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Irreversible[Q1] Electroporation of the Pancreas: Definitive Local Therapy Without Systemic Effects

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Background: The use of thermal tumor ablative techniques in the pancreas is limited due to the risk of pancreatitis and damage to major vascular structures. Irreversible electroporation (IRE) is a non-thermal ablation technique that could allow ablation in the pancreas while preserving vital surrounding blood vessels. The aim of this study was to assess the safety and ablation volume of IRE in porcine pancreatic tissue.
Methods: IRE of swine pancreases was performed using an open technique and ultrasound guidance and the animals were followed for 72 hr, 7 days, and 14 days post-IRE.
Results: All pigs underwent IRE, with one pig was unsuccessful after three attempts using at 3,000 V due to inability to achieve a stable current. All animals recovered well and revealed only mild adhesions, no ascites, and no pancreatic necrosis. All animals had transient increases in amylase and lipase that normalized on post-IRE day 3. Pathologic analysis revealed that ablation defects (median size 3 cm × 2.8 cm) were seen in electroporated areas with significant destruction of the pancreatic tissue with patent vascular structures.
Conclusions: This animal model demonstrates that IRE of the pancreas performed at an optimal voltage is well tolerated, with rapid resolution of pancreatic inflammation and preservation of vascular structures.
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KEY WORDS: pancreas; irreversible electroporation; ablation; cancer

INTRODUCTION

Ablative techniques such as radio frequency ablation (RFA) and microwave ablation have been widely used in treatment of patients with liver, lung, and kidney tumors. Ablative methods are limited in that they rely upon the indiscriminate use of thermal energy to induce necrosis of tumor cells, a process that can result in damage to nearby structures including blood vessels, bile ducts, and nerves. In addition, the blood flow of large vessels creates a heat sink effect that severely inhibits the ability to ablate cancer cells in the vicinity of large vessels [1]. These limitations are especially relevant to the pancreas which lies immediately adjacent to the superior mesenteric artery, portal vein, and common bile duct. Furthermore, the use of ablative therapies in the pancreas has largely been avoided altogether due to the possibility of thermal injury induced pancreatitis [2].

Irreversible electroporation (IRE) is an emerging technology for non-thermal tumor ablation. Electroporation utilizes targeted delivery of millisecond electrical pulses to induce permeabilization of cell membranes through nanoscale defects. Reversible electroporation has long been used as a technique for electotransfection of genetic material or intracellular drug delivery [3–6]. When the energy of the pulses is increased above a certain electric field threshold, the permeabilization becomes irreversible resulting in apoptosis [7]. This process leads to cell death but does not injure the extracellular matrix; thus allowing cellular tumor ablation while preserving structural components of tissues such as blood vessels [8–11]. IRE has been demonstrated to be successful in ablating cutaneous tumors in mice [12]. Animal models of tumor ablation using IRE have also been studied in rat and pig liver[Q2] [9,10], canine prostate [13], and canine brain [14].

IRE might potentially be used to safely ablate tumors of the pancreas with minimal damage to vascular or biliary structures. In the present study, a porcine model is used to investigate the safety, feasibility, and pathologic results of IRE ablation in the pancreas.

METHODS

Animals

This study was conducted in accordance with the National Institutes of Health guidelines for the care and use of animals in research, and the protocol was approved by the Animal Care and Use Committee of the University of Louisville. The University of Louisville animal care and use program is fully accredited by the American Association for Accreditation of Laboratory Animal Care, International. Six female all-white Yorkshire x Landrace swine (*Sus scrofa*) from Oak Hill Genetics, (Ewing, IL) were obtained at 8–10 months of age, weighing 85–90 kg at the time of arrival. During quarantine and acclimation animals were group-housed in pens with elevated fiberglass slatted floors providing a minimum 20 ft^2 per animal in a temperature (20.0–22.0°C) and humidity (30–70%) controlled room on a 12:12 hr light:dark cycle. Animals were housed individually in pens providing a minimum 24 ft^2 post-operatively, however, visual contact with conspecifics was maintained. Pens were cleaned daily and animals were fed 5084 Laboratory Porcine Diet Grower (LabDiet®, PMI® Nutrition International, Richmond, IN) twice daily in amounts recommended by the manufacturer. Animals were provided with filtered tap water ad libitum from arrival

