Impact of Freezing Time and Balloon Size on the Thermodynamics and Isolation Efficacy During Pulmonary Vein Isolation Using the Second Generation Cryoballoon

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Background—The differences in ablation characteristics of freezing time and balloon size using second generation cryoballoon are still unknown.

Methods and Results—Twenty-six dogs underwent pulmonary vein (PV) isolation. Balloon and tissue temperatures (left atrial–PV junction, phrenic nerve, and internal esophagus) were monitored. The ablation duration was randomized to either 3 or 4 minutes, which did not show significant differences in temperature profiles, PV isolation success rate, complications, or histological changes. Twenty dogs underwent cryoablation using 28-mm cryoballoon, 6 dogs were done using the 23-mm cryoballoon. Positioning of the 23-mm cryoballoon was more distal in the PV, which resulted in better PV occlusion. Temperature profiles showed lower temperatures in the 23-mm cryoballoon than in the 28-mm cryoballoon (inner balloon, median [range]: −51.5 [−66.0 to −31.0] versus −43.0 [−64.0 to −26.0]°C, P<0.001; balloon surface: −43.0 [−60.0 to −15.8] versus −6.5 [−46.2 to 28.9]°C, P<0.001; left atrial–PV junction: −6.7 [−20.0 to 21.4] versus 15.8 [−14.4 to 35.1]°C, P<0.001), and trended toward a higher PV isolation success rate in the 23-mm cryoballoon. Histologically, deeper extensions of ablative lesions into the PV were seen with 23-mm cryoballoon, and larger ablative lesions were seen in the left atrial antrum using 28-mm cryoballoon.

Conclusions—The efficacy of 3-minute ablation was not significantly different from 4-minute ablation in dogs. The 23-mm cryoballoon had a greater cooling effect than the 28-mm cryoballoon for small PVs, but showed narrower ablative lesions in the left atrial antrum. (Circ Arrhythm Electrophysiol. 2015;8:836-845. DOI: 10.1161/CIRCEP.115.002725.)

Key Words: atrial fibrillation ■ cryoballoon ■ esophagus ■ freezing ■ pulmonary vein ■ thermodynamics
WHAT IS KNOWN
• The second generation cryoballoon has shown high success rates for pulmonary vein (PV) isolation.
• Three- or 4-minute ablation duration has been clinically chosen using second generation cryoballoon. The 28-mm cryoballoon is more frequently used than the 23-mm cryoballoon.

WHAT THE STUDY ADDS
• The balloon and tissue temperature profiles were not significantly different between 3- and 4-minute ablation.
• The success rate of PV isolation was not significantly different between 3- and 4-minute ablation.
• The 28-mm cryoballoon showed significantly larger ablation lesion in left atrium antrum.

allow implantation of thermocouples on the LA–PV junction (3 thermocouples around each PV). In procedures performed on the right superior PV, 1 or 2 thermocouples were implanted along the margin of the phrenic nerve (PN). Esophageal lumen temperature was monitored via freeze was applied. During right superior PV ablation, the PN was occluded right and left anterior oblique views with the 15 F sheath and balloon diameters serving for calibration.

Catheterization
Sheaths were placed in right external jugular vein and left or right femoral vein and artery. A 6 F decapolar catheter was placed in the coronary sinus. A 10 F 5.5 to 10 MHz intracardiac echocardiography (ICE) catheter (Acuson-Siemens, Mountain View, CAUSA) was introduced via the external jugular vein. LA size and PV ostium diameter were measured using ICE and PV venography. ICE was used to facilitate contact of the balloon with tissue around the PV orifice and to reduce blood flow leaks around the balloon.

Cryoballoon Ablation
Ablation using the second generation cryoballoon in a canine model has been reported from our laboratory. After transseptal puncture, a 12 F deflectable sheath (FlexCath; Medtronic CryoCath LP) was introduced. A 3.3 F 15-mm circular catheter (Achieve mapping catheter; Medtronic) was used through the central lumen of a 10.5 F 28-/23-mm cryoballoon catheter (Arctic Front Advance; Medtronic) was used for comparisons of normally distributed variables, such as animal weight and LA size. Mann–Whitney U test was used for group comparisons of non-normally distributed variables, such as balloon and tissue temperatures, thawing time, distance from balloon to thermocouples, and ablation lesion size. Fisher exact test was used for comparisons of categorical variables. Whenever multiple measurements had been obtained per animal (PV or ablation level), generalized estimating equation models were used for continuous or categorical variables. Correlation between temperature and distance to each profile was fit using a logarithmic transformation. A P<0.05 was used to indicate statistical significance.

Results
Ablation Duration: 3 versus 4 minutes
Twenty-six dogs were randomized to 3- and 4-minute ablation. The baseline PV diameter and LA size were not significantly different between the 2 groups (Table 1).

