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Novel Ablation Therapy Using Endoscopic Irreversible Electroporation in the Bile Duct: A Pilot Animal Study

Kang Won Lee^{1*}, Jae Min Lee^{1*}, Hyuk Soon Choi¹, Eun Sun Kim¹, Bora Keum¹, Yeon Seok Seo¹, Yoon Tae Jeon¹, Soon Ho Um¹, Hong Sik Lee¹, Hoon Jai Chun¹, Chang Duck Kim¹, Chi Hyuk Oh² and Hong Bae Kim³

¹Division of Gastroenterology and Hepatology, Department of Internal Medicine, Korea University College of Medicine, Seoul, ²Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kyung Hee University College of Medicine, Seoul, ³Department of Biosystems and Biomaterials Science and Engineering, Seoul National University, Seoul, Korea

Background/Aims: Irreversible electroporation (IRE) is a relatively new ablation method. However, the application of IRE ablation in the treatment of biliary disease has not been attempted. A minimally invasive approach using endoscopic retrograde cholangiopancreatography (ERCP) can be a novel therapeutic modality for IRE ablation. In this study, we aimed to investigate the feasibility of endoscopic IRE for the biliary tract using an animal model.

Methods: A new catheter-type electrode was developed for endoscopic IRE ablation of the biliary tract. We performed ERCP and endoscopic IRE ablations in the normal common bile duct of Yorkshire pigs. The experimental setting of IRE was 500 V/cm (50 pulses, 100- μ s length). The animals were sacrificed after 24 hr, and the ablated bile duct was examined.

Results: Well-demarcated focal color changes were observed on the mucosa of the common bile duct. The depth of change after IRE was confined to the mucosal and submucosal layers. Apoptotic changes in the bile duct were observed only around the IRE ablation area. Immunohistochemistry assay showed cell death in the bile duct along the electrode.

Conclusions: Endoscopic IRE ablation using ERCP was successfully performed in the common bile duct. It can be a potential option for the treatment of biliary tumors. **Clin Endosc 2021;54:413-419**

Key Words: Ablation; Bile duct; Endoscopic retrograde cholangiopancreatography; Irreversible electroporation

INTRODUCTION

Cholangiocarcinoma is among the types of cancer with a poor prognosis, and only a few patients with this diagnosis can undergo surgery with complete resection.¹ Several chemotherapeutic drugs have been introduced; however, the poor prognosis and short survival period limit the treatment

of cholangiocarcinoma.² Under these circumstances, several studies evaluating local treatments for cholangiocarcinoma in addition to systemic chemotherapy have been conducted.³ As a prime example of ablation therapy, irreversible electroporation (IRE) can destroy cancer cells in a nonthermal manner.⁴ Previous electrical treatments used radiofrequency applications; however, their clinical applications have been limited owing to the thermal effect.⁵ IRE can disrupt cellular homeostasis confined to the target lesion area, without damaging the surrounding vascular structures, thus inducing apoptosis of the parenchyma.⁶

IRE is based on the principle of electroporation. Electroporation is a technology that introduces electrical pulses with a width from 1 ns to 1 ms and an energy intensity from 0.4 to 1 kV/cm to the cell, forming a nanometer-sized pore on the surface of the cell membrane. This technology has been applied to various biotechnology fields for decades since its first discovery in the 1970s.⁷ The micropore on the surface of a cell mem-

Received: May 8, 2020 Revised: July 7, 2020

Accepted: July 9, 2020

Correspondence: Jae Min Lee

Division of Gastroenterology and Hepatology, Department of Internal Medicine, Korea University College of Medicine, Korea University Medical Center, 73 Goryeodae-ro, Seongbuk-gu, Seoul 02841, Korea

Tel: +82-2-920-6555, Fax: +82-2-953-1943, E-mail: jmlee1202@gmail.com

ORCID: <https://orcid.org/0000-0001-9553-5101>

*These authors contributed equally to this study.