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TABLE I. All Porcine Animals Who Underwent IRE of Pancreas

Animal no.	Harvest interval	Electrode	Probe spacing (cm)	Probe length (cm)	Volts	Pulse length (μsec)	Current range (amp)		Comments	Pulses delivered
							Low	High		
1	3 days	Monopolar	2.0	3.0	3,000	100	40	50	Over current shut off	33
		Monopolar	2.0	3.0	3,000	50	18	50	Over current shut off	44
		Monopolar	2.0	2.0	3,000	50	Lost	Lost	Aborted due to movement	30
		Monopolar	2.0	2.0	2,400				Charge/discharge error	0
2	3 days	Bipolar	0.8	0.7	2,700	70	14	24		90
3	7 days	Monopolar	1.5	3.0	2,300	100	28	32		90
4	7 days	Bipolar	0.8	0.7	2,700	70	11	20		90
5	14 days	Monopolar	2.0	2.0	3,000	100	25	50	Over current shut off	18
		Monopolar	2.0	2.0	2,000	100	17	22		90
6	14 days	Monopolar	1.5	2.0	2,300	100	23	47	Over current shut off	25

throughout the end of the study. Animals were specific pathogen free for the following pathogens: *Actinobacillus pleuropneumoniae*, *Mycoplasma pneumoniae*, Porcine Reproductive and Respiratory Syndrome, Atrophic Rhinitis, Pseudorabies virus, and Acute Malignant Hypothermia. Animals were free of internal and external parasites. Before any experimental manipulations were initiated animals were allowed to acclimate for at least 7 days. Veterinary staff assessed the animals before and after each procedure for general health, vital signs, oral intake, urine and fecal output, and for pain every 2 hr for the first 6 hr post-operatively and then every 12 hr until euthanasia. Baseline complete blood cell counts (CBC) and serum biochemistries were performed prior to surgery.

Ablation Technique

Fentanyl transdermal patches (50 μg/hr, Ortho-McNeil-Janssen Pharmaceuticals Inc., Raritan, NJ) were applied over the shoulders of the animals the evening before surgery. The animals were fasted for 12 hr, access to water was not restricted. The animals were anesthetized with subcutaneous 4.4 mg/kg Telazol® (Fort Dodge Animal Health®, Overland Park, KS), 2.2 mg/kg Ketamine (Fort Dodge Animal Health®), and 2.2 mg/kg Xylazine (Butler Animal Health®, Dublin, OH). The animals were then intubated and maintained on 1–3% Isoflurane (Butler Animal Health®) and 100% oxygen. Artificial Tears® ophthalmic ointment (Butler Animal Health®), was applied to the surface of both eyes. Body temperature was maintained with a warm air circulating blanket (Bair Hugger®, Arizant Healthcare®, Eden Prairie, MN). A 20-gauge intravenous catheter was placed in the marginal ear vein and 10 ml/kg/hr Normosol-R® (Hospira®, Lake Forest, IL) was administered to maintain blood pressure. Each animal received 2 mg/kg Carprofen IV (Pfizer Animal Health®, New York, NY), and 0.04 mg/kg Buprenorphine IV (Butler Animal Health®) for analgesia prior to surgery. The animals were placed on mechanical ventilation and heart rate, body temperature, SpO_2, reflexes, and blood pressure were monitored continuously throughout the anesthetic procedure to maintain the animals in a surgical plane of anesthesia. To control muscle twitches during IRE, 30 mg succinylcholine (Butler Animal Health®) was administered IV and was readministered as needed during the procedure based on muscle twitches of the animal. Each animal was placed in dorsal recumbency; a 7 French polysulfone vascular access port (VAP, Access Technologies®, Skokie, IL) was implanted in the external jugular vein for refinement of blood collection, as well as drug and fluid administration post-operatively. The VAPs were exteriorized on the dorsal neck due to the relatively short duration of the study and to minimize potential pain and distress to the animals of repeated daily access. Access to the pancreas was made through a ventral midline incision.