Ablation and Temperature Data
Sixty-seven cryoablations of 52 PVs were performed. Table 1 shows the comparison of ablation data during PVI between 3- and 4-minute groups. Total ablation duration per PV in the 4-minute group was significantly longer than in the 3-minute group (310±111 s versus 249±89 s; P<0.001). Figure 1 shows the representative time-course temperature changes in 3- and 4-minute ablation cases during PVI. Table 1 also shows the comparison of the lowest balloon and tissue temperatures between the 2 groups. There was no significant difference in temperature profiles between the 2 groups. In 4-minute groups, temperature drop from 3 to 4 minutes was analyzed. The mean temperature drop from 3 to 4 minutes was −1.0±1.6°C in inner imbedded surface thermocouples, 1 in each balloon quadrant. These were placed equidistant between the largest balloon diameter and its tip in both 23- and 28-mm cryoballoon.

Distance Analysis
Measurement of distance from balloon surface to thermocouples was reported previously. Distances were calculated from digitally acquired right and left anterior oblique views with the 15 F sheath and balloon diameters serving for calibration.

Follow-Up
In all dogs, esophageal endoscopy was performed within 3 to 5 days post ablation. All animals underwent an electrophysiological follow-up study at 30 to 60 days after ablation to assess chronic PVI using a circular mapping catheter and to rule out PV stenosis. If right superior PV ablation was done at first procedure, PN function was evaluated. All dogs were euthanized for histological analysis.

Pathology
Triphenyltetrazolium chloride was used to delineate the cryoablation lesions. Heart, lungs, trachea, and esophagus were removed. Gross pathology was assessed. Each LA–PV junction was cut into 8 quadrants and each of these segments was examined microscopically. The esophagus and PN segments were cut cross-sectionally with 2-mm thickness and stained with hematoxylin–eosin and Masson trichrome.

Statistical Analysis
Statistical analysis was performed using JMP 10.0 statistical software (SAS Institute Inc). Continuous variables which are normally distributed are presented as mean±SD. Continuous variables which are not normally distributed are presented as median (range). Two-sample t test was used for group comparisons of normally distributed variables, such as animal weight and LA size. Mann–Whitney U test was used for comparisons of normally distributed variables, such as balloon and tissue temperatures, thawing time, distance from balloon to thermocouples, and ablation lesion size. Fisher exact test was used for comparisons of categorical variables. Whenever multiple measurements had been obtained per animal (PV or ablation level), generalized estimating equation models were used for continuous or categorical variables. Correlation between temperature and distance to each profile was fit using a logarithmic transformation. A P<0.05 was used to indicate statistical significance.
balloon, $-1.2\pm3.1^\circ C$ at balloon surface, and $-1.0\pm2.0^\circ C$ at LA–PV junction. Time to PVI was recorded during 5 of 67 (7%) cryoablations, 2 of 5 PVs were 3-minute freezes, and 3 of 5 PVs were 4-minute freezes. Time to PVI was 43 and 70 s in 3-minute freezes, and 37, 45, and 120 s in 4-minute freezes.

### Efficacy

Table 2 shows acute and chronic success rates of PVI. Acute PVI success rates were 26 of 26 (100%) PVs in the 3-minute group, and 22 of 26 (85%) PVs in the 4-minute group ($P=0.05$). Acute success rate of different balloon size in