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brane produced under appropriate electrical pulse conditions has a reversible characteristic; that is, it disappears again after a period of time (reversible electroporation). On the contrary, a micropore produced under stronger electrical pulses remains open, which compromises the cellular homeostasis and eventually destroys the cell. This method is referred to as IRE.⁸

IRE was approved with restrictions by the Food and Drug Administration in 2011 after it was theoretically introduced in 2005. Considering the results of previous studies, IRE could be an effective and feasible treatment option for advanced cholangiocarcinoma. In fact, previous studies have reported that combining IRE and systemic chemotherapy showed good results in pancreatic cancer.⁹ Therefore, IRE is clinically applicable in solid organs such as the liver and pancreas. However, the IRE procedure has a threshold when inserting the IRE probe directly into the lesion with the patient's abdomen opened in the operating room.¹⁰ As open surgery under general anesthesia can cause a burden to patients with advanced cancer, IRE is not commonly performed in real clinical practice even if this procedure has a certain level of efficacy. As it requires a computed tomography-guided percutaneous approach, which is invasive, it has certain limitations in terms of methodology. In the case of pancreatic cancer, another study has shown the feasibility of endoscopic ultrasound-guided IRE ablation in an animal experiment.¹¹ Therefore, we devised a method using endoscopic retrograde cholangiopancreatography (ERCP) as an alternative to insertion of an IRE ablation electrode into the

bile duct without open surgery. First, an IRE ablation electrode in the form of a catheter, which can be inserted into the lesions in the bile duct after cannulation during ERCP, was developed. In this study, we conducted an experiment using an animal model to determine whether this method is effective and whether IRE treatment can be performed in the bile duct using ERCP.

MATERIALS AND METHODS

Animals

This study was conducted in three Yorkshire pigs (Orient Laboratory Animal Company, Seongnam, Korea) weighing 35 ± 5 kg. All pigs were housed for acclimation in an animal facility for 7 days. They were kept in the facility in 12-hr night and day intervals in a controlled room where constant temperature (22°C – 24°C) and humidity (55%) were maintained. The animals were fasted the night before the experiment. After the intravenous injections of midazolam 0.5 mL/kg and ketamine 25 mg/kg, the procedure was performed under general anesthesia. This study was approved by the Institutional Animal Care & Use Committee of Korea University (approval no. KOREA-2019-0146).

Device

The experiment was conducted using the newly designed

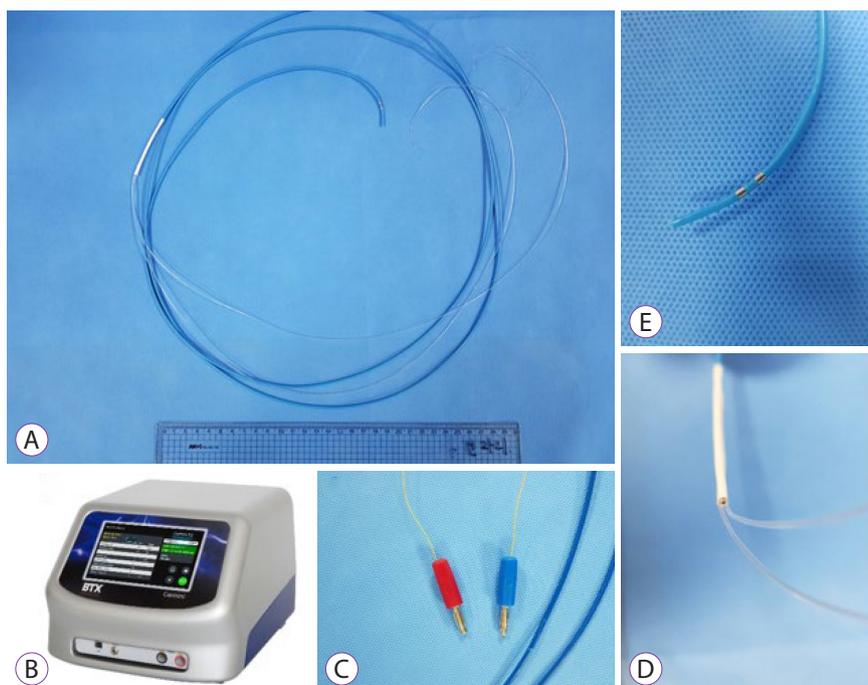


Fig. 1. Components of the newly designed endoscopic catheter-type irreversible electroporation electrode. Catheter-type irreversible electroporation (A) connecting to the main generator system. The Gemini Twin Wave BTX electroporation system (BTX Genetronics, San Diego, CA, USA) (B) consists of the banana connector (C) along with insulated wires (D) and two electrodes arranged at 5-mm intervals on the tip (E) transmitting energy to the tissue.

