Effects of Angiotensin-Converting Enzyme Inhibition on the Development of the Atrial Fibrillation Substrate in Dogs With Ventricular Tachypacing–Induced Congestive Heart Failure

Danshi Li, MD, PhD; Kaori Shinagawa, MD; Li Pang, MD; Tack Ki Leung, MD; Sophie Cardin, BSc; Zhiguo Wang, PhD; Stanley Nattel, MD

Background—Atrial structural remodeling creates a substrate for atrial fibrillation (AF), but the underlying signal transduction mechanisms are unknown. This study assessed the effects of ACE inhibition on arrhythmogenic atrial remodeling and associated mitogen-activated protein kinase (MAPK) changes in a dog model of congestive heart failure (CHF).

Methods and Results—Dogs were subjected to various durations of ventricular tachypacing (VTP, 220 to 240 bpm) in the presence or absence of oral enalapril 2 mg · kg\(^{-1}\) · d\(^{-1}\). VTP for 5 weeks induced CHF, local atrial conduction slowing, and interstitial fibrosis and prolonged atrial burst pacing–induced AF. Atrial angiotensin II concentrations and MAPK expression were increased by tachypacing, with substantial changes in phosphorylated forms of c-Jun N-terminal kinase (JNK), extracellular signal–regulated kinase (ERK), and p38-kinase. Enalapril significantly reduced tachypacing-induced changes in atrial angiotensin II concentrations and ERK expression. Enalapril also attenuated the effects of CHF on atrial conduction (conduction heterogeneity index reduced from 3.1 ± 0.4 to 1.9 ± 0.2 ms/mm, \(P<0.05\)), atrial fibrosis (from 11.9 ± 1.1% to 7.5 ± 0.4%, \(P<0.01\)), and mean AF duration (from 651 ± 164 to 218 ± 75 seconds, \(P<0.05\)). Vasodilator therapy of a separate group of VTP dogs with hydralazine and isosorbide mononitrate did not alter CHF-induced fibrosis or AF promotion.

Conclusions—CHF-induced increases in angiotensin II content and MAPK activation contribute to arrhythmogenic atrial structural remodeling. ACE inhibition interferes with signal transduction leading to the AF substrate in CHF and may represent a useful new component to AF therapy. (Circulation. 2001;104:2608-2614.)

Key Words: arrhythmia ■ remodeling ■ atrium ■ electrophysiology ■ heart failure

Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice. Although antiarrhythmic agents promote sinus rhythm maintenance, they can cause potentially serious proarrhythmia.\(^1\) A potential new approach to AF therapy is to target the underlying substrate.\(^2\) We recently found that dogs with ventricular tachypacing (VTP)–induced congestive heart failure (CHF) have a substrate for AF maintenance,\(^3\) with interstitial fibrosis that resembles atrial pathology in many patients with AF.\(^4\)

The renin-angiotensin system is involved in myocardial fibrosis in hypertensive heart disease,\(^5\) CHF,\(^6\) myocardial infarction,\(^7\) and cardiomyopathy.\(^8\) Angiotensin II (Ang II) stimulates collagen synthesis in rat cardiac fibroblasts.\(^9,10\) Mitogen-activated protein kinases (MAPKs) are important mediators of Ang II effects on tissue structure.\(^11,12\) The 3 best-characterized MAPK subfamilies are extracellular signal–regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK), and p38-MAPKs.\(^12,13\)

Recent studies showed ERK activation\(^14\) and angiotensin receptor changes\(^15\) in patients with AF. We speculated that Ang II and MAPK activation might be involved in AF-promoting atrial structural remodeling. We therefore designed this study to determine (1) the changes in atrial Ang II and MAPKs during the development of VTP-induced CHF in dogs, (2) the effect of ACE inhibition on atrial Ang II and MAPKs, and (3) the impact of ACE inhibition on AF substrate development.