Ablations were performed using 19-gauge monopolar electrodes or 16-gauge bipolar electrodes. The electrodes were place within the pancreas in 1-mm proximity of the portal vein or mesenteric artery using continuous ultrasound guidance. Monopolar electrodes were spaced at varying intervals of 1.5 cm or 2.0 cm using an attached spacing device. The depth of exposed electrode was also varied depending on the vascular anatomy and size of the pancreas available.

The IRE current generator (NanoKnife™; AngioDynamics®: Queensbury.[Q3] Energy output – 3 kV, 50-amp maximum energy output) was synchronized to deliver electrical pulses coordinated with the pig's cardiac rhythm in order to prevent cardiac dysrhythmias. Each ablation began with a test pulse at 10% planned energy output to assess that an adequate current was achieved. Following successful test pulse, the therapeutic pulses were then delivered. As has been previously reported in prior animal models, the goal of treatment is to deliver 90 electrical micro-second pulses in groups of 10, with a pulse duration of 100 μsec and a pulse interval of 250 msec. These parameters were chosen as a conservative estimate

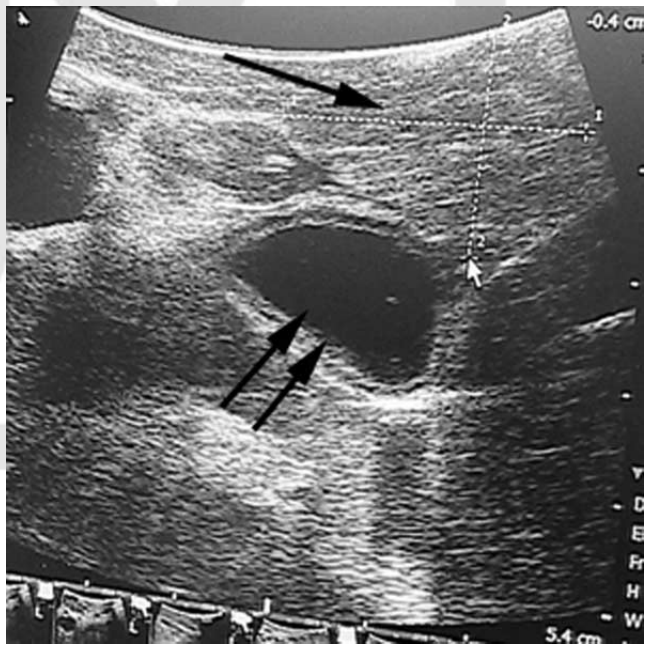

Fig. 1. Ultrasound image after IRE to porcine pancreas with degree of electroporation (single arrow) and intake portal vein (two arrows).

of the electrical field needed to induce IRE in a pancreatic tumor. The high number of pulses tested the greatest potential thermal damage as well. Ablation sizes were measured as longest length (i.e., along the probe) and longest width, with depth not being able to be assessed since all porcine pancreases are only approx 1.5–2.0 cm deep.

Animal Evaluation

The animals were monitored continuously after the surgical procedure until they were able to be extubated and were fully recovered from anesthesia. The animals were only allowed water in the first 12 hr post-procedure, but they were then advanced to solids as tolerated. Blood glucose measurements were performed at 1, 3, and 5 hr post-ablation to monitor for hypoglycemia. The pigs were followed with daily assessment of activity, pain, and dietary intake. All animals received 4 mg/kg carprofen orally, once daily for 48 hr post-operatively in addition to transdermal Fentanyl patches which remained in place for 72 hr post-operatively for analgesia. VAPs were maintained by daily flushing with sodium chloride (Butler Animal Health®) following blood collection and were locked with a 2% taurolidine-citrate lock solution (Access Technologies®). Daily laboratory analysis was conducted that included liver enzymes, amylase, lipase, cholesterol, complete blood count, BUN, and creatinine. Two pigs each were euthanized at intervals of 72 hr, 7 days, and 14 days post-procedure. Pigs were anesthetized with Telazol, Ketamine, and Xylazine subcutaneously as previously described, intubated and maintained on 1–3% Isoflurane. Each animal received 20,000 IU Heparin IV (Butler Animal Health®), and was then euthanatized with 2 mmol/kg Potassium chloride IV (Butler Animal Health®). Necropsies were performed and pancreatic tissue was harvested, along with portions of the adjacent portal vein, mesenteric artery, and bile duct.

