancreas was exposed within the abdomen and mobilized to enable carrying out of the IRE. IRE ablation was performed after manual puncturing using endoscopic needle-electrode. In the EUS-guided endoscopic approach, the linear EUS endoscope (GF-UCT180, Olympus, Tokyo, Japan) was advanced into the stomach; therefore, the endoscopic needle-electrode was inserted through the working channel of the scope. Needle puncturing and passing of the needle-electrode into the pancreas was done toward the body or tail. The energy delivered was a pulse wave of 10 sequences with five pulses, pulse duration of 100 µs, amplitude of 2000 V, and a repetition frequency of 1 Hz with a 2-s pause between the five pulse sequences.

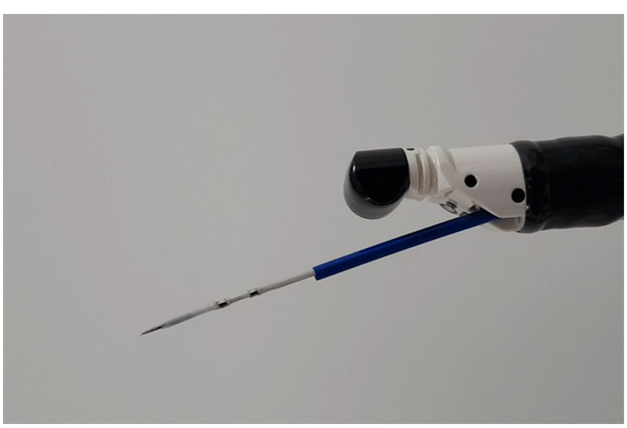

Fig. 1 Endoscopic electrode for EUS-guided IRE of the pancreas. A 19-gage (1.1 mm) endoscopic IRE electrode and tip of the IRE electrode in the EUS endoscope

Post-procedure assessment

Pigs were killed at 24 h after the procedure, and the pancreas was resected for histopathologic evaluation. After formalin fixation, sagittal segments of each pancreas were taken at approximately 2–3 mm of thickness at the level of the puncture site. Paraffin-embedded segments were sectioned and stained with hematoxylin and eosin (H&E). In addition, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate digoxigenin nick end-labeling (TUNEL) assay was conducted. Digitalized slides were obtained from a scanner (Leica SCN400 Image Viewer, Leica, Germany) and were analyzed using its viewer software.

Results

Ablation and clinical course

Six female Yorkshire pigs underwent IRE ablation for the pancreas; three pigs with the surgical approach and three pigs with the EUS-guided endoscopic approach. In the EUS-guided endoscopic approach, the device was well visualized as a hyperechoic line with significant bands of electrodes on EUS. IRE ablation for the pancreas was technically successful and effective using the needle-electrode delivered by laparotomy or endoscopic approach. In both the approaches, no spark or short circuit in the procedure using the device with linearly arranged electrodes was observed. IRE ablation was successfully performed in all cases with 2000 V. During IRE ablation, muscle contraction occurred for a short time. Immediately following IRE, the ablated pancreas showed no significant change except focal hemorrhage on the puncture site. All pigs tolerated the procedure at the time of the procedure and during the 24 h after procedure. Any severe complication during the study was not encountered.

Histopathology

No significant finding of the pancreas was found, except for the small hematoma at the puncture site (Fig. 2A). Pathological examination after sagittal sectioning revealed a well-demarcated focal lesion of the pancreas (Fig. 2B). The ablation site had a circular shape and measured 10–15 mm in size. Round, small hematoma near the ablation site was found in one pig. Histopathological examination showed a well-demarcated necrosis of the pancreatic parenchyma. The H&E-stained pancreatic tissue showed a diffuse cell death with circular shape at the center of the puncture site (Fig. 3D). Inflammatory change with necrosis was shown with residual pancreatic acini on the ablation site (Fig. 3C). Pancreatic tissues far from the puncture site were less damaged and preserved of normal acinar cells (Fig. 3A). It presented a clear demarcation between the viable and non-viable tissues in the pancreas after IRE ablation (Fig. 3B). At high magnification, extracellular matrix, ducts, and blood vessels, such as small arteries and arterioles, were preserved. Positive results of TUNEL assay in the ablated zone indicate involvement of apoptotic cell death (Fig. 4). In the round ablated zone, pyknosis and karyorrhexis of pancreatic parenchyma were observed. No significant difference was found in the result of ablation between the surgical approach and EUS-guided endoscopic approach.

