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Efficacy and Safety of Circumferential Pulmonary Vein Isolation Using a Novel Cryothermal Balloon Ablation System

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OBJECTIVES We sought to evaluate the efficacy and safety of a novel cryothermal balloon ablation system in creating pulmonary vein (PV) isolation.

BACKGROUND Pulmonary vein isolation using standard radiofrequency ablation techniques is limited by procedure-related complications, such as thrombus formation and PV stenosis. Cryothermal ablation may reduce the risk of such complications.

METHODS Eight dogs underwent circumferential ablation of both superior PVs for either 4 or 8 min using a cryothermal balloon catheter (CryoCath Technologies Inc., Kirkland, Canada). Both fluoroscopy and intracardiac ultrasound (ICE)-guided balloon and Lasso catheter positioning at the PV ostia assessed short-term PV integrity. In six additional dogs, long-term PV integrity was assessed by computed tomography at 16 weeks after ablation.

RESULTS Successful electrical isolation was achieved acutely in 14 of 16 (87.5%) PVs and was confirmed in one-week survival studies in 10 of 12 (83%) PVs. Successful isolation was higher in the absence of any peri-balloon flow leak as seen by ICE ($p = 0.015$), and with balloon temperatures $\leq -80°C$ ($p = 0.015$). Cryolesions were located at the veno-atrial junction and were homogeneous, with intact endothelium and free of thrombus formation. Although limited angiographic PV narrowing was noted in the early follow-up period, no significant PV narrowing was seen long-term. Right phrenic nerve injury was seen in 50% of the animals studied at one week.

CONCLUSIONS This novel cryothermal balloon ablation system is effective for isolating PVs, but injury to the right phrenic nerve was noted in this early experience. Further studies are needed to assess the long-term efficacy and safety of this technique. (J Am Coll Cardiol 2005;46:1902–12)

Pulmonary veins (PVs) play an important role in the pathogenesis of atrial fibrillation (AF), and their electrical isolation has been shown to be an effective treatment for this arrhythmia (1–5). Although the success rate for radiofrequency AF ablation has improved as new anatomically and electrophysiologically based treatment strategies have been introduced (1–5), a variety of procedure-related complications, including thromboembolic events, PV stenosis, and esophageal-atrial fistula formation, remain a significant concern and limit an even wider application of these techniques (1–6). Therefore, alternative energy sources and new ablative strategies are being investigated (7–10).

Previous clinical and experimental investigations have shown that cryoablation is a safe method of eliminating cardiac arrhythmias (11,12). More recently, catheter-based techniques have been developed and used for circumferential ablation around the PV ostia (7,8). Although initial results have been favorable, cryoablative procedures have been lengthy and efficacy variable (7,8). Safety also remains to be established. These findings provide the incentive for further development of cryothermal energy delivery systems to increase efficacy, reduce procedure times, and allow wider application of this approach in the clinical arena.

We speculated that delivering cryoenergy at the veno-atrial junction via an occlusive balloon catheter would facilitate the rapid isolation of PVs. The purposes of this study therefore were to evaluate the efficacy and safety of a novel cryothermal balloon ablation system and establish the key contributors to ablative success.

METHODS

General. The experimental protocol was approved by the Mayo Foundation Institutional Animal Care and Use Committee. Eight mongrel dogs weighing between 30 and 40 kg were used for this study. Animals were anesthetized with intravenous ketamine (10 mg/kg) and diazepam (0.5 mg/kg), intubated, and maintained on 1% to 3% isoflurane with positive-pressure ventilation. The body temperature was maintained at 37°C. The surface electrocardiogram, temperature, and blood pressure were continuously monitored during the procedure.

Catheterization and PV mapping. Using tissue cut-downs and the Seldinger technique, hemostatic sheaths were placed in the right and left external jugular veins and the

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Abbreviations and Acronyms

AF = atrial fibrillation
CT = computed tomography
ICE = intracardiac echocardiography
LA = left atrium
LSPV = left superior pulmonary vein
PV = pulmonary vein
RA = right atrium
RSPV = right superior pulmonary vein
SVC = superior vena cava

right femoral vein and artery. A 6-F decapolar catheter was advanced to the coronary sinus for anatomical guidance and pacing. A 7-F quadripolar catheter was positioned at the right atrium (RA)/superior vena cava (SVC) junction for high-output pacing of the phrenic nerve. Right heart hemodynamic monitoring was assessed by a Swan-Ganz catheter. Double transseptal catheterization was accomplished under both fluoroscopic and intracardiac ultrasound (ICE) guidance using an 8-F Mullins sheath and a Brockenbrough needle (13). Intravenous heparin was given to attain an activated clotting time >250 s. Before ablation, each targeted PV was mapped with a deflectable, circumferential decapolar catheter (Lasso catheter, Biosense Webster, California), positioned at the PV ostium guided by biplane fluoroscopy.

Selective PV angiography and ICE imaging. Selective PV angiography was performed through a multipurpose angiocatheter by hand injection of 10 ml of contrast medium into the targeted vein. Pulmonary vein ostial diameters were measured by angiography using the technique described by Lin et al. (14). The ICE imaging of the left atrium and PVs was performed via a 10-F, 5.5- to 10.0-MHz imaging frequency, deflectable phased-array ultrasound catheter, with pulsed and color Doppler flow capabilities (AcuNav, Acuson Siemens Corp., Mountain View, California) advanced into the RA.

Cryothermal balloon catheter. The cryothermal balloon ablation system (CryoCath Technologies Inc., Kirkland, Canada) consisted of a non-deflectable, over-the-wire 10-F two-lumen catheter with double inner-outer cooling balloons (outer balloon maximum diameter, 23 mm; total length, 20 mm) (Fig. 1A). The refrigerant N_2O was delivered under pressure from the console into the inner balloon chamber via a lumen within 2 mm of the catheter tip, where it undergoes a liquid-to-gas phase change resulting in inner balloon cooling to temperatures ≤−80°C. During cryotherapy, temperature was monitored via a thermocouple located at the inner balloon.

