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A randomized trial comparing effects of radiofrequency and cryoablation on the structural integrity of esophageal tissue

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Abstract
Background Esophageal injury is a rare, but catastrophic complication of radiofrequency (RF) pulmonary vein isolation. It is not known if cryoablation is less likely to injure esophageal tissue. The purpose of this study is to compare the effects of RF and cryoablation on the structural integrity of esophageal tissue.
Methods and results Porcine esophageal tissue was sectioned into 396 strips measuring 3 mm in width by 30 mm in length. Samples were randomly assigned to receive no ablation (149 specimens in the control group), RF ablation (126 specimens) or cryoablation (121 specimens). A single ablation was administered in the center of the tissue sample. A force gauge was used to measure the tensile strength of the tissue sample in Newtons. Groups were compared using ANOVA and a Bonferroni post-test. The mean tensile strength in the control group was 2.19 N (SD, 2.17), 1.66 N (SD, 0.88) for RF ablated tissue and 1.96 N (SD, 1.68) for cryo. RF ablation resulted in a significant reduction in esophageal tensile strength when compared to control (t= 2.59), however cryo did not (t=1.11). On microscopic evaluation RF ablation disrupted elastic fiber architecture whereas cryoablation did not.
Conclusions Cryoablation has no significant adverse impact on the structural integrity of esophageal tissue. Cryoablation may be a safer alternative to RF for left atrial ablation and reduce the risk of esophageal injury and atrial-esophageal fistula formation.

Keywords Catheter ablation · Atrial fibrillation · Esophageal perforation

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1 Introduction

Over the last decade percutaneous catheter ablation of atrial fibrillation has become a common procedure. It is becoming increasingly effective in eliminating the arrhythmia [1, 2] and has been shown to improve quality of life when compared to pharmacologic rhythm management [3, 4]. Pulmonary veins have been targeted for isolation, since they are frequently the source of focal activity that triggers AF episodes [4, 5]. As the procedure has evolved, extra-ostial pulmonary vein isolation or left atrial circumferential ablation has largely replaced focal ablation within the pulmonary vein because of increased efficacy and the reduced risk of pulmonary vein stenosis [6, 7]. However, ablation at these sites carries a risk of collateral injury to adjacent structures, including esophagus and phrenic nerve [8]. Esophageal injury is a rare, but catastrophic complication of LA ablation. Patients typically present several days-to-weeks after ablation and may complain of fever, neurologic symptoms, chest pain, odynophagia, or hematemesis [9–13]. The incidence has been reported to be as high as 1.2% following operative AF ablations using RF energy [14]. Atrial-esophageal fistula following RF catheter ablation of AF was first noted in 2004. It is estimated to occur in at least 0.05% of cases, but is frequently fatal [15].

The mechanism of RF ablation is thermal injury to the tissue created by direct heating from the RF source and

conductive heating to deeper tissue layers [16]. High tissue temperatures cause thermal denaturation of structural proteins, including collagen [17]. In contrast, cryothermal ablation causes tissue injury by damage to organelles and the sarcolemmal membrane by ice lattice formation and dissolution [18]. Structural cellular elements are left intact [19]. We hypothesized that lesions created with cryoablation might be less prone to tissue disruption than those formed with RF ablation, and that this would, in turn, decrease the likelihood of esophageal tissue disruption. This finding might ultimately translate into a lower risk of esophageal perforation after ablation of the contiguous left atrium.

2 Materials and methods

Sample preparation The study was approved by the William Beaumont Hospital Animal Care Committee. Eight common farm swine, which were being euthanized as part of another unrelated protocol, were utilized. In seven pigs the esophagus was harvested immediately following euthanasia (*in-vitro* portion of study). The porcine esophageal tissue was sectioned longitudinally into strips measuring 3 mm in width by 30 mm in length and placed in a saline solution. Two additional 1×1 cm tissue specimens were prepared for histopathologic examination of *in-vitro* ablation with RF and cryo. In one pig RF and cryoablation was performed prior to euthanasia (*in-vivo* portion of study) on the serosal surface of the esophagus, under general anesthesia, through an abdominal incision accessing the esophagus as it crosses the diaphragm.