### Table 1. Comparison of Ablation Data, Temperature Profiles Between 3- vs 4-minute Ablation

<table>
<thead>
<tr>
<th></th>
<th>3-min Ablation (13 Dogs)</th>
<th>4-min Ablation (13 Dogs)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veins ablated (n)</td>
<td>26</td>
<td>26</td>
<td>...</td>
</tr>
<tr>
<td>Targeted PV: LSPV/LIPV (n)</td>
<td>5/8</td>
<td>5/8</td>
<td>1.00</td>
</tr>
<tr>
<td>RSPV/RIPV (n)</td>
<td>5/8</td>
<td>5/8</td>
<td>1.00</td>
</tr>
<tr>
<td>PV size (mm)*</td>
<td>12.7±1.7 (n=26)</td>
<td>13.2±1.6 (n=26)</td>
<td>0.24</td>
</tr>
<tr>
<td>LA size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid to lateral (mm)*</td>
<td>27.7±3.1 (n=13)</td>
<td>27.7±3.1 (n=13)</td>
<td>0.98</td>
</tr>
<tr>
<td>Superior–Inferior (mm)*</td>
<td>34.1±3.1 (n=13)</td>
<td>33.6±4.2 (n=13)</td>
<td>0.57</td>
</tr>
<tr>
<td>Mean ablations number/Vein (n)*</td>
<td>1.4±0.5 (n=26)</td>
<td>1.3±0.5 (n=24)</td>
<td>0.12</td>
</tr>
<tr>
<td>Total ablation time/ Vein (s)*</td>
<td>249±89 (n=26)</td>
<td>310±111 (n=24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Complete PV occlusion (%)</td>
<td>15/36 (42)</td>
<td>14/31 (45)</td>
<td>0.74</td>
</tr>
<tr>
<td>Lowest inner balloon temperature ($^\circ C$)‡‡</td>
<td>$-46.5$ (−69.0 to −28.0; n=36)</td>
<td>$-43.0$ (−66.0 to −26.0; n=31)</td>
<td>0.81</td>
</tr>
<tr>
<td>Lowest balloon surface temperature ($^\circ C$)‡‡</td>
<td>$-23.4$ (−56.5 to 4.8; n=37)</td>
<td>$-25.9$ (−60.0 to 28.9; n=32)</td>
<td>0.88</td>
</tr>
<tr>
<td>Lowest LA–PV junction temperature($^\circ C$)‡‡</td>
<td>0.3 (−19.0 to 29.4; n=34)</td>
<td>4.7 (−20.0 to 24.4; n=24)</td>
<td>0.66</td>
</tr>
<tr>
<td>Distance from balloon to LA–PV junction (mm)†</td>
<td>2.6 (0.7 to 16.2; n=33)</td>
<td>2.8 (0.5 to 11.5; n=24)</td>
<td>0.61</td>
</tr>
<tr>
<td>Lowest phrenic nerve temperature ($^\circ C$)‡‡</td>
<td>29.8 (13.6 to 36.2; n=9)</td>
<td>35.1 (23.5 to 36.2; n=5)</td>
<td>0.49</td>
</tr>
<tr>
<td>Distance from balloon to phrenic nerve TCSs (mm)†</td>
<td>14.6 (8.5 to 24.3; n=9)</td>
<td>14.1 (9.3 to 24.6; n=5)</td>
<td>0.44</td>
</tr>
<tr>
<td>Lowest internal esophagus temperature ($^\circ C$)‡‡</td>
<td>30.7 (18.3 to 38.0; n=19)</td>
<td>36.0 (9.2 to 37.8; n=15)</td>
<td>0.74</td>
</tr>
<tr>
<td>Distance from balloon to esophagus TCSs (mm)†</td>
<td>12.0 (4.5 to 22.9; n=19)</td>
<td>12.8 (5.1 to 22.4; n=15)</td>
<td>0.56</td>
</tr>
</tbody>
</table>

LA indicates left atrium; LIPV, left inferior pulmonary vein; LSPV, left superior pulmonary vein; PV, pulmonary vein; RIPV, right inferior pulmonary vein; RSPV, right superior pulmonary vein; and TCSs, thermocouples.

*Mean±SD.
‡Median (range).
‡‡Lowest tissue temperature in the all tissue temperatures during each ablation.

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### Figure 1. Time-course temperature changes in balloon and tissue during cryoablation. Blue line indicates inner balloon temperature. The 4 green lines depict balloon surface temperatures. The 3 red lines show tissue temperatures at the left atrium–pulmonary vein junction (LA–PV jct). Red triangle denotes ablation start and end points. A, During ablation duration of 3 minutes. Lowest inner balloon temperature was $-45.0^\circ C$, lowest balloon surface temperatures were $-43.0^\circ C$, $-25.8^\circ C$, $-18.0^\circ C$, and $-15.1^\circ C$. Lowest tissue temperatures at LA–PV junction were $-14.6^\circ C$, $-6.4^\circ C$, and $15.2^\circ C$. B, During ablation duration of 4 minutes. Lowest inner balloon temperature was $-49.0^\circ C$, lowest balloon surface temperatures were $-41.6^\circ C$, $-45.3^\circ C$, $-33.4^\circ C$, and $12.7^\circ C$. Lowest tissue temperatures at LA–PV junction were $-14.4^\circ C$, $-8.6^\circ C$, and $11.3^\circ C$. Balloon inner indicates inner balloon temperature; and Surface, balloon surface temperatures.
3-minute group was 20 of 20 (100%) with 28-mm cryoballoon and 6 of 6 (100%) with 23-mm cryoballoon. Success rate in 4-minute group was 16 of 20 (80%) with 28-mm cryoballoon and 4 of 4 (100%) with 23-mm cryoballoon (**P**=0.75).

A follow-up study was performed after a mean of 46±14 days postablation. Chronic PVI success rates were 20 of 26 (77%) in the 3-minute group versus 16 of 20 (80%) in the 4-minute group (**P**=0.83). Chronic success rate of different balloon size in 3-minute group was 15 of 20 (75%) with 28-mm cryoballoon and 5 of 6 (83%) with 23-mm cryoballoon. Success rate in the 4-minute group was 12 of 16 (80%) with 28-mm cryoballoon and 4 of 4 (100%) with 23-mm cryoballoon (**P**=1.00).