endoscopic catheter-type electrode. The device consisted of two electrodes (anode and cathode) arranged at 5-mm intervals on the tip and a long flexible catheter (shaped like an endoscopic nasobiliary drainage catheter) that can be inserted through the instrument channel of the endoscope. The cross section of the catheter is the structure in which the center hole through the guidewire and two insulated wires lead to the banana connector. The Gemini Twin Wave BTX electroporation system (BTX Genetronics, San Diego, CA, USA) was used for IRE ablation of the bile duct (Fig. 1). ERCP-guided IRE was performed using a duodenoscope (TJF-260V; Olympus, Tokyo, Japan).

Procedure

In this study, the catheter-shaped electrode was inserted into the bile duct during ERCP. The ERCP-guided approach was performed by placing the duodenoscope on the duodenum and subsequently inserting the guidewire into the bile duct after cannulation. After inserting the guidewire into the center hole of the electrode, the electrode was inserted through the instrument channel of the scope (Fig. 2). Through the C-arm X-ray view, the electrode inserted along the guidewire was confirmed to be at a suitable location in the bile duct. The IRE

protocol was proceeded following a setup that was considered to be appropriate in a previous pilot study.¹² The energy was delivered in a pulse wave form in 10 sequences of 5 pulses, with a pulse duration of 100 μ s, a pulse interval of 100 ms, an amplitude of 500 V/cm, and a repetition frequency of 1 Hz, with a 2-sec pause between the 5-pulse sequences.

Recent studies about the therapeutic effects of IRE for human or animal subjects were often conducted with a setting of 1,500–2,000 V/cm energy level. However, according to references, initial studies to check the safety of IRE began with a setting of 500–600 V/cm, which is the minimum energy level for IRE.^{6,13,14} As this study was an animal experiment with the aim of investigating the feasibility of a method of inserting the IRE probe into bile duct through ERCP, not through a percutaneous approach, the experiment was conducted with the minimum IRE energy of 500 V/cm rather than high energy setting of $\geq 1,000$ V/cm.

Postprocedure assessment

The pigs were sacrificed 24 hr after the IRE procedure. The bile ducts of all sacrificed pigs were resected for histopathologic evaluation. First, the samples were visually checked and subsequently formalin fixed. The bile duct resected in the axial