Ablation methods All testing was performed at room temperature. For *in-vitro* testing each 3×30 mm sample was selected from the saline solution and clamped proximally and distally with hemostats. Using a random numbers table, the sample was then assigned to receive no ablation (control), RF ablation or cryoablation. RF ablation was performed in a half normal saline solution to provide adequate impedance for energy delivery. An 8 Fr 5 mm tipped ablation catheter (Blazer II HTD, Boston Scientific Corporation, Natick, MA, USA) was used. RF energy was delivered using temperature–feedback power control, with a target temperature of 70°C, a maximum power of 20 W, and duration of 60 s. A single lesion was placed in the center of each tissue sample. The electrode was positioned perpendicular to the axis of the tissue sample in order to assure that the lesions created extended the entire width of the sample. The tissue was returned to room temperature and tensile strength testing was performed within 5 min. Cryoablation was performed in a similar fashion at room temperature in a bath of half normal saline solution. A 7 Fr 6-mm tip cryoablation catheter (Cryocath Freezor Xtra 5, CryoCath Technologies Inc., Montreal, Quebec, Canada) was used to create the lesion using temperature feedback control, with a target temperature of −80°C and duration of 300 s.

Tensile strength measurements The tensile strength of each tissue specimen was used as an indicator of tissue integrity. Each longitudinal tissue sample was secured on each end with a clamp. One clamp was connected to a force gauge hanging from a table stand, and the opposite clamp was connected by a noncompliant wire to a reel. The reel was gradually turned, and increasing linear tension was progressively applied with increasing tension applied to the longitudinally oriented tissue specimen until it avulsed into two pieces. The maximum tension measured in Newtons (N) prior to tissue avulsion was recorded.

Statistical methods Data are reported as a mean, standard deviation, and 95% confidence intervals (CI). An ANOVA test was used to evaluate for a difference among the three groups and additional comparisons between groups were performed using the Bonferroni post-test.

Histopathology Tissue blocks prepared for histopathologic examination of *in-vitro* and *in-vivo* ablation using RF and cryoablation were immediately fixed with 10% neutral buffered formalin. Specimens were then dehydrated in alcohol and embedded in paraffin wax. Five-micron slices were prepared and stained using hematoxylin and eosin (H&E), Masson's Trichrome (MT), and Elastica-Van Gieson's (EVG) stains. Multiple segments of each pathological specimen were examined, and observed histopathological abnormalities were recorded.

3 Results

A total of 396 swine esophageal specimens were prepared for tensile strength testing. Using the random number table for assignment, 149 specimens were randomized to the control group, 126 were randomized to the RF ablation group, and 121 were randomized to the cryoablation group. The mean tensile strength of the tissue specimens in the control group was 2.19 N, SD 2.17, 95% CI: 1.92–2.46. The tensile strength of tissues ablated using RF energy was 1.66 N, SD 0.88, 95% CI: 1.36–1.96. The mean tensile strength of tissues subjected to cryoablation was 1.96 N, SD 1.68, 95% CI: 1.66–2.26. Using an ANOVA test there was a statistically significant difference between the three

treatment groups (p=0.036). When comparing RF ablation to control using the Bonferroni post-test there was a significant reduction in esophageal tissue strength (t= 2.59). In contrast, cryoablation did not appear to have a statistically significant effect on esophageal tensile strength acutely when compared to control (t=1.11). See Fig. 1. The dominant site of avulsion was at the lesion midpoint in the RF ablation specimens, but frequently occurred at the site of tissue clamping in the controls and the cryoablation specimens.