Tissue Histology

Following harvest, tissues were serially sectioned at 5-mm intervals, fixed in 10% neutral buffered formalin, and embedded in paraffin blocks. Sections were cut and stained with hematoxylin and eosin for microscopic analysis. In order to assess for areas of apoptosis, terminal transferase dUTP nick end labeling (TUNEL) assays were performed on select sections using a In Situ Apoptosis Detection Kit (ApopTag; Intergen Company, Purchase, NY) according to the manufacturer's protocol. The TUNEL-positive cells were counted against negative cells under a light microscope at a magnification of 40×, and 6 visual fields were chosen on the ablated areas, areas adjacent

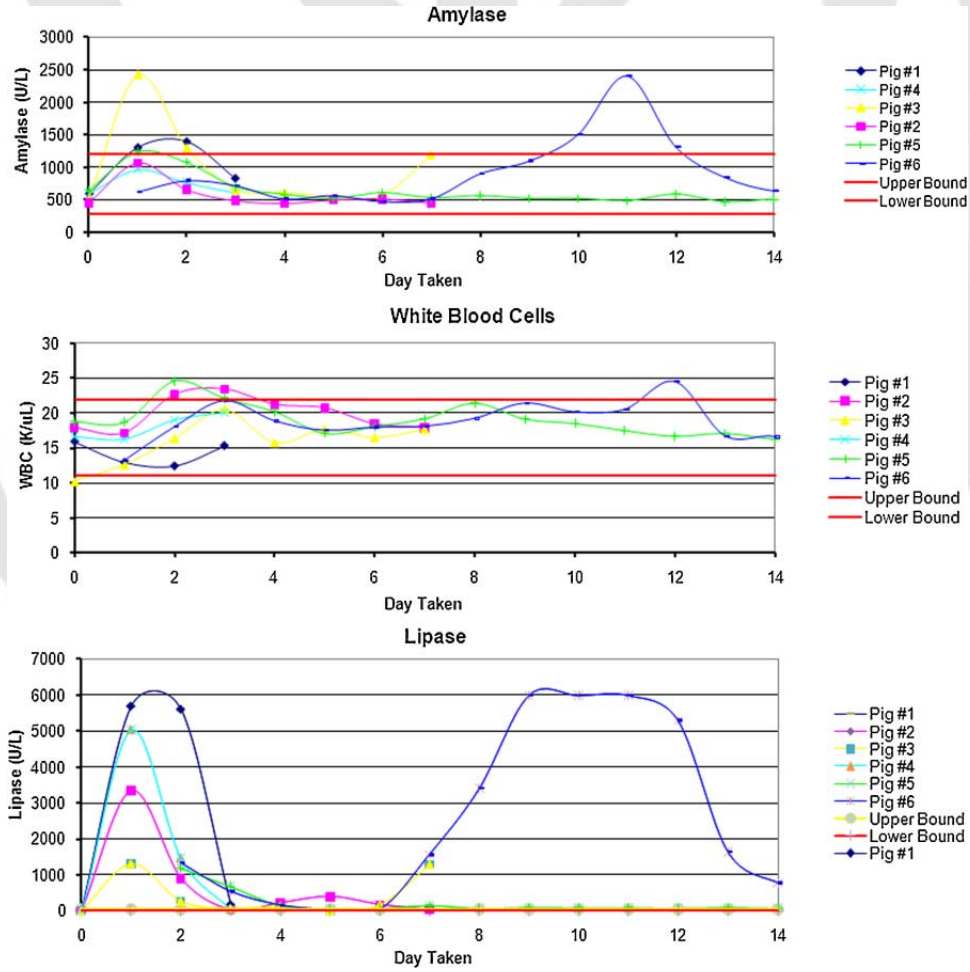

Fig. 2. Serial amylase, lipase, and white blood cell count in all animals on the day of IRE or Pancreas (Day 0) and until euthanasia. Single animal had wound infection on day 8, which required wound opening and antibiotics with resolution of elevated levels.

to the ablation, and random normal pancreatic parenchyma. An apoptotic index (the number of epithelial nuclei labeled by the TUNEL method/the number of total nuclei × 100) was calculated.