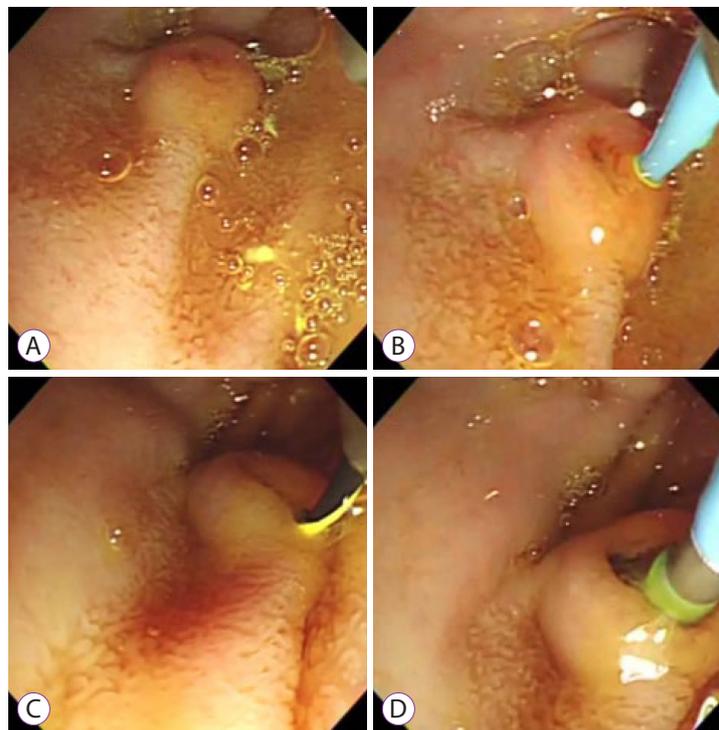


Fig. 2. Process of endoscopic retrograde cholangiopancreatography-guided endoscopic irreversible electroporation ablation. (A) After inserting the duodenoscope and targeting the ampulla of Vater, (B) cannulation was performed. (C) The guidewire was inserted, and (D) the electrode was inserted through the guidewire.

plane, near the IRE application point, was sectioned again in the axial plane at 2–3 mm thickness intervals for tissue slides. Paraffin-embedded tissue slides were stained with hematoxylin and eosin (H&E). Additionally, a terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate digoxigenin nick end labeling (TUNEL) assay was performed. A digital slide was created using a slide scanner (Leica SCN400 Image Viewer; Leica, Wetzlar, Germany), and a precise analysis was performed using its viewer software.

RESULTS

Ablation and clinical course

The bile ducts of the three female Yorkshire pigs were examined. All three pigs underwent IRE ablation using the ERCP-guided approach. Under ERCP guidance, the IRE catheter was well visualized on an X-ray view with the band of the electrode. The process of inserting the catheter-shaped electrode through ERCP also did not significantly differ from that of a typical ERCP procedure. IRE ablation targeting the bile duct was technically successful. No technical problems, such as an electric short circuit, occurred during the IRE ablation of the bile duct. The IRE ablation, performed using an amplitude of 1,000 V/cm, was completed in three pigs. The procedure was completed in all pigs under general anesthesia. The pigs were observed for 24 hr after the procedure, and no complications were noted.

Histopathology

The pigs were euthanized 24 hr after the experiment. Thereafter, the bile ducts were resected and the luminal mucous membranes of the specimens were visually checked. The size of the ablated region in specimen #1 was 10×5 mm and the depth was 3.1 mm (Fig. 3). The size and depth of the ablated region in specimen #2 and #3 were 13×6 mm and 3.3 mm and 8×5 mm and 2.6 mm, respectively. The average length and width were 10.3×5.3 mm, and the average depth was 3 mm (Table 1). Well-demarcated focal color changes were observed on the mucosa of the bile duct where IRE was applied. The boundary of the ablated region was also visually apparent, as seen after formalin fixation (Fig. 4).

At a low magnification of the H&E-stained slides, well-demarcated cellular changes were observed in the epithelium and lamina propria layer of the internal luminal wall of the bile duct. In the TUNEL assay, in which the tissue was dyed brown to detect apoptosis, apoptotic change was observed in the direction of the long axis of the bile duct. TUNEL immunohistochemistry showed cell death in the bile duct mucosa and submucosa along the electrode (Fig. 5). A positive TUNEL

assay suggests a cellular change resulting from apoptosis rather than necrosis.

At higher magnifications, the IRE-ablated region showed loss of nucleus and fibrotic changes compared with the normal epithelium (Fig. 6). However, the ablation depth was within 3 mm from the lumen, which means that it barely reached the mucous membrane and the submucosal layer. This finding indicates that the ablation depth was still insufficient.

Histopathology revealed loss of epithelium and fibrotic change in the IRE-ablated area, as well as increased lymphocyte numbers and inflammatory cell infiltration around the ablated zone. The presence of edema of the duct wall was not clearly identified.



Fig. 3. Gross specimen of the bile duct after endoscopic retrograde cholangiopancreatography-guided endoscopic irreversible electroporation (IRE). In the red circle, the ablated region in the bile duct was 1 cm long and 5 mm wide. Well-demarcated focal color changes were observed on the mucosa of the bile duct where IRE was applied. No evidence of burn, bleeding, or any mucosal damage was observed in areas where IRE ablation was not applied.