Discussion

This experimental study revealed that EUS-guided IRE ablation of the pancreas was feasible and effective. The entire procedure could be performed under real-time observation on EUS. During the endoscopic procedure, it was possible to do accurate targeting, avoid vascular structures, and puncture the needle-electrode into the pancreas. Although we performed experimental procedures in only six animals, no severe complications (e.g., perforation, burn, procedure-related bleeding) were observed.

The laparotomy and endoscopic approach for IRE ablation were both effective and beneficial. It can be applied as local or palliative therapy through minimally invasive endoscopic approach. And also, new flexible and slim electrode will be able to use for IRE ablation therapy by laparoscopy or minimal incision surgery.

Several limitations in this study should be noted. First, the number of animals used was relatively small, with only 24-h follow-up. Safety and effectiveness evaluation would require various follow-up times. Second, given that twitching during IRE ablation occurred, endoscopic IRE ablation is at risk for needle dislocation throughout the procedure. Third, the ablation site was only limited to the body and tail. Given that the porcine had a different

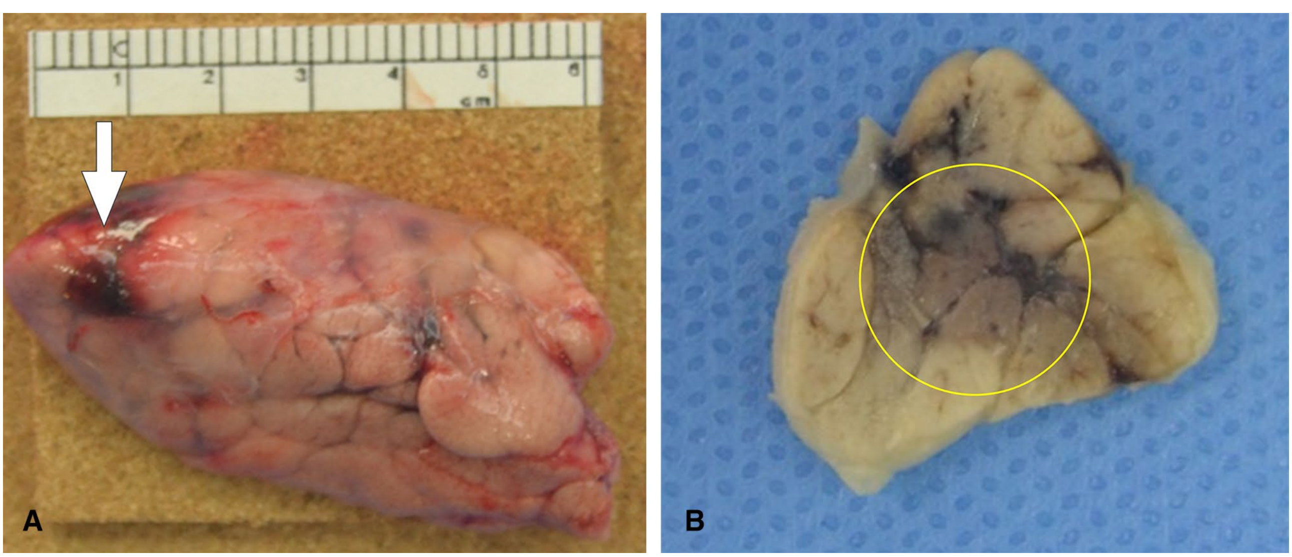

Fig. 2 Specimen of the pancreas after EUS-guided endoscopic IRE. **A** Gross specimen with small hematoma on the puncture site (white arrow: needle puncture site). **B** Well-demarcated, round ablated lesion of about 10–15 mm in diameter (yellow circle: ablation site)