Esophageal lesions created with RF ablation showed a gross appearance of a central zone of pallor with volume loss at the lesion center. In contrast, the cryolesion was not perceptible on gross examination (Fig. 2). Histopathological examination of the specimens with H&E and MT staining revealed evidence of coagulation necrosis and nuclear pyknosis in the ablated region of the RF specimens. The cryoablated tissue displayed minimal evidence of cellular injury (Fig. 3). Staining with EVG stain displayed clear disruption of elastic fibers within the RF lesion with sparing of elastic fibers in the non-ablated areas. The specimen ablated with cryothermal energy had normal appearing elastic fibers (Fig. 4). Evaluation of the *in-vivo* specimens revealed myofiber palor, early inflammatory infiltration, and hemorrhage in the RF ablation specimen with H&E staining while the cryoablation specimen demonstrated minimal pallor. With MT staining the cryoablated segment was indistinguishable from non-ablated tissue. The RF lesion was well demarcated and involved the outer longitudinal and inner circular of the muscularis propria and extended into the muscularis mucosa (Fig. 5). The disruption of elastic fibers noted with *in-vitro* RF ablation was also demonstrated with EVG staining of the *in-vivo* ablation while the elastic fibers in the cryoablated specimen appeared normal (see Fig. 6).

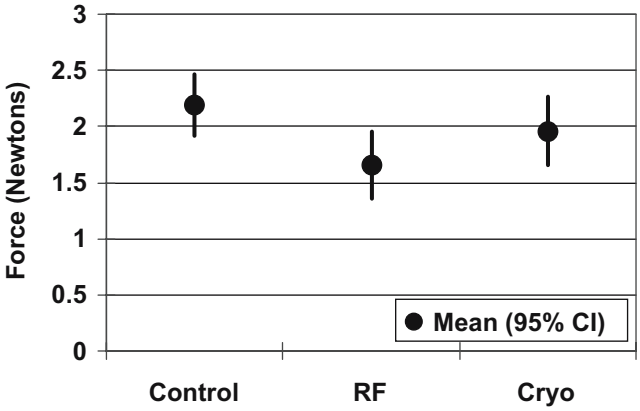

Fig. 1 Displays the tensile strength of tissues in the control group, RF ablation and cryoablation groups. The *points* represent mean tensile strength in Newtons and the *bars* represent 95% confidence intervals

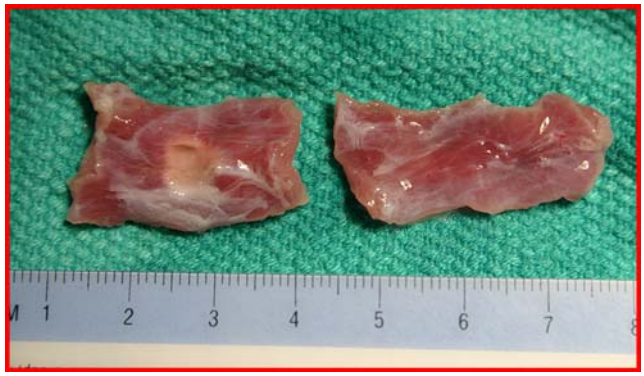

Fig. 2 Presents the gross appearance of *in-vitro* ablation with the RF lesion on the left showing pallor and volume loss. In contrast the cryolesion shown on the right is not perceptible

4 Discussion

The present study demonstrates that the response of the esophagus to ablation using hypothermia with cryoablation is different than the response to hyperthermic injury with RF ablation. The tensile strength of esophageal tissue acutely after ablation was not significantly different between cryoablated samples and control samples with no ablation at all. The microscopic appearance of elastic fibers in the region of cryoablation appeared preserved. In contrast, RF energy appeared to result in acute degradation of esophageal tissue strength. Clear evidence of disruption of elastic fiber architecture was observed in the RF lesions created *in-vitro* and *in-vivo*. Thus, it appeared that hyperthermic ablation was associated with greater degradation of the structural integrity of the esophageal tissue.

Radiofrequency has been the traditional energy source for catheter ablation of most arrhythmias since 1986, but may not be the ideal energy source for catheter ablation of AF. Lesions are produced through resistive and conductive heating, which causes irreversible myocytes injury at temperatures exceeding 50°C [16]. Temperatures exceeding 60°C have been shown to result in denaturation of collagen and loss of elasticity and compliance in pulmonary veins [17]. RF creates myocardial lesions with intralesional hemorrhage and disruption of endothelial cell integrity. [20, 21] Lesion size and depth depend on the complex interaction of catheter tip design, power and temperature settings, duration of ablation, effects of cavitary blood flow, patient anatomy (characterized by variable myocardial thickness at the LA/PV junction), and stability and contact pressure at the tissue/catheter interface. [22] Because of the numerous variables, it is sometimes difficult to assure creation of a transmural lesion without applying excess energy. The excess energy delivery can, in turn lead to injury of adjacent structures (esophagus or phrenic nerve), pulmonary vein stenosis [8], or char and thrombus formation [23].