Ablation. The balloon catheter was advanced over a guidewire through a 12-F, 65-cm-long sheath (Daig, St. Jude Medical Inc., Minnetonka, Minnesota) to the PV orifice, as shown in Figure 1B. The balloon was then inflated and the balloon-PV seal verified by both Doppler flow examination and repeated contrast injection through the central lumen of the catheter (Figs. 1B and 1C). The location of any peri-balloon flow leak was recorded and referenced to the position of the PV orifice. Cryoenergy was delivered to the coldest achievable balloon temperature (~−80°C) to both right and left superior PVs, for either 4 or 8 min according to pre-established randomization. Ablation was attempted up to two times, or until complete elimination of all PV potentials, as established by Lasso mapping repeated after every cryoablation.

One-week follow-up study. Two dogs were euthanized immediately after the ablation for histopathological examination. The remaining six animals were again instrumented at 1 week, before euthanasia. Ventricular fibrillation was induced with high-rate burst pacing, and the animal was exsanguinated.

Gross pathology and histologic examination. The heart, lungs, esophagus, trachea, and phrenic nerves were examined immediately for evidence of ablation effects and collateral damage. The left atrium was carefully dissected, and the PVs were then opened longitudinally to examine the endocardial surface with an incision along the 12 o'clock point of the vessel. After fixation with formalin, each ablated PV was embedded in paraffin and stained with hematoxylin-eosin and Masson trichrome. The samples were examined histologically and the results correlated with the key PV angiography, ICE, and Lasso catheter findings.

Long-term assessment of PV orifice dimension. In an additional six dogs, cryothermal ablation of both superior and inferior PVs was accomplished with the same size balloon for 2 to 16 min. Long-term PV orifice size was assessed with contrast-enhanced spiral computed tomography (CT) imaging, performed at baseline and repeated at 4 and 16 weeks after ablation. The CT images were obtained after 125 ml of contrast injection, and the acquired set of images was reconstructed with a 0.6-mm interval (Vitrea, Analyze software). Reconstructions of the ablated PVs were evaluated in two orthogonal planes, axial and coronal.

Statistical analysis. Continuous variables are reported as mean ± 1 SD and were compared using the Mann-Whitney test or a *t* test for paired data, as appropriate. The analysis of variance test was used to compare baseline, 4-week, and 16-week CT scan measurements. Categorical variables were compared using the Fisher exact test. Two observations from each dog were made in this study. An attempt was made to adjust for the potential correlation between these observations using generalized estimating equation models. However, given the small number of observations and the results of the data, the models could not be estimated. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Cryoablation of PVs. A total of 25 cryoapplications were delivered to 16 PVs, including 14 applications to 8 right superior pulmonary veins (RSPV) and 11 cryoapplications

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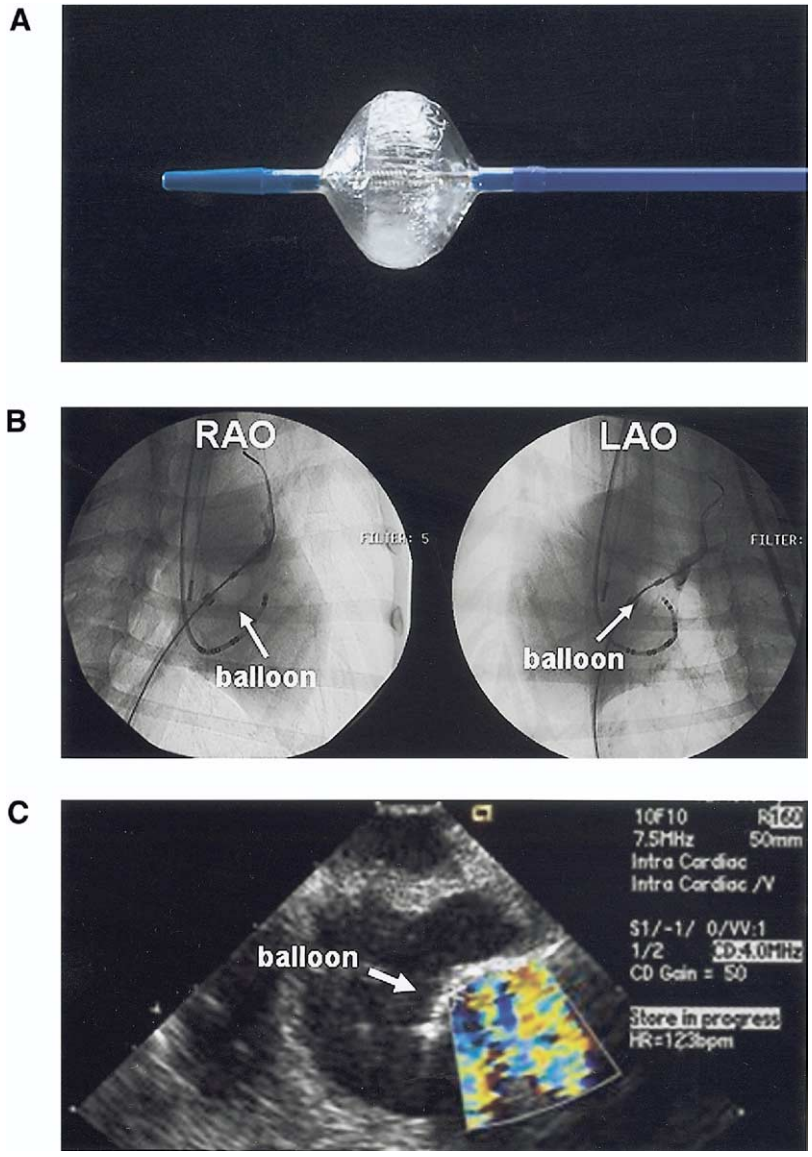

Figure 1. **(A)** Photograph of the cryothermal balloon catheter. **(B)** Fluoroscopic image of the inflated balloon engaged at the left superior pulmonary vein (LSPV) orifice **(arrows)**. **(C)** Intracardiac echocardiographic image of the inflated balloon positioned at the orifice of the LSPV **(arrow)**, illustrating an example of an unsuccessful occlusion of the pulmonary vein orifice, with a peri-balloon flow leak as seen by color Doppler flow. LAO = left anterior oblique projection; RAO = right anterior oblique projection.

to 8 left superior pulmonary veins (LSPV). The details of the ablative procedure and its results are summarized in Table 1. The total duration of cryoablation per vein was 8 ± 3 min. In acute studies, successful electrical PV isolation, as indicated by elimination of PV potentials, was achieved in 14 of the 16 (87.5%) attempted veins, as shown in Figure 2.