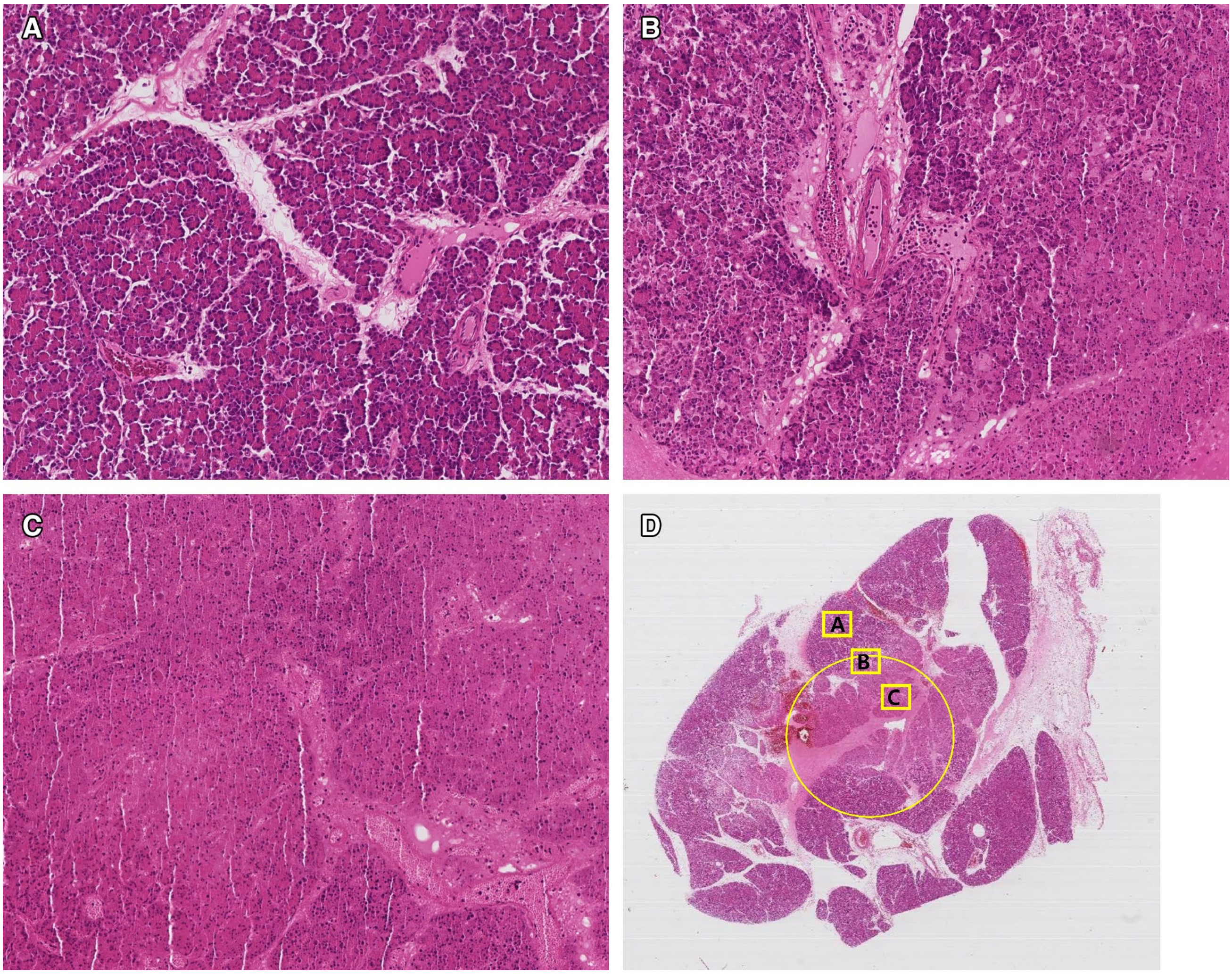

Fig. 3 Histopathologic findings of the pancreas parenchyma. **A** Normal area of unremarkable change. **B** Well-demarcated margin between the normal pancreatic parenchyma and necrotic tissue. **C** The ablation zone characterized by completely necrotic area with nuclear dust of the acinar pancreatic tissue. **D** Scout image of pancreas (H&E)