RESULTS

Treatment

A complete treatment was delivered in 4 out of 6 pigs (Table I). Delivery in one pig was unsuccessful using a 3-cm length, 2-cm spaced monopolar probe at 3,000 V due to inability to induce a stable current after three treatment attempts yielded 33, 44, and 30 pulses. The reason for these failed attempts was both the probe exposure (>2 cm in length) and the amount of voltage that was attempting to be delivered. The greater probe exposure increases the resistance and thus leads to greater voltage and a high current warning. Total delivery also failed in a 14-day pig with too closely spaced (1.5 cm) monopolar probes used in the attempt to deliver 2,300 V, with only 25 pulses given. An additional pig had an incomplete delivery at 3,000 V using monopolar electrodes spaced 2 cm, yielding only 18 pulses. A repeat ablation in the same pig at 2,000 V was successful, yielding 90 pulses.

At the conclusion of each ablation, ultrasound evaluation revealed changes consistent with treatment effect (Fig. 1). All pigs tolerated the procedure without immediate complications, and there were no associated cardiac dysrhythmias detected.

Physiologic Outcome

All animals recovered well during the early post-operative phase, and were drinking liquids by 6 hr post-procedure. Pigs in the 7- and 14-day groups received 20 mg/kg Cefazolin (Butler Animal Health®) IV, twice daily beginning on POD 3 due to increases in body temperature and elevations in WBC. The pig that failed multiple delivery attempts became anorexic and sluggish while all other animals were active and eating by post-operative day 2. Supportive care for the anorexic animal included intravenous sodium chloride. All animals developed only transient increases in WBC, amylase and lipase that began to normalize by post-operative day 3 (Fig. 2). One animal that was euthanized at day 14 developed a secondary rise in amylase thought to be related to a wound infection which returned to normal after the wound was opened and packed. BUN and creatinine remained within normal limits for all animals. ALT levels remained elevated throughout for all pigs at 0 to 60 U/L above normal. All animals experienced a transient hypoglycemia at 1 and 3 hr post-operatively that began resolving by 5 hr and was normalized by the first post-operative day. Only two animals required a dose of 100 ml intravenous 5% Dextrose in Sodium Chloride (Butler Animal Health®) for blood glucose levels of 40 mg/dl at 3 hr post-operatively. The animals responded well to the dextrose supplement and there were no complications associated with the VAP implants throughout the study.

Pathologic Outcome

Gross findings at necropsy revealed mild adhesions, no ascites, and no pancreatic necrosis. Otherwise there were no significance differences noted between the pigs at the 72-hr, 7-day, and 14-day harvest intervals. On histologic staining there where sharp demarcations of the margins between necrotic ablated areas and the surrounding non-ablated parenchyma (Fig. 3). There was also significant replacement of destroyed pancreatic tissue with infiltrating foamy macrophages. Ablation volumes were consistent across all animals when a monopolar or bipolar probe was used (Table I). Necrosis of pancreatic cells was seen immediately adjacent to vascular structures. Venous as well as arterial structures were widely patent without any evidence of thrombosis at the 72-hr, 7-day, and 14-day time intervals. Results of the TUNEL assay revealed that areas of cell death due to apoptosis were induced by the IRE throughout the ablated areas. The apoptotic index in the ablated tissue was significantly elevated compared to the non-ablated pancreas (Ablation field: 95.3 ± 18.6 vs. Distal field: 3.2 ± 1.5, $P < 0.05$) (Fig. 4).