Determinants of successful PV isolation at one-week follow-up. In animals that survived to one week, successful electrical isolation was seen in 10 of the 12 (83%) treated PVs. Successful electrical isolation was obtained using 4-min cryoapplications in 2 PVs and >8-min of cryoablation in 8 PVs (7 PVs [8 min], 1 PV [16 min]). The outcome of ablation was not dependent on the treated PV ($p = 0.455$) or the number ($p = 0.455$) or duration ($p = 1.0$) of freezing, despite the occurrence of unsuccessful electrical isolation of the LSPV in two dogs. These two animals had residual PV potentials at the inferior portion of the LSPV ostium. The success rate for chronic PV isolation was significantly higher in the absence of any peri-balloon flow leak as evaluated by ICE (no leaks, 100% success; vs. any leak, 0% success; $p = 0.015$). Furthermore, the success rate for chronic PV isolation was significantly higher with achievement of balloon temperatures colder than −80.0°C (temperature ≤−80°C: success in 10 of 10 veins, 100%; vs. temperature >−73°C: success 0 of 2 veins, 0%; $p = 0.015$). In these animals, the minimum balloon temperature achieved during successful PV isolation was −81 ± 1°C (range, −80°C to −83°C) and −67 ± 6°C (range, −63°C to −72°C) during unsuccessful PV ablation ($p < 0.0001$) (Fig. 3).

Table 1. Ablative Approach and Results of Cryothermal Balloon Ablation of Pulmonary Veins

Dog No.	Vein	No. Cryoenergy Application	Total Duration Application (min)	Minimum Ablation Temperature (°C)	Angiography Leak Pre-Ablation*	ICE Leak Pre-Ablation	Immediate Success†	One-Week Success‡	Histopathologic Evidence of Complete PV Lesion
Group I									
1	RSPV	2	8	−82	None	No	Yes	NA	NA
	LSPV	2	8	−54	Mod	Yes	No	NA	NA
2	RSPV	2	8	−83	None	No	Yes	NA	NA
	LSPV	1	4	−81	Mild	No	Yes	NA	NA
Group II (one-week survival dogs)									
3	RSPV	1	4	−82	None	No	Yes	Yes	Yes
	LSPV	2	16	−72	Mod	Yes	Yes	No	No
4	RSPV	2	8	−81	None	No	Yes	Yes	Yes
	LSPV	1	8	−82	Mild	No	Yes	Yes	Yes
5	RSPV	2	16	−82	None	No	Yes	Yes	Yes
	LSPV	1	8	−80	Mild	No	Yes	Yes	Yes
6	RSPV	1	4	−81	None	No	Yes	Yes	Yes
	LSPV	2	8	−63	Mod	Yes	No	No	No
7	RSPV	2	8	−81	None	No	Yes	Yes	Yes
	LSPV	1	8	−83	None	No	Yes	Yes	Yes
8	RSPV	2	8	−81	Mild	No	Yes	Yes	Yes
	LSPV	1	8	−80	Mild	No	Yes	Yes	Yes
Mean ± SD		1.5 ± 0.5	8.2 ± 3.4	−78 ± 8			14/16	10/12	10/12

*Angiographic peri-balloon flow leaks were classified as none: during contrast injection absence of any peri-balloon flow leak, with persistence of the contrast agent inside the PV for minutes; mild leak: presence of a temporary mild peri-balloon flow leak related to the increased pressure inside the vein during contrast injection, with persistence of the contrast agent inside the PV for minutes; moderate leak: during contrast injection presence of a peri-balloon flow leak, with quickly expelling of the contrast agent, but still allowing visualization of the venous structure; severe leak: during contrast injection presence of a peri-balloon flow leak, with quickly expelling of the contrast agent resulting in poor visualization of the venous structure. †Persistence of PV entrance block 30 min after the last cryoapplication. ‡Persistence of PV entrance block at one-week after ablation.

ICE = intracardiac echocardiography; LSPV = left superior pulmonary vein; NA = not available; RSPV = right superior pulmonary vein.

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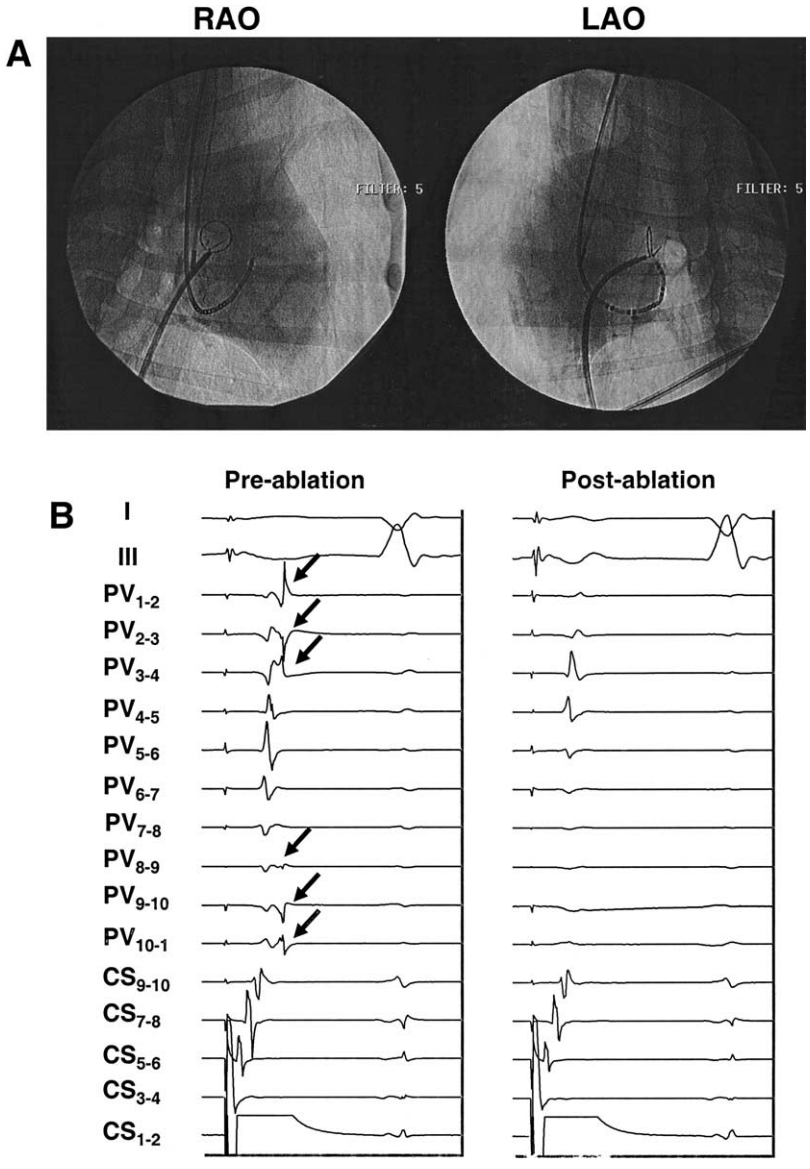

