En Bloc Exclusion of the Pulmonary Vein Region in the Pig Using Off Pump, Beating, Intra-Cardiac Surgery: A Pilot Study of Minimally Invasive Surgery for Atrial Fibrillation

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Background. **Off-pump, closed, beating heart, minimally-invasive surgery in patients with lone atrial fibrillation (AF) must be effective to become the preferred alternative to catheter ablation. Because of the inherent anatomical limitations of the epicardial access, we explored the feasibility of an intracardiac approach.**

Methods. **We report an acute study of en bloc, cryoexclusion of the pulmonary vein region in 7 pigs. The left atrial appendage (LAA) was approached via a left thoracotomy. Electrodes were attached to the posterior wall of the left atrium (LPA) and right atrial appendage (RAA) for pacing and electrophysiological monitoring. A modified Surgifrost probe was introduced via the LAA and positioned using transesophageal (TEE) and intracardiac (ICE) echocardiographic guidance to generate encircling cryolesions (3 minutes, −105°C) of the pulmonary vein region.**

Results. **A complete two-way block was achieved in 6 pigs and an incomplete block in 1. The excluded segment had very slow idiosyncratic rhythm or was electrically silent. In all pigs before isolation, sustained AF was inducible with the most rapid rhythms and fractionated electrograms recorded from the LA. While sustained AF was induced before exclusion, it was not after isolation in either the exclude or non-excluded segments. All tissue samples taken along the encircling cryolesions had transmural cryolesions on pathological examination.**

Conclusions. **We conclude that off-pump, closed heart, beating, intracardiac AF surgery is feasible, reliable and can duplicate the accuracy and precision of the open-heart approach. However, further developments are needed to make this novel approach an alternative to current approaches for catheter ablation.**

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Surgery for cardiac arrhythmias has a well established history of effectiveness with the off pump, epicardial approach for the surgical ablation in patients with Wolff-Parkinson-White syndrome [1–3]. However, surgical side effects have either discouraged the use of surgery to correct arrhythmia [4–6] or nullified the benefit of arrhythmia control as in direct surgery for ventricular arrhythmias after acute myocardial infraction [7, 8].

Minimally-invasive surgery for lone atrial fibrillation (AF) has an opportunity to become the intervention of choice if the very high efficacy of the conventional open heart approaches [9–12] could be achieved with minimal surgical invasiveness [13, 14]. This goal requires an off-pump, closed, beating heart intervention with minimal surgical trauma in terms of access.

Left atrial surgery for AF is currently performed under those conditions using an epicardial approach [15, 16]. However, the epicardial approach has inherent limitations in terms of access due to the complex anatomy of the posterior left atrium and pericardial sac, and in terms of energy delivery with limited effectiveness of some energy sources to attain transmural lesions [17–19]. Despite recent advances in technology, the epicardial approach does not duplicate the effectiveness of open heart surgical techniques [15, 16].

The intracardiac approach is not associated with any limitations in term of access of the entire left atrium but has challenges in terms of safe intracardiac access,

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absence of direct vision, navigation of tools and effective energy delivery.

We report a feasibly study of en bloc exclusion of the pulmonary vein region in the pig using closed heart, off-pump, beating, intracardiac surgery with cryoablation.

Material and Methods

The pig was selected because it is used routinely in cardiac experiments [15] it has been used as a model of experimental AF [15, 16], and has an anatomy and size comparable to humans. The protocol was approved by the Animal Care Committee of the University of Western Ontario and followed the Guidelines of the Canadian Council on Animal Care.

Surgical Preparation

The 7 animals were tranquillized with Telazol and Rompun before transportation to the laboratory. After intubation they were ventilated mechanically and anaesthesia was maintained with nitrous oxide and 1% to 2% Isoflurane during the intervention. They received Buprinorphine analgesic and Lidocaine was given subcutaneously on the site of the incision line followed by neuromuscular blockade with Pancuronium bromide. The heart was exposed via a small left thoracotomy in the fifth intracostal space, and the left lung was deflected and covered by a cotton sponge. The pericardium was opened longitudinally, posterior to the phrenic nerve. The two edges of the pericardial sac were attached to the chest wall with silk sutures to expose the heart, which was supported in the pericardial sling. Right and left atrial quadripolar electrode plaques were attached with monofilament sutures.