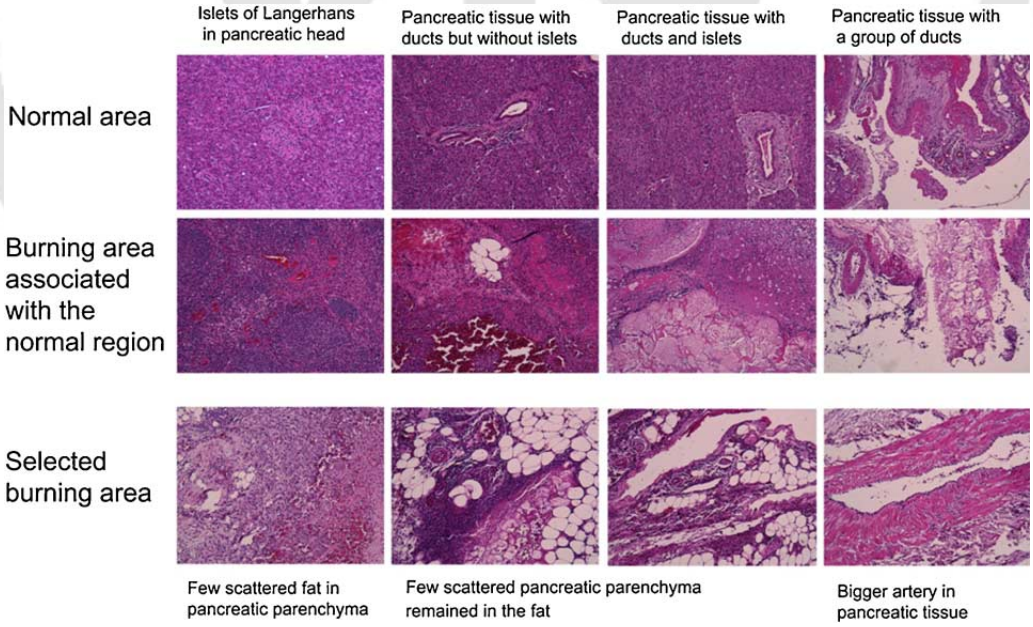

Fig. 3. Day 14 animal H&E staining demonstrating resolving histologic changes in the pancreas as well as intake artery, vein, and pancreatic duct.