Figure 2. Successful circumferential cryoablation of the left superior pulmonary vein. **(A)** Fluoroscopic image of the Lasso catheter positioned at the pulmonary vein (PV) ostium. **(B)** Tracings of the surface electrocardiographic leads I and III and PV ostial electrograms as seen by the Lasso catheter (PV_{1-2} to PV_{10-1}) during distal coronary sinus pacing, and recorded before ablation **(left)** and after ablation **(right)**. LA = left atrium; other abbreviations as in Figure 1.

PV angiography, ICE imaging, and Doppler flow studies. In all six surviving dogs, selective angiograms were repeated before euthanasia in 11 of the 12 treated PVs (Table 2). Under baseline conditions, the mean ostial diameter of the ablated PVs was 10.5 ± 1.0 mm, which decreased to 7.2 ± 1.1 mm after the ablative procedure ($p < 0.001$). In contrast, PV-LA mean pressure gradient and right heart pressures were similar at baseline and after ablation.

On ICE imaging, a modest reduction of the PV orifice dimension was also noted after ablation (baseline 13.8 ± 1.6 mm vs. post-ablation 12.9 ± 2.1 mm; $p < 0.05$). The PV flow velocities measured at one week post-ablation were not significantly different from baseline in all ablated (0.47 ± 0.09 vs. 0.52 ± 0.06, $p = 0.586$) and non-ablated veins (0.53 ± 0.07 vs. 0.55 ± 0.09, $p = 0.332$).

Long-term assessment of PV orifice dimension. To further assess the long-term impact of early PV narrowing on outcome, we performed a second experiment in six additional dogs. Fifteen PVs were ablated (six LSPV, six RSPV and three LIPV), with a mean duration of cryoenergy delivery of 6 ± 5 min. The minimum balloon temperature achieved was −71 ± 7°C (range, −62°C to −80°C). On CT scan, PV orifice diameter was similar at baseline, at 4 weeks, and at 16 weeks after ablation at both the axial view (baseline, 13.4 ± 2.2 mm; 4 weeks, 12.6 ± 3.3 mm; and 16 weeks, 13.8 ± 2.0; $p = 0.48$) and coronal view (baseline, 15.0 ± 3.2 mm; 4 weeks, 14.5 ± 4.2 mm; and 16 weeks, 15.2 ± 3.6 mm; $p = 0.47$).

Pathological findings. In the six surviving dogs, gross and histologic examination showed a complete circumferential

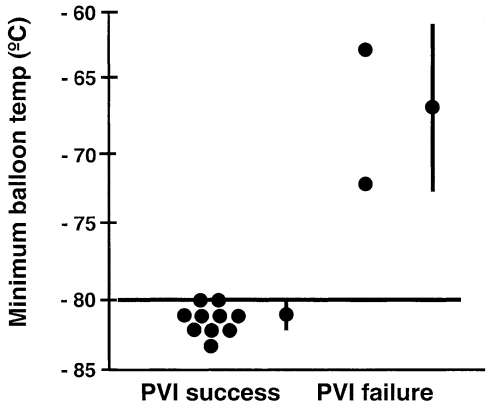

Figure 3. Impact of minimum balloon-tip temperature on outcome of ablation in 12 pulmonary veins. The minimum balloon-tip temperature levels related to successful or unsuccessful pulmonary vein isolation (PVI) are shown. Temperature values in some pulmonary veins overlap.

and transmural lesion at the veno-atrial junction in all instances in which successful PV electrical isolation was obtained (Fig. 4). Conversely, in the two unsuccessfully isolated LSPVs, a non-circumferential lesion gap at the inferior aspect of the PV orifice was noted (Fig. 5). Interestingly, the position of the lesion discontinuity corresponded to both the location of the remaining PV potentials as seen by the Lasso catheter and the peri-balloon flow leak as seen by PV angiography and ICE imaging (Fig. 5).

At one week after ablation, cryolesions had well-demarcated borders with inflammatory infiltrate, necrotic myocardial cells, a mild degree of fibrosis, and a discrete subendothelial proliferation. In all lesions there was no disruption of the endothelial surface and no evidence of thrombus formation.

Collateral damage to adjacent structures. Failure to capture the right phrenic nerve despite maximum pacing output (20 mA) was seen in four of the eight dogs in the immediate post-ablation evaluation, and remained unsuccessful in all three animals surviving to one week. In these four animals, evidence of right phrenic nerve injury was observed on gross inspection, as shown in Figure 6A. There was no correlation between phrenic nerve injury and either the number or total duration of freezing. There was no evidence of injury to the esophagus or trachea on gross examination. A minor lung injury adjacent to the RSPV ostium was noted in one of the eight studied dogs.

DISCUSSION

This study provides substantial information regarding the efficacy and safety of novel cryothermal balloon technology in creating PV isolation. First, cryoablation was highly effective, although this is a short-term study and our results may not represent the long-term response of cryolesions. Second, optimal balloon-tissue contact was required for successful PV isolation. Third, electrical isolation of PVs correlated closely with circumferential and transmural lesions at the veno-atrial junction. Fourth, cryolesions were homogeneous, with intact endothelium, and were free of thrombus formation. Fifth, although limited angiographic PV narrowing was noted in the early follow-up period, no significant PV narrowing was seen on long-term follow-up. Finally, right phrenic nerve injury was common in the early experience with this technique.

