Acute and Long-Term Effects of Full-Power Electroporation Ablation Directly on the Porcine Esophagus

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Background—Esophageal ulceration and fistula are complications of pulmonary vein isolation using thermal energy sources. Irreversible electroporation is a novel, nonthermal ablation modality for pulmonary vein isolation. A single 200 J application can create deep myocardial lesions. Acute and chronic effects of this new energy source on the esophagus are unknown.

Methods and Results—In 8 pigs (±70 kg), the suprasternal esophagus was surgically exposed. A linear suction device with a single 35-mm long and 6-mm wide protruding linear electrode inside a plastic suction cup was used for ablation. Single, nonarcuring, nonbarotraumatic, cathodal 100 and 200 J applications were delivered at 2 different sites on the anterior esophageal adventitia. No proton-pump inhibitors were administered during follow-up. Esophagoscopy was performed at days 2 and 7. After euthanasia at day 60, the esophagus was evaluated visually and histologically. All ablations were uneventful. Esophagoscopy at day 2 showed small white densities in the ablated areas, which appeared to be small intraepithelial vesicles. No epithelial erythema, erosions, or ulcerations were seen. At day 7, all densities had disappeared, and all esophaguses appeared completely normalized. After euthanasia, there were no macroscopically visible lesions on the adventitia or epithelium. Histologically, a small scar was observed at the outer part of the muscular layer, whereas the mucosa and submucosa were normal.

Conclusions—Esophageal architecture remains unaffected 2 months after irreversible electroporation, purposely targeting the adventitia. Irreversible electroporation seems to be a safe modality for catheter ablation near the esophagus.


Key Words: ablation ■ complication ■ esophagus ■ irreversible electroporation ■ tissue specificity

Thermal catheter ablation may cause collateral damage to extracardiac structures. Various manifestations of esophageal damage, like esophageal ulceration and atrial-to-esophageal fistula, by radiofrequency energy, cryo-energy, and various other thermal catheter ablation methods have been described.1–5

Circular electroporation ablation via a multielectrode catheter is a novel, nonthermal ablation technique. A few milliseconds short high-current density impulse creates microlesions in the cell membrane. The increased cellular permeability results in disruption of cellular hemostasis and ultimately in cell death.6,7 Electroporation ablation has been shown to create sufficiently large and deep lesions for treatment of cardiac arrhythmias.8–10 With large and deep lesions in or around pulmonary vein ostia, the risk of esophageal damage may be greater than with currently used thermal ablation methods, like radiofrequency or cryoablation. This risk needs to be assessed before clinical implementation of electroporation ablation.

In the present study, the esophagus was targeted purposely with electroporation ablation, using an energy level capable of creating ≥7-mm deep lesions in myocardial tissue.10,11

Methods

This porcine study was performed in 8 animals (60–75 kg) after prior approval from the Animal Experimentation Committee of Utrecht University and was in compliance with the Guide for Care and Use of Laboratory Animals.12

Animal Preparation

Each pig was intubated and anesthetized following standard procedures. According to the study protocol, 5000 International Units of intravenous heparin were administered. Arterial pressure and capnogram were continuously monitored throughout the whole procedure.

Surgical Exposure of the Esophagus and Electroporation Ablation

After a suprasternal incision, an ≥12-cm long section of the esophagus was surgically exposed. Ablation was performed with a custom linear suction device comprising a 35-mm long and 6-mm wide linear electrode inside a 42-mm long and 7-mm wide plastic suction cup (Figure 1). The suction device was sucked with a constant partial vacuum of 50 to 60 cm H₂O parallel to the esophageal axis on the anterior esophageal adventitia and caused firm contact between the metal strip electrode inside the suction cup and esophagus (Figure 2). In the first
animal, a single, 6-ms, 100-J cathodal application was then delivered. In absence of adverse events in the first animal, a single, 6-ms, 200-J cathodal application was delivered in all other animals because maximal effect was aimed for. The energy was generated by a monophasic external defibrillator (Lifepak 9; Physio-Control, Inc, Redmond, WA). A large skin patch (7506; Valleylab Inc, Boulder, CO) on the back served as indifferent electrode. The ablation was repeated at a second site on the anterior esophageal adventitia, as described earlier. Voltage and current waveforms of all applications were recorded as previously described.9 With the suction device still in position after the application, both far ends of the device were marked with sutures and surgical clips (for future fluoroscopic identification of the ablated segment).