anatomic structure than humans, EUS-guided IRE was not performed on the pancreatic head and uncinated process.

This experimental study suggests that EUS-guided IRE is a feasible and safe local therapy for pancreatic ablation. Endoscopic needle-electrode was useful to perform endoscopic IRE on EUS. The further challenges of EUS-guided IRE will be to standardize needle device placement for target lesion and to optimize the efficacy endpoints with appropriate electronic parameters. We expect that EUS-guided IRE can be widely applicable to treat not only malignant but also benign lesions in pancreas. Moreover, further studies to demonstrate its clinical application would be required.

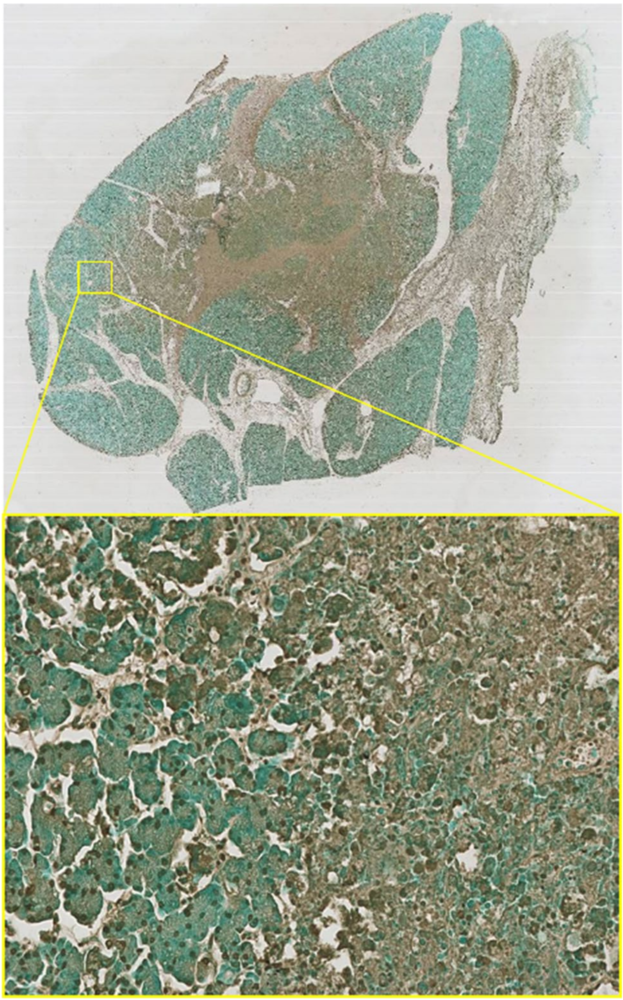

Fig. 4 Immunohistopathology with TUNEL assay in the pancreas parenchyma. Round ablated zone with apoptosis and visualized pyknosis and karyorrhexis (TUNEL, ×100)

Acknowledgements This study was supported by a College of Medicine, Korea University Grant (No. K1523601) and the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2018R1C1B6006044).

Compliance with ethical standards

Disclosures Authors Jae Min Lee, Hyuk Soon Choi, Hoon Jai Chun, Eun Sun Kim, Bora Keum, Yeon Seok Seo, Yoon Tae Jeen, Hong Sik Lee, Soon Ho Um, Chang Duck Kim, and Hong Bae Kim have no conflicts of interest or financial ties to disclose.

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