Electrical, Morphological, and Ultrastructural Remodeling and Reverse Remodeling in a Canine Model of Chronic Atrial Fibrillation

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Background—In patients with recurrent persistent atrial fibrillation (AF), vulnerability to AF persists indefinitely despite presumed completion of reverse electrical remodeling within days of return to normal sinus rhythm. Atrial electrical and anatomic remodeling and reverse remodeling were studied in a canine model of chronic AF.

Methods and Results—Chronic AF was induced in 8 dogs by creating moderate mitral regurgitation and rapidly pacing the right atrium at 640 bpm for >8 weeks. Measurements performed at baseline, after establishment of chronic AF, and then at 4 hours and again at 7 to 14 days after cardioversion to sinus rhythm included atrial effective refractory periods, AF cycle lengths, left atrial dimensions, premature atrial contraction (PAC) frequency, and atrial vulnerability to atrial extrastimuli. After establishing chronic AF, atrial effective refractory period shortening, increases in spontaneous PAC frequency, increases in left atrial size with loss of contractility, and multiple ultrastructural abnormalities were demonstrated. Complete reverse electrical remodeling and decreases in PACs were observed after 7 to 14 days of sinus rhythm, but there was no resolution of anatomic and ultrastructural abnormalities. Occurrence of spontaneous AF paralleled PAC frequency, but vulnerability to AF induction persisted (75% immediately after conversion versus 63% at 4 hours and 50% at 7 to 14 days) despite reverse electrical remodeling.

Conclusions—After conversion from chronic AF to sinus rhythm in this canine model, electrical remodeling occurs rapidly. However, gross and ultrastructural anatomic changes persist, as does vulnerability to induced AF. Vulnerability to AF initiation 7 to 14 days after cardioversion is more dependent on persisting structural abnormalities than on electrophysiological abnormalities. (Circulation. 2000;102:1454-1460.)

Key Words: arrhythmia ■ atrial fibrillation ■ remodeling ■ atrium

Atrial fibrillation (AF) is a common disease that is characterized by multiple wavelets of reentry that are both functionally and anatomically determined.1,2 As AF persists, it often develops into chronic AF. Studies have shown that the atria undergo electrophysiological changes, or electrical remodeling, during AF, which promotes the continuation of AF. These changes include shortening of the AF cycle lengths (AFCLs) and shortening of the atrial effective refractory period (AERP).3–9 After restoration of sinus rhythm, recovery of atrial mechanical function is delayed depending on the duration of AF,10 and the vulnerability to AF persists, with the highest risk of AF recurrence occurring within the first week after cardioversion.11–14 After long periods of AF, it has been shown that the atrial tissue develops structural abnormalities, which can be observed on gross examination (evidenced by atrial enlargement) and histologically (on the ultrastructural level).4,15 It is not clear what role the persistent structural abnormalities seen after cardioversion play in the recovery of atrial function and in the spontaneous recurrence of AF. The purpose of the present study was to develop an animal model of chronic AF and to assess the relative contributions of electrical and morphological remodeling to AF propagation and vulnerability.

Methods

Establishment of Chronic AF

Chronic AF was established with the combination of rapid atrial pacing and creation of moderate mitral regurgitation as previously described.16 Catheters were introduced into the left and right heart of female mongrel dogs via femoral venous and arterial sheaths. Baseline hemodynamic measurements were recorded. A 7F steerable catheter with a stiff 2-mm wire hook at its terminus was placed in the left ventricle and manipulated until mitral chordae tendineae were ensnared and then avulsed. This procedure was repeated until the mean wedge pressure increased 5 mm Hg with associated V waves on the wedge pressure tracing and there was an audible mitral regurgitation murmur. The catheters were removed, and the wounds were repaired.
An active-fixation atrial J permanent pacemaker lead (Teletronics Pacing Systems, Inc, or Medtronic Inc) was placed in the right atrial appendage by a jugular venous approach. The lead was connected to a specially modified Teletronics or Medtronic implantable pulse generator, and appropriate atrial pacing was confirmed. The pacemakers were programmed to a rate of 640 bpm (Teletronics) or 400 bpm (Medtronic) and an output of 2 to 3 times atrial diastolic threshold. After 6 weeks and weekly thereafter, the pacemakers were reprogrammed to low rates and to subthreshold outputs. After 24 hours without pacing, a 6-lead surface ECG was obtained to verify the presence of AF. If AF was present, a 24-hour ambulatory ECG recording was obtained (SpaceLabs Inc) to confirm that the rhythm was sustained.

Electrophysiological Testing
A roving standard ablation catheter (EP Technologies) was introduced to 5 sites in the atria: right atrial appendage, anterior right atrium, posterior right atrium, anterior left atrium, and posterior left atrium. AERP was determined at each of the 5 atrial locations by pacing the atria at twice diastolic threshold with an 8-beat drive train at 350 ms, followed by a single extrastimulus. The atria were considered vulnerable to fibrillation if the arrhythmia occurred spontaneously or was easily induced. Spontaneous AF was defined as AF that occurred spontaneously and required cardioversion for termination. Easily induced AF was defined as AF induced by a single atrial extrastimulus that required a cardioversion to terminate. Only episodes of AF lasting >5 minutes requiring cardioversion were recorded. After each AERP was determined, AF was briefly induced with burst pacing to determine AFCL at each location. AFCL was defined as the mean of 25 consecutive activation intervals.

All cardioversions were performed with 3-ms biphasic intra-atrial shocks. After a successful cardioversion, the rhythm was observed, and the frequency of premature atrial contractions (PACs) was recorded.

Intracardiac Ultrasound
A 6F 12.5-MHz intravenous ultrasound catheter (Hewlett-Packard) was introduced into the left atrial appendage of the dogs through the transeptal sheath. Imaging of the left atrium was performed in sinus rhythm (with use of the Hewlett-Packard SONOS intracardiac ultrasound system) as the catheter was withdrawn through the sheath at 0.5-cm intervals. Images were recorded on videotape for offline analysis.

An estimate of left atrial volume and surface area was calculated by planimetry of the intracardiac ultrasound images recorded at 0.5-cm intervals. At each interval, the maximum diastolic and minimum systolic area along with the diameter were planimetered. The left atrial volume was calculated by an Euler approximation of the integration of the areas multiplied by the pullback distance. An atrial ejection fraction was calculated by the percent volume differences between atrial diastole and systole divided by diastolic volume.

Experimental Protocol

Pre-AF Measurements
During the pacemaker implant procedure, before initiation of pacing, baseline electrophysiological testing and an intracardiac ultrasound were performed. An additional 2 normal dogs underwent baseline electrophysiological testing and intracardiac ultrasound and were then euthanized as control animals for pathological examination.

After Establishment of Chronic AF
All of the dogs underwent comprehensive testing. Cardioversion was performed in each animal after ongoing AF was observed for a minimum of 60 minutes. Before cardioversion, a roving standard ablation catheter was introduced to 5 sites in the atria: right atrial appendage, anterior right atrium, posterior right atrium, anterior left atrium, and posterior left atrium. AF was recorded at each of these sites for regional AFCL determination. After the initial AF record-

ings were obtained, atrial defibrillation was accomplished. After cardioversion, electrophysiological testing for AERP and AF vulnerability assessment and an intracardiac ultrasound were performed.

After Restoration of Sinus Rhythm
The dogs were observed for a minimum of 4 hours. Dogs experiencing episodes of spontaneous AF in this interval were immediately subjected to cardioversion. At the end of the 4-hour observation period, repeat electrophysiological testing and intracardiac ultrasound were performed. AF was briefly reinitiated with rapid pacing, and the AFCL was measured at the 5 atrial recording sites. The animal was subjected to cardioversion and returned to the vivarium in sinus rhythm for observation.

Follow-Up Testing
After a 7- to 14-day period of sinus rhythm, a 24-hour ambulatory ECG was repeated with use of a Holter monitor. Electrophysiological testing and intracardiac ultrasound measurements were again performed. AF was initiated with rapid pacing, and the regional AFCLs were recorded. After these measurements, a thoracotomy was performed.

Pathology
At the completion of the protocol, the beating heart was rapidly extracted, and a small portion of the right atrial appendage, right atrial free wall, left atrial appendage, and posterior left atria was excised and fixed for pathological analysis. Microscopic analysis was performed on 2 normal dogs, 2 dogs with chronic AF, and 4 dogs after 7 to 14 days of recovery. The tissue was immediately minced to \(\approx 1 \text{ mm}^2\) in size in a room temperature solution containing 4.0% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer at pH 7.4. After overnight fixation (4°C), the samples were equilibrated to 24°C, washed in 0.1 mol/L phosphate buffer, postfixed for 1 hour in 2.0% osmium tetroxide, dehydrated in acetone, embedded in EPOX 812 (Ernest F. Fullam, Inc), and polymerized for 48 hours at 60°C.

Light Microscopy
After polymerization, thin sections (0.5 mm) were cut from 2 tissue blocks representing 2 different locations within each atrial region with the use of glass knives and a Reichert Ultracut-E microtome. Sections were collected on glass slides, stained with toluidine blue, and examined with a conventional compound microscope to determine the suitability of the tissue areas for subsequent ultrathin sectioning and light level morphometry.

Light Level Morphometry
Digital images (512x512 pixels) were obtained from the thin sections from each block with a \(x \times 63\) (numerical aperture 1.4) Zeiss objective and a Zeiss LSM 410 laser scanning confocal microscope. By use of a linear measurement program embedded in the Zeiss LSM 410 software (release 3.98), 10 measurements (in microns) of myocyte diameters were made on the digitized images from each tissue block. Each measurement was selected. These measurements were averaged for reporting of the mean diameters for myocytes in control and experimental dog atria.

Electron Microscopy
Ultrathin sections (70 to 80 nm) were cut with a diamond knife and ultramicrotome. The sections were collected on 200 mesh copper grids (Ernest F. Fullam, Inc), contrast-stained with uranyl acetate (50% in acetone) and lead citrate,17 and examined in a JEOL 100-CX transmission electron microscope. Tissue in a 1.5-mm\(^2\) area was visually evaluated and given a score from 1 to 4 based on its degree of abnormality, with 1 being normal and 4 being highly abnormal.

Data Analysis
Electrophysiological data and clinical data were stored in a master computer file. Statistical analysis was conducted by use of RS/1
Results

Baseline measurements were obtained in 12 dogs with a mean weight of 26.6 ± 3.4 kg. Ten dogs continued with the induction of AF by rapid pacing and chronic testing. Eight dogs underwent testing at the follow-up study. The animals underwent high-rate atrial pacing for 115 ± 61 days (median ± quartile range) before achieving chronic AF. All animals that underwent the mitral avulsion and pacing protocol demonstrated sustained AF during the entire 24-hour period of ambulatory ECG monitoring before subsequent testing. Electrical conversion to sinus rhythm was successful in all dogs with persistent AF.

Intracardiac Ultrasound

A characteristic finding in dogs with induced persistent AF was an increase in left atrial size and a decrease in left atrial ejection fraction (Figure 1). There was a significant increase in left atrial end-diastolic volume and a decrease in left atrial ejection fraction between measurements at baseline and those after establishment of persistent AF (P < 0.04). These changes persisted at the follow-up study despite cardioversion to sinus rhythm was successful in all dogs with persistent AF.

Electrophysiological Analysis

The mean AERP values for the 5 individual measurement sites for each time period tested are shown in Figure 2. After establishment of persistent AF, the mean AERP for all sites was 106 ms (25%) shorter than the pre-AF values (P < 0.0001). No significant change in the mean multisite AERP was observed after 4 hours of sinus rhythm, but after maintaining sinus rhythm for 7 to 14 days, the AERP returned to pre-AF values (139 versus 141 ms, respectively). No difference in the magnitude or time course of atrial electrical remodeling and reverse electrical remodeling was seen among the 5 mapped sites, except for higher AERP values after 4 hours of sinus rhythm at the anterior right atrial sites (P < 0.05).

Figure 3 shows that the mean AFCL decreased from 125 ms at baseline to 113 ms in chronic AF (P < 0.001) and returned to baseline values over time after restoration of sinus rhythm. A significant prolongation of AFCL was observed after just 4 hours of sinus rhythm in the absence of a prolongation of the AERP at the 5 sites measured, indicating that the time course of reverse electrical remodeling for the functional refractory period may be shorter than that for the AERP.
The time course and magnitude of observed AFCL changes were similar among the 5 mapped sites. Immediately after cardioversion from chronic AF, spontaneous sustained AF recurred in 6 of 8 dogs. After maintaining sinus rhythm for 4 hours, only 1 of 8 dogs had spontaneous AF, and after 7 to 14 days of sinus rhythm, no animal had spontaneous AF during the follow-up procedure. In contrast, AF could be easily induced with single extrastimuli in 5 of 8 dogs immediately after cardioversion from chronic AF, in 5 of 8 dogs 4 hours after cardioversion, and in 4 of 8 dogs after 7 to 14 days of sinus rhythm (Figure 4). In comparison, normal dogs demonstrated neither spontaneous episodes of AF nor AF induction with single atrial extrastimuli. After cardioversion from persistent AF, the PAC frequency was 4 ± 3 per minute, decreasing to 1 ± 1 per minute after 4 hours of sinus rhythm (P = 0.005). There were no PACs observed at the follow-up procedure (Figure 4).

**Pathological Examination**

**Gross Analysis**
No pericardial inflammation, effusion, or hemorrhage was noted in any dog. The chronic AF group demonstrated a significant increase in left atrial size in all dogs. All dogs had small septal defects from the transeptal punctures and fibrosis in the right atrial appendage at the pacer lead implant site. Within the control group, the heart and intracardiac structures appeared normal.

**Electron Microscopy**
Tissue from normal dogs (Figure 5A) was compared with tissue from dogs with chronic AF (Figure 5B) and with dogs that had completed the 7- to 14-day follow-up period of sinus rhythm after cardioversion from chronic AF (Figure 5C). The semiquantitative analysis of observations from these specimens is presented in the Table. Several ultrastructural abnormalities were observed in the samples from animals with persistent AF. In both the right and left atria, the intercalated disks were disrupted in some areas, and the sarcomeres were at various stages of contraction. There was increased space surrounding the myofibrils (previously shown to contain large accumulations of glycogen). The mitochondria were disrupted in some areas, and the sarcomeres were at various stages of contraction.
greatly increased in number and size, and the sarcoplasmic reticulum was partially destroyed and indistinct. The nuclei appeared active (euchromatic). Although these abnormalities were seen in both the left and right atria, the tissue from the right atrium displayed significantly more abnormalities than did the tissue from the left atrium. Notably, there was no resolution of any of these findings after restoration of sinus rhythm for 7 days and completion of reverse electrical remodeling.

**Light Microscopy**

Three hundred twenty randomly selected atrial myocytes were examined and measured. The atrial myocytes from control dogs revealed a normal composition of sarcomeres distributed throughout the cell, and the intercellular space also appeared normal (Figure 6A). In contrast, myocytes from the chronic AF dogs (Figure 6B) and the follow-up dogs (Figure 6C) were smaller in diameter than the myocytes from normal dogs. The myocytes measured 17.0±3.3 μm in chronic AF dogs versus 18.8±3.5 μm in normal dogs (P=0.001) and 16.3±3.9 μm in follow-up dogs versus 18.8±3.5 μm in normal dogs (P<0.0001). The light microscopic analysis also showed a loss of some contractile elements, particularly around the nucleus. In addition, the intercellular space was hyperexpanded in most samples from the chronic AF dogs and follow-up dogs. No inflammatory cells were present.

**Discussion**

The present study examined the electrophysiological and histological natural history of remodeling and reverse remodeling in a canine model of persistent AF that combined mitral regurgitation and high-rate atrial pacing. As anticipated, shortened AERPs and AFCLs consistent with electrical remodeling were observed after persistent AF was achieved. Evidence of reverse electrical remodeling was seen as early as 4 hours after cardioversion with an increase in the AFCLs, but AERPs did not normalize until later. The right atrium appeared to begin recovery sooner than did the left atrium, as evidenced by the AERP and the AFCL data after 4 hours of sinus rhythm. Reverse electrical remodeling for all regions was complete at the follow-up study 7 to 14 days after cardioversion. However, despite the restoration of sinus rhythm, marked anatomic abnormalities persisted through the time of the follow-up study, possibly accounting for the decreased contractile function of the atria. Thus, although the atria showed evidence of reverse electrical remodeling, there was no evidence of concomitant reverse anatomic remodeling with restoration of sinus rhythm.

With restoration of sinus rhythm, the propensity for spontaneous recurrence of AF diminished over time and was correlated with a decrease in the frequency of PACs observed. However, the animals remained vulnerable to AF initiation with single atrial extrastimuli. Even though restoration of normal atrial refractoriness should have been protective against AF propagation, the structural abnormalities of the atria, especially their increased size, likely accounted for their continued vulnerability to programmed electrical stimulation. Therefore, the high prevalence of spontaneous recurrence of AF early after cardioversion in this model is likely to be due to a combination of both increased ectopic activity and the propensity for reentrant AF wavelet propagation.

**Previous Research**

**Animal Models of AF Remodeling**

Several animal models of chronic sustained AF have been developed to assess the utility of potential therapies for AF and to further characterize the arrhythmia. In a model of pacing-induced (1- to 3-week) AF in instrumented goats, AERP and AFCL decreased, and vulnerability to induced AF increased significantly. The reverse electrical remodeling of the atrial tissue was almost complete by 1 week of sustained sinus rhythm and complete after 2 weeks, with the AERPs, AFCLs, and inducibility of AF returning to levels comparable to baseline measurements. Another study examined the acute time course of atrial electrical remodeling with a protocol using 7 hours of rapid atrial pacing. Shortening of AERP began within 30 minutes of the onset of pacing, and reverse electrical remodeling was observed in a similar time period. The rapid time course of changes in refractoriness in that study suggest that these changes were likely due to neurohumoral changes rather than changes in the properties of the atrial myocytes. Two studies monitored the atrial electrophysiological changes after restoration of sinus rhythm in dogs that had been conditioned with 2 to 8 weeks of rapid atrial pacing. In follow-up, some AERP shortening persisted up to 1 week after return to sinus rhythm, and animals had persisting vulnerability to AF induction.

The AF model presented in the present study showed electrophysiological remodeling and reverse electrophysiological remodeling similar to those previously described.
Complete reverse electrical remodeling was not observed until >4 hours after restoration of sinus rhythm. With increasing time in sinus rhythm, both the prevalence of spontaneous AF and PAC frequency decreased in a parallel fashion. However, despite complete normalization of the AERP, the atria remained vulnerable to AF induction. Thus, it would appear that vulnerability to induced AF remains for 7 to 14 days after sinus rhythm conversion in this model, perhaps because of the persisting left atrial enlargement and cellular abnormalities. But the propensity for spontaneous AF decreases as the prevalence of spontaneous triggers for initiation decreases.

**Structural Changes**

Electron microscopic analysis of atrial structural changes has been studied in 2 models of chronic AF. Ultrastructural changes of atrial myocytes observed in those models and confirmed in the present study included enlarged nuclei with dispersed chromatin, an increase in number and size of the mitochondria, and disruption of the sarcoplasmic reticulum. Also observed were disintegration of contractile structures and an increase in glycogen in the myolytic space. Morphometric analysis reported by Ausma et al showed slight enlargement of myocytes after establishing AF with rapid atrial pacing in goats. In contrast, some myocytes were smaller and appeared hypercontracted in the present study. This may be due to a difference in species tested or methodology for achieving sustained AF. Importantly, the present data demonstrate that despite complete reverse electrophysiological remodeling at 7 to 14 days after conversion to sinus rhythm, no corresponding reverse remodeling of the cellular ultrastructure was observed.

**Atrial Function**

Studies of patients undergoing cardioversion for persisting AF have shown that recovery of atrial mechanical function after restoration of sinus rhythm is delayed and that the time of recovery of mechanical function is dependent on the duration of AF before cardioversion. The present animal model is consistent with clinical observations in that the atrial function was markedly depressed after establishment of chronic AF and these changes persisted for at least 1 week.

**Study Limitations**

The model chosen included a combination of mitral regurgitation and rapid atrial pacing to emulate the clinical syndrome of AF in the setting of left atrial hypertension. Thus, it was not possible to discern the relative contributions of the 2 interventions to the electrophysiological and structural/functional changes observed. The follow-up time period of this study was limited to 7 to 14 days. Longer follow-up time periods could be used to identify the time period for the return
of atrial function and to determine whether any reverse structural remodeling occurs.

**Conclusions**
The present study introduced a canine model of chronic AF with mitral regurgitation that demonstrated both electrical remodeling and reverse electrical remodeling. It was shown that reverse electrical remodeling is complete 7 to 14 days after cardioversion. The left atrium remained enlarged, and atrial function was depressed 7 to 14 days after cardioversion. The occurrence of spontaneous AF decreased with reverse electrical remodeling, but the vulnerability to easily induced AF persisted in the setting of complete reverse electrical remodeling and continued anatomic abnormalities. This suggests that an atrial myopathy in response to chronic AF is the dominant factor in the recurrence of AF, not electrical remodeling.

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**References**

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