The left atrial appendage was excluded using a large Satinski vascular clamp. The atrial appendage was opened longitudinally and its edges were then reenforced using Dacron® fabric. Before introducing the probe, full heparinization was established.

The porcine left atrial anatomy differs from human anatomy. There is a single ostium with two to four pulmonary vein orifices that is situated on the "posterior inferior" (dorso-caudal) segment of the left atrium. The right and left pulmonary vein orifices are very close to each other and only separated by a crest. The posterior wall is therefore above (cephalad) the pulmonary vein ostium (Fig 1). There is a sizable cuff of left atrium between the pulmonary vein ostium and the mitral valve annulus over the inferior wall that comprises the "left atrial isthmus" [17]. Beneath runs the coronary sinus that is the terminal segment of a patent left superior vena cava.

The coronary sinus comprises a thick layer of myocardium in its adventitia that constitutes an inter-atrial connection that remained attached to the nonexcluded segment after isolation. Therefore the line of block was located dorsal (or posterior) to the coronary sinus to achieve complete reproducible isolation.

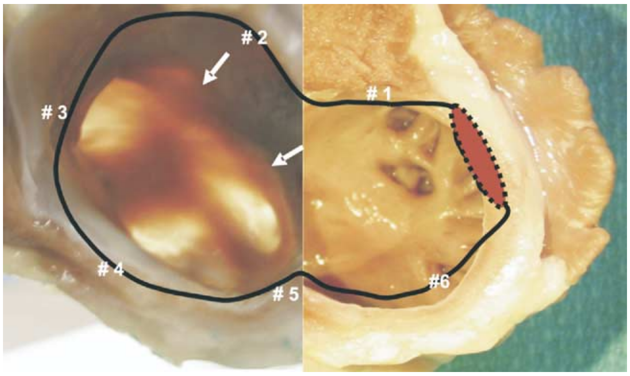

Fig 1. *A composite photograph with two adjacent views of the same formalin preserved porcine left atrium viewed through the mitral valve orifice. The left view shows the pulmonary vein ostium that is located transversally over the posterior inferior (dorso-caudal) segment of the left atrium. The limits of the ostium are well delineated* (arrows)*. There is one pulmonary vein on the right and two veins on the left. Transillumination shows well the network of myocardial bundles within the ostium and around the veins. The posterior wall lies above the ostium. The right view shows a large left appendage orifice with its trabeculations. The* filled, dash lined oval *facing the appendage represents the atrial port access through the appendage, whereas the* solid line *represents the line of overlapping cryoablations. The* numbers *indicate the sequence of the overlapping applications of the cryoprobe.*

The Bachmann bundle [18] is a thick inter-atrial bundle, whose width and thickness can preclude transmural cryoablation. Therefore the cryoablation line was located dorsal to the bundle over the posterior wall, while the Bachmann bundle was left attached to the nonexcluded left atrial cuff.

The encircling line of block ran from the upper segment of the left atrial appendage, then along the left atrial posterior wall dorsal to the Bachmann bundle, circumscribing the right pulmonary vein orifice. It then traveled along the left atrial inferior wall at a distance from the mitral valve annulus and posterior to the coronary sinus, to end at the inferior segment of the left atrial appendage (Fig 1).

Manipulation of the cryoprobe was guided using echocardiography and the effect was assessed by monitoring of interatrial conduction delay. In particular, the latter was of value in positioning the probe dorsal to the Bachmann bundle. The correct application of the cryoprobe to attain uniform contact and optimal freezing was assessed using a mark on the tool, the pattern of temperature drop and surgeon's experience. The overlapping applications were conducted in a clockwise fashion from the left superior quadrant contiguous to the superior segment the left atrial appendage, to the left inferior quadrant contiguous to the inferior segment of the left atrial appendage (Fig 1).

Image Guidance

The probe for intracardiac echocardiography was introduced after a cut down via the left internal jugular vein into the right atrium and complemented by transesophageal echocardiography.

Cryoablation

Cryoablation was carried using a modified SurgiFrost (CryoCath Inc, Kirkland, Quebec, Canada) cryoprobe introduced intra-atrially via the opening in the left atrial appendage. The cryoprobe was cooled at maximum capacity between −95°C to −135°C for 3 minutes for each application.

Electrophysiological Studies

A custom designed quadripolar electrode plaque was attached to the right atrial appendage (RAA) and another was positioned over the posterior wall of the left atrium (LPA). In the pig the posterior wall of the left atrium is in direct contact with the pulmonary artery trunk. A small pouch was constructed between the two, the plaque electrode was inserted and the pouch closed by a 4-0 monofilament stitch. This maneuver allowed insertion of the plaque at the center of the excluded segment at a distance from the cryoablation lines.

A surface electrocardiogram (ECG) signal, two bipolar electrograms from each of the RAA and LPA electrodes and the arterial blood pressure were continuously monitored by a Quinton EPAmp recording system (Quinton Electrophysiology Corp, Seattle, WA), while selected segments were recorded on optical disk or paper. Right and left atrial pacing thresholds were determined, and the atria were then paced at twice threshold to determine interatrial conduction delays.

Before ablation, burst pacing (2 seconds at 60 Hz) of the RAA and LPA electrodes was used to induce atrial arrhythmia: fibrillation or flutter. During cryoablation, RAA and LPA electrograms were monitored to assess pulmonary vein region isolation. Occurrence of right to left atrial delay, dissociation of the right and left electrograms, or disappearance of the left electrograms was of particular interest. Pacing of the posterior left atrium was used to verify the electrical isolation of the pulmonary vein region.

Following cryoablation, a repeat electrophysiological study was performed 15 min or more after completion of isolation. Stimulation of either the excluded pulmonary vein region or the non-excluded atrial segment (RAA), while recording surface and electrical activity of the other segment, was employed to assess a two-way isolation of the pulmonary vein region. Burst pacing (60 Hz) was also delivered to the RAA and LPA, to assess the impact of isolation on the inducibility of atrial arrhythmias (atrial vulnerability).

Pathological Examination

After completion of isolation and the final electrophysiological study, the heart was excised en bloc, with care being taken to keep the left atrium with its posterior wall and pulmonary veins intact. Macroscopic visual and photographic examination of the left atrium was conducted via the left atrial appendage, the mitral valve orifice after partial resection of the left ventricle and/or via a small window constructed through the posterior wall. The heart was subsequently fixed in 10% buffered formalin solution, prior to being sent to the pathology department, where, after fixation, 5 to 7 tissue samples were obtained along the circular cryoablation line (Fig 2). Alternate tissue slides were stained with Hematoxylin-Eosin and Movat's for examination by the pathologist (CG).

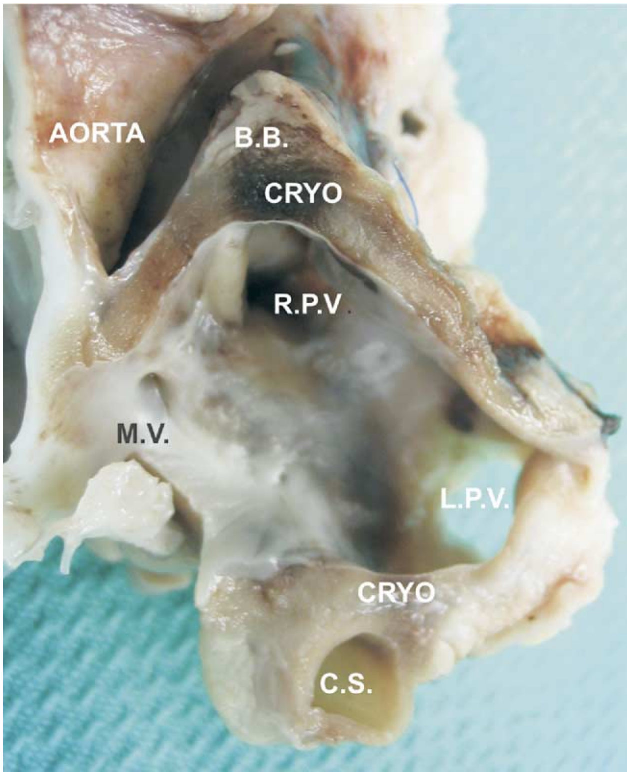

Fig 2. A left panoramic view of left atrium after excision of the appendage. The cryolesions are identified by the transparent band drawn over the endocardial surface. The CRYO are near B.B. superiorly, then circumscribe the pulmonary vein ostium (R.P.V. and L.P.V.), but stay posterior to the large C.S. and distant from the M.V. (B.B. = *Bachmann's bundle;* CRYO = *cryolesions;* C.S. = *coronary sinus;* L.P.V. = *left pulmonary vein;* M.V. = *mitral valve;* R.P.V. = *right pulmonary vein.)*

Results

No complications resulted from the surgical procedure in any of the pigs.

Image Guidance

Echocardiographic imaging provided critical guidance, with a good correlation to the surgeon's estimated position of the cryoprobe. No iceball was observed around the intra-atrial probe during freezing. Change associated with frozen atrial myocardium was not obvious by echocardiographic imaging during or after ablation. Various modes of ultrasonic imaging were employed to help

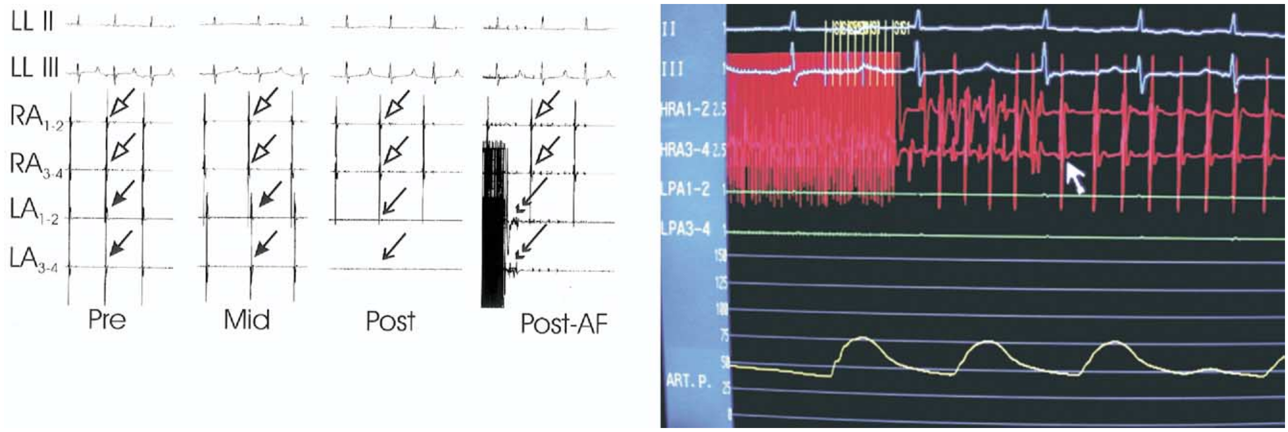

Fig 3. Electrophysiological recordings from LLs I and II (LLI, LLII), RAA, and LPA electrodes. (A) Representative spontaneous recordings: prelesion (Pre) *with simultaneous electrograms from the RAA* (open arrows) *and LPA* (closed arrows); *after the second lesion* (Mid) *with a delay between the RAA and LPA electrograms; after completion of the cryolesions* (Post) *showing sinus rhythm with no activation in the LPA* (thin arrows), *as well as 60-Hz burst induced short run of electrograms* (Post-AF) *from LPA electrodes on the isolated segment* (double-headed arrows) *without conduction to the RAA tissue* (open arrows). *(B) Photograph from the computer screen during burst (60 Hz) stimulation of the RAA that induced atrial flutter recorded in the RAA* (white arrow) *but without response in the LPA electrodes.* (ART.P. = *arterial pressure;* HRA = *right atrial electrograms;* LA = *left atrium;* LL = *limb leads;* LPA = *left posterial atrial region;* RA = *right atrium;* RPA = *right posterior atrial region.)*

visualize the probe within the left atrium and guide catheter manipulation. Initially two-dimensional (2D) transesophageal echocardiographic method was used for guidance, whereas four-dimensional (4D) imaging reconstructed from off line serial 2D images was used to create a three-dimensional (3D) image set for catheter visualization with respect to the pulmonary veins and mitral valve. However, 4D imaging proved difficult and therefore live 3D imaging with an epicardial probe was employed with good result.

Pulmonary Vein Isolation by Electrophysiological Assessment

A good marker for the adequate positioning of the probe dorsal to the Bachmann bundle was the recording of a significant right to left atrial delay (Fig 3A) when applying the second or third cryoapplication involving the right upper quadrant of the left atrium (Fig 1). This delay suggested that the nonexcluded segment remained in direct connection with the Bachmann bundle while the activation now took a "longer or slower" conducting route to reach the posterior wall (Table 1).

A complete en bloc isolation of the pulmonary region was obtained in 6 animals, while an incomplete result was observed in pig 5. In this pig, 2 additional cryoablation applications were performed, associated with temporary isolation within less than 20 sec of application, but conduction returned although the "isolated segment" had exit block and 3 to 2 or 3 to 1 entry conduction.

Atrial Arrhythmias Before Isolation of Pulmonary Vein Region

Sustained AF with a pattern consistent with left atrial origin was inducible using burst pacing of the LPA and often several seconds after atrial flutter induced with burst pacing of the RAA (Table 2). Two animals with sustained atrial fibrillation had to be cardioverted because of low blood pressure. The shortest cycle length varied from 87 to 135 ms when measurable. Reproducible fractionated electrograms were recorded from the LPA, and rarely from the RAA electrodes. RAA burst pacing induced non-sustained atrial flutter in 3 animals with a cycle length that varied from 92 ms to 137 ms.

Table 1. Sequence of Cryoapplications and the Degree of Isolation of the Left Posterior Atrial Region

Animal	Time of Significant R-L Delay	Time of Complete Isolation
Pig #1	80 ms at 3rd application. Right upper quadrant	6th application
Pig #2	60 ms delay after 2nd and then 90 ms after 3rd application	6th application
Pig #3	50 ms at right upper quadrant (2nd application)	Inferior LAA (4th application)
Pig #4	60 ms at right upper quadrant (2nd application)	Inferior LAA
Pig #5	45 ms inferior LA wall (4th application)	Had some intermittent conduction
Pig #6	39 ms 1st application	Needed repeat application over LA inferior wall
Pig #7	50 ms 2nd application	5th application

LA = left atrium; LAA = left atrial appendage; R-L = right to left atrial.

Table 2. Electrophysiological Characteristics of the Pigs Following Recording From, and Stimulation of the Right Atrial Appendage and Left Posterior Atrium

	Preoperative		Postoperative		
Animal	RA Pacing	LA Pacing	Rhythm in Isolated LA	Isolated LA Pacing	RA Pacing
Pig #1	Afl	Sustained AF	Very slow spontaneous activity	No AF fractionation for < 2.5 sec	No Afl
Pig #2	Afl turning into AF	Sustained AF	Slow spontaneous activity	No AF	Nonsustained Afl
Pig #3	Afl-137 ms	Sustained AF with fractionation	No spontaneous activity	One single beat after burst	Nonsustained Afl 130 ms
Pig #4	3–5 beats of Afl- 150 ms	AF with fractionation 87 ms	No spontaneous activity	Short lived fragmentation after burst	Afl during 3 Sec at 110 ms
Pig #5	Afl- 92 ms	Sustained AF 135 ms burst terminated	No activity or synchronous to right atrium	1–2 beats after burst	No inducible arrhythmias
Pig #6		Sustained AF with fractionation difficult to terminate	No spontaneous activity	1–2 beats after burst	Afl 150 ms less than 3 sec
Pig #7		Sustained Afl turning into AF-125 ms		Spontaneous narrow potentials activity	Few beats after burst for 1.5 sec

The numbers represent the R-R cycle length recorded from that electrode location. Cycles of fractionated local electrograms and typical surface lead morphology were considered indicative of atrial fibrillation.

AF = atrial fibrillation; Afl = atrial flutter; LA = left atrium; RA = right atrium.

Arrhythmia in the Excluded and Nonexcluded RA Segments After Isolation

No spontaneous electrical activity was recorded in 3 animals. In pig 4 , there were intermittent narrow potentials at a very slow rate. While burst pacing did not induce AF in the isolated segment in any animal, it nevertheless induced short run of fragmented electrograms or 1 to several beats with short time intervals.

In the nonexcluded segment, short runs of atrial flutter (≤ 3 seconds) were induced by RAA pacing in 5 animals (Fig 3B), but no atrial arrhythmia was inducible in 3. The animal with intermittent isolation exhibited a response to stimulation similar to that observed in pigs with the fully isolated LPA.

Pathological Findings

MACROSCOPIC EXAMINATION. Shortly after excision and opening of the left atrium, most of the lesions were not readily visible, although a subtle reddish discoloration could be detected in some locations around the pulmonary vein region. There was neither erosion of the endocardium nor thrombi. After fixation, the lesions were readily visible, with a much darker band of endocardium circumscribing the pulmonary vein region (Fig 2). The left atrial wall thickness varied significantly from 7 mm over the superior wall in the prolongation of the Bachmann bundle, to 1.5 mm at the inferior wall posterior to the coronary sinus. The line of cryoablation was positioned posterior to the coronary sinus as planned (Fig 2).

Histological examination documented typical acute cryolesions (Fig 4). Transmural lesions were obtained with myocytes necrosis, vascular congestion and interstitial haemorrhage. Lesions were completely transmural in the thinner segments, while the epicardial fat was spared in thicker segments with some hemorrhage at the surface. In the endocardium, hemorrhage and edema were a constant feature.

Comment

Feasibility of Off-Pump Beating Intracardiac Surgery

Left atrial surgery for atrial fibrillation is currently performed using an epicardial approach on the off-pump beating heart [19, 20]. Because of inherent limitations in

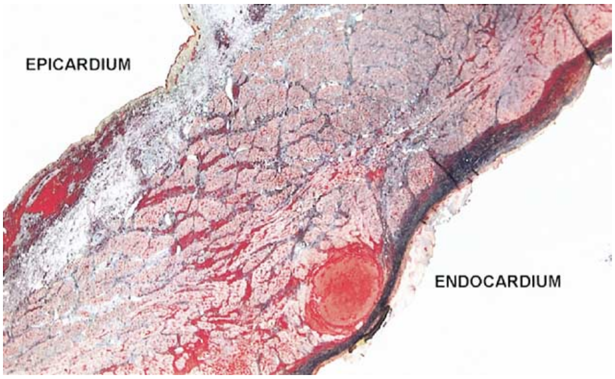

Fig 4. A histological section of a transmural cryolesion in the left atrium. There is transmural hemorrhage, thinning of subendocardial elastic layers and a subendocardial hematoma, transmural necrosis of cardiomyocytes, and edema and hemorrhage of the epicardium. This specimen shows thin endocardial fibrinous layers.

terms of access to the complex anatomy of the pericardial sac, and in terms of transmurality of lesions [21–23], the epicardial approach does not duplicate the effectiveness of open heart surgical techniques [19, 20].

We have demonstrated that an open-heart surgical procedure could be duplicated in the off-pump, closed, beating heart without complications or limitations associated with the challenges of this novel approach. This study is part of a multidisciplinary project which includes bioengineering imaging [24–26], haptics and other artificial intelligence tools and manipulators to offer beating, intracardiac, off-pump surgery as a preferred alternative to open-heart surgery for most of its current targets. At this stage of the evolution of this technique, the most important priority is the development of a reliable 4D image guidance environment, to compensate for the absence of direct vision, along with the integration of robotic and haptic tools.

Image Guidance

Echocardiographic imaging provided good guidance to ascertain the location of the probe in relation with the pulmonary vein orifices, the Bachmann bundle, the mitral valve orifice and the coronary sinus. However, the quality of the contact of the probe was left to the surgeon's touch. In support of this procedure and other intracardiac interventions, we have developed an extensive virtual cardiac environment to permit preoperative dynamic compute tomographic (CT) or magnetic resonance (MR) patient images to be registered to and synchronized with the patient. Preliminary work towards this goal has been reported [24–28]. We are currently addressing the issues of image-to-patient registration, as well as the mapping of electrophysiological data, acquired via a tracked (CARTO System; BioSense Webster, Diamond Bar, CA) probe onto the model. In addition we plan to integrate tracked intracardiac ultrasound with the virtual model in order to give the intraoperatively acquired images their appropriate context with respect to the model. We believe that the realization of this environment, when integrated with intra-cardiac procedures such as that described in this paper, will result in enhanced planning, more precise targeting, and increased efficacy of the intracardiac, off-pump procedures.

Cryoablation

Transmural lesions were reliably obtained using modified SurgiFrost probes. Failure to achieve complete isolation was more likely associated with misguided application [21], and the difficulty of precisely deploying the full length of the 6-cm probe to assure contiguous contact with the atrial wall, particularly in the convoluted regions of the septum to pulmonary vein junction.

Criteria for Isolation

Electrical isolation can only be documented using electrophysiological testing to confirm a bidirectional bock. Because cryoablation is associated with three zones: a central zone of irreversible myocardial damage, a zone of reversible cellular change and a distal zone of intact tissue, observed electrical isolation may be only temporary. Based on our clinical experience of reversible cryogenic damage [21], we allowed 15 minutes before reassessment. Macroscopic and/or microscopic examination can only suggest or document incomplete isolation when borderline transmurality or a gap of viable myocardium is observed. Because the entire circumference of the ablation zone cannot realistically be examined using serial histological slides, the histological examination is used only in support of electrophysiology and to document unwanted effects on tissue or vasculature.

Conclusion

These acute studies produced electrophysiological features very similar to those observed in surgical patients with chronic or persistent lone AF [12]. Before isolation, all animals exhibited evidence consistent with sustained left sided atrial fibrillation and right-sided atrial flutter, a condition similar to patients with lone AF. Acute effects of isolation of the pulmonary vein region was associated with inability to induce sustained AF in the excluded segment, while only short lived atrial flutter could be induced in the non-excluded segment. These findings are very similar to those of patients with persistent or paroxysmal AF and suggests that the mechanism of acutely induce AF in "normal" porcine heart and that of patients may be similar and that the pig is a valid experimental model for AF.

Further developments need to address the study of the pathophysiology of AF in a chronic isolation model, while at the same time developing sophisticated technology to transfer this approach to a "user-friendly" clinical setting. Additional developments will be needed in ultrasound guidance, as well as improvements in the design of the lesioning probe to optimize the efficacy of this procedure, while developing the 4D virtual reality platform [24–28].

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