ORIGINAL RESEARCH

Intrapulmonary Vein Ablation Without Stenosis: A Novel Balloon-Based Direct Current Electroporation Approach

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***Background*—**Current thermal ablation methods for atrial fibrillation, including radiofrequency and cryoablation, have a suboptimal success rate. To avoid pulmonary vein (PV) stenosis, ablation is performed outside of the PV, despite the importance of triggers inside the vein. We previously reported on the acute effects of a novel direct current electroporation approach with a balloon catheter to create lesions *inside* the PVs in addition to the antrum. In this study, we aimed to determine whether the effects created by this nonthermal ablation method were associated with irreversible lesions and whether PV stenosis or other adverse effects occurred after a survival period.

***Methods and Results*—**Initial and survival studies were performed in 5 canines. At the initial study, the balloon catheter was inflated to contact the antrum and interior of the PV. Direct current energy was delivered between 2 electrodes on the catheter in ECG-gated 100 μs pulses. A total of 10 PVs were treated demonstrating significant acute local electrogram diminution (mean amplitude decrease of 61.2±19.8%). After the survival period (mean 27 days), computed tomography imaging showed no PV stenosis. On histologic evaluation, transmural, although not circumferential, lesions were seen in each treated vein. No PV stenosis or esophageal injury was present.

***Conclusions*—**Irreversible, transmural lesions can be created inside the PV without evidence of stenosis after a 27-day survival period using this balloon-based direct current ablation approach. These early data show promise for an ablation approach that could directly treat PV triggers in addition to traditional PV antrum ablation. **(*J Am Heart Assoc*. 2018;7:e009575. DOI: 10.1161/JAHA.118.009575.)**

Key Words: animal study • atrial fibrillation • direct current ablation • electroporation • pulmonary vein stenosis

Atrial fibrillation (AF) is an epidemic problem in the United States and around the world.[1,2] While radiofrequency catheter ablation is effective for some patients with paroxysmal AF, the long-term freedom from arrhythmia is limited and outcomes for patients with persistent AF are even worse.[3] Surgical treatments for AF have traditionally shown better outcomes than radiofrequency ablation.[4] This disparity in outcomes may be caused by inadequate lesions created with catheter-based radiofrequency. While radiofrequency ablation has the ability to create full thickness destruction of atrial myocardium, power is deliberately limited to avoid known potential complications such as atrioesophageal fistula, steam pops, and coagulum formation, which can occur with high temperatures.[5] Furthermore, the lesions are typically placed outside of the pulmonary veins (PVs). While there may be specific benefits of treating this tissue in the antrum of the PV, triggers from muscle inside the PVs are not treated directly because of concern for PV stenosis. Cryoablation, another common method of thermal ablation, has not been immune to these complications and is also directed at tissue outside of the PV.[6]

Irreversible electroporation (IRE) is a method of tissue ablation using microsecond pulses of direct current (DC) energy, which creates cell death by altering the stability of the cell membrane and disrupting the homeostasis of the cell.[7,8] While some small amount of heat may be created, the

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Clinical Perspective

What Is New?

- This study demonstrates a novel ablation approach using pulses of direct current electricity from a balloon catheter to directly eliminate triggers of atrial fibrillation inside the pulmonary veins without causing pulmonary vein stenosis.

What Are the Clinical Implications?

- With further validation in future studies, this approach might offer a useful addition to current atrial fibrillation ablation techniques and limit concerns about pulmonary vein reconnection.

mechanism of cell death does not rely on heat formation, thus removing the need for high temperatures. The absence of a large thermal effect may allow direct treatment of vessels, where the muscular cell layer is destroyed but the structural integrity of the vessel is preserved, as has been demonstrated in prior studies.[9,10]

IRE is approved by the US Food and Drug Administration for clinical use in the treatment of solid tumors. Further, it has been tested in animal models for treatment of cardiac disease by our group and others.[10–18] Preliminary studies suggest that IRE may be a promising alternative ablation method for AF management. First, IRE could potentially be used to directly treat the triggers in the PVs without causing stenosis.[10,11,18] Second, risk of other collateral damage, such as esophageal injury, may be lower as well because of the absence of high temperatures. Prior studies using IRE have shown no damage to the esophagus and no fistula formation even with direct treatment of the esophageal wall.[19] The phrenic nerve, often injured with cryoenergy, also appears to be spared using IRE.[20]

In this study, we aimed to evaluate the feasibility of IRE for ablating within the PVs without creating PV stenosis or damage to neighboring structures. To achieve this, we designed a balloon-based catheter with a titratable DC energy delivery protocol to deliver an ablation lesion on the interior of the PV in addition to the PV antrum.