Follow-Up and Esophagoscopy
After surgical closure of the wound, the animal was allowed to recover. No proton-pump inhibitors were administered during follow-up.
In 5 out of 8 animals, after intubation and anesthesia after standard procedures, esophagoscopy using a video endoscope (Q160AL; Olympus CF, Tokyo, Japan) was performed at days 2 and 7. An experienced gastroenterologist performed the esophagoscopy, and the entire video endoscopy was digitally saved for post hoc analysis. The other 3 animals were euthanized at day 2 to study the acute effects of electroporation ablation. In these animals, no esophagoscopy was performed.

Results
All recorded voltage and current waveforms were smooth, demonstrating the absence of arcing.9 Average peak current of the 200-J applications was 21.2±2.6 amperes (Table). Visual inspection of the ablation area and the electrode directly after the energy application never revealed any blood clots or charring. Acutely, the suction device caused minimal local hematoma because of bursting of superficial small blood vessels, as has been described before.11 These hematomas were also observed prior to energy delivery. All animals survived the procedure and the follow-up period without complications. There were no signs of anorexia, vomiting, weight loss, hematemesis, or melena, suggesting adverse clinical effects on the esophagus or pain.

Esophagoscopy was guided by fluoroscopic identification of the surgical clips and area of interest. The surgical clips that marked both far ends of the ablation line allowed easy identification of the ablated areas (Figure 3). At day 2, esophagoscopy showed normal esophageal motility, no stenosis, and multiple whitish, circumscribed, clear fluid–containing elevations with a diameter of several millimeters, resembling vesicles in the ablated areas and, to a lesser degree, on the opposite esophageal wall. No epithelial erythema, erosions, or ulcerations were seen. These findings were macroscopically confirmed (Table and Figure 4). At day 7, esophagoscopy again showed normal esophageal motility and no stenosis. All elevations had disappeared, and all esophagi appeared completely normalized (Table).
After euthanasia at day 60, there were no macroscopically visible lesions on the adventitia or epithelium. However, the muscular layer showed a scar in all animals (Table).

Three animals were euthanized as planned at day 2. Microscopy of the ablated tissue showed 3 layers: mucosa consisting of nonkeratinizing squamous epithelium, submucosa with mucus glands, and the muscular layer. An intraepithelial vesicle was present, compatible with the macroscopic observation (Figure 5). Further analysis showed degeneration of the superficial part of the epithelium, with intact basal epithelial layers. The muscular layer showed a lymphohistiocytic inflammatory infiltrate in the outer muscular layer with degeneration of some striated muscle cells. The submucosa in between this inflammatory infiltrate and the epithelial vesicle was unremarkable. There were no signs of thermal injury.

Five animals were euthanized as planned at day 60. A superficial scar was present in the outer part of the esophagus, in the outer part of the muscular layer at the location where the

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Voltage and current values are recorded peak values. NA indicates not available.
*No esophagoscopy was performed in these animals; findings are based on direct visual inspection after termination.

Figure 3. A, Fluoroscopic image of an endoscope (E) in the esophagus during esophagoscopy at day 2. An endotracheal ventilation tube (T) is visible. Also, the 3 surgical clips (C) are clearly visible. They mark the far ends of the 2 esophageal ablation sites. Using this fluoroscopic guidance, the gastroenterologist knows exactly where the area of interest is. B, Endoscopic view of an esophageal lesion area at day 2; the distal esophagus is on the far side. Multiple whitish, circumscribed, clear fluid–containing elevations with a diameter of several millimeters, resembling vesicles in the ablated areas (lower left side of B) and on the opposite esophageal wall (upper side of B) are seen. No epithelial erythema, erosions, or ulcerations were found.
Inflammatory infiltrate was observed after 2 days follow-up (Figure 6). The epithelium of the mucosa was intact.

