Correlation Between Anatomy and Electrical Activation in Canine Pulmonary Veins

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Background—The roles of complex muscle sleeve geometry and fiber orientation in the pulmonary veins (PVs) in wave-front propagation are poorly understood.

Methods and Results—We mapped the left superior PV (LSPV, n=7) and left inferior PV (LIPV, n=4) of dogs with 420 bipolar electrodes (1-mm resolution) and performed detailed histological examination. In the anterior LSPV–left atrial (LA) junction, myocardial muscle fibers were oriented perpendicular to PV blood flow. A wedge filled with connective tissues led to a complete muscle separation or an abrupt increase in muscle thickness between the PV and LA (0.42±0.12 versus 2.0±0.31 mm, P<0.01). Distal LSPV pacing resulted in conduction block at the anterior PV-LA junction, with double potentials. In contrast, the posterior LSPV-LA junction showed gradual muscle thickening and a fiber orientation parallel to the blood flow. The maximum PV muscle thickness in the anterior PV-LA junction is thinner than that in the posterior junction (0.83±0.15 versus 1.3±0.38 mm, P<0.01). Distal LIPV pacing showed multiple PV-LA breakthroughs, with segmental conduction block in the anterior PV-LA junction. The conduction block corresponded to segmental PV-LA muscle disconnection. Complex fiber orientations in the PV muscle sleeves away from the PV-LA junction were responsible for intra-PV conduction delay or block during rapid PV pacing.

Conclusions—We conclude that segmental muscle disconnection and differential muscle narrowing at PV-LA junctions and complex fiber orientations within the PV provide robust anatomical bases for conduction disturbance at the PV-LA junction and complex intra-PV conduction patterns. (Circulation. 2003;107:1550-1555.)

Key Words: fibrillation ■ mapping ■ electrophysiology ■ immunohistochemistry ■ pacing

Catheter ablation of triggers originating from the pulmonary veins (PVs) may successfully terminate paroxysmal atrial fibrillation (AF).1,2 Because ablation within the PVs may result in stenosis, the junctions between the PVs and the left atrium (LA) have become new radiofrequency ablation targets for achieving electrical PV isolation.3-5 Segmental ablation can successfully isolate the PV by placing radiofrequency lesions in only 21% to 59% of the circumference of the PV ostia.5 The anatomic basis for this segmental ablation technique is unclear. Previous studies showed that ectopic beats originating from the PV propagated to the LA with characteristically long conduction time, often with conduction delay or block within the PV or at the PV-LA junction.6,7 The complex arrangement of myocardial fibers in the PV and/or in the PV-LA junction8-11 is a possible reason for conduction delay or block in the PV-LA junction and within the PV.7 Ho et al10 reported that a differential thickness of muscle sleeves could also account for a variable safety factor of propagation across the PV-LA junction. Hocini et al11 reported that zones of activation delay were observed in canine pulmonary veins and correlated with abrupt changes in fascicle orientation. However, the detailed conduction patterns within the PV and across the PV-LA junction remained unclear. We hypothesize that the breakthrough sites do not occur randomly around the PV ostia. Rather, certain underlying anatomical structures determine the segments of preferential conduction and block between the LA and the PV. If these segments always occur in fixed and known locations, then these sites should be targeted for segmental ablation to create complete LA-PV conduction block. In the present study, we performed detailed histological examinations of the PV-LA junction. We also performed high-density computerized mapping studies of the patterns of impulse propagation from the PV to the LA during distal PV pacing and from the LA to the PV during sinus rhythm in the same veins. The purpose of this study was to test the hypothesis that the patterns of conduction along the PV and at the PV-LA junction correlate with underlying anatomical structures and that the anatomical structures associated with breakthrough predictably occur in fixed and known locations.

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Methods

Computerized Mapping
Seven open-chest dogs (18 to 25 kg) were studied. The anterior PV-LA junctions of the left superior PV (LSPV, n=7) and the left inferior PV (LIPV, n=4) were mapped by use of a computerized mapping system (Unimap, Uniservices). The electrode patch contained 420 bipolar electrodes in 15 columns and 28 rows, with a 1-mm interelectrode distance. Wave-front propagation in the PV and the PV-LA junction was analyzed during sinus rhythm and during regular, burst, and premature stimulation applied to the middle of the anterior wall of the distal PV not covered by the mapping electrodes. The output of the stimulus was 5 to 7 mA, with a pulse width of 0.5 ms. Surface ECGs and atrial electrograms were recorded using bipolar hook electrodes placed on the LA appendage.

Histological Examinations
We took photographs of the tissues after each experiment. The mapping electrodes were machine-made, with a 1-mm interelectrode distance. Therefore, the electrodes were distributed at fixed and known locations. By comparing the photograph with the mapping electrodes, we could accurately compare the histological findings with the results of computerized mapping.

The hearts were excised together with the PVs and fixed in 4% formalin for 1 hour, followed by immersion in 70% alcohol. The PVs were cut transversely, 3 to 5 mm above the PV-LA junction, and dissected into multiple transverse blocks. At the PV-LA junctions, sections were cut longitudinally (parallel to the blood flow). For the LSPV, multiple blocks of PV-LA junctions were taken around the PV orifice, both from the mapped anterior aspect of the PV-LA junction and from the posterior aspect of the PV-LA junction. The tissue blocks were routinely processed and embedded in paraffin. Multiple sections (5 μm thick) from each tissue block were stained either with hematoxylin and eosin or with Masson’s trichrome stain. The sympathetic nerves and gap-junctional proteins at the PV-LA junctions were visualized by immunohistological methods with anti-tyrosine hydroxylase and anti-connexin 40 antibodies, respectively.

Data Analyses
The data obtained from the computerized mapping system were analyzed with methods similar to those reported previously. Student’s t tests were used to compare the means of 2 groups. ANOVAs were performed to compare the means among 3 or more groups. A probability value of P<0.05 was considered significant.

Activation Patterns at the Anterior LSPV-LA Junction
Figure 1A shows that distal LSPV pacing (cycle length, 400 ms) initiated an activation wave front that propagated from 1 branch of the LSPV to the main LSPV (1 to 14 ms) and then to its other branch (18 to 27 ms). However, the propagation from the PV to the LA was delayed or blocked in the PV-LA junction (between 10 and 50 ms). At 50 ms, a breakthrough occurred at the left edge of the mapped region, resulting in activation of the LA (50–66 ms). Three LSPVs showed PV-LA activation patterns similar to that shown in Figure 1A. In comparison, the conduction delay was less apparent during sinus rhythm (Figure 1C).

Figure 1B shows bipolar electrograms recorded from the episode in Figure 1A. Double potentials were recorded at the PV-LA junction (sites e and g) and at the boundaries between 2 PV branches (site b). At site f, triple potentials (red arrows) were observed, because that site was near both the PV-LA junction and the boundaries of the 2 PV branches. The interval between the PV potential and the LA potential in all dogs averaged 35.3±8.2 ms. The PV-LA conduction time was further prolonged during burst pacing at shorter cycle lengths in 4 LSPVs. However, during sinus rhythm (Figure 1D), the LA and PV potentials merged together. The time between the local LA potential and the sharp PV deflection at the site with the maximum PV-LA conduction time (site f) was 8 ms in this dog and 7.7±5.1 ms in all dogs.

Histological Findings at the Anterior LSPV-LA Junction

Fiber Orientation
At the root of the LA appendage, which is anterior to the LSPV (Figure 2A), fiber orientation (yellow arrows in Figure 2B) was aligned perpendicular to the PV blood flow, roughly parallel to the ostium of the LSPV. The anterior PV-LA junctions can be identified by a fold (Figure 2B). The arc
formed by the fold defines a circle. The intersection of that circle with the posterior wall of the LA is the posterior PV-LA junction. Figure 2C is a transverse section of the neighboring LSPV branches. An arrow points to a gap in muscle fibers between the 2 branches, which accounts for the conduction delay and double potentials registered in Figure 1. Sections at the PV-LA junction longitudinal to the blood flow showed that the fiber orientation of both the LA and the LSPV at the anterior PV-LA junction was perpendicular to the blood flow (Figure 2, D and F). In contrast, the muscle fiber orientation in the posterior PV-LA junction (Figure 2, E and G) was parallel to the PV blood flow (red arrow in Figure 2B). Parts of those fibers coursed in a circular way surrounding the LSPV ostium. The right inferior (RI) PV and LIPV formed a common ostium (Figure 2B) located inferior to the LSPV.

Wedge in Anterior LSPV

A wedge filled with connective tissues (light blue stain) but lacking muscle fibers was observed in all dogs between the LSPV and the LA. This wedge served as the microscopic definition for the PV-LA junction. This wedge can completely separate the anterior PV and LA (between arrows in Figure 2H) or partially separate the 2 (arrows in Figure 2I). The wedge might also be present between 2 PVs, such as between the LSPV and the RIPV.

We measured the thickness of the muscle sleeves at the PV-LA junction according to methods presented in Figure 2, D and E. The solid red line segment in Figure 2D indicates the narrowest portion of the PV muscle sleeve at the PV-LA junction. The muscle sleeves at this narrowest portion had a thickness of 0.42 ± 0.12 mm. In contrast, the LA muscle thickness 0.5 mm from the PV-LA junction (dotted red line in Figure 2D) was 2.0 ± 0.31 mm (P < 0.01). In 2 LSPVs studied, there was a large gap filled with connective tissues between the muscle sleeves in the PV and the muscle in the LA, as shown in Figure 2H. In 1 case, the gap between the LSPV muscle sleeve and the LA myocardium reached a maximum of 340 μm. Serial sections showed that a complete separation was present over 100 consecutive sections (at least 500 μm). The actual length could be even longer. In another dog, the gap (310 μm at maximum) was present in 55 consecutive slices (275 μm).

The muscle thickness between the PV and the LA at the posterior PV-LA junction also differed, but to a lesser extent than that at the anterior junction (Figure 2E), with a smooth transition and no wedge. However, there was usually a sudden increase of muscle thickness going from the PV into the LA. The microscopic definition of the posterior PV-LA junction was therefore defined as the site at which there is an abrupt change of muscle thickness (red solid line segment in Figure 2E). The thickness of the PV muscle sleeve at that site averaged 1.3 ± 0.38 mm, which was thicker than the maximum PV muscle sleeve (white solid line segment in Figure 2D) at the anterior junction (0.83 ± 0.15 mm, P < 0.01). Furthermore, the fiber orientation in the posterior PV-LA junction was parallel to the blood flow.

Connexin and Sympathetic Nerves

Connexin-40 (CX40) staining showed abundant connexin within the PV. Figure 2F shows a longitudinal (parallel to blood flow) section of the anterior LSPV at the PV-LA junction, where muscle fibers were uniformly perpendicular to the blood flow. The brown color shows immunolabeled connexin 40. Figure 2E shows the longitudinal section of the posterior wall of the LSPV at the PV-LA junction, where muscle fibers were parallel to the blood flow. There were large sympathetic nerve trunks in the adventitia at the anterior LSPV-LA junction and anterior LIPV-LA junction. In agreement with previous reports, large sympathetic nerve trunks were noted in the ligament of Marshall (LOM), which is located near the LIPV orifice.

Activation Patterns at the Anterior LIPV-LA Junction

In general, the conduction between the LIPV and the LA during distal LIPV pacing showed less conduction delay than that in the LSPV-LA junction (11 ± 3.8 versus 35.3 ± 8.2 ms, P < 0.005). Figure 3 shows a typical example. The wave front induced by distal PV pacing propagated directly into the LA through the anterior PV-LA junction except at the center, where there was significant conduction delay (Figure 3E, line
Histological findings showed that there was a complete disconnection of the LIPV muscle sleeves from the LA at that site. The bipolar recording during LIPV pacing (400-ms cycle length) showed double potentials at site c.

In 2 cases, the mapped area of the LIPV-LA junction included the LOM, which originated from the coronary sinus and coursed toward the LSPV. Figure 3, H and I and N and O, shows that the LOM was activated from right (coronary sinus direction) to left (LA free wall direction) in the mapped area after the local LA had already activated. The bipolar recording at sites g and h show LOM potentials. During sinus rhythm (Figure 3, right), the anterior wall of the LIPV was activated by wave fronts from the right and left borders, not from the LA.

**Histological Findings at the LIPV-LA Junction**

Figure 4 shows the muscle connections between the LIPV and the LA of the same dog as that shown in Figure 3. Figure 4, A to C, shows that there is a large difference in the thickness of muscle sleeves between the PV and the LA in the anterior junction. There were longitudinally oriented fibers connecting the LIPV and the LA at site 1 (Figure 4, A and E). However, at site 2, there was a complete separation of PV muscle sleeves from the LA musculature (yellow arrowheads in Figure 4, B and F). The muscle separation between the PV and LA (532 μm at maximum) was observed throughout the tissue block (≈2 mm). These findings explained the conduction block in only a small segment along the LIPV-LA junction, as shown in Figure 3. Figure 4D shows the histological findings in the posterior wall of the LIPV. The posterior walls of the LIPV and RIPV connect to a common ostium. The muscle sleeves were contiguous, without abrupt changes in muscle thickness. The fiber orientation was parallel to blood flow in the PVs. Figure 4, C and G, shows the coronary sinus and the vein of Marshall within the LOM.

**Figure 3.** Activation patterns at LIPV-LA junction during distal LIPV pacing and during sinus rhythm. A, Mapped LIPV. Vertical line segments on activations indicate time of local activation on that electrogram. CS, coronary sinus.

**Figure 4.** Trichrome stains of LIPV-LA junction. Objective magnification was ×1.25 in A through D and H and ×4 in other panels. I, Sites from which sections were taken. Yellow arrowheads in B, C, and F show a gap filled with connective tissues between LIPV and LA. Black arrowheads in all panels show nerve trunks. CS, coronary sinus; VOM, vein of Marshall.
These structures are adjacent to the LIPV. Figure 4H shows that at the distal end of the LIPV, the muscle sleeves form individual muscle bundles (red arrowheads) separated by connective tissues (blue color). Black arrowheads in Figure 4G show nerve trunks in the adventitia at the LIPV-LA junction and in the LOM.

Conduction Patterns Within the PV

The muscle orientation within the PV was highly anisotropic, which resulted in local intra-PV conduction block in 3 LSPVs during rapid pacing or premature stimulation. Figure 5A shows rapid pacing at 120 ms, resulting in 1-to-1 capture near the site of stimulation (site a). There was 2:1 conduction between site a and other parts of the PV. Red asterisks show the change in the polarity of the bipolar electrogram, which reflects the change in the direction of activation direction at the site. Figure 5B shows conduction block within the PV and the PV-LA junction during premature stimulation. During pacing at 200 ms, intra-PV conduction delay or block was observed, resulting in a change of activation within the PV. Double PV potentials (red arrows at sites b and c) were observed along the line of block within the PV. The premature beat at an interval of 170 ms activated only a small portion of the PV, and the impulse was also blocked at the PV-LA junction.

Figure 6 shows histological sections at the sites of conduction block or delay in Figure 5A. The double-headed white arrow in Figure 6C indicates horizontal fiber orientation, corresponding to the site of conduction block in Figure 5A. Abrupt fiber orientation changes (Figure 6, D–F) were also observed at the site of conduction block and/or delay in the LSPV. In those sites, fibers going left to right (double-headed white arrows in Figure 6, E and F) bordered fibers going in a perpendicular direction (asterisks). An abrupt change of myocardial fiber orientation was also observed at the sites of conduction block within the PV shown in Figure 5B.

Discussion

In this study, we documented that there is an anatomical basis for the segmental electrical breakthrough and conduction block around the ostia of LSPV and LIPV. Conduction block is usually
observed in the anterior PV-LA junction because of a thin or nonexistent connection between the muscle sleeve and the LA at that region. Another major finding is the presence of abrupt fiber orientation changes within the PV, associated with conduction block at short pacing cycle lengths.

**Wedge in Anterior Wall of PV-LA Junction**

The anterior PV-LA junction showed a fiber orientation perpendicular to blood flow, and a wedge of connective tissue was present at the junction. Because of the presence of this wedge, the PV and LA musculature either was totally disconnected or was connected via a narrow isthmus. There were also large and abrupt changes of fiber orientation in the middle portion of the PV. These findings predict conduction slowing or conduction blocks at the anterior PV-LA junction and in the PV itself. Detailed computerized mapping studies confirmed these predictions. These findings might provide an anatomical explanation for the observation that segmental PV ablation procedures can result in electrical isolation of the PV without the need to encircle the entire ostia with radiofrequency lesions.1,3

**Conduction Blocks Within the PV**

Another important finding of this study is the intra-PV conduction block observed during rapid pacing and premature stimulation. Histological sections at the site of conduction block showed the presence of abrupt changes in myocardial fiber orientation. It is well known that the anisotropic myocardial structure is an important factor in promoting conduction blocks and reentrant excitations.1,3,18–20 The presence of anisotropic structures within the PV might promote reentry formation within the PVs during AF.

**Complex Electrogoms in the PV**

We demonstrated that the double or multiple potentials could be recorded at the LA-PV junction, at the junction of 2 PV branches, and when there were conduction blocks within the PV. During ectopy originating from the PV, complex conduction patterns were frequently observed in the PVs in humans.2 In this study, we demonstrated that distal PV pacing induced conduction block within the PV, resulting in complex conduction patterns. Because histological sections showed complex fiber orientations within the PV, an ectopic rhythm originating from the PV could propagate with variable velocities to different regions and exhibit conduction blocks, resulting in complex activation patterns within the PV.

**Limitations of the Study**

Because of technical difficulties, we were not able to map the right PVs or the posterior aspect of the PV. Whether conduction blocks are also present in the right PVs and in the posterior aspects of the left PVs is unclear. Whether the theoretical benefit of first ablating the posterior LSPV and LIPV translates into actual benefit in patient care needs further studies. Fiber orientation may change as these structures expand and contract in systole and diastole, and we did not attempt to distend these structures by perfusion fixation before sectioning. We measured the LA muscle 0.5 mm from the PV-LA junction. Because LA muscle thickness is highly variable, the results might vary depending on where the measurements are done. Finally, because of the atrial size differences, the distances between the PVs and the muscle thickness reported in the present article might not be applicable to humans.

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**References**


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