Cryoablation through various systems has been used successfully for PV isolation (7,8). For example, one study used a catheter-based technique for cryoablation with circumferential point-by-point ablation around the PV ostia guided by a Lasso catheter (7). The long-term efficacy with this approach was 71% without evidence of PV stenosis. However, the technique required high interventional expertise and was associated with long procedure times (7 to 8 h). In comparison with current radiofrequency ablation ap-

Table 2. Distribution of Pulmonary Vein Ostial Diameters and Doppler Flow Velocities

Dog No.	PV	Angiography Mean PV Ostial Diameter (mm)		Angiography % Reduction Diameter	ICE Mean PV Ostial Diameter (mm)		ICE PV Doppler Flow Velocity (m/s)	
		Baseline	Post-Ablation		Baseline	Post-Ablation	Baseline	Post-Ablation
Group II (one-week chronic studies)								
3	Ablated*	10.9	6.1	44	13.2	11.6	0.54	0.50
4	Ablated*	9.8	7.4	24	12.4	10.6	0.35	0.61
5	Ablated*	11.2	8.8	21	14.5	15.2	0.45	0.45
6	Ablated*	8.7	6.0	31	13.7	12.6	0.38	0.48
7	Ablated*	11	8.1	26	16.7	15.8	0.56	0.56
8	Ablated*	11.2	6.6	41	12.4	11.5	0.57	0.55
Mean ± SD		10.5 ± 1.0	7.2 ± 1.1‡	31 ± 9	13.8 ± 1.6	12.9 ± 2.1‡	0.47 ± 0.09	0.52 ± 0.06
3	Non-ablated†	NA	NA	NA	11.9	11.8	0.65	0.50
4	Non-ablated†	NA	NA	NA	11.4	13.6	0.47	0.74
5	Non-ablated†	NA	NA	NA	13.5	12.0	0.49	0.55
6	Non-ablated†	NA	NA	NA	11.7	11.0	0.50	0.48
7	Non-ablated†	NA	NA	NA	12.9	12.5	0.52	0.51
8	Non-ablated†	NA	NA	NA	12.8	15.2	0.57	0.53
Mean ± SD		NA	NA	NA	12.4 ± 0.8	12.7 ± 1.5	0.53 ± 0.07	0.55 ± 0.09

*Right and left superior pulmonary veins. †Right and left inferior pulmonary veins. ‡$p < 0.05$.
ICE = intracardiac echocardiography; NA = not available; PV = pulmonary vein.

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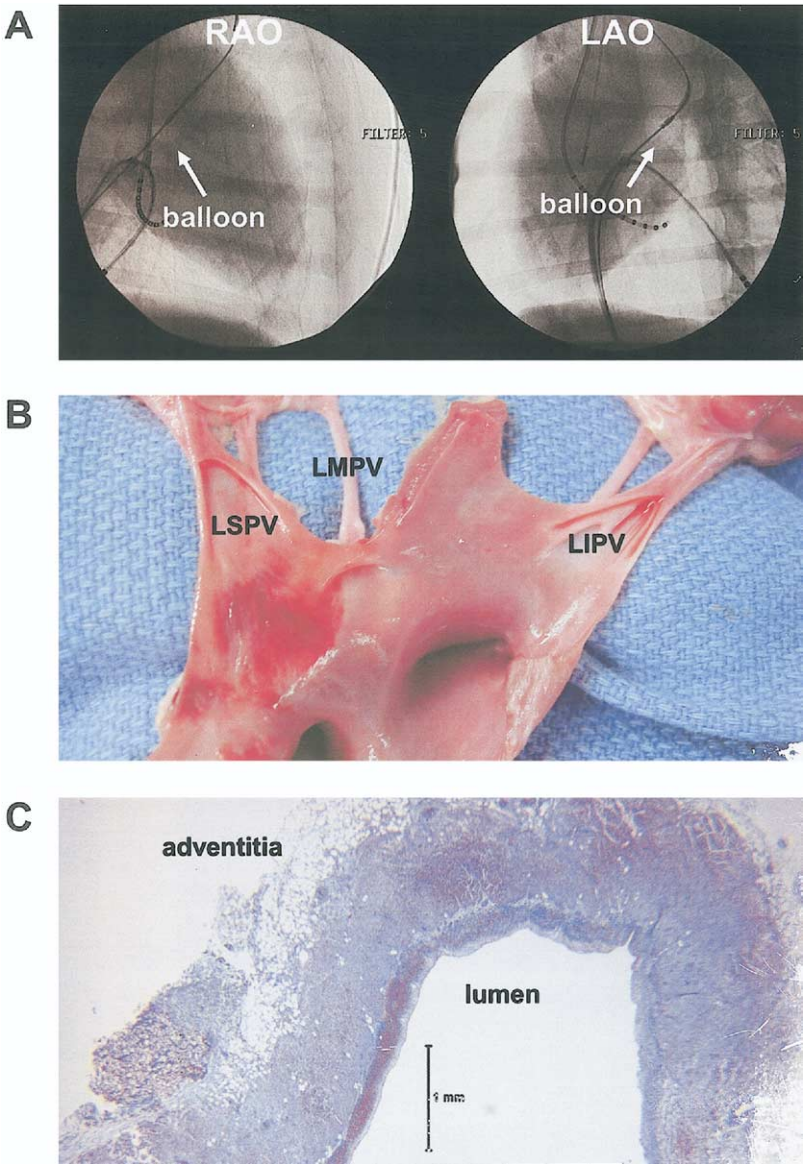

Figure 4. Complete circumferential and transmural lesion at the veno-atrial junction of the left superior pulmonary vein (LSPV). Same ablated pulmonary vein as seen in Figure 2. **(A)** Fluoroscopic image of the inflated balloon engaged at the LSPV orifice **(arrows)**. **(B)** Photograph of the left atrium open showing a well-demarcated lesion at the veno-atrial junction that extended to a wide area outside the LSPV ostium. **(C)** (Masson trichrome stain; 2.5×; bar = 1 mm), photomicrograph of a transverse section at the veno-atrial junction; a circumferential and transmural lesion is shown. LIPV = left inferior pulmonary vein; LMPV = middle branch of the left superior pulmonary vein; other abbreviations as in Figure 1.

proaches for paroxysmal atrial fibrillation (1–4), the success rate was lower and the procedure times were longer. These limitations prompted investigation of novel technologies such as balloon-based systems that would be less dependent on operative expertise and decrease procedure times (9,15).