Methods

A total of 5 studies were performed, with a 7- to 44-day survival period between the initial procedure and the follow-up study. The procedures were performed on 30- to 40-kg mongrel canines, which were cared for by study investigators, veterinarians, and veterinary technical staff at the Mayo Clinic animal facilities. All procedures were performed with approval and guidance from the Mayo Clinical Institutional Animal Care and Use Committee in accordance with the *Guide for the Care and Use of Laboratory Animals* issued by the National Institutes of Health. The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure. Some of the materials used are proprietary catheters still under development and it would not be feasible to provide all materials used in the study.

Preprocedure and Sedation

Animals were fasted overnight before the procedure. Sedation was provided with intravenous diazepam (5 mg) and ketamine (10 mg) for induction followed by inhaled isoflurane (1–3%) throughout the procedure. Intramuscular buprenorphine (0.3 mg) and morphine (1 mg/kg) were given for analgesia. While intravenous vecuronium (0.1 mg/kg) was available for muscle paralysis within the protocol, it was only given during a single procedure as it was typically not found to be necessary. Intravenous cefazolin (1000 mg) was given during the procedure followed by oral cefpodoxime (10 mg/kg) postprocedure to prevent infection.

Left Atrial Access

Vascular access was obtained with a cutdown approach in the femoral artery and vein as well as the external jugular vein. After vascular access was secured, intravenous heparin was given as a 100-U/kg bolus and followed with repeated doses to maintain an activated clotting time >300 seconds. Transseptal access to the left atrium was obtained using a transseptal needle through a DIREX long steerable sheath (Boston Scientific) under fluoroscopic and intracardiac echocardiography guidance. The novel balloon catheter (Figure 1) was then placed into the left atrium. This catheter was connected to the CardioLab electrogram recording system (GE), as well as the NanoKnife electroporation delivery device (Angiodynamics) with ECG gating.

Catheter Setup and Energy Delivery Parameters

An Orion catheter along with the Rhythmia mapping system (Boston Scientific) was used to create an electroanatomic map to assist in navigation of the catheter. This map, along with intracardiac echocardiography and fluoroscopy, were used to guide placement of the catheter in the desired PV for treatment. The balloon was then inflated with the intention of creating contact with and treating the inside of the PV and antrum. Local electrogram signals were measured with electrodes on the catheter. For the purposes of quantifying the amplitude of electrogram change, the averages of 3 local

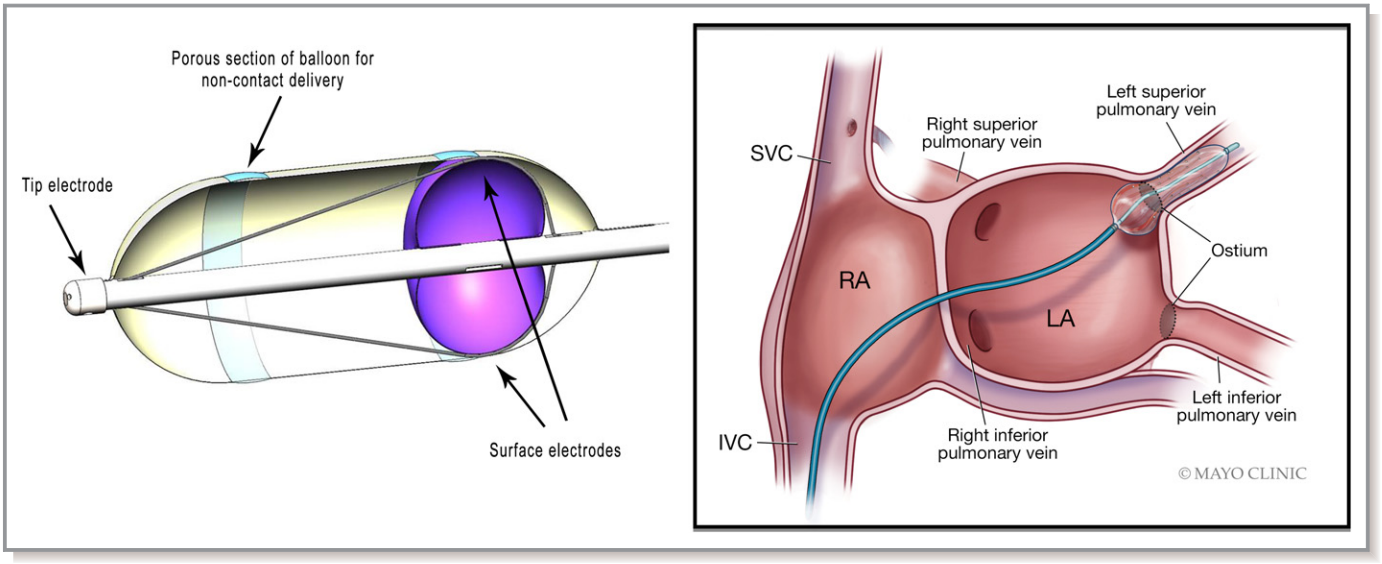

Figure 1. Prototype balloon catheter for delivery of direct current ablation energy, shown alone (left) and in the intended ablation location (right). IVC indicates inferior vena cava; LA, left atrium; RA, right atrium; SVC, superior vena cava.

electrograms before and after the treatment were measured on the bipole between the distal and proximal electrodes on the balloon.

Based on our observations of local electrogram effects in prior acute studies, 100 pulses was thought to be an appropriate number to see significant change; however, 10 and 200 pulses were also attempted. Pulse duration was set at 100 μs. All pulse deliveries in this experiment were ECG-gated to the occurrence of the QRS to prevent ventricular fibrillation. DC energy was delivered between 2 local electrodes of the balloon catheter. There was no body surface or indifferent electrode in the circuit. The local electrodes were at the surface of the balloon, the tip of the catheter, or what we term as a "virtual electrode," an electrode within the balloon that is not in contact with the tissue. This electrode delivers electricity that is conducted to the tissue at the surface of the balloon through a 0.9% saline solution, which is of low impedance. The balloon is porous at select locations to allow a circular band of saline to contact the tissue and thus create a circular "virtual electrode."

Energy was delivered while monitoring the animal's vital signs and the ECG. After energy delivery, the local electrogram was again recorded. Two to 3 veins were treated per experiment with the other vein(s) left as controls. In 1 experiment, only a single vein was treated because of concerns regarding the health of the canine after an inadvertent cardiac perforation during transseptal access. After energy delivery and electrogram recording, the vascular access sheaths were removed with surgical closure of the vessel access sites. The animal was monitored closely in recovery and throughout the survival period.

CT Scan for Evaluation of PVs

A contrast-enhanced ECG-gated computed tomography (CT) scan was performed before the follow-up procedure in each canine to determine whether there was evidence of PV stenosis. Iodinated contrast was given through an intravenous line at 5 mL per second for a total of 80 mL. The scan was triggered after a 7-second delay when 150 Hounsfield units were present at the ascending aorta. CT scans were also performed before the initial procedure in 2 experiments but were not continued in later studies as a result of logistic issues with procedure duration.

Follow-Up Study and Histologic Analysis

The survival period ranged from 7 to 44 days. Initial studies were scheduled to be ≈6 weeks of survival, but the final 2 studies were shortened to a 1-week survival period for logistic reasons involving laboratory time and delays in histologic evaluation. The follow-up procedure was performed in a similar fashion to the initial procedure although no ablation was performed. After completion of the follow-up study, the animal was euthanized by induction of ventricular fibrillation. The intrathoracic contents were removed en bloc. In the first 2 studies, the lungs and esophagus were examined grossly but trimmed away before preservation. In the latter studies, all structures were preserved and examined by the veterinary pathologist. The tissue was immersion fixed in 10% neutral buffered formalin. The left atrium, PVs, and esophagus were sectioned at 1000-μm intervals and stained with hematoxylin and eosin as well as Masson's trichrome.

Table. Lesion Specifics: Treatment Protocol, Acute ECG Changes, and Key Histology Findings

Experiment	Survival Period, d	Location of Treatment	Voltage	Total Number of Pulses At Location	Acute Change in Voltage, %	Presence of a Transmural Lesion	Percentage of Circumference Ablated	Length of Lesion Parallel to the PV, mm
1	40	LSPV*	2000	200	−54.9%	Yes	NC	3
		LIPV	2000	100	−78.5%	Yes	NC	3
2	36	LIPV	1000	100	−55.3%	Yes	NC	5
		LSPV	1000	10	−19.7%	Yes	NC	7
		RSPV	1000	10	−59.1%	Yes	NC	8
3	44	LIPV	2000	200	−39.7%	Yes	80	8
4	9	RSPV	2000	100	−71.0%	Yes	70	15
		LIPV	2000	200	−75.6%	Yes	100	16
		LSPV	2000	100	−75.0%	Yes	60	17
5	7	LSPV*	2000	100	−83.2%	Yes	30	5

The percentage of the circumference was not quantified in these lesions as a result of the nonaxial method of slide preparation, but the lesions were demonstrated to effect less than the full circumference of the vein. LIPV indicates left inferior pulmonary vein; LSPV, left superior pulmonary vein; NC, not circumferential; PV, pulmonary vein; RSPV, right superior pulmonary vein.

*Treatments using the virtual electrode. All other treatments were surface electrode-to-tip configuration.

Statistical Analysis

The results of this study consist primarily of descriptions of the treatment effects including mean values for continuous variables where appropriate. Acute treatment effects were compared within and between groups using a paired and unpaired *t* test on GraphPad (GraphPad Software).

Results

There were a total of 10 individual PVs treated in 5 canine studies followed by a mean survival period of 27 days (7–44 days). The Table demonstrates the details of each therapy delivered. The left inferior and left superior PVs were the most frequent sites of treatment because of ease of access inside the small canine atrium. Energy was delivered between the balloon surface electrodes and the tip of the catheter for the majority of treatments, although the "virtual" electrode was used in 2 veins. With this energy delivery between local poles, there was minimal contraction of skeletal muscle and a paralytic was only given in a single study.

The NanoKnife device (Angiodynamics) was typically set to deliver 2000 V/cm, although 1000 V/cm was used during 1 experiment. The mean current delivered per each 2000 V/cm, 100 μs pulse was 2.11 amps for a mean energy delivery of 0.42 J. For the 1000 V pulses, the current and total energy per pulse were 2.46 amps and 0.25 J. Each area was most commonly treated with 100 total pulses. However, in 3 veins, an additional 100 pulses was delivered in the same area and in 2 veins only 10 pulses were used. The overall energy delivered during a 100 pulse treatment with 2000 V/cm was ≈42 J.

Treatment Effect in the PVs and Antrum

All initial treatments in an area, whether 10 or 100 pulses, resulted in a decrease in electrogram amplitude (Table). The change in local electrogram amplitude after the entire treatment in each vein resulted in a mean decrease in amplitude of 61.2±19.8%. IRE treatment was also often associated with fractionation of the electrogram.

There were no major complications related to IRE delivery. No ventricular arrhythmias occurred at any point. There were a few short runs of AF/flutter immediately after treatment, but none required any treatment and all resolved spontaneously within 2 minutes. There was a cardiac perforation during the transseptal puncture on the last experiment that required pericardiocentesis, but this was before and not related to the IRE treatment. We were able to proceed with treatment of 1 vein and the animal survived to the planned follow-up experiment without further incident. There was no evidence of PV stenosis on any of the follow-up CT scans.

Histologic Analysis

On histologic analysis, a transmural lesion was seen in a segment of all of the treated PVs (Figure 2B and D). The lesion pattern was typically that of decellularization with only collagen scaffolding remaining. In a minority of lesions, there was also some fibrosis, meaning the appearance of an increased fibrotic reaction. Only 1 treatment demonstrated a lesion that was fully circumferential.

There was no significant luminal narrowing in any PV on histologic examination, with all lesions classified in the 0% to 1% range except for 1 lesion, with 5% narrowing. There was

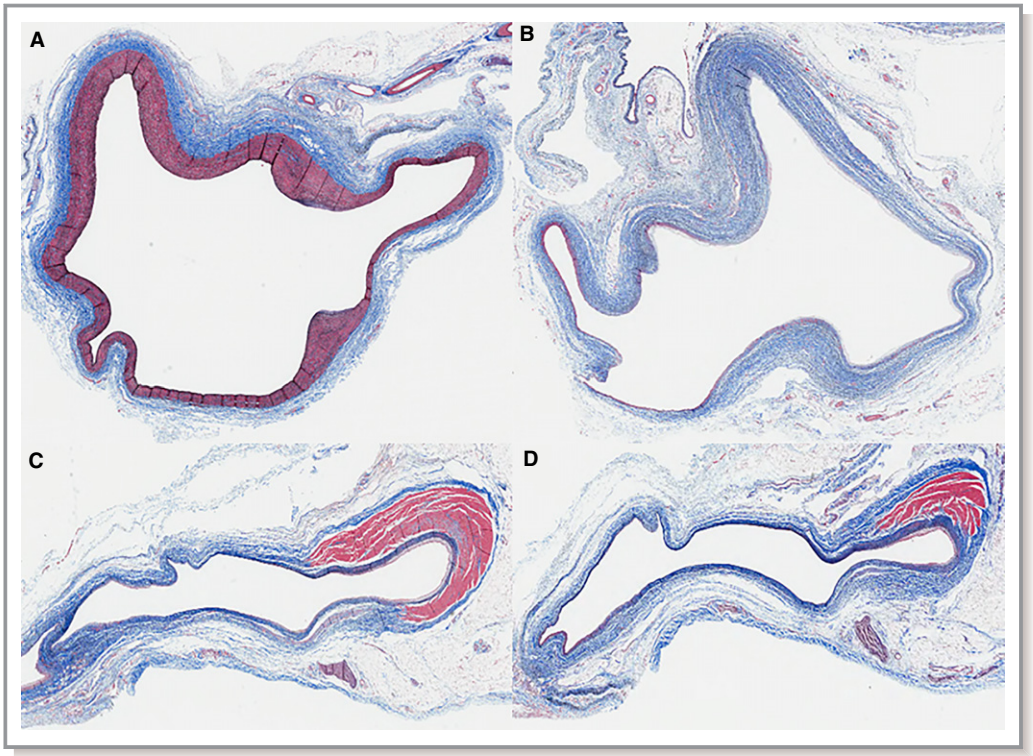

Figure 2. Slide sections with elastic Masson's trichrome stain demonstrating: (A) a control canine pulmonary vein without ablation; (B) a canine pulmonary vein after treatment with direct current ablation showing near-complete absence of cardiomyocytes with preserved structural collagen and no stenosis; and (C and D) 2 consecutive sections of treated canine pulmonary vein with sharp demarcation between an area of transmural cardiomyocyte loss and an area with relatively unaffected myocardium.

minimal intimal hyperproliferation seen in some veins. There was mild endocardial thickening seen at the antrum in the first 2 studies that was believed to be caused by pressure/stretch from overinflation of the balloon and was absent in the later studies.

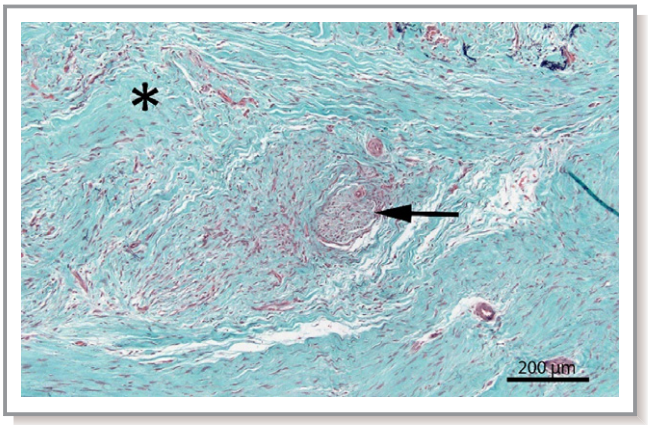

Figure 3. Slide section demonstrating an autonomic nerve fiber with atrophy and fibrosis in a treated region (arrow). (Hematoxylin-eosin and trichrome staining, magnification 10×; bar=200 µm.) * indicates epicardium.

Gross examination of surrounding structures in the thorax, including the lungs and esophagus, did not reveal any treatment effect. Histologic analysis confirmed a lack of any effect seen in the esophagus or lungs. While no damage to the phrenic nerve was noted, there was damage seen to nerves on the posterior atrium at the location of the ganglionated plexus/intrinsic cardiac autonomic nervous system (Figure 3).

Discussion

This study demonstrates that transmural ablation can be performed in the PVs without significant adverse effects noted after a 1-month survival period. This was accomplished with standard left atrial access procedures, a prototype balloon catheter, and a Food and Drug Administration–approved electroporation delivery device gated to the ECG.

Acutely, application of the IRE treatment created a reduction in the electrogram amplitudes that have been found to be consistent with transmural tissue ablation with radiofrequency in prior studies. A decrease in the electrogram amplitude of 50% to 60% is typical of transmural radiofrequency ablation lesions and the average decrease per

treatment region in this study was 61.2%.[21,22] Furthermore, histology from this study confirmed that there was a transmural lesion present in each treated PV. Interestingly, transmurality was even seen in some portion of the lesions where the electrogram did not have this level of reduction, potentially because the area of transmural ablation was not sufficient to decrease the electrogram from the relatively large local bipole. The widely spaced electrodes on the ablation balloon catheter likely record electrical activity from far-field atrial sites that are not in the treatment zone, thus reducing the sensitivity of the reduction of local electrogram amplitude in predicting lesion size.

Histologic evaluation of these lesions shows that the myocardial cells are destroyed with preservation of the underlying collagen scaffolding. There was a mild increased fibrosis present in some lesions. While the importance of this is unclear, the clinical significance would likely be minimal, but further study is needed. We did not directly measure the temperature of the catheter, but significant heating was unlikely. The balloon catheter design, with a soft membrane and 2 of the electrodes (tip and virtual) being noncontact, is not conducive to thermal ablation. Moreover, the relatively low amount of energy delivered over the extremely short pulses would be unlikely to create any significant ablative effect even if heat were created. Each pulse delivered energy for 100 μs and, therefore, with all pulses combined, energy is only being delivered for a total of 10 milliseconds for a typical treatment.

Despite these large, transmural ablation lesions in the PV and antrum, there were no significant adverse effects noted. The postprocedure CT scans performed before the end experiment in each dog did not show any significant stenosis. While this is not as sensitive as the histology, it is more representative of how we assess for PV stenosis clinically. Histology confirmed that no significant PV stenosis was seen even with transmural lesions inside the PV. There were small amounts of endothelial fibrosis seen in some lesions, although this typically accounted for only 0% to 1% stenosis, with 5% being the most seen and in only 1 lesion. This small level of change may be related to physical contact with the endothelium. These findings suggest that PV stenosis would not occur clinically with IRE in the PV, but longer-term follow-up with final clinical catheter designs will be important before the initiation of human studies.

There was also no damage seen in other structures that have been shown to be damaged in clinical radiofrequency and cryoablation, such as the lungs, phrenic nerve, or esophagus. The esophagus was grossly examined in all studies and histology performed in 3 animals. There was no evidence of a treatment effect. Notably, with treatment inside the veins it is possible that the esophagus is less likely to be in the ablation field. Even if it were, it is unlikely that there would be fistula formation because of the preservation of structural collagen by nonthermal IRE. A recent study demonstrated that even with electroporation directly on the surface of the esophagus, there was no loss of structural integrity in the esophageal wall.[19]

While we did not see any damage to the phrenic nerve in these studies, we did see some ablation of small nerves associated with the intrinsic cardiac autonomic nervous system and ganglionated plexus. Although there is still considerable debate regarding the importance of ganglia ablation for treatment of AF, this finding suggests that endocardial IRE could potentially create concurrent autonomic nerve ablation similar to radiofrequency. This has not been seen with prior endocardial electroporation studies to our knowledge, but was noted in prior experiments with epicardial IRE.[13]

The QRS gating used in this study seemed to work as intended and was similar to what is used in clinical electroporation of tumors in the thorax where proximity to the heart can lead to ventricular arrhythmias.[23] We did not see any ventricular arrhythmias in these studies. We did see brief atrial arrhythmias, however, which would not likely be an issue in the setting of AF ablation. Although, in other settings, a method for gating to both the P and QRS may be beneficial.

If shown to be feasible and safe in future studies, the approach demonstrated here could provide an added benefit to our current techniques for AF ablation. This method creates an ablation lesion in the traditional PV antrum location and, in addition, creates a lesion directly inside the PV. While direct treatment of the triggers inside the PV may be beneficial in itself, it could also limit the negative consequences of "reconnection of the veins" caused by a break in the isolation line.

While procedural time is not the most important factor in an ablation procedure, any increased length of the procedure is riskier to the patient and increases the cost of health care. The lesions delivered in this study take only a few minutes. The lesions are ECG-gated and therefore treatment using 100 pulses only takes as long as 100 heart beats, typically between 1 and 2 minutes for most people. Even if several treatments were used, the procedural time would be much less than that seen with radiofrequency and possibly less than that of cryoablation.

A group in the Netherlands has laid much of the groundwork for study in the field of cardiac electroporation and previously performed DC ablation in the PVs.[18] Results from our study and their previous studies validate the same concept: that IRE can be delivered in the PVs without significant adverse effects. However, the system used in their studies was different from what we have described here, although both may be viable clinical options. In their studies, a large amount of energy (200 J) from a defibrillator between a

circular multielectrode catheter in the PV and an indifferent grounding patch on the body surface has generally been delivered as a single pulse of 6 milliseconds. In our studies, a smaller energy level per pulse was used with a shorter pulse length and the overall energy delivered was lower. However, it must be considered that the dose delivered in their studies was spread over multiple electrodes in the circular catheter and therefore a lower dose per electrode was delivered. Furthermore, the electrodes in our bipole were both local (within the PV) so less energy was lost. With these factors considered, it is possible that the actual "dose" delivered was similar.

In these studies, which all used 2 local electrodes for the poles of DC delivery, there was only minimal muscular contraction noted. Traditionally, with DC delivery between an endocardial electrode and a body surface patch, there is capture of the skeletal muscles and prominent convulsion of the animal. For this reason, a paralytic is typically used. In these experiments, the muscle contraction was small or, at times, imperceptible. It is possible that IRE with local electrodes only may be performed without a paralytic. This is important because it could potentially remove the necessity of intubation and thus significantly improve the safety and length of ablation procedures. It is also conceivable that energy may be delivered in even shorter (nanosecond) pulses, as has been demonstrated in prior studies of high-frequency IRE.[24]

Limitations

There were several limitations noted in this study. As with all studies using an animal model, there are differences in anatomy and physiology of humans and canines that may limit translation of these results. Furthermore, there were only 5 animals included in the study. While we felt that follow-up in the first 3 studies was reasonably long, future studies will ideally include a longer period to monitor for late adverse effects.

It is also notable that while several lesions were 60% to 80% circumferential, only 1 delivery resulted in a 100% circumferential lesion. Our speculation is that the partially circumferential lesions occur as a result of a lack of proximity of 1 or a portion of an electrode to the myocardial surface, which allows the energy to focus in 1 area rather than the full circumference. The ablation technique used here is not solely for isolation of the PVs, and therefore a less than circumferential lesion may not be a treatment failure. Ideally, however, the lesions would be fully circumferential to prevent any triggers in the PV distal to the ablation zone from affecting the atrium and ultimately all of the arrhythmogenic muscle in the PV would be treated. If accomplished, the single balloon catheter could be used to both isolate the PV and directly treat the triggers inside the vein. We anticipate that with further catheter and protocol optimization, this is an imminently achievable result, as was seen in one of the later studies.

It also seems apparent that higher levels of energy could be attempted, as there is no indication of adverse effects at this level. The few variations in the parameters in this study do not allow a rigorous analysis of the settings, which would be most effective and still maintain adequate safety. Ongoing study will be important to identify an optimal "dose" for the most effective IRE in the PV.

No significant PV stenosis was seen in this study; however, longer-term follow-up will be important for further assessment. There were some areas of minor endothelial thickening, however, which may be related to catheter trauma.

This study was performed to assess key principles of IRE in the PV, but also to identify optimal approaches to this type of therapy. These ongoing innovations lead to some differences between individual canine experiments. While many factors were standardized between studies, the variability in location and energy delivery and between animals does not allow much comparison between experiments. With further identification of optimal approaches, longer and more standardized studies will be beneficial.

Conclusions

This study demonstrates that IRE can be performed inside the PVs without major adverse effects noted after a 1-month survival period. Transmural ablation was created with DC energy delivered from a clinically available electroporation system through 2 local electrodes on a prototype balloon catheter within the canine PV. No significant PV stenosis or esophageal injury was noted. These findings merit further study in a larger cohort of survival animals and, if consistent, could provide a new technique in the treatment of AF.

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Disclosures

Drs Witt, Padmanabhan, Desimone, Kapa, and Asirvatham have intellectual property claims involving the catheter and techniques used in this study. Dr Gruba and Mr Rohl have intellectual property claims involving the catheter and techniques used in this study. They are also employees of Boston Scientific. Boston Scientific supplied catheters for the procedures but did not design the studies, nor did they control the

final publication of the findings. The remaining authors have no disclosures to report.

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