Table 1. The Size of Ablated Site in Each Animal's Bile Duct Specimen

Specimen	Length (mm) ^{a)}	Width (mm) ^{a)}	Depth (mm) ^{b)}
Specimen #1	10	5	3.1
Specimen #2	13	6	3.3
Specimen #3	8	5	2.6
Average	10.3	5.3	3.0

Values are presented as number.

^{a)}The visual findings of specimen were measured with a ruler. The result was round from the first decimal place.

^{b)}The deepest depth was measured using a microscope ruler. The number is rounded to the second decimal place.

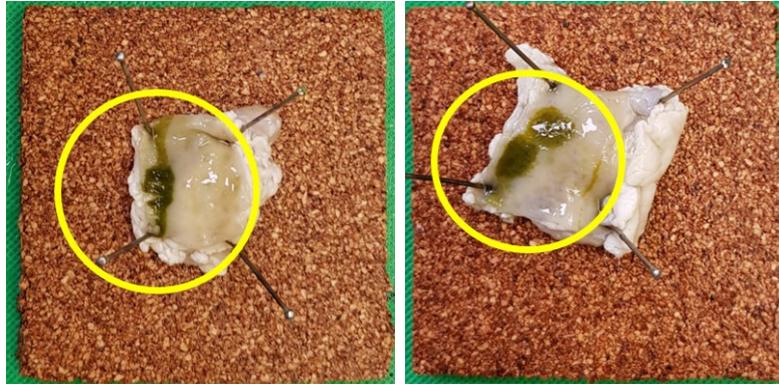


Fig. 4. Specimen with formalin fixation. In the yellow circle, the boundary of the ablated region was also visually apparent, as seen after formalin fixation.

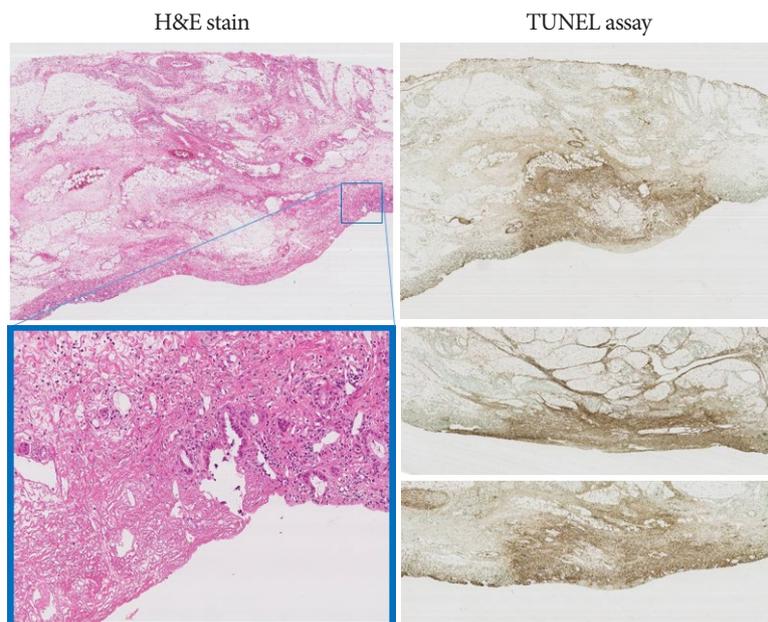


Fig. 5. Histopathologic findings of the bile duct in hematoxylin and eosin (H&E) staining and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate digoxigenin nick end labeling (TUNEL) assay ($\times 40$, $\times 100$). In the areas where irreversible electroporation was applied, the nucleus inside the cells were not observed (left). The area where irreversible electroporation was applied could be seen as a dark brown area in immunohistopathology with the TUNEL assay (right).