Our findings extend the results of previous experimental studies (9,15) reporting the use of cryoballoon technology for PV isolation. In prior studies, cryoballoon ablation was successful in reducing P-wave activity in the ablation zone, decreasing inducibility of atrial fibrillation, and reducing local PV electrogram amplitudes (9,15). Although these preliminary studies suggested a potential benefit from cryoballoon-based systems, our observations clarify the clinical efficacy using a more rigorous elimination of all PV activity as an end point. These data also document the prerequisites for successful PV isolation and potential limitations of this approach. Furthermore, they show the value of balloon occlusion as measured by intracardiac echocardiography on procedural success.

In animals surviving to one week, successful electrical isolation was possible in 83% of the treated PVs. In animals with electrical PV isolation, a complete circumferential cryolesion was observed at the veno-atrial junction. However, the Lasso catheter specifically identified the location of the histologic lesion gap. The rate of PV electrical isolation observed in this study is similar to or better than that achieved with anatomic-based approaches using radiofrequency energy (2,16).

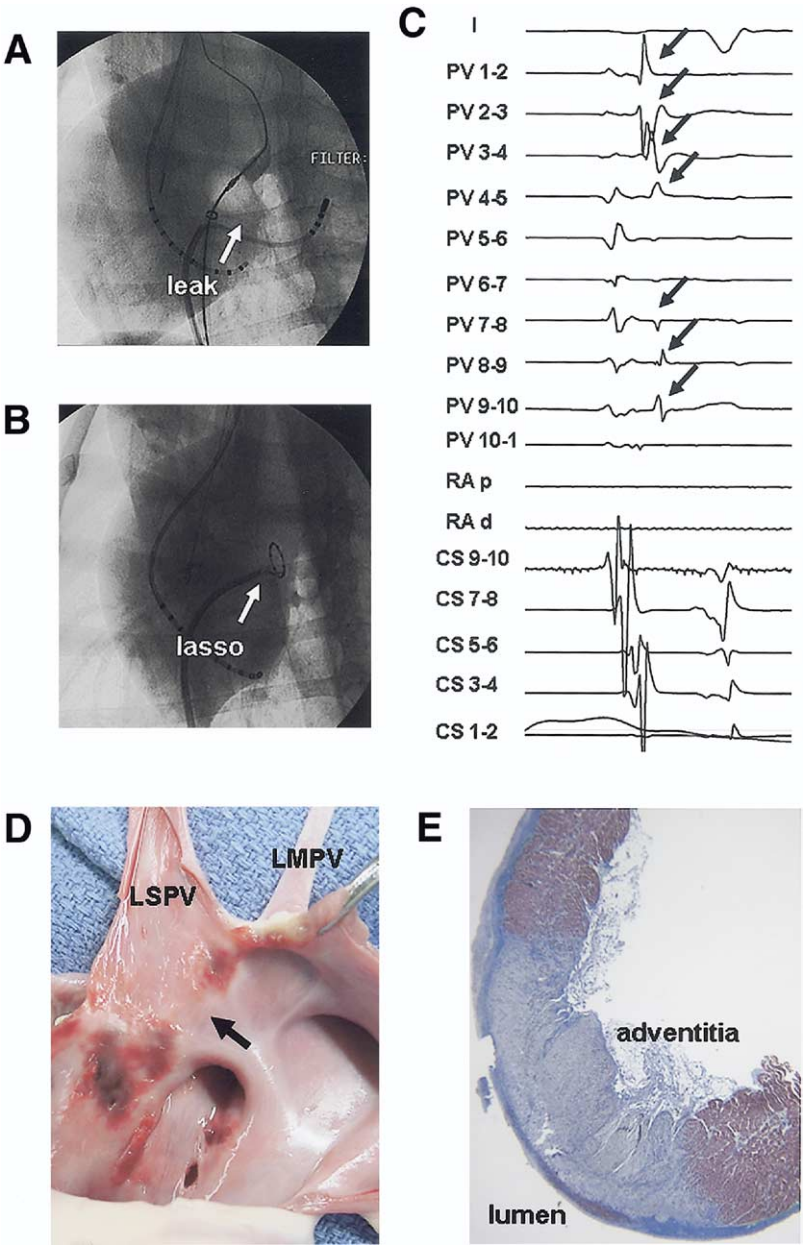

Figure 5. Example of unsuccessful cryoablation of the left superior pulmonary vein (LSPV). **(A)** Venography through the inflated balloon positioned at the LSPV orifice shows an unsuccessful occlusion of the pulmonary vein (PV) orifice, with a peri-balloon flow leak at its inferior aspect. **(B)** Position of the Lasso catheter at the LSPV orifice during repeated mapping at one week after ablation. **(C)** The PV potentials recorded by the Lasso catheter (PV_{1-2} to PV_{10-1}) as shown in **panel B**. Note that the remaining PV potentials **(arrows)** predominated at the inferior aspect of the vein. **(D)** Photograph of the endocardial surface of the left atrium showing a non-circumferential lesion at the veno-atrial junction, with the arrow pointing to the lesion discontinuity. Note that the position of the lesion gap corresponded to both the location of the remaining PV potentials and the peri-balloon flow leak. **(E)** (Masson trichrome stain; 2.5×), photomicrograph of a transverse section at the veno-atrial junction. Abbreviations as in Figure 5.

The long-term impact of lesion gaps is uncertain. Ouyang et al. (17) reported outcomes in 41 patients with symptomatic paroxysmal atrial fibrillation undergoing continuous circular lesions around the PVs. After six months, recurrent atrial fibrillation developed in 24% with gaps found in the circumferential lines in all patients studied. Furthermore, in eight of nine (89%) patients, closure of these gaps resulted in freedom from recurrent atrial fibrillation. The impact of incomplete isolation of PVs with cryotherapy on successful elimination of arrhythmia remains to be elucidated in long-term models, because others have suggested that complete elimination of all gaps may not be necessary for successful elimination of AF (2,16).