Analyzing the 5 PVs with recorded time to PVI, 2 PVs were reconnected and 3 PVs were isolated. Time to PVI in chronic isolated PVs (37, 43, and 45 s) was relatively shorter than in reconnected PVs (70 and 120 s). In histology, maximal ablation lesion length was not significantly different between the 2 groups (Table 2). Figure 2 shows representative transmural ablation lesions of the LA–PV junction in both 3- and 4-minute group.

### Safety and Complications
Tables 1 and 2 show temperature profiles of PN and internal esophagus and complication rates.

### PV Stenosis
Although 2 moderate PV stenoses (60% and 55% stenoses) in the 3-minute group and 1 moderate PV stenosis (60% stenosis) in the 4-minute group were seen, no severe PV stenosis was observed in either group.

### Phrenic Nerve
Lowest PN temperature during ablation was not significantly different between the 2 groups (Table 1). Figure 3 shows cooling delay in the PN. Tissue temperature continued to decrease after termination of cryoablation. Time to achieve lowest temperature at the PN was 196±11 s in 3-minute ablations and 254±12 s in 4-minute ablations. Neither temporary nor chronic PN palsy was observed in either group (Table 2).

### Esophagus
Lowest internal esophageal temperature during ablation was not significantly different between the 2 groups (Table 1). Cooling delay was also observed at the internal esophagus during ablation (Figure 3). Time to achieve lowest temperature was 212±37 s for 3-minute ablations and 279±35 s for 4-minute ablations. Postablation esophageal endoscopy showed an esophageal ulcer in 2 of 13 (15%) dogs in the 3-minute group and in 1 of 12 (8%) dogs in the 4-minute group (**P**=1.00; Table 2). No esophageal LA fistula was observed in either group. Follow-up endoscopy after 30 days showed healing of these lesions in all dogs.

### Balloon Size: 23- versus 28-mm Cryoballoon
Fourteen cryoablations using the 23-mm cryoballoon were performed in 12 PVs (6 left inferior PVs [LIPV] and 6 right

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**Table 2. Comparison of PVI Success Rate, Complications, and Histological Changes Between 3- vs 4-minute Ablation**

<table>
<thead>
<tr>
<th></th>
<th>3-min Ablation (13 Dogs)</th>
<th>4-min Ablation (13 Dogs)</th>
<th><strong>P</strong> Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute PVI (%)</td>
<td>26/26 (100)</td>
<td>22/26 (85)</td>
<td>0.05*</td>
</tr>
<tr>
<td>Chronic PVI (%)</td>
<td>20/26 (77)</td>
<td>16/20 (80)†</td>
<td>0.83</td>
</tr>
<tr>
<td>Esophageal ulcerated lesion (%)</td>
<td>2/13 (15)</td>
<td>1/12 (8)†</td>
<td>1.00</td>
</tr>
<tr>
<td>Phrenic nerve palsy (%)</td>
<td>0/13 (0)</td>
<td>0/12 (0)†</td>
<td>1.00</td>
</tr>
<tr>
<td>PV stenosis (moderate) (%)</td>
<td>2/26 (8)</td>
<td>1/24 (4)†</td>
<td>0.59</td>
</tr>
<tr>
<td>Maximal ablation lesion length to LA (mm)‡</td>
<td>10.9 (1.5–34.1; n=24)</td>
<td>10.5 (2.1–29.0; n=22)</td>
<td>0.51</td>
</tr>
<tr>
<td>Maximal ablation lesion length to PV inside (mm)‡</td>
<td>5.7 (0–12.0; n=23)</td>
<td>5.4 (0–8.8; n=22)</td>
<td>0.61</td>
</tr>
<tr>
<td>Remaining myocardial sleeve length (mm)‡</td>
<td>3.3 (0–11.1; n=24)</td>
<td>4.4 (8–8.8; n=21)</td>
<td>0.39</td>
</tr>
<tr>
<td>Circumferential transmural lesion (%)</td>
<td>20/26 (77)</td>
<td>16/24 (67)†</td>
<td>0.46</td>
</tr>
</tbody>
</table>

LA indicates left atrium; and PVI, pulmonary vein isolation.

*Generalized estimating equation model could not be computed for this comparison.

†One dog in 4-min ablation died after first procedure because of the bleeding from the thermocouple suture site, therefore, the 12 dogs (24 PVs) were done for follow-up study.

‡Median (range).

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**Figure 2.** Microscopic histological changes at the left atrium–pulmonary vein (LA–PV) junction. **Top,** A representative ablation lesion of 3-minute group. **Bottom,** An ablation lesion of 4-minute group. Both pictures depict a transmural lesion at the LA–PV junction.
inferior PVs (RIPv) in 6 dogs. Data were compared with that of 24 cryoablations using the 28-mm cryoballoon for 20 PVs (10 LIPVs and 10 RIPv) in 20 dogs. Baseline PV and LA size were not significantly different between the 23- and 28-mm cryoballoon group (Table 3).

**Balloon Positioning**

Figure 4 shows 2 representative cases of balloon positioning during PVI of the LIPV using 23- and 28-mm cryoballoon. Cryoballoon-tissue contact point occurred at a more proximal position (atrial side) in the 28-mm cryoballoon than in the 23-mm cryoballoon.

Positional relationship between balloon-tissue contact point and LA–PV junction were analyzed by fluoroscopy. In 14 cryoablations using 23-mm cryoballoon, 9 of 14 (64%) showed that the balloon-tissue contact point was at the LA–PV junction, and 5 of 14 (36%) showed the contact point in the PV. In 24 cryoablations using 28-mm cryoballoon, 14 of 24 (58%) showed the balloon-tissue contact point at the LA–PV junction, 3 of 24 (13%) showed the contact point in the PV, and 7 of 24 (29%) showed the contact point was more atrial than the LA–PV junction.

**Blood Flow Leak Around Balloon**

Table 4 shows a comparison of ablation data. Complete PV occlusion during PVI was more often seen in 23-mm cryoballoon than in 28-mm cryoballoon (10/14 [71%] versus 8/24 [33%; P=0.01], and moderate blood flow leak was more often seen in 28-mm cryoballoon than in 23-mm cryoballoon.

**Temperature Data**

Figure 5 shows 2 representative cases of time-course temperature changes during PVI. Table 4 compares temperature profiles between the 2 balloon sizes. Lowest inner temperature,
balloon surface temperature, and LA–PV tissue temperature in 23-mm cryoballoon were significantly lower than those in 28-mm cryoballoon. Balloon thawing time (from final temperature to +20°C) was longer in ablations using 28-mm cryoballoon.

**Relationship Between Temperature and Distance**

Figure 6 shows the relationship between lowest tissue temperature and distance from balloon surface. Both the balloon sizes showed good correlation between lowest tissue temperature and distance from the balloon surface (23-mm cryoballoon: \( r=0.90; P<0.0001 \) and 28-mm cryoballoon: \( r=0.89; P<0.0001 \)). Figure 7 shows a schematic view of lowest temperature distribution in both the groups. Lowest temperature distribution was different between 23- and 28-mm cryoballoon.

**Efficacy**

Table 5 shows a comparison of the efficacy between 23- and 28-mm cryoballoon. Acute PVI success rate was 12 of 12 (100%) in 23-mm cryoballoon and 16 of 20 (80%) in 28-mm cryoballoon (\( P=0.27 \)). Chronic PVI success rate was 9 of 10 (90%) in 23-mm cryoballoon group and 11 of 16 (69%) in 28-mm cryoballoon group (\( P=0.21 \)). Figure 8 shows representative ablation lesions using 23- and 28-mm cryoballoon. The 28-mm cryoballoon created a larger lesion in the LA antrum than the 23-mm cryoballoon. Table 5 also depicts differences of ablation lesions between the 2 groups. Maximal ablation lesion extension outside of the PV orifice to the LA endocardial surface was 10.0 (5.0–24.8) mm for 28-mm cryoballoon and 4.2 (1.5–11.9) mm for 23-mm cryoballoon (\( P=0.001 \)). In contrast, the maximal ablation lesion extension into the PV was 5.3 (0–17.0) mm for 28-mm cryoballoon and 6.8 (5.6–8.5) mm for 23-mm cryoballoon (\( P=0.006 \)). Mean length of the remaining myocardial sleeve was 4.7 (0–7.0) mm for 28-mm cryoballoon, and 1.9 (0–4.0) mm for 23-mm cryoballoon (\( P<0.001 \)). In the 28-mm cryoballoon group, histological gap was observed in 9 PVs. Four of 9 PVs had multiple or wide gaps (>25% of PV ostium). Analyzing the gap location at the PV ostium, the gap was seen inferriorly for 6 PVs (2 LIPVs and 4 RIPVs), anterior for 4 PVs (1 LIPV and 3 RIPVs).

**Table 4. Comparison of Ablation Data and Temperature Profiles Between the 23- and 28-mm Cryoballoon Ablations**

<table>
<thead>
<tr>
<th></th>
<th>23-mm Cryoballoon (6 Dogs)</th>
<th>28-mm Cryoballoon (20 Dogs)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veins ablated (n)</td>
<td>12</td>
<td>20</td>
<td>...</td>
</tr>
<tr>
<td>Targeted PV: LIPV/RIPV (n)</td>
<td>6/6</td>
<td>10/10</td>
<td>1.00</td>
</tr>
<tr>
<td>Veins ablated with 3-/4-min ablation (n)</td>
<td>6/6</td>
<td>10/10</td>
<td>1.00</td>
</tr>
<tr>
<td>Ablations number/vein (n)*</td>
<td>1.2±0.4 (n=12)</td>
<td>1.4±0.5 (n=20)</td>
<td>0.33</td>
</tr>
<tr>
<td>Total ablation time/vein (s)*</td>
<td>240±63 (n=12)</td>
<td>282±130 (n=20)</td>
<td>0.38</td>
</tr>
<tr>
<td>Complete PV occlusion (%)</td>
<td>10/14 (71)</td>
<td>8/24 (33)</td>
<td>0.01</td>
</tr>
<tr>
<td>Blood flow leak: small (%)</td>
<td>3/14 (21)</td>
<td>7/24 (29)</td>
<td>0.55</td>
</tr>
<tr>
<td>Blood flow leak: moderate (%)</td>
<td>1/14 (7)</td>
<td>9/24 (38)</td>
<td>0.05</td>
</tr>
<tr>
<td>Lowest inner balloon temperature (°C)‡</td>
<td>-51.5 (-66.0 to -31.0; n=14)</td>
<td>-43.0 (-64.0 to 26.0; n=24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lowest balloon surface temperature (°C)‡</td>
<td>-43.0 (-60.0 to -15.8; n=14)</td>
<td>-6.5 (-46.2 to 28.9; n=23)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lowest LA–PV junction temperature (°C)§</td>
<td>-6.7 (-20.0 to 21.4; n=14)</td>
<td>15.8 (-14.4 to 35.1; n=24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distance from balloon to LA–PV junction TCs (mm)¶§</td>
<td>2.3 (0.7 to 8.0; n=12)</td>
<td>5.1 (0.5 to 15.2; n=22)</td>
<td>0.10</td>
</tr>
<tr>
<td>Lowest internal esophagus temperature (°C)¶§</td>
<td>31.0 (9.2 to 38.0; n=14)</td>
<td>34.7 (15.5 to 37.8; n=20)</td>
<td>0.73</td>
</tr>
<tr>
<td>Distance from balloon to esophageal TCs (mm)¶§</td>
<td>13.7 (7.5 to 21.5; n=14)</td>
<td>13.6 (5.1 to 22.4; n=20)</td>
<td>0.75</td>
</tr>
<tr>
<td>Thawing time (s)¶§</td>
<td>24 (4 to 93; n=14)</td>
<td>35 (9 to 72; n=22)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

LA indicates left atrium; LIPV, left inferior pulmonary vein; PV, pulmonary vein; RIPV, right inferior pulmonary vein; and TCs, thermocouples.
*Mean±SD.
†Median (range).
‡Lowest tissue temperature in the all tissue temperatures during each ablation.
RIPVs), posterior for 3 PVs (2 LIPVs and 1 RIPV), and superior for 3 PVs (1 LIPV and 2 RIPVs). In 23-mm cryoballoon group, histological gap was observed in 1 PV at the inferior portion of the RIPV.

Safety and Complications

PV Stenosis

Two of 10 PVs showed moderate PV stenosis (60% and 55% stenoses) in the group ablated with 23-mm CB. No PV stenosis was seen in 28-mm cryoballoon group (P=0.10; Table 1). Analyzing ablation data in the 2 moderately stenotic veins, balloon-tissue contact points during ablation were located inside the PV. Lowest inner balloon temperature was −55.0 and −65.0°C, respectively. Lowest tissue temperature at LA–PV junction was −17.0 and −12.9°C in these cases. Histological analysis revealed that ablation lesion extension from the PV orifice into the PV was 5.8 and 5.3 mm, respectively.

Esophageal Complications

Lowest internal esophageal temperature during ablation was not significantly different between the 2 groups (Table 4). Esophageal endoscopy showed an ulcer in 3 of 5 (60%) for 23-mm cryoballoon group, and 1 of 20 (5%) in the 28-mm cryoballoon group (P=0.02; Table 5). In 3 dogs with esophageal ulcer lesions using 23-mm cryoballoon, the PV-diameter/balloon-diameter ratio was 0.62 (14.3/23.0), 0.53 (12.1/23.0), and 0.52 (12.0/23.0), respectively. Lowest internal esophageal temperatures during cryoablation were 21.4°C, 9.2°C, and 37.1°C, respectively. In 1 dog with an ulcer using 28-mm cryoballoon, the PV-diameter/balloon-diameter ratio was 0.56 (15.8/28.0). Lowest internal esophageal temperature was 15.5°C. Follow-up esophageal endoscopy at 4 weeks showed healed lesions in all dogs.

Discussion

Major Findings

This study demonstrates the following findings: (1) balloon and tissue temperature profiles, acute and chronic PVI success rate, complications, and histological changes were not significantly different between 3- and 4-minute ablation durations; (2) cooling delay after cryoablation termination was observed in PN and internal esophagus; (3) positioning of 28-mm cryoballoon tended to be more proximal at the LA–PV junction than that of 23-mm cryoballoon; (4) better PV occlusion was seen using 23-mm cryoballoon; (5) tissue and balloon temperature profiles revealed significantly lower temperatures in 23-mm cryoballoon than in 28-mm cryoballoon, with a corresponding trend toward higher PVI success rates in 23-mm cryoballoon; and (6) histologically, deeper extension of the balloon-diameter ratio was 0.62 (14.3/23.0), 0.53 (12.1/23.0), and 0.52 (12.0/23.0), respectively. Lowest internal esophageal temperatures during cryoablation were 21.4°C, 9.2°C, and 37.1°C, respectively. In 1 dog with an ulcer using 28-mm cryoballoon, the PV-diameter/balloon-diameter ratio was 0.56 (15.8/28.0). Lowest internal esophageal temperature was 15.5°C. Follow-up esophageal endoscopy at 4 weeks showed healed lesions in all dogs.

Figure 5. Two representative cases of time-course temperature changes during PVI (A) temperature profiles using 23-mm cryoballoon, and (B) 28-mm cryoballoon ablation. Lowest inner temperature and balloon surface temperature in the 23-mm cryoballoon ablation were lower than that in 28-mm cryoballoon. Tissue temperatures at the left atrium–pulmonary vein junction (LA–PV jct) were also lower when using 23-mm cryoballoon. Balloon thawing time (from final temperature to +20°C) was longer when using 28-mm cryoballoon. Balloon inner indicates inner balloon temperature; and Surface, balloon surface temperatures.

Figure 6. Relationship between the lowest tissue temperature and distance from balloon surface. Lowest tissue temperature was significantly dependent on distance from the balloon. A, For 23-mm cryoballoon with a correlation: r=0.90, P<0.0001, described by the equation: y=17.242 ln (x)−14.157. The R² value for this model is 0.81. B, For 28-mm cryoballoon with a correlation: r=0.89, P<0.0001, described by the equation: y=15.543 ln (x)−8.0707. The R² value for this model is 0.79. *r Value derives from the logarithmic transformation.
of ablative lesions into the PV was seen in 23-mm cryoballoon and larger ablation lesions were seen in the LA antrum in 28-mm cryoballoon.

**Ablation Duration**

Although preset ablation duration using second generation cryoballoon is 240 s by the manufacturer, the adequate ablation duration to achieve chronic PVI remains unknown. In this study, lowest balloon and tissue temperatures were not significantly different between 3- and 4-minute ablations. During 4-minute ablations, additional mean temperature drop in balloon and tissue from 3 to 4 minutes was only −1°C. In addition, ablation lesion size was not significantly different between the 2 groups. Dubuc et al.\(^1\) reported that cryoablation lesion size measured by ICE grew progressively during the first 3 minutes of freezing in atrial myocardium. However, they did not find difference in lesion size after 3 minutes. Considering results from previous and present studies, additional 1-minute ablation duration (from 3 to 4 minutes) might not cause additional tissue damage at the LA–PV junction. Therefore, 3 minutes ablations using second generation cryoballoon might be sufficient to achieve acute and chronic PVI. Impact of ablation duration on tissue temperature profiles in PN and internal esophagus was not significantly different between 3- and 4-minute ablations. Previously, we showed the spread of conductive cooling for each direction from the balloon-tissue contact point,\(^6\) explaining latent cooling that was seen in PN or esophagus even after termination of cryoablation. No severe PN or esophageal complications were observed in this study. Hence, it is not clear whether shorter ablation durations of 3 minutes reduce complications compared with 4-minute ablation. The protocol without bonus freeze in this study was intended to investigate

<table>
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<th>Table 5. Comparison of PVI Success Rate, Complications, and Lesion Characteristics Between 23- and 28-mm Cryoballoon</th>
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<td>23-mm Cryoballoon (6 Dogs)</td>
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<td>Maximal ablation lesion length to LA (mm)(^‡)</td>
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<td>Maximal ablation lesion length to PV Inside (mm)(^‡)</td>
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<td>Remaining myocardial sleeve length (mm)(^‡)</td>
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LA indicates left atrium; LIPV, left inferior pulmonary vein; PVI, pulmonary vein isolation; and RIPV, right inferior pulmonary vein.\(^*\) Generalized estimating equation model could not be computed for this comparison.\(^†\) One dog in 23-mm cryoballoon group died after first procedure because of the bleeding from the thermocouple suture site, therefore, the 5 dogs (10 PVs) were done for follow-up study.\(^‡\) Median (range).
Cryoballoon Size

There are 2 sizes of cryoballoons (23 and 28 mm) which can be used. In this study, the biophysics using the 2 different balloon sizes were clarified and use of 28-mm cryoballoon led to fewer complications.

Different Thermodynamics Between 23- and 28-mm Cryoballoons

Tissue and balloon temperatures were lower using 23-mm cryoballoon. There may be several reasons for this. First, balloon surface area is different between the balloons. The number of refrigerant jets within the balloons is the same for both sizes. Therefore, although there is a difference in refrigerant flow rate between 23-mm cryoballoon (6.3 L/min) and 28-mm cryoballoon (7.2 L/min), cooling effect of the balloon surface per unit area is still stronger for 23-mm cryoballoon. Second, canine PVs are smaller than human PVs. Therefore, relationship between PV size and balloon size might affect thermodynamics. The 28-mm cryoballoon tended to position more proximally (atrial side) at LA–PV junction, thus, a larger balloon surface area was exposed to blood flow in the LA. This would cause a warming effect of the inner balloon and surface temperatures. In addition, balloon positioning might affect the distance from balloon surface to thermocouples at LA–PV junction, which might cause higher tissue temperature at the LA–PV junction in 28-mm cryoballoon. Third, better PV occlusion with use of 23-mm cryoballoon was seen. We reported that blood flow leaks around the balloon cause a warming effect on balloon and tissue temperatures. Higher complete vein occlusion rates with use of 23-mm cryoballoon may explain lower balloon and tissue temperatures. However, we observed a trend toward longer balloon thawing time in 28-mm cryoballoon than in 23-mm cryoballoon. This might be because of bigger ice formation on the larger surface of 28-mm cryoballoon, taking more time to thaw.

Ablation Lesion Characteristics Between 23- and 28-mm Cryoballoons

This study elucidated different characteristics of ablation lesions. These findings could simply result from differences in positioning of the 2 sizes of cryoballoons. The bigger balloon might not only be able to ablate for PVI but also could perform PV antral modification. Wide area circumferential ablation has been recommended because ganglionated plexi, non-PV foci, and arrhythmogenic substrate have been observed in the PV antrum. The 28-mm cryoballoon might be better to create wide area lesion in LA. Although lower balloon and tissue temperatures, and higher acute and chronic PVI success rates were seen in 23-mm cryoballoon, further extension of ablation lesion into the deep PV was observed after 23-mm cryoballoon ablation. Previous studies have shown that radiofrequency application within the vein results in frequent occurrence of PV stenosis. Although the energy source is different, positioning deeper in the PV using 23-mm cryoballoon might produce deeper extension of the ablation lesion into the PV, which might cause higher rate of moderate PV stenosis in 23-mm cryoballoon group.

Histological gap was most often observed in the inferior portion of PV ostium. In dogs, the angle between the LA and PV is steep; therefore, blood flow leaks around cryoballoon were most often seen in inferior portion of PV ostium. Our previous article showed correlation between location of blood flow leak around the balloon and chronic histological gaps at the PV ostium.

Esophageal Complication

Esophageal ulcers were more often observed in 23-mm cryoballoon. Distance from balloon surface to esophageal thermocouples was not significantly different between 23- and 28-mm cryoballoon. Lower inner balloon and balloon surface temperature profiles in 23-mm cryoballoon might cause higher incidence of esophageal ulcer. However, lowest internal esophageal temperature in dogs that had esophageal lesions was different in each dog (from 9.1 to 37.1°C). Therefore, relationship between internal esophageal temperature and occurrence of esophageal ulcerative lesions could not be delineated in this study.

Limitations

This study was done in an in vivo canine model. Although dogs are often used as an animal model for catheter ablation
studies, anatomy of the PVs is different than that found in humans; the mean ostial diameter of human PVs is larger than that in dogs.14 Therefore, balloon positioning, balloon and tissue thermodynamics, and ablation lesion size might translate to different results in humans. In this study, cryoablation using 23-mm cryoballoon was performed only in the inferior PVs (LIPV and RIPV); temperature data for LSPV, right superior PV, and PN between 23- and 28-mm cryoballoon could not be compared. Only 3 thermocouples were implanted at each LA–PV junction, 1 or 2 thermocouples in PN, and 4 thermocouples in esophagus. Therefore, we cannot exclude possibility that there might be lower tissue temperatures. Time to PVI during ablation was recorded in only 5 (7%) cryoablations, because positioning of the circular mapping catheter was deep in PV to achieve better PV occlusion. Therefore, correlation between time to PVI and outcomes was not clear in this study. Comparison of complication rates should be viewed with caution as the number of animals ablated with 23-mm cryoballoon was small.

Clinical Implications

To minimize procedure and fluoroscopy time, ablation duration may need to be reconsidered. The efficacy of 3-minute ablation was not significantly different from 4-minute ablation. Therefore, 3-minute ablation seems to be a viable option when using the second-generation cryoballoon. Conductive cooling delay was seen in PN and internal esophageal thermocouples. Thus, in the clinical arena, continuing to check PN capture and internal esophageal temperature even after stop of cryoablation would be reasonable. Clinically, target PVs are sometimes small, especially the LIPV. In this case, it might be hard to achieve total occlusion using the 28-mm cryoballoon and, therefore, choosing the 23-mm cryoballoon for ablation would be an option. However, meticulous balloon positioning and monitoring of temperature achieved should be performed.

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Disclosures

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