Discussion
One of the worst possible complications of pulmonary vein isolation using currently available ablation techniques is thermal damage to the esophagus, resulting in atrial-to-esophageal fistula.

Thermal injury to the esophagus is mainly being caused by catheter ablation on the adjacent left atrial wall, but esophageal heating can also be caused by catheter ablation in the posterior right atrium.13 The ablation energy (heat or cold) does not stop at the pericardial side of the atrial myocardium and can cause collateral damage to neighboring structures, like the esophagus.1,14 Typically, the anterior part of the esophagus at a distance of ≈25 to 35 cm from incisors is endangered.15 Injury to the esophagus can be caused by all presently used ablation modalities: radiofrequency ablation, cryoballoon ablation (Arctic Front and Arctic Front Advance), irrigated radiofrequency multipolar ablation (nMARQ), duty-cycled phased radiofrequency ablation (PVAC), visually guided laser balloon catheter (CardioFocus), and catheter ablation by robotic navigation (Sensei).3,14,16–20 Several theories have been developed as to why esophageal injury and even atrial-to-esophageal fistula do happen.18 One theory sees the direct thermal injury with subsequent necrosis of the esophageal wall as the culprit.21–24 Another theory suggests injury to the periesophageal nerves, (increased) gastric acid regurgitation, and acid etching of the lesion, leading to formation of ulcer or fistula, but the data on this matter is ambiguous.24,25

The occurrence of this complication may not be recognized because there is a delay of ≤6 weeks between pulmonary vein...
isolation and onset of aspecific symptoms, like dysphagia, chest pain, fever, seizures or cerebrovascular accidents, and melena. Urgent esophageal stenting or operation is indicated, but often the atrial-to-esophageal fistula is lethal.

In 2008, Hong et al performed esophageal electroporation ablation using a modified, commercially available epicardial ablation clamp, as well as radiofrequency ablation. Esophageal lesions from electroporation energy were characterized by myocyte and interstitial damage practically identical to the cardiac lesions. The lesions were restricted to the muscle layer. The luminal epithelial layer and the delicate lamina muscularis mucosae (a small rim of smooth muscle cells beneath the epithelial basal layer) were left without pathological changes. In contrast, the radiofrequency ablations compressed the esophageal wall and destroyed the epithelial and muscular layers, and the adventitia.

Irreversible electroporation can create deep myocardial ablation lesions, while sparing the coronary arteries and the phrenic nerve. It can also persistently isolate the pulmonary veins without occurrence of acute or chronic pulmonary vein stenosis. Because of the combination of deep lesions and the close proximity of the posterior left atrial wall to the esophagus, it is of paramount importance to exclude possible adverse effects on the esophagus. Therefore, this study purposely targeting the esophagus was conducted. Linear electroporation ablation using 200 J applications directly on the outer esophageal wall was regarded as a worst-case scenario for human electroporation ablation near the esophagus. Because of the design of the ablation electrode inside a suction cup, optimal electrode–tissue contact is guaranteed. In the absence of a surrounding blood pool or other conducting tissue, the energy delivered is forced to go entirely through the esophageal tissue. The energy application was only delivered when the suction cup was placed and remained firmly attached to the esophagus.

**Main Findings**

The main findings of the present study are that direct electroporation ablation on the outer esophageal wall seems to be asymptomatic in pigs and causes harmless, self-limiting vesicles on the nonkeratinizing squamous epithelium at the ablation site. After 7 and 60 days follow-up, the epithelium has completely normalized. There are no signs of ulceration or other adverse reactions. Microscopy after 2 and 60 days follow-up shows evidence of ablated muscular tissue, proving that the delivered energy reached its target area. The epithelial layer at 60 days follow-up shows intact epithelium and an intact extracellular matrix, proving that the architecture of the esophageal tissue remains chronically intact.