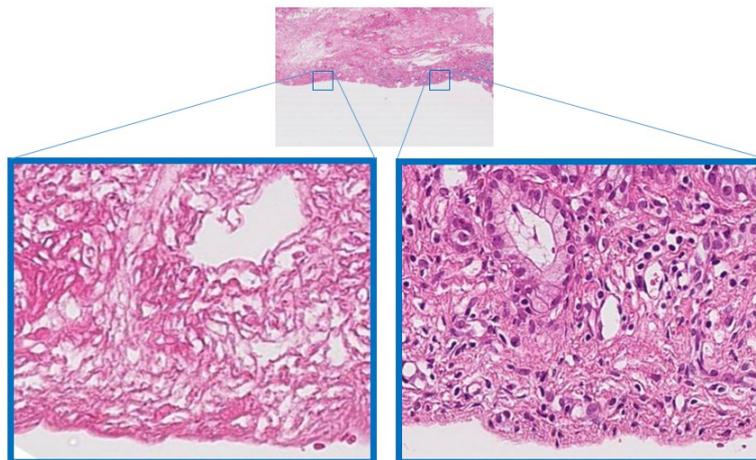


Fig. 6. Magnified images of the irreversible electroporation ablation area and the nonablated area in hematoxylin and eosin-stained sections ($\times 40$, $\times 400$). Intracellular nuclei were observed in normal cells (right). However, the nuclei were not observed in the irreversible electroporation-ablated area (left).

DISCUSSION

The current treatment based on systemic chemotherapy has limitations in improving the survival rate of patients with advanced cholangiocarcinoma.¹ ERCP is an effective treatment option for bile duct stenosis, which is one of the major issues in patients with cholangiocarcinoma, through endoscopic decompression of the stenosis in unresectable cholangiocarcinoma. This procedure is frequently performed in clinical practice. However, it is difficult to expect an improvement in the survival rate with this endoscopic treatment. Moreover, this method should only be used as a palliative and supportive treatment.² In addition to various studies using thermal or freezing methods such as radiofrequency ablation or cryotherapy, attempts of selective radioembolization using microspheres containing yttrium-90 have been made. However, these were only experimental trials owing to concerns about the possible occurrence of complications.¹⁵ Recent studies have been conducted to confirm whether IRE, a nonthermal method, could serve as a local tumor controller, rather than a thermal or freezing method with a high complication risk.¹⁶ Therefore, this study was planned with the expectation that IRE could be an effective treatment method if applied to the correct target lesion using a noninvasive approach via ERCP.

Cases of IRE performed in the pancreas, liver, prostate, and lungs have been reported in humans. Usman et al. reported an unsuccessful IRE ablation conducted using a computed tomography-guided percutaneous approach in two patients with unresectable lung tumors.¹⁷ The authors speculated that because the lung is not a solid organ, the air inside the lungs may have prohibited the deposition of energy. For this reason, IRE may have technical limitations when used as a local treatment for lung tumors. In previous human case studies, IRE ablation was effective for the treatment of tumors of the pancreas, liver, and prostate. A systematic review including four IRE studies conducted in patients with pancreatic cancer showed statistically significant differences in progression-free survival and overall survival between the IRE group and the non-IRE group.¹⁸ The review pointed out that a thermal procedure, such as radiofrequency ablation, for treating pancreatic cancer can damage the surrounding vascular structures; hence, scalability is limited. IRE does not cause a heat sink effect because it is a nonthermal-based ablation technique. Therefore, the structure and connective tissues of the target organ remain intact after the treatment. In Sutter et al., an IRE ablation targeting 75 hepatocellular carcinoma lesions in 58 patients showed an 87% and 70% progression-free survival at the 6-month and 12-month follow-up, respectively.¹⁹ Of 58 patients, 11 (19%) developed complications, whereas 3 (5.2%) had severe complications of grade III or higher. On the basis of this result,

the authors argued that IRE ablation is a safe and effective treatment method for patients who are not suitable for conventional radiofrequency ablation. In a previous clinical study using IRE ablation for prostate cancer, although the treatment had negative effects on urinary and erectile function, cancer cells were identified in the ablated zone on a follow-up biopsy 6 months after treatment induction.²⁰ Moreover, no residual cancer cells were noted in the ablated zone. Whether IRE ablation can improve the patients' survival is evident only when data are sufficient to confirm statistical significance. IRE ablation has received large attention from many researchers, as it is a promising option for cancer treatment.