In this study, 17% of the PVs were not successfully isolated, which allows analysis of cryoballoon therapy characteristics associated with efficacy. First, our data underscore the need of optimal balloon-tissue contact for successful electrical isolation of PVs. Complete balloon-tissue contact allows circumferential delivery of cryotherapy, which, as seen in this study, impacts the minimum temperature achieved. Optimal contact is de-

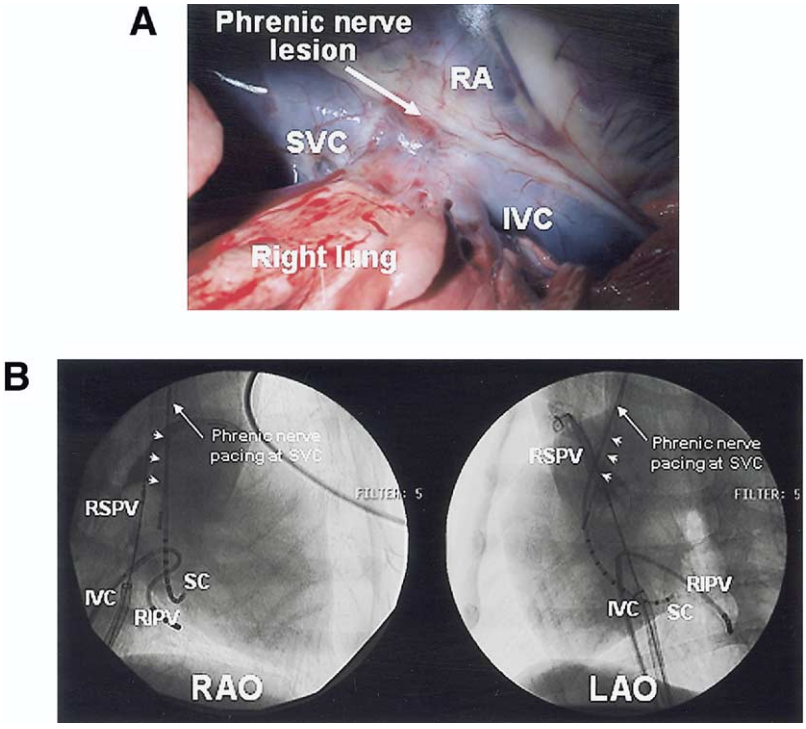

Figure 6. Phrenic nerve injury as seen on gross inspection. **(A)** Right phrenic nerve course is visible after deflecting the right lung rightward. A lesion at the right superior pulmonary vein (RSPV) ostium is visible, extending to adjacent structures and encompassing the right phrenic nerve. **(B)** Fluoroscopic image illustrating the relative proximity of the right phrenic nerve course to the inflated balloon engaged at the RSPV antrum. **Arrows** at the anterolateral wall of the superior vena cava (SVC) depict the site where successful phrenic nerve capture was obtained. The plausible course of the right phrenic nerve is marked with **arrowheads**. CS = coronary sinus; IVC = inferior vena cava; RA = right atrium; RIPV = right inferior pulmonary vein; other abbreviations as in Figure 1.

pendent on the size of the balloon and its relationship to PV shape and size. Specifically, in two animals in which the balloon size was much larger than the LSPV orifice, incomplete occlusion of the vein orifice resulted in an ineffective ablation. In this study, ICE was successful in each case in the identification of peri-balloon leak that was directly correlated with PV isolation failure. These data suggest that ICE use to identify peri-balloon leak and the availability of variable-sized balloons may overcome inherent anatomic barriers and enhance procedural success.

These data show that cryoapplications with balloon temperatures colder than −80°C were important to maximize successful PV isolation. Low temperatures were obtained with uniform and complete contact between the balloon and the PV wall. This would be expected to limit passive convective heating of the balloon and downstream atrial tissue by leaking blood flow. These data also expand other previous in vivo studies that reported therapy success when temperatures ranging from −30°C to −80°C were achieved at the PV orifice (8,9,15). The end points of this study requiring complete electrical isolation of PVs support a specific balloon temperature of −80°C.

In this study, cryolesions were extensive and located outside the PV orifice, which underscores another potential advantage of cryothermal balloon technology in creating PV isolation. With cryoablation, once complete PV occlusion is achieved, the orientation of the balloon at the PV orifice may be less relevant for successful PV isolation than for other balloon ablation technologies, such as ultrasound (10) and laser-based (18) balloon ablation systems, with a focused energy source. With the present cryoballoon system, provided temperature is uniform throughout the balloon, a circumferential lesion should be feasible at the PV orifice once complete balloon-tissue contact is achieved around the vein.

We sought to rigorously assess procedural-related complications resulting from this cryoballoon ablation system. The cryolesions had well-demarcated borders, with replacement of the muscular sleeve with inflammatory infiltrate and fibrotic tissue and without endothelial surface thrombus formation. These findings were consistent with previous observations using cryoenergy for ablating cardiac tissue (9,11,15).

In this study, modest angiographic PV ostial narrowing was seen in the early follow-up period, although these changes were not associated with untoward physiological sequelae. However, an additional chronic safety study conducted in our laboratory showed no long-term narrowing on repeated CT scanning over the course of four months after ablation. This apparent discrepancy may be related to transient edema or spasm occurring early after cryoablation, with resolution on long-term follow-up. Our observations confirm the results obtained in a previous experimental study using another balloon technology and repeated cryoenergy applications at the PV-LA junction (15), which

reported no PV stenosis or narrowing over the course of three months after ablation.

Phrenic nerve injury has been reported in patients undergoing radiofrequency-based (5) and cryoenergy-based catheter isolation of PVs (7) as well as with newer ablation balloon technologies (10). The incidence of phrenic nerve injury in our study was higher than expected. A potential explanation for this may be that the ablation procedure was performed with an undersized balloon positioned well into the RSPV antrum, and as a consequence cryoenergy had to be delivered twice, targeting each RSPV branch individually, to successfully isolate the RSPV antrum. Because the phrenic nerve courses closely to the RSPV orifice (Figs. 6A and 6B), it is conceivable that positioning the cryoballoon inside the RSPV antrum and delivering repeated cryoenergy might have increased the risk of phrenic nerve injury. A larger balloon catheter will be required to avoid this complication.

Second, balloon techniques may create a local environment conducive to enhanced "cold" transfer to deeper tissues. In vitro studies have shown that the distance of freezing from the energy source is dependent on the speed of ice growth, which in turn is related to initial temperature achieved, and inversely related to the tissue thickness and temperature gradient (19). In our study, the balloon occluded flow completely in all but one RSPV. This characteristic of the device in the RSPV allowed rapid freezing of the tissue to levels at or below −80°C. Because the balloon was within the RSPV antrum in four cases, the distance from the balloon to the phrenic nerve and the amount of tissue between the nerve and balloon were decreased. Expected phrenic nerve recovery could not be assessed over the one week of follow-up in this study.

Finally, although a minor lung injury adjacent to the RSPV ostium was observed in one of the eight dogs studied, none of the surviving animals had any symptoms attributable to lung injury such as hemoptysis.

Study limitations. The findings of this early experience with this new cryoballoon technology should be interpreted in light of several limitations. First, cryothermal ablation of PVs was evaluated using a single shape and size of balloon catheter without a deflectable tip for guidance. Because the anatomy of the dog does not generally allow positioning of the balloon catheter within inferior PVs without deflecting its tip or using specialized deflectable or curved sheaths, ablation of inferior PVs was not addressed in our study. Future balloon catheter models would require different shapes and sizes and a deflectable tip to overcome inherent anatomic barriers and enhance procedural success. This study also fails to precisely clarify the amount of cryoenergy that should be delivered to successfully isolate PVs. It remains unknown whether cryoapplications lasting <4 min are equally as effective as cryoapplications ≥ 4 min duration while limiting phrenic nerve injury risk. Another limitation of the present study is that the pathological effects of cryotherapy were investigated only at one week after ablation period, whereas prior studies have shown that cryolesions may take up to 12 weeks to fully maturate. Thus, it should be stressed that this is a short-term study and that our results may incompletely represent the long-term response of cryolesions. Further studies are needed to assess the long-term efficacy and safety of this novel technique. Finally, data from an in vivo canine model may vary from human tissue characteristics and responses in patients with atrial fibrillation.

Clinical implications. The efficacy of the novel cryoballoon ablation system for isolating PVs seen in the present study supports the utility of this technique in ablating PVs in the clinical electrophysiological laboratory. A cryoballoon approach to PV isolation is attractive because isolation of PVs can be quickly performed through a simple anatomical approach based on balloon positioning at the PV orifice. However, because of the variability of the PV anatomy, a family of balloon catheters with variable shapes and sizes will be required to fully implement this technique. The impact of this new technique on collateral injury remains to be elucidated completely.

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REFERENCES

1. Haissaguerre M, Shah DC, Jais P, et al. Electrophysiological breakthroughs from the left atrium to the pulmonary veins. Circulation 2000;102:2463–5.
2. Pappone C, Oreto G, Rosanio S, et al. Atrial electroanatomic remodeling after circumferential radiofrequency pulmonary vein ablation: efficacy of an anatomic approach in a large cohort of patients with atrial fibrillation. Circulation 2001;104:2539–44.
3. Oral H, Knight BP, Tada H, et al. Pulmonary vein isolation for paroxysmal and persistent atrial fibrillation. Circulation 2002;105:1077–81.
4. Marrouche NF, Martin DO, Wazni O, et al. Phased-array intracardiac echocardiography monitoring during pulmonary vein isolation in patients with atrial fibrillation: impact on outcome and complications. Circulation 2003;107:2710–6.
5. Adragao PP, Cavaco DM, Santos KR, et al. Percutaneous ablation of atrial fibrillation: assessment of outcomes at 1-year follow-up. Rev Port Cardiol 2003;22:1301–8.
6. Pappone C, Oral H, Santinelli V, et al. Atrio-esophageal fistula as a complication of percutaneous transcatheter ablation of atrial fibrillation. Circulation 2004;109:2724–6.
7. Tse HF, Reek S, Timmermans C, et al. Pulmonary vein isolation using transvenous catheter cryoablation for treatment of atrial fibrillation without risk of pulmonary vein stenosis. J Am Coll Cardiol 2003;42:752–8.
8. Rostock T, Weiss C, Ventura R, Willems S. Pulmonary vein isolation during atrial fibrillation using a circumferential cryoablation catheter. Pacing Clin Electrophysiol 2004;27:1024–5.
9. Avitall B, Urboniene D, Rozmus G, Lafontaine D, Helms R, Urbonas A. New cryotechnology for electrical isolation of the pulmonary veins. J Cardiovasc Electrophysiol 2003;14:281–6.
10. Saliba W, Wilber D, Packer D, et al. Circumferential ultrasound ablation for pulmonary vein isolation: analysis of acute and chronic failures. J Cardiovasc Electrophysiol 2002;13:957–61.
11. Khairy P, Chauvet P, Lehmann J, et al. Lower incidence of thrombus formation with cryoenergy versus radiofrequency catheter ablation. Circulation 2003;107:2045–50.

12. Friedman PL, Dubuc M, Green MS, et al. Catheter cryoablation of supraventricular tachycardia: results of the multicenter prospective "frosty" trial. Heart Rhythm 2004;1:129–38.
13. Johnson SB, Seward JB, Packer DL. Phased-array intracardiac echocardiography for guiding transseptal catheter placement: utility and learning curve. Pacing Clin Electrophysiol 2002;25:402–7.
14. Lin WS, Prakash VS, Tai CT, et al. Pulmonary vein morphology in patients with paroxysmal atrial fibrillation initiated by ectopic beats originating from the pulmonary veins: implications for catheter ablation. Circulation 2000;101:1274–81.
15. Avitall B, Lafontain D, Rozmus G, et al. The safety and efficacy of multiple consecutive cryo lesions in canine pulmonary veins-left atrial junction. Heart Rhythm 2004;1:203–9.
16. Stabile G, Turco P, Rocca VL, Nocerino P, Stabile E, DeSimone A. Is pulmonary vein isolation necessary for curing atrial fibrillation? Circulation 2003;108:657–60.
17. Ouyang F, Banish D, Ernst S, et al. Complete isolation of the left atrium surrounding the pulmonary veins. New insights from the double-lasso technique in paroxysmal atrial fibrillation. Circulation 2004;110:2090–6.
18. Reddy VY, Houghtaling C, Fallon J, et al. Use of a diode laser balloon ablation catheter to generate circumferential pulmonary venous lesions in an open-thoracotomy caprine model. Pacing Clin Electrophysiol 2004;27:52–7.
19. Bischof JC. Quantitative measurement and prediction of biophysical response during freezing in tissues. Annu Rev Biomed Eng 2000;2:57–88.