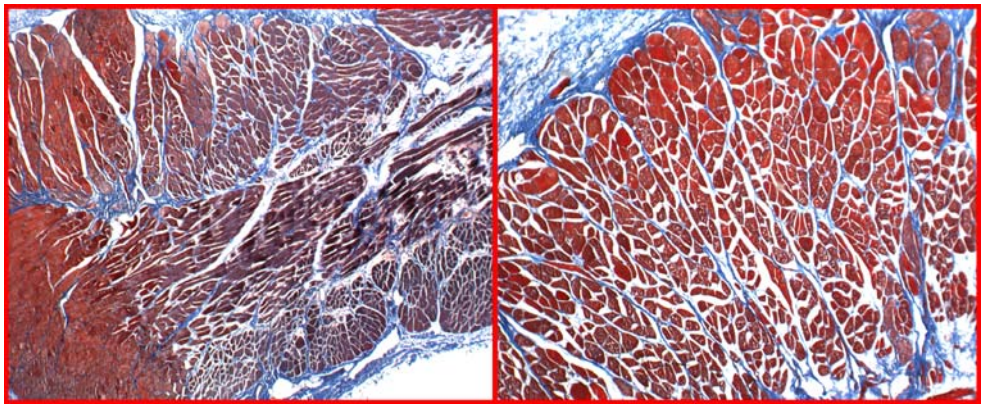

Fig. 3 Shows Masson's Trichrome staining of *in-vitro* esophageal lesions created using RF energy (*left*) and cryothermal energy (*right*). Note the dark staining of the entire muscularis propria within the RF lesion. In contrast the cryolesion is not well demarcated from the surrounding normal tissue

Several new catheter designs with alternative energy technologies are emerging which may be useful for catheter ablation of atrial fibrillation in the future. Like radiofrequency energy, laser [24] and microwave [25] rely upon hyperthermia to cause coagulative necrosis. In contrast, cryothermal energy produces lesions through hypothermic exposure, with a very different mechanism of tissue injury. Cryothermal energy has been used for cardiac ablation since 1977 [26], but catheter cryoablation has been a more recent development. Cooling cardiac tissues results in conduction slowing and eventually block when 0 to −20°C is approached [27, 28]. These electrophysiologic modifications are temporary allowing cryomapping prior to permanent lesion creation. This may be advantageous in the right superior pulmonary vein where the phrenic nerve is in close proximity to the vein and nerve injury resulting in hemidiaphragm paralysis with RF ablation can occur [29, 30]. Permanent cryolesions are created when temperatures are reduced to −60 to −80°C [31–33]. Interestingly the cell membrane remains intact, but ice crystal formation increases mitochondrial membrane permeability, de-energizing the mitochondria, leading to cellular ischemia and coagulative necrosis [18]. Lesions created with cryothermal energy are generally well circumscribed and homogeneous with less disruption of the extracellular matrix architecture [19, 34]. Several studies have demonstrated the absence of pulmonary vein stenosis following pulmonary vein isolation (PVI) utilizing cryotherapy [29, 35–37]. In addition, endothelial cell integrity is preserved which makes the lesions less thrombogenic [20, 38]. This may result in a lower embolic risk acutely and during follow-up. With the development of a cryoballoon catheter, rapid and efficient pulmonary vein isolation using cryothermal energy may soon be feasible [36].