Irreversible electroporation is the first ablation technique that can create large myocardial ablation lesions without severely damaging the esophagus, acutely or chronically. This can have important future implications, in the sense that for the first time in the history of cardiac ablation, there is a fast and powerful endocardial ablation technique available without the feared flip side of the coin: collateral damage to surrounding extracardiac structures. The absence of severe esophageal damage after ablating with an energy level able to create ≈7-mm deep myocardial

**Figure 5.** Esophageal electroporation ablation lesion at 2 days follow-up. A, Overview of the wall of the esophagus showing 3 layers (from top to bottom): mucosa consisting of nonkeratinizing squamous epithelium (*), submucosa with mucus glands (†), and the muscular layer (‡). An intraepithelial vesicle is present. Hematoxylin and eosin stain, bar=1 mm. B, Higher magnification of the vesicle shown in A. Bar=500 μm. C, Higher magnification of the rectangle in B, showing degeneration of the superficial part of the epithelium with intact basal epithelial layers. Bar=150 μm. D, Higher magnification of the muscular layer of A, showing an lymphohistiocytic inflammatory infiltrate in the outer muscular layer with degeneration of some striated muscle cells. The submucosa in between this inflammatory infiltrate and the epithelial vesicle is unremarkable. Bar=300 μm.
lesions could mean that safe and effective myocardial ablation at the posterior side of the atria is within reach.

Tissue specificity of electroporation ablation has been well described in the past. Over the last 2 decades, reversible electroporation has been intensively pursued as a promising technique for the treatment of cancer, especially hepatic and prostatic cancer cells are sensitive to electroporation ablation.\textsuperscript{30,31} On the other hand, blood vessels and nerves are much less susceptible to electroporation ablation.\textsuperscript{28,32–34} The exact reason for this tissue specificity is unknown. This low susceptibility to electroporation ablation could also be a possible explanation for our findings: one of the proposed mechanisms of late esophageal fistula formation is ischemic damage to the esophagus. Because of the low susceptibility of blood vessels and nerves to electroporation ablation, the blood vessels stay intact, and therefore, the proposed mechanism of ischemia to the esophageal wall is less likely to occur.

Limitations
This is an animal study in which the porcine esophagus was ablated. Although no animal showed signs of esophageal discomfort (anorexia, vomiting, and hematemesis) or pain, we cannot exclude that humans would experience discomfort or even pain after electroporation ablation near the esophagus.

Using a porcine animal model, we realize that the effects of electroporation ablation on the human esophagus may be different.

The finding of scarring in the muscular layers may be a precursor to esophageal stricture. We do not know the long-term effects on the esophageal musculature.

In this study, we only investigated one energy setting. However, previous studies have showed that this energy level and mode of delivery can create deep and wide myocardial ablation lesions, able to create transmural lesions.

Histologically, we never saw any signs of thermal damage. Especially, the border zones of lesions do not resemble thermal lesions at all. When calculating the change in temperature (Joule heating effect) per 6-ms application, we estimate a maximal temperature increase of \( \approx 15^\circ\text{C} \). Given the short duration of the application, it is highly unlikely that there is conductive heating. But (the extend of) this cannot be fully ruled out. To the best of our knowledge, there are no methods to measure the (changes in) tissue temperature after a single 6-ms application in an ex vivo model.

Conclusions
Esophageal architecture remains unaffected 2 months after electroporation ablation, purposely targeting the adventitia. Data of this study suggest that electroporation ablation is a safe modality for catheter ablation near the esophagus.

Disclosures
Dr Wittkampf and H. van Wessel are the inventors of circular electroporation. The other authors report no conflicts.

References


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