Pathological Effects of Extensive Radiofrequency Energy Applications in the Pulmonary Veins in Dogs

Gregg W. Taylor, MD; G. Neal Kay, MD; Xiangsheng Zheng, MD; Sanford Bishop, DVM, PhD; Raymond E. Ideker, MD, PhD

Introduction—The long-term complications of catheter ablation within the pulmonary veins are unknown. The development of pulmonary vein stenosis has recently been described after catheter ablation to treat either chronic or paroxysmal atrial fibrillation. The purpose of this study was to examine the pathological and hemodynamic effects of radiofrequency (RF) energy application within the pulmonary veins.

Methods and Results—Right heart and transseptal catheterization were performed in 9 anesthetized mongrel dogs. The pulmonary vein ostia were cannulated and pulmonary venous pressure was measured before RF energy application in up to 4 separate pulmonary veins. Animals were euthanized at intervals of 2 to 4 weeks (n = 3), 6 to 8 weeks (n = 3), or 10 to 14 weeks (n = 3) after ablation. Repeat catheterization before euthanasia demonstrated statistically significant differences in pulmonary capillary wedge pressure, cardiac output, pulmonary vascular resistance, and systemic vascular resistance (P < 0.05) compared with the baseline. Luminal narrowing was observed in 22 of 33 pulmonary veins to which RF energy was applied. Of these, 7 were totally occluded, 7 had severe stenosis, and 8 were only minimally narrowed. Histological examination revealed intimal proliferation with organizing thrombus, necrotic myocardium in various stages of collagen replacement, endovascular contraction, and a proliferation of elastic lamina.

Conclusions—Applications of RF current within the pulmonary veins may result in pulmonary vein narrowing or complete occlusion. These observations should be considered in treatment of arrhythmias originating within the pulmonary veins.

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Key Words: catheter ablation • fibrillation • lung • veins

Radiofrequency (RF) ablation within the pulmonary veins has been used to treat focal sites that may initiate paroxysmal atrial fibrillation.1 RF energy has also been used to create lines of conduction block within the atria of patients with chronic atrial fibrillation by withdrawing the ablation electrode across predetermined portions of atrial endocardium.2–4 The development of progressive dyspnea with or without pulmonary hypertension as a consequence of pulmonary vein stenosis has been an unexpected complication after both of these procedures.5 The mechanism by which pulmonary vein stenosis may develop after ablation is unclear but could be due to thrombus formation, vascular wall stricture secondary to scar contraction, smooth muscle activation leading to fibrocellular hyperplasia, or a combination of these pathological processes. The purpose of the present study was to characterize the pathological and hemodynamic sequelae of RF energy application within the pulmonary veins in dogs.

Methods

All animals were managed in accordance with the guidelines established in the Position of the AHA on Research Animal Use adopted by the AHA on November 11, 1984.6 The University of Alabama at Birmingham Institutional Animal Care and Use Committee approved the experimental protocols.

Surgical Preparation

Ten mongrel dogs of either sex weighing 25 to 35 kg were sedated with intravenous diazepam (0.075 mg/kg) and butorphenol (0.075 mg/kg) and then anesthetized with thiopental 12 mg/kg and isoflu- rane 2% to 3%. A mechanical ventilator (Ohio Corp) supplied a tidal volume of 12 to 15 mL/kg at 15 respirations per minute. The left femoral artery was percutaneously cannulated with a 6F sheath to monitor arterial blood pressure. The right femoral and jugular veins were percutaneously cannulated, and a balloon-tipped 7F thermolysis catheter (Abbott Corp) was advanced to the pulmonary artery. Cardiac output was measured by thermodilution (Hewlett Packard Corp). A hexapolar catheter was inserted into the right jugular vein and advanced into the coronary sinus. Transseptal catheterization was performed with an inner 8F and an outer 11F sheath (model 406893, Daig Corp) that was advanced into the left atrium. After the measurement of right heart and left atrial pressures, a 7F steerable ablation catheter (EP) with a 4-mm distal electrode was advanced through the inner guiding sheath into the left atrium.

For each animal, an attempt was made to cannulate up to 4 pulmonary veins. The guiding sheath–ablation catheter assembly was advanced 2 cm into a pulmonary vein, and bipolar atrial
electrograms were recorded with the ablation catheter. The ablation catheter was withdrawn toward the pulmonary vein ostium until an electrode could be recorded with both the distal and proximal bipolar electrode pairs. After pulmonary venous pressure was measured, a baseline pulmonary venous angiogram (Figure 1) was performed by hand injection of 10 to 15 mL of iodinated intravenous contrast through the 8F inner sheath. Next, RF energy was delivered (EPT generator) to the ablation catheter lying within the pulmonary vein. Tissue contact was assessed by monitoring impedance and electrode temperature with a thermocouple in the distal electrode. An increase in electrode temperature to ≥60°C could typically be produced with <10 W of power.

During RF application, the ablation catheter was withdrawn 2 to 3 mm every 30 seconds until it entered the left atrium. This process was repeated until three or four 120-second RF energy applications were delivered to each pulmonary vein. The power delivered was varied so that the temperature recorded at the thermocouple was maintained between 60°C and 80°C. After RF energy was applied to the pulmonary veins, the catheters were removed, hemostasis was ensured, and the animal was allowed to recover. Aspirin and cephalaxin were given to each animal. Systemic heparinization was performed by hand injection of 10 to 15 mL of iodinated intravenous contrast through the 8F inner sheath. Next, RF energy was delivered to each pulmonary vein. The power delivered was 10 W of power.

At necropsy, the pulmonary veins were classified as free of luminal narrowing, stenosed, or occluded. The degree of stenosis was classified as severe if there was >70% luminal area narrowing, moderate if 40% to 70%, and mild if <40% narrowed. Intimal proliferation was graded as severe, moderate, or mild if it covered >50%, 20% to 50%, or <20% of the cross-sectional luminal area, respectively. Endocardial contraction was present when the normal endocardial surface became tortuous and enfolded on itself secondary to scar retraction. The degree of contraction was graded as severe, moderate, or mild if the cross-sectional area was reduced by >30%, 10% to 30%, or <10%, respectively. The degree of elastic lamina proliferation was determined by comparison of the stenosed pulmonary vein with a normal pulmonary vein and was assessed qualitatively as previously described.

### Statistical Analysis
Differences between preablation and postablation data were analyzed with a paired Student’s t-test, with P<0.05 considered significant.

### Results

#### Hemodynamic Findings

The hemodynamic data before ablation and before euthanasia are presented in Table 1. Compared with baseline, the pulmonary capillary wedge pressure, systemic and pulmonary vascular resistance, and cardiac output were found to be significantly different at the final study. The mean pulmonary artery and right ventricular systolic pressures were also increased but only approached statistical significance. No animal developed more than mild pulmonary arterial hypertension, with a maximum pulmonary artery systolic pressure of 34 mm Hg and a mean pulmonary artery systolic pressure of 28±5 mm Hg at the final study. However, on average, the pulmonary vascular resistance doubled before euthanasia.

Of the 36 pulmonary veins targeted for ablation, the venous pressure could not be measured at the initial study in 7 segments because of an inability to engage the pulmonary...
TABLE 2. Ablation Results

<table>
<thead>
<tr>
<th>Survival Duration, wk</th>
<th>2 to 4</th>
<th>6 to 8</th>
<th>10 to 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veins targeted</td>
<td>12</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Total occlusion</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Severe stenosis (&gt;70% luminal narrowing)</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Mild/no stenosis (&lt;40% luminal narrowing)</td>
<td>9</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

Ablation Results and Pathological Findings

On gross examination, the pulmonary vein endocardium, which did not receive RF energy, was light tan and pliable where atrial myocardium was present and nearly translucent and paper-thin distally. The endocardial surface, where RF energy produced tissue necrosis and resulting scar, was white, firm, thickened, and contracted, resulting in luminal narrowing or total occlusion. The pulmonary parenchyma drained by completely stenosed pulmonary veins was atrophic and firm.

The ablation results are summarized in Table 2. Of the 33 pulmonary veins to which RF energy was applied, ablation lesions were found in 22, whereas 11 pulmonary veins had no grossly visible lesion. Of these 11, a scarred area was found in the atrial myocardium just adjacent to the ostia of 7 pulmonary veins. Of the 22 veins with identifiable lesions, 7 were totally occluded, 7 were severely stenotic, and 8 were only mildly narrowed (none were moderately stenotic). Of the 10 pulmonary veins examined in animals killed 10 to 14 weeks after ablation, 6 were either occluded or severely stenotic, compared with 5 of 11 veins in animals that survived for 6 to 8 weeks and 3 of 12 veins in animals that survived for 2 to 4 weeks.

The histological findings are summarized in Table 3. At 2 to 4 weeks after ablation, the atrial myocardium where RF energy was applied was in various stages of collagen replacement. At 2 weeks, strands of remaining atrial myocardium were interspersed with red blood cells (RBCs), macrophages, and collagen fibrils (Figure 2A). By 4 weeks, the majority of necrotic muscle was replaced with collagen (Figure 2B). On the intimal surface, endothelial cells were absent at 2 weeks but were observed at 4 weeks. Organizing thrombus was present within the thickened intima, resulting in luminal narrowing. In addition, the thickened intima and underlying elastic lamina were contracted over regions of necrotic myocardium that was replaced by collagen (Figure 2C). Disruption and thickening of the internal elastic lamina were present at regions bordered by necrotic myocytes (Figure 2B). By 4 weeks, the thickened intimal region appeared organized, as indicated by the presence of small vascular channels (Figure 2B). In some regions, there were chondroblasts, chondroclasts, and cartilage formation in the thickened intima (Figure 2C), underlying vascular media, and adjacent myocardium.

The histological changes seen at 6 to 8 weeks were similar to those seen at 4 weeks but were more mature. Necrotic myofibers had been completely replaced, leaving only sparse normal fibers among a mature collagen matrix. In the media
and underlying tissue of several pulmonary veins, there were regions of cartilaginous metaplasia with chondroblasts and chondroclasts. Pulmonary veins that were completely occluded or severely narrowed contained small intimal vascular channels (Figure 3A). Although elastic lamina thickening was typically only mild to moderate, it was occasionally severe, resulting in marked luminal narrowing (Figure 3B). In many sections, multiple layers of elastic lamina were interspersed with small clusters of smooth muscle cells within the media, but few or no smooth muscle cells were identified in the thickened intima (Figure 3C).

The histological changes seen at 10 to 14 weeks are demonstrated in Figure 4. Severely stenotic or occluded veins were markedly reduced in size (Figure 4A). Intimal and surrounding tissue collagen was mature and well organized, with neovascularization (Figure 4B). The necrotic atrial myocardium was replaced by a collagenous matrix containing...
small islands of surviving myocardium (Figure 4C). Compared with the changes seen at 6 to 8 weeks, the replaced myocardium was less cellular and the collagen more dense. The thickened intima revealed multiple well-developed endothelialized channels as well as occasional highly organized regions of osseous metaplasia and bone marrow (Figure 4D). In addition, variable amounts of smooth muscle were seen in the media of the larger pulmonary veins, whereas the smaller branching vessels appeared arterialized secondary to smooth muscle proliferation (Figure 4E).

The lung parenchyma draining the pulmonary veins contained macrophages, lymphocytes, RBCs, and a lacy thin matrix of collagenous material at 2 weeks. By 4 weeks, RBCs were nearly absent, but macrophages, some of which contained hemosiderin, were present. The lung parenchyma adjacent to pulmonary veins where RF energy

Figure 4. Histological findings 10 to 14 weeks after RF energy application (GAFT and H&E staining). A, Severely stenosed pulmonary vein (secondary branch) that was markedly reduced in size (arrow). B, Occluded intima and surrounding tissue collagen have matured, and neovascularization is present (arrows). C, Necrotic atrial myocardium, which was replaced by a collagenous matrix containing islands of surviving myocardium (arrows). D (H&E staining), osseous metaplasia (small arrow) and bone marrow production (large arrow) were present in thickened intima, while smaller branching vessels (large arrow) appeared arterialized (E) secondary to smooth muscle proliferation. High-power examination of pulmonary parenchyma revealed hemosiderin-laden macrophages at sites adjacent to pulmonary veins where RF was applied (F). Bars: ~1.5 mm (A), 200 \( \mu \)m (B), 100 \( \mu \)m (C and D), 400 \( \mu \)m (E), and 50 \( \mu \)m (F).
was applied was replaced by variable amounts of collagen. In some locations, the distance from a narrowed pulmonary vein to normal-appearing lung parenchyma was <2 to 3 mm, whereas in other locations, the lung tissue undergoing collagen replacement was >1 cm from the stenosed pulmonary vein. Lung lobes draining pulmonary veins that were totally occluded demonstrated patchy, scattered areas of collagen that were most dense near the occluded pulmonary vein but extended to more remote portions of the involved lobe infiltrating alveoli and bronchioles. At high magnification, hemosiderin-laden macrophages were present (Figure 4F).

Discussion
This study demonstrates that extensive RF energy applied in a series of linear withdrawals from the pulmonary veins into the left atrium in dogs produces pulmonary vein stenosis, which is predominantly due to intimal thickening, thrombus formation, endocardial contraction, and proliferation of elastic laminae. These changes are associated with significant increases in pulmonary vascular resistance and a decrease in cardiac output. However, no animal developed pulmonary hypertension. These observations suggest that extensive ablation within the pulmonary veins may be associated with long-term physiological effects.

Pathophysiological Mechanisms
Much is unknown about the histological response to thermal injury within the pulmonary veins. In infants and children with congenital or acquired pulmonary vein stenosis undergoing balloon angioplasty or surgical repair (mechanical injury to the pulmonary veins), Lock et al described the presence of prominent intimal proliferation at the restenosed angioplasty site that was composed largely of collagen and disorganized elastic fibers. In addition, van Son et al described intimal smooth muscle proliferation, fibrosis, and partially recanalized thrombus formation within the pulmonary veins. Short-term and long-term studies examining the pathophysiological effects of RF energy application within the coronary sinus in dogs have typically not described intimal thickening, and only occasionally has thrombus been observed. In a study by Huang and associates, 50 RF applications were delivered within the coronary sinus in 10 dogs that were studied 2 to 12 weeks later. In their study, in which systemic anticoagulation was not given, no luminal narrowing was demonstrated at necropsy. In contrast, Langberg and colleagues described luminal narrowing (2) or complete obstruction (1) of the coronary sinus in 3 of 13 animals after ablation within the coronary sinus.

Because the thickened intimal tissue revealed endothelialized vascular channels and only small amounts of smooth muscle in the intima, a mechanism likely to be responsible for the proliferation of fibrocellular material on the luminal side of the internal elastic lamina in the present study is a cicatrical response to thermal injury. Intimal proliferation was present, at least to some extent, in all pulmonary veins with luminal narrowing. This may frequently have been a response to thrombus formation. Differences in the incidence of intimal thickening in the present study and those mentioned above could be due to multiple factors, including the presence of atrial myocardium and fully oxygenated blood within the pulmonary veins, as well as differences in the method of RF energy application.

Although intimal proliferation accounted for much of the luminal narrowing, endovascular contraction involving the media (Figures 2C and 4A) was also observed in 18 of the 22 pulmonary veins with luminal stenosis. Little is found in the literature regarding vessel contracture leading to luminal narrowing after RF energy application to the vasculature. A description of luminal contracture after angioplasty has been mentioned in reviews of restenosis but is poorly characterized. Studies of wound contracture in the skin of animals have revealed that wound size can be reduced by up to 90%, with the mechanism being contraction of modified wound fibroblasts. In the present study, vessel wall contracture was observed in various degrees in nearly all lesions, typically at the site at which RF energy application produced necrosis and resulting scar formation.

Proliferation of elastic lamina with medial hypertrophy of the pulmonary veins may occur after chronic venous congestion. In the present study, elastic lamina proliferation was observed in 16 of the 22 pulmonary veins with luminal narrowing. Whether elastic lamina proliferation was a result of a localized response to vessel injury or a sequel of pulmonary venous hypertension in the stenosed pulmonary vein is uncertain.

Absence of Pulmonary Hypertension
Although the average pulmonary vascular resistance doubled in this study, significant pulmonary artery hypertension did not develop in any animal. However, typically 1 to 3 arteries were completely or severely stenosed in these animals. Unlike humans, dogs typically have 7 separate lung lobes that drain into the left atrium via 6 separate pulmonary veins. In previous studies of experimentally induced pulmonary hypertension in dogs, it was necessary to resect multiple lung lobes in conjunction with the creation of a high-flow state in the remaining pulmonary vasculature to induce pulmonary hypertension.

Implications for Catheter Ablation in the Pulmonary Veins
Current investigational techniques to restore sinus rhythm in patients with paroxysmal or chronic atrial fibrillation have used the delivery of RF energy within the pulmonary veins. The development of pulmonary hypertension or pulmonary vein stenosis was not described in the early reports of this technique. However, isolated stenosis of a single pulmonary vein may result in localized pulmonary congestion, cough, hemoptysis, and dyspnea. In the patients who developed pulmonary hypertension, as described by Robbins et al, RF was applied to all 4 pulmonary veins, resulting in severe stenosis at the pulmonary vein–left atrial junction. These sequelae occurred despite careful monitoring of temperature and anticoagulation.

Limitations
The findings in the present study must be interpreted in light of several limitations. First, systemic anticoagulation was...
used in only 1 animal. It is uncertain whether the routine use of anticoagulation would have changed the pathological effects of ablation. Intimal thickening due, at least in part, to a response to thrombus formation may have been modified by anticoagulation. Another limitation of the study is that extensive RF energy application using a series of linear withdrawals from within the pulmonary veins into the left atrium may not result in the same pathophysiological consequences as focal RF energy applications within the pulmonary veins. Therefore, these findings may not represent the sequelae of a more limited ablation procedure. Finally, the response to injury in the healthy canine atrium may be different from that in the dilated fibrillating human atrium.

Conclusions
Delivery of extensive RF current within the pulmonary veins may result in pulmonary vein narrowing or complete occlusion secondary to fibrocellular intimal proliferation, thrombus formation, endocardial contraction, and elastic lamina proliferation. These observations should be considered in treatment of arrhythmias originating within the pulmonary veins.

Acknowledgments
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References
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