Our experimental study was conducted to evaluate if ERCP-guided IRE ablation with insertion of a catheter-type electrode is feasible, safe, and effective. We verified the feasibility of the procedure; that is, whether the catheter-shaped electrode could be inserted through the instrument channel of the duodenoscope without damaging the surrounding tissues. C-arm X-ray confirmed that the electrode can be inserted into the target location in the bile duct of pigs. This technique has achieved technical advancement and can be directly applied to ERCP procedures in real clinical practice. It has also shown exceptional outcomes in terms of feasibility. After the IRE ablation, the pigs were closely monitored by experienced veterinarians every 8 hr for 24 hr, and no critical complications were observed. Subsequent necropsy showed no evidence of perforation, peritonitis, or injury of vascular structures, similar to other studies conducted in different organs.²¹⁻²³ Apoptosis, the result of electroporation, was confirmed by histopathologic evaluation; however, the evaluation showed insufficient depth of the ablated ductal wall. Compared with other studies, our study used lower energy levels as the wall of the bile duct with a tunnel-type structure is thinner than that of other solid organs such as the liver and pancreas. If the setting of IRE ablation is adjusted, it will cause sufficient apoptosis up to the target depth. On the basis of the results of this study, endoscopic IRE treatment using the newly developed catheter-type electrode is feasible and safe.

However, some limitations have been reported. First, the catheter-type electrode could not be used for ablation of the entire bile duct lumen. When ERCP-guided IRE ablation is used as a local treatment for bile duct tumors in the future, it is unclear if the electrodes can be evenly applied to an internal lumen narrowed by tumors. A solution is being considered to allow the electrodes to bond to the ballooning area of the catheter so that it can be applied evenly to the internal lumen of the bile duct. Another concern is the insufficient energy depth. To apply IRE ablation to cholangiocarcinoma lesions through ERCP in the future, setting to a low energy level for treatment should be considered a priority because of the narrow tunnel

shape of the bile duct, the thin ductal wall, and the adjacent major vessels such as the portal vein. As confirmed by H&E staining and the TUNEL assay, significant cellular changes induced by IRE ablation were only observed 2–3 mm from the lumen and were limited to the mucosal and submucosal layers. To apply this technique to real-world practice, the ablation depth should be >3 mm to induce transmural fibrosis and apoptosis. However, additional experiments are needed, taking into consideration the safety of the procedure at a higher energy intensity applied to reach the deeper layers. The IRE intensity used in this experiment did not cause any complications, such as burns. However, necrosis is expected to occur instead of apoptosis if higher energy intensities are used.²⁴

Endoscopic IRE ablation using ERCP was successfully performed in the common bile duct of pigs using a catheter-shaped electrode. If sufficient data can be collected to allow statistical analysis for optimization of the applied parameters, IRE can be a potential minimally invasive ablation technique for the treatment of biliary tumors. Further efforts are needed to improve the current technique of ERCP-guided IRE ablation for the treatment of bile duct tumors.

Conflicts of Interest

The authors have no potential conflicts of interest.

Funding

This research was supported by a 2018 Weolbong grant from the Korean Gastrointestinal Endoscopy Research Foundation and by a National Research Foundation of Korea grant funded by the Korean government (no. 2018R1C1B6006044).

ORCID

Kang Won Lee:	https://orcid.org/0000-0002-5902-9700
Jae Min Lee:	https://orcid.org/0000-0001-9553-5101
Hyeok Soon Choi:	https://orcid.org/0000-0002-4343-6950
Eun Sun Kim:	https://orcid.org/0000-0003-1820-459X
Bora Keum:	https://orcid.org/0000-0003-0391-1945
Yeon Seok Seo:	https://orcid.org/0000-0003-4171-6331
Yoon Tae Jeon:	https://orcid.org/0000-0003-0220-3816
Soon Ho Um:	https://orcid.org/0000-0002-4545-7907
Hong Sik Lee:	https://orcid.org/0000-0001-9726-5416
Hoon Jai Chun:	https://orcid.org/0000-0002-5539-361X
Chang Duck Kim:	https://orcid.org/0000-0003-1027-7890
Chi Hyeok Oh:	https://orcid.org/0000-0002-4382-5876
Hong Bae Kim:	https://orcid.org/0000-0002-1661-6585

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