Over the past decade, the trend in catheter ablation of atrial fibrillation has been to destroy more atrial tissue in ever widening circles around the pulmonary veins [39]. As the volume of ablated LA increases, the concern about collateral injury to adjacent structures also increases. Esophageal injury and atrial-esophageal fistula formation is a rare complication of this elective procedure, but is frequently fatal. Presently, efforts have been made to visualize the esophagus during ablation procedures and avoid RF energy delivery directly contiguous to this

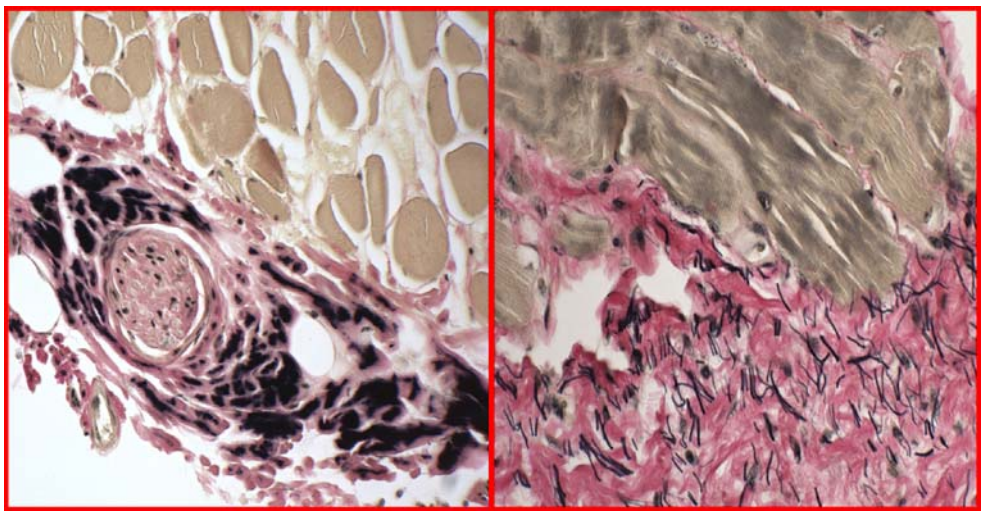

Fig. 4 Shows Elastica–Van Gieson's staining (250×) of *in-vitro* esophageal lesions created using RF energy (*left*) and cryothermal energy (*right*). Note the normal appearing, sharply demarcated spindle shaped elastic fibers in the cryoablation specimen compared to the blurred, indistinct, disrupted elastic fibers in the RF lesion

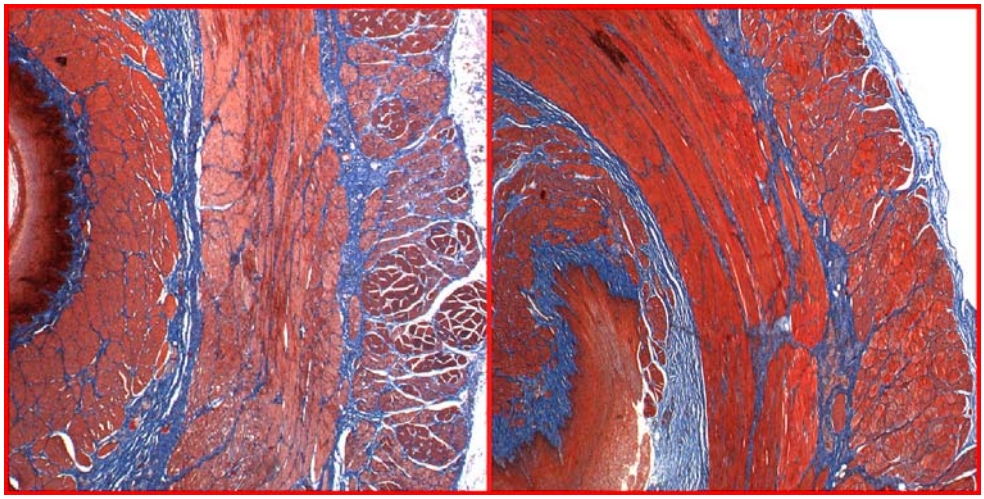

Fig. 5 Shows Masson's Trichrome staining of *in-vivo* esophageal lesions created using RF energy (*left*) and cryothermal energy (*right*). Note the pallor of the entire muscularis propria within the RF lesion. In contrast the cryolesion is not well demarcated from the surrounding normal tissue

structure [40]. However, even the most careful placement of ablative lesions still exposes the patient to a small risk of esophageal injury. Based on the findings of this study, cryoablation as an alternative to hyperthermic ablation may be associated with less tissue disruption and may ultimately lead to a lower risk of esophageal injury.

Study limitations We did not examine the electrophysiologic effects of the two energies on atrial tissue. It is not known if AF recurrence rates after cryoablation PVI are comparable to those after RF ablation. We used conventional RF and cryoablation catheters applied directly to the adventitial surface of the esophageal tissue strips. Of course in clinical practice interspersed atrial myocardium would be present which would likely modify energy delivery to the esophagus. We chose this design for consistency and predicted that this would result in the worst case scenario for both catheters. The preset 70°C used for the RF lesions exceeds what would be commonly used in clinical practice, but many operators are using a cooled tip RF catheter which would be expected to result in similar deep tissue temperatures 3 mm from the irrigated tip where the esophagus lies. We did not test RF ablation at lower temperatures or using an irrigated tip catheter which may have resulted in different findings. The present study employed tensile strength of esophageal tissue as a surrogate indicator of tissue integrity after *in-vitro* ablation. The linear force applied to the esophagus was non-physiologic and may or may not represent the force generated within a living esophagus. A limited number of *in-vivo* lesions were created to examine histology only. In order to preserve tissue architecture no tensile strength testing was performed in these specimens. In addition, sub-acute and chronic tissue/lesion strength was not addressed by our study methodology. Given the late presentation of clinical symptoms of esophageal injury, many times weeks after ablation, it is likely that the ensuing inflammatory reaction is a critical component of the complication. Our study lacks long-term follow up of *in-vivo* esophageal lesions which limits conclusions about the safety of cryoablation over RF.

Conclusions Ablation with cryothermy did not result in a statistically significant reduction in tensile strength of porcine esophagus when compared to control. Ablation with RF energy clearly did reduce esophageal tensile strength acutely. These different energies yielded disparate histopathological findings acutely. Cryothermal ablation did

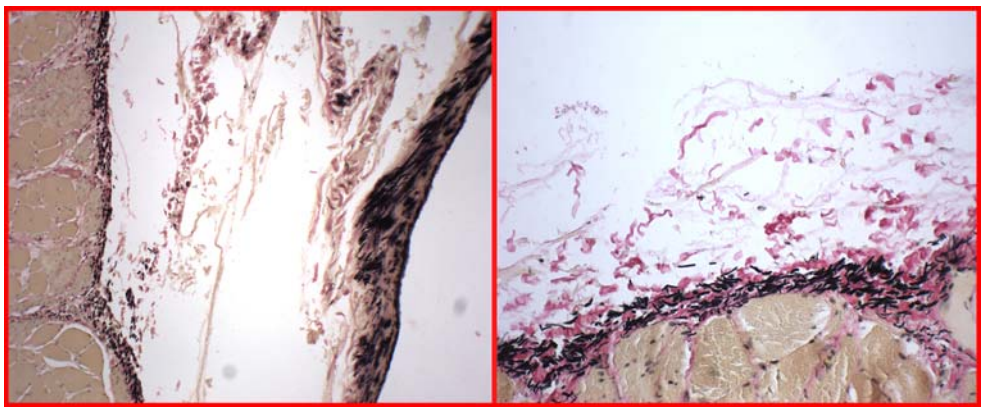

Fig. 6 Shows Elastica–Van Gieson's staining (125×) of *in-vivo* esophageal lesions created using RF energy (*left*) and cryothermal energy (*right*). Note the normal appearing, sharply demarcated spindle shaped elastic fibers in the cryoablation specimen compared to the blurred, indistinct, disrupted elastic fibers in the RF lesion

not appear to disrupt elastic fiber architecture whereas RF energy did. Cryoablation may ultimately be a safer alternative to RF energy for left atrial ablation and reduce the risk of clinically apparent esophageal injury, but further study evaluating chronic lesion strength with conventional RF, irrigated tip RF and cryoablation is required.

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