Methods

Enalapril Effects on the CHF-Induced AF Substrate

The following groups of mongrel dogs (25 to 33 kg) were studied: controls (n=7), placebo-VTP group (n=10), and enalapril-VTP group (n=10). The placebo and enalapril groups were subjected to VTP\(^9\) for 5 weeks (240 bpm for 3 weeks, 220 bpm 2 weeks) and given either placebo or enalapril (2 mg · kg\(^{-1}\) · d\(^{-1}\), Merck Frosst)
during VTP. Investigators were blinded to treatment assignment until all analyses were completed. Five additional dogs were subjected to the same protocol with oral hydralazine (50 mg twice daily) and isosorbide mononitrate (30 mg/d) given throughout the VTP period. Heart failure was established by lethargy, loss of appetite, dyspnea, peripheral edema, and/or ascites associated with appropriate hemodynamic abnormalities.

On study days, dogs were anesthetized with morphine (2 mg/kg SC) and α-chloralose (120 mg/kg IV, 29.25 mg·kg⁻¹·h⁻¹) and ventilated. Body temperature was maintained at 37°C, and a femoral artery and femoral veins were cannulated for pressure monitoring and drug administration. A median sternotomy was performed, and bipolar Teflon-coated stainless steel electrodes were hooked into the atrial appendages. A programmable stimulator was used to deliver 2-ms twice-threshold pulses. Atrial electrograms were recorded to confirm that atrial rate was not affected by ventricular pacing. The implanted pacemaker was then deactivated.

**Electrophysiological Study**

Stimulation and recording were performed and atrial activation was mapped with 248 epicardial electrodes as previously described. The effective refractory period (longest S₃S₄ failing to capture) was measured at left atrial (LA) and right atrial (RA) appendages, with 15 basic (S₁) stimuli followed by a premature (S₄) stimulus, with S₃S₄ decreasing by 5 ms decrements. The mean of 3 effective refractory period measurements was used for analysis. Conduction velocity was measured, as previously described, in the RA and LA free wall after 2 minutes at each basic cycle length. Spatial inhomogeneities in conduction were evaluated by phase analysis as previously reported.

The Pᵥᵥ (difference between 5th and 95th percentiles of the phase-difference distribution) indicates absolute heterogeneity. The variation coefficient (Pᵥᵥ/Pᵥᵥ) is an index of conduction heterogeneity independent of conduction velocity.

AF was induced by atrial burst pacing (10 Hz, 1 to 5 seconds). AF >20 minutes requiring electrical cardioversion for termination was considered persistent. To estimate mean AF duration, AF was induced 10 times for AF duration <10 minutes and 5 times for AF of 10 to 20 minutes. If persistent AF was induced twice, no further AF inductions were performed. A 30-minute rest period was allowed after cardioversion.

**Microscopic Examination**

Animals were euthanized by α-chloralose overdose. Atria were immersed in 10% neutral buffered formalin for 24 hours. Samples were obtained from Bachmann’s bundle, the appendages, the LA posterior and inferior walls, the crista terminalis, and the RA free wall. From each zone, tissue blocks were collected along the longitudinal and transverse planes. Sections (5-μm thickness) were cut at room temperature and stained with Masson’s trichrome, and cut at room temperature and stained with streptavidin–biotinylated antibodies. Sections were blocked in 10% neutral buffered formalin for 24 hours. Samples were processed for immunohistochemistry and for Masson’s trichrome staining.

**Studies of Changes in Tissue Ang II and MAPK Expression**

Sixty additional dogs were divided into 5 groups (12 per group): controls (no pacing) and groups subjected to 1 day and 1, 2, and 5-week tachypacing (time-course studies) and control, 1-day, and 1-, 2-, and 5-week tachypacing (without drug) plus 1-day and 1- and 5-week tachypacing with enalapril (enalapril studies).

The following primary antibodies were used for immunodetection: rabbit anti-P44/P42 polyclonal IgG (phosphorylