Dynamic Nature of Atrial Fibrillation Substrate During Development and Reversal of Heart Failure in Dogs

Kaori Shinagawa, MD; Yan-Fen Shi, MD; Jean-Claude Tardif, MD; Tack-Ki Leung, MD; Stanley Nattel, MD

**Background**—Clinical atrial fibrillation (AF) often results from pathologies that cause atrial structural remodeling. The reversibility of arrhythmogenic structural remodeling on removal of the underlying stimulus has not been studied systematically.

**Methods and Results**—Chronically instrumented dogs were subjected to 4 to 6 weeks of ventricular tachypacing (VTP; 220 to 240 bpm) to induce congestive heart failure (CHF), followed by a 5-week recovery period leading to hemodynamic normalization at 5-week recovery (Wk5rec). The duration of burst pacing–induced AF under ketamine/diazepam/isoflurane anesthesia increased progressively during VTP and recovered toward baseline during the recovery period, paralleling changes in atrial dimensions. However, even at full recovery, sustained AF could still be induced under relatively vagotonic morphine/chloralose anesthesia. Wk5rec dogs showed no recovery of CHF-induced atrial fibrosis (3.1 ± 0.3% for controls versus 10.7 ± 1.0% for CHF and 12.0 ± 0.8% for Wk5rec dogs) or local conduction abnormalities (conduction heterogeneity index 1.8 ± 0.1 in controls versus 2.3 ± 0.1 in CHF and 2.2 ± 0.2 in Wk5rec dogs). One week of atrial tachypacing failed to affect the right atrial effective refractory period significantly in CHF dogs but caused highly significant effective refractory period reductions and atrial vulnerability increases in Wk5rec dogs.

**Conclusions**—Reversal of CHF is followed by normalized atrial function and decreased duration of AF; however, fibrosis and conduction abnormalities are not reversible, and a substrate that can support prolonged AF remains. Early intervention to prevent fixed structural abnormalities may be important in patients with conditions that predispose to the arrhythmia. (Circulation. 2002;105:2672-2678.)

**Key Words:** arrhythmias ▪ remodeling ▪ heart failure

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Experimental congestive heart failure (CHF) produces a substrate for atrial fibrillation (AF) maintenance characterized by atrial interstitial fibrosis similar to atrial histopathology in clinical CHF and in mitral valve disease. CHF and valvular dysfunction can be improved by medical therapy and valve surgery, respectively; however, the extent to which the AF substrate subsequently resolves is unknown. We previously showed that atrial tachycardia (AT) remodeling effects in the presence of CHF differ from those in normal hearts; whether AT effects are modified by previous CHF is unknown. The present study was designed to evaluate time-dependent changes in the AF substrate on development and reversal of ventricular tachypacing (VTP)–induced CHF and to compare AT remodeling in dogs with active versus previous CHF.

**Methods**

**Animal Preparation**

Mongrel dogs (weight 24 to 38 kg) were subjected to serial electrophysiological studies (EPSs), with 14 of 25 dogs completing a protocol of 5-week VTP followed by 5-week recovery (Wk5rec) results from 11 dogs with technical failure or death before protocol completion were excluded from analysis). Under diazepam (0.25 mg/kg IV)/ketamine (5.0 mg/kg IV)/isoflurane (1.5%) anesthesia, unipolar leads were inserted in the right ventricular apex and connected to a pacemaker implanted in the neck. Two bipolar leads were inserted in the right atrial (RA) appendage for EPS. After 24 hours of recovery, a control EPS was performed, and then the ventricular pacemaker was programmed to capture the right ventricle at 240 bpm for 3 weeks, followed by 1 to 3 weeks at 220 bpm. After CHF was fully developed (lethargy, dyspnea, and edema), VTP was discontinued, and the dog was followed up for another 5 weeks. Of 14 dogs that completed the protocol, 7 were then allocated to open-chest study (1 of 7 died before open-chest study completion, leaving results from 6 dogs) and 7 to subsequent ATP (see below).

On EPS days, dogs were pretreated with ketamine (5 mg/kg) and diazepam (0.25 mg/kg), anesthetized with isoflurane (1.5%), and ventilated mechanically. The ventricular pacemaker was deactivated and EPS performed with the indwelling atrial electrodes (1 for programmed stimulation and the other to monitor response). A programmable stimulator was used to deliver 2-ms twice-threshold current pulses. Atrial effective refractory period (ERP) was measured with 15 S1 stimuli at various basic cycle lengths (BCLs), followed by an S2 extrastimulus with decrementing S1-S2 intervals.
(ERP=longest S1-S2 that failed to capture). The mean of 3 ERP values at each BCL was used for data analysis. AF was induced by atrial burst pacing (10 Hz, 4 times threshold, 1 to 10 seconds). Mean AF duration in each dog (DAF) was based on 10 inductions for AF ≤20 minutes and 5 for 20- to 30-minute AF. AF >30 minutes was considered sustained and was terminated by synchronized DC cardioversion. Two-dimensional echocardiographic studies were performed in apical 4-chamber, 2-chamber, and parasternal long-axis views.

Open-Chest Study

The day after closed-chest study at Wk5rec, dogs were anesthetized (morphine 2 mg/kg SC and α-chloralose 120 mg/kg IV load, 29.25 mg · kg⁻¹ · h⁻¹) and instrumented for mapping.1 ERP, DAF, and conduction velocity (CV; regression of electrode distance on activation time) were determined. Hemodynamic data were obtained with Masson trichrome. Microscopic images were analyzed with SigmaScan 4.0 (Jandel Scientific).1 

Evaluation of AT Remodeling

RA pacing (400 bpm) was performed as previously described1 in 12 normal dogs (AT only), 13 CHF dogs (CHF + AT, 1-week AT superimposed on last week of VTP), and 7 Wk5rec dogs (1-week AT beginning 5 weeks after VTP; 1 death left a study population of 6 dogs). Atrial vulnerability (percentage of sites in each dog at which AF was induced by a single extrastimulus) and ERP were measured at open-chest study.

Data Analysis

Phase-delay analysis was used to evaluate local conduction abnormalities as described previously1: P95 indicates conduction times between bipolar electrode sites at regions of slowest conduction; P5 to 95/P50 is a conduction heterogeneity index independent of mean CV changes. Statistical comparisons for intergroup mean differences were by ANOVA, with range tests for single comparisons. Contingency comparisons were by χ² analysis. Average results are given as mean±SEM; 2-tailed P<0.05 was considered statistically significant.

Results

Hemodynamic Indices

Ventricular diastolic and atrial pressures were higher and ventricular and arterial systolic pressures were lower in CHF dogs (Table). Wk5rec and control dogs were hemodynamically comparable.

<table>
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<tr>
<th>Hemodynamic Indices at Open-Chest Study</th>
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<tr>
<td>Control Group</td>
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<tr>
<td>Systolic BP</td>
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BP indicates arterial blood pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LAP, LA mean pressure; and RAP, RA mean pressure. All values are mm Hg.

*P<0.05; †P<0.01 vs control group.

Figure 1. Mean±SEM DAF and ERP values at repeated EPS under diazepam/ketamine/isoflurane anesthesia in 14 dogs subjected to VTP followed by 5-week recovery period. A, DAF at control EPS (Day0), followed by EPS at 3 (Day3VTP) and 7 (Day7VTP) days of VTP, mid-VTP period (middle), and end of VTP (CHFmax) and at 3 (Day3rec) and 7 (Day7rec) days and 2 (Wk2rec), 3 (Wk3rec), 4 (Wk4rec), and 5 (Wk5rec) weeks after VTP was stopped. B, Percentage of dogs that had sustained AF at least once on each study day. C, ERP values during VTP and recovery interval.

Time-Dependent Changes in Results of Closed-Chest EPS During Development and Recovery of CHF

Figure 1 shows the time course of DAF changes. CHFmax indicates the time when CHF was fully developed (mean 4.8±0.2 weeks). “Middle” indicates the average of DAF values from 2 weeks after VTP onset until CHFmax. DAF remained relatively short until CHF was fully developed (Figure 1A) and remained significantly greater than baseline until 2 weeks after VTP. Figure 1B shows the percentage of dogs that had sustained AF at least once on each study day. A significant increase occurred before CHFmax and persisted until 2 weeks after VTP cessation. Although the percentage of dogs showing sustained AF decreased during the recovery period, at least 1 dog had sustained AF at all evaluation points, as opposed to none at baseline. Figure 1C shows ERP values at various BCLs during and after VTP. No significant changes were observed.

Closed-chest studies were performed under diazepam/ketamine/isoflurane anesthesia to allow repeated assessments,
but open-chest studies were performed with morphine/chloralose anesthesia to reproduce more closely the physiological vagotonic rested state of the dog. To relate results during closed-chest studies to those during complete EPS under open-chest conditions, DAF was measured under morphine/α-chloralose anesthesia at Wk5rec before thoracotomy in 4 dogs and averaged 1575±225 seconds (P<0.01 versus baseline, 0.3±0.2 seconds). ERP at BCL 360 ms decreased from 127±5 ms under diazepam/ketamine/isoflurane to 105±3 ms (P<0.01) under morphine/chloralose anesthesia. These results indicate that although DAF decreases during recovery from VTP-induced CHF, a substrate for AF remains and can be unmasked by the use of a different anesthetic. Changes in left atrial (LA) and left ventricular dimensions and function over the course of the study are illustrated in Figure 2. LA systolic and diastolic areas increased progressively, by a maximum of 68.1±5.3% and 96.2±6.9%, respectively, at CHFmax and decreased substantially subsequently (Figure 2A). At Wk5rec, they remained 18.4±3.8% and 18.0±3.9% above baseline, respectively. LA fractional shortening decreased significantly by day 3 of VTP, reached a minimum at CHFmax and returned to values not significantly different from baseline by day 7 after VTP. Left ventricular fractional shortening decreased significantly within 3 days of VTP onset and required 4 weeks after VTP to normalize. Combined with the hemodynamic data, the echocardiographic data indicate virtually complete recovery from CHF-induced contractile dysfunction and dilatation. DAF over the course of the study correlated significantly with LA systolic (r=0.28, P<0.01, n=12) and diastolic (r=0.30, P<0.001, n=12) areas.

Results of Terminal Open-Chest Studies and Histology

Figures 3A through 3C show representative examples of atrial histopathology in control, CHF, and Wk5rec dogs. In CHF and Wk5rec dogs, there was extensive interstitial fibrosis. Figure 3D shows mean data in each group. In each of 6 regions, fibrous tissue content was similar for CHF and Wk5rec dogs, and both were significantly greater than for controls. Overall, mean fibrous tissue content averaged 10.7±1.0% for CHF, 12.0±0.8% for Wk5rec, and 3.1±0.3% for control dogs (P<0.01).

Figures 4 and 5 provide mean data regarding electrophysiological properties of control, CHF, and Wk5rec dogs at open-chest EPS. DAF in CHF and Wk5rec dogs was similar and was significantly greater than in controls (Figure 4A). In keeping with the interstitial fibrosis seen on histopathology, the absolute extent of local conduction slowing (Figure 4B) and the conduction heterogeneity index (Figure 4C) were both significantly and equally greater in CHF and Wk5rec dogs versus controls. ERP was slightly greater in CHF dogs at various BCLs (Figure 5A) and regions (Figure 5B) versus controls and Wk5rec dogs, but overall CV was similar (Figures 5C and 5D).

Effects of AT-Induced Remodeling

We have previously shown that in the presence of CHF, AT remodeling effects on ERP and atrial vulnerability are greatly attenuated compared with changes in normal hearts. We could not determine in that study if attenuation was due to the CHF state per se or to effects of CHF-induced atrial structural remodeling on the response to AT. The present study indicates that the atrial fibrotic substrate that characterizes CHF is maintained in Wk5rec dogs at a time when hemodynamics, cardiac dimensions, and cardiac function have recovered, i.e., when CHF and CHF-induced atrial architectural remodeling (interstitial fibrosis) are dissociated. We therefore compared the effects of 1-week AT in control, CHF, and Wk5rec dogs. In control dogs, AT substantially reduced ERP and ERP rate adaptation in both RA (Figure 6A) and LA (Figure 6B). In CHF dogs, AT-induced changes in ERP were greatly attenuated (Figures 6C and 6D). In Wk5rec dogs, AT decreased ERP and ERP rate adaptation (Figures 6E and 6F) in a fashion similar to control dogs. Figure 6G shows associated changes in atrial vulnerability. AT substantially increased vulnerability to AF induction in control dogs but did not significantly alter atrial vulnerability in CHF dogs. In contrast, atrial vulnerability was substantially enhanced by AT in Wk5rec dogs, producing atrial vulnerability even greater than that noted in control dogs with AT remodeling.
Discussion
We have shown that the atrial fibrotic changes caused by 5 weeks of VTP are not reversed during a subsequent 5-week recovery period that allows for reversal of CHF-associated changes in hemodynamics, ventricular function, atrial emptying, and atrial dimensions. Persistent arrhythmogenic atrial structural remodeling is manifested as prolonged AF under morphine/chloralose anesthesia and by enhanced atrial vulnerability with AT remodeling. On the other hand, under diazepam/ketamine/isoflurane anesthesia, cessation of VTP is associated with a return of DAF toward baseline, despite unchanged fibrosis, which indicates that atrial fibrosis is not the only determinant of AF in CHF.

Relationship to Previous Observations Regarding Reversibility of AF Substrate
The reversal of AT-induced electrical remodeling on return to sinus rhythm has been reported in previous experimental and clinical studies. Changes compatible with full recovery of AT-induced ERP alterations were seen. Everett et al. studied reversal of remodeling in dogs with chronic AF produced by creation of mitral regurgitation and by pacing of the RA at 640 bpm for >8 weeks. During a 2-week recovery period, they observed complete reversal of electrical remodeling but no resolution of anatomic and ultrastructural abnormalities, along with incomplete reversal of AF vulnerability. However, mitral regurgitation was not reversed, and therefore the stimulus to structural remodeling was likely unchanged during the recovery period. Thijssen et al. suggested that slow reversal of cellular ultrastructural changes may account for slower recovery of AF-induced contractile versus electrical remodeling. We could not identify any experimental studies of reversibility on removal of the initiating stimulus for arrhythmogenic atrial structural remodeling of the type caused by CHF.

Potential Significance
CHF and rheumatic valve disease strongly predispose to AF, and both produce prominent interstitial fibrosis in humans. The hemodynamic consequences of both potentially may be reversed, CHF by appropriate medical therapy and rheumatic valve disease by corrective surgery. The effects of such reversal on the substrate for AF is poorly understood. Cardioversion of AF after corrective mitral valve surgery permits restoration and maintenance of sinus rhythm in some patients, but many relapse into AF. The results of the present study suggest that once fibrotic atrial structural remodeling has occurred, it is irreversible. It may therefore be
very important to intervene early, if possible, to prevent atrial structural remodeling before it occurs. This notion is consistent with the results of a recent study that found that early mitral valve replacement significantly reduces the long-term incidence of chronic AF.17

We have previously provided evidence for a role of atrial fibrosis in the AF promotion associated with experimental CHF.1 Atrial fibrosis is also seen in a variety of clinical conditions that predispose to AF, such as mitral valve disease,2 CHF,15 and senescence.18 The results of the present study indicate that atrial fibrosis is not the only determinant of prolonged AF, because DAF decreased substantially (Figure 1) with the recovery of hemodynamic function and atrial dimensions after cessation of VTP (Figure 2), despite the complete absence of recovery in tissue fibrosis at Wk5_{rec} (Figure 3). Potential factors other than fibrosis and conduction abnormalities that could contribute to the AF substrate in CHF include atrial dilation, tissue stretch, and neurohumoral factors. In an ovine CHF model, increases in AF duration corresponded to the time of greatest LA dilatation.19 In a previous study,2 as well as in the present work, DAF was significantly correlated with atrial dimensions in CHF. In animal models, acute atrial stretch strongly promotes AF maintenance,20–22 an effect that appears to require intact stretch-activated, nonselective cation channels.23 In humans, variable changes in atrial ERP have been reported with atrial stretch.24,25 CHF is associated with important neurohumoral activation.26 Atrial natriuretic peptide can affect ion channel function,27 and alterations in sympathetic function can play an important role in AF.28 Neurohumoral activation is greater in CHF patients with AF than in those in sinus rhythm29 and could play a role in AF maintenance.

Despite the decrease in DAF under diazepam/ketamine/chloralose anesthesia with hemodynamic recovery, the potential importance of the atrial fibrotic substrate was indicated by the occurrence of prolonged AF under morphine/chloralose anesthesia and the increased atrial vulnerability of dogs subjected to AT after recovery from CHF (Figure 6G). These results point to the dynamic and multifactorial nature of the AF substrate.

We3 have previously shown that in the presence of CHF, AT remodeling changes in ERP are greatly attenuated. In that study, it was impossible to determine whether the interaction was due to CHF-induced atrial fibrosis or to some feature of the CHF state per se (eg, cross talk in signal transduction systems). In the present study, cessation of VTP allowed us to dissociate CHF (which resolved completely) from atrial fibrosis (which remained unchanged). AT in Wk5_{rec} dogs produced ERP changes very similar to those in normal dogs, whereas changes were greatly attenuated in CHF dogs (Figure 6). These results indicate that the attenuation of AT remodeling in CHF is due to the CHF state per se and not to CHF-induced interstitial fibrosis. Furthermore, they imply
that the occurrence of atrial tachyarrhythmias will have quite different remodeling effects in subjects with active CHF, in whom effects on ERP are expected to be minor, compared with subjects with atrial fibrosis due to previous pathology (eg, repaired mitral valve disease), in whom important ERP reductions combined with residual fibrosis might lead to substantial increases in atrial vulnerability (Figure 6).

The treatment of AF with antiarrhythmic drugs is limited by incomplete efficacy and the risk of significant adverse effects, particularly proarrhythmia. Prevention of the development of the AF substrate is an attractive alternative and has been shown to be feasible in animal models.30 The results of the present study suggest that such interventions may need to be applied early, before irreversible fibrotic changes occur.

Potential Limitations
We studied a specific model of CHF produced by sustained VTP. Additional work is needed to determine whether similar phenomena occur with other causes of CHF and with other entities associated with atrial fibrosis, such as mitral valve disease. To obtain reproducible results in serial stimulation studies in the same animal, we used electrodes fixed in a single RA appendage region during development and recovery of CHF. We did not evaluate ERP in other atrial regions and therefore cannot exclude undetected changes in such regions and in spatial ERP heterogeneity. In previous studies, we have found qualitatively similar ERP responses to CHF in different atrial regions and no change in ERP heterogeneity with CHF.1,3

The discrepancy between the results under diazepam/ketamine/isoflurane compared with morphine/chloralose anesthesia was striking. We found that morphine/chloralose anesthesia decreased atrial ERP, consistent with its tendency to produce a vagotonic state.5 Although decreases in ERP promote AF by decreasing the reentrant wavelength,6 we cannot exclude the possibility that other mechanisms might have been involved. Isoflurane has been reported to increase the AF threshold10 and ketamine to have potential effects on intra-atrial conduction.32 In any case, these observations point to the dynamic nature of the AF substrate after recovery from CHF.

We examined tissue fibrosis and local conduction abnormalities after 5 weeks of recovery from CHF. The fact that neither showed any change despite full hemodynamic recovery suggested that they were irreversible. We cannot exclude the possibility that reversal could occur but proceeds extremely slowly and would require observation for much longer recovery intervals to be detected.

Conclusions
Arrhythmogenic atrial structural remodeling may be irreversible on withdrawal of the initiating stimulus. Structural remodeling is not the only factor that determines maintenance of AF in experimental CHF, but persistent atrial interstitial fibrosis leads to a substrate that can support prolonged AF under the appropriate conditions and results in enhanced atrial vulnerability in response to long-term AT. This study highlights the complexity and potential interactions among factors that determine the substrates for AF, as well as the need to intervene early in the prevention of atrial structural remodeling, before irreversible changes have developed.

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References


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