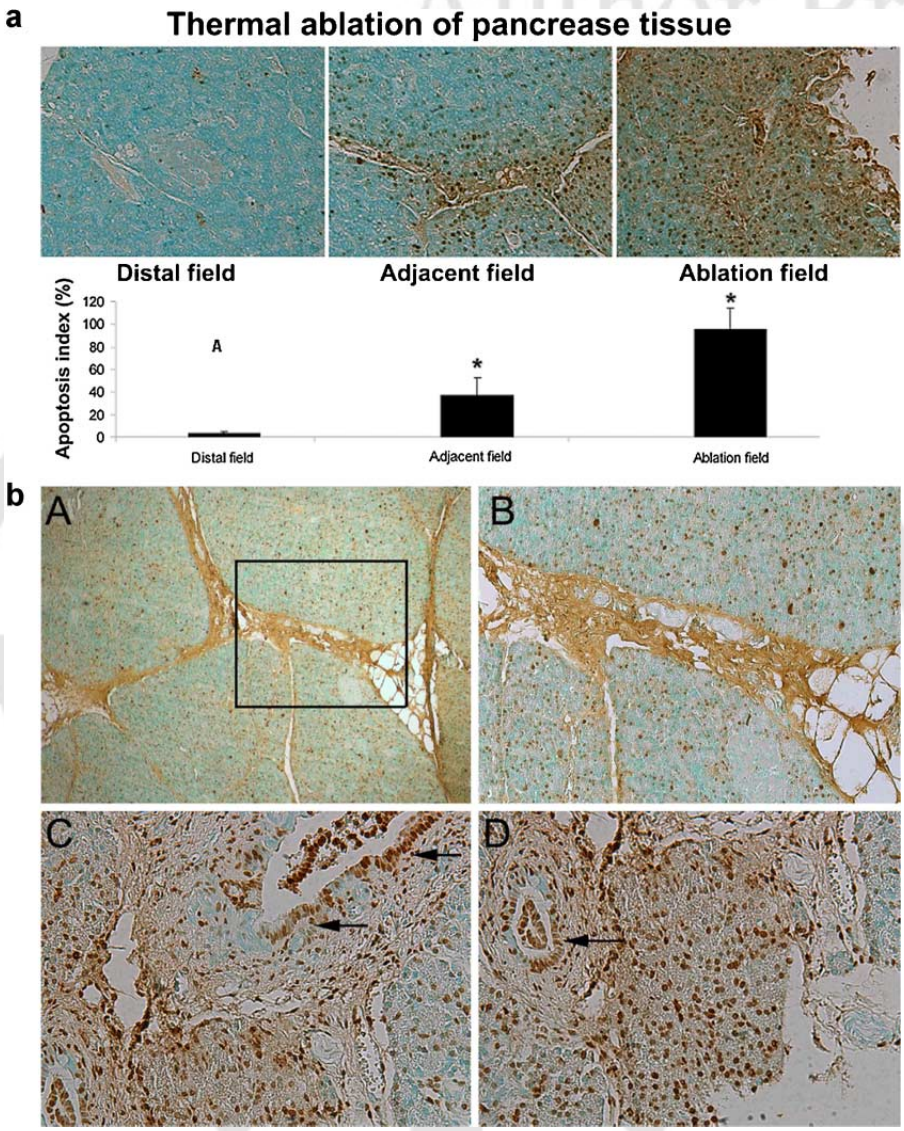

Fig. 4. (**a**) Extent of Apoptotic index at pancreatic ablated sections from a seven days survival animal. (**b**) Representative slides of apoptotic pancreatic tissue at low power (**A**), high power (**B**), with representative intact pancreatic duct (**C**, 2 arrows), and higher power pancreatic duct (**D**, one arrow) with obvious apoptosis ongoing from a 14 day animal.

DISCUSSION

The results of this study provide preliminary evidence that IRE may be safely performed upon the pancreas. This data suggest that adequate pancreatic IRE tumor ablations can be performed without significant risk of inducing clinically significant pancreatitis or surrounding vascular injury-thrombosis. IRE can generate some degree of thermal injury when the resistance to voltage generated reaches a threshold, commonly seen when over electroporation is performed, or when the resistance is over ridden by the treating physician (Fig. 5), which was the etiology of the adverse event in this animal. IRE parameters are designed to minimize the extent of thermal injury as long as precise spacing is confirmed prior to initiating therapy.

We did not observe any discernible differences in outcome based upon probe type and placement other than that the bipolar probes are placed more easily. The goal of electrode placement is to induce an IRE field that encompasses the entire tumor. The outcome of IRE is influenced by number of parameters including pulse shape, length, frequency, electrical field strength, voltage applied to the electrodes, electrode shape, electrode size, and tissue type [15–17]. However, accurate field prediction based on mathematical models and in vitro studies has been performed for IRE in some tumor types. The predicted IRE parameters are designed to allow prediction of the ablation field, determine maximum ablation, and minimize the thermal effects [17–19]. Such studies have yet to be conducted for tumors of the pancreas.

Accurate prediction of the IRE field may be less of an issue due to the fact that ultrasound imaging reveals tissue changes of the ablation. We were able to easily note a defect in the pancreas tissue by ultrasound following the ablation. Lee et al. performed IRE in porcine livers and visualized the treatment effect by ultrasound. In their experience, the measured ultrasound changes corresponded accurately with the extent of the ablation by histology [20]. Recent

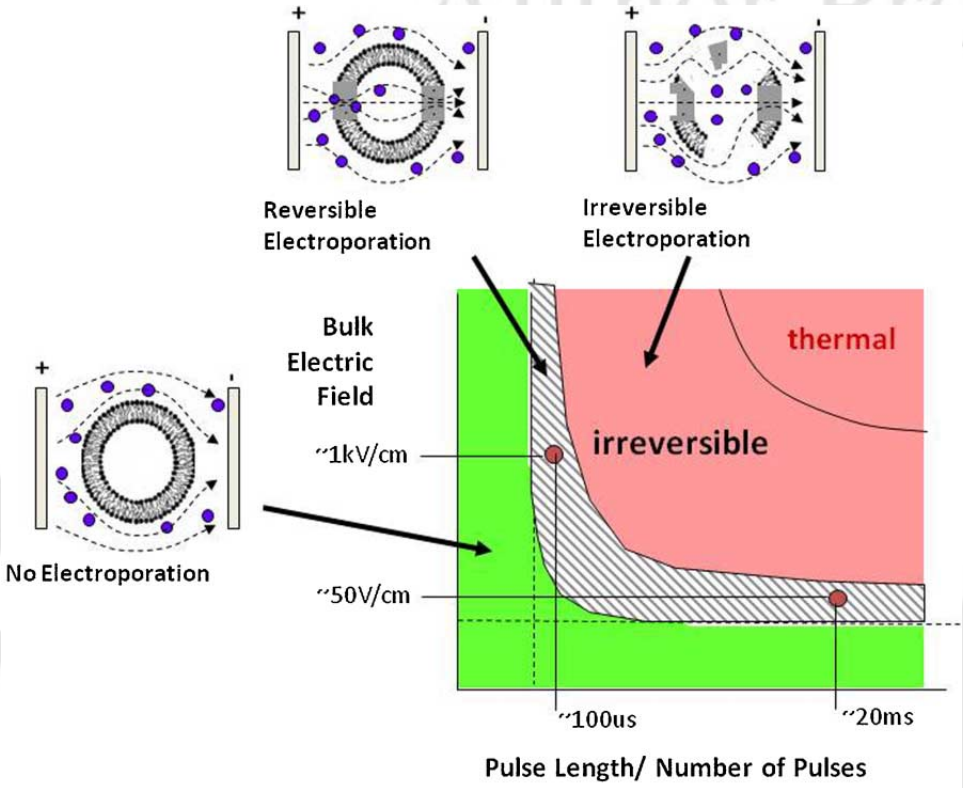

Fig. 5. Zones of reversible, irreversible, and thermal ablation based on pulsed length and electric field.

studies have also offered the intriguing possibility that the effectiveness of tumor ablation may be assessed by post-ablation conductivity measurements, or even determined in real-time imagery of the ablation process using electrical impedance tomography [21].

It is also noteworthy that placement of 16- to 19-gauge electrodes into the pancreas does not lead to pancreatic leak or pancreatitis. Core needle biopsy of the pancreas by open technique is generally avoided due to the high risk of pancreatic leak and pancreatitis. The IRE process itself may have potentially minimized this risk.

Previous ablative therapies have also been utilized in the pancreas with mixed results. The initial results from Goldberg et al. demonstrated that EUS-guided radio frequency can be used in the establishing discrete zones of coagulative necrosis in a porcine model [22]. Similarly, EUS-guided Nd:YAG laser ablation has also been utilized with inducing tissue necrosis with a tissue ablation volume of 314 to 483 mm^3 [23]. Alcohol ablation has also been utilized, again with reports of tissue necrosis and EUS guidance [24]. The key difference in all these reported studies and others is that with IRE, immediate tissue necrosis is not the method of action. This could potentially lead to safer application and the ability to avoid relying on the diffusion of therapy (ethanol) or the passive distribution of heat (radio frequency) [2,25–27]. Additional studies, comparisons, and, most importantly, patient validation will remain the deciding factor in determining which of these ablative therapies are optimal.

CONCLUSION

The primary outcome of the present study was establishing the safety and potential use of IRE in the minimally invasive treatment of pancreatic tumors. The histologic results confirm that IRE of the pancreas leads to cell death with preservation of vascular structures. Furthermore, apoptosis played a key role in the cell death induced by IRE. Cell death through apoptosis potentially avoids the fibrosis, scaring, and loss of tissue architecture that result from coagulative thermal necrosis caused by radio frequency techniques. Future investigations should be conducted to establish the electrical field threshold required for IRE of pancreatic cancer cells. Optimized treatment IRE parameters and protocols for the pancreas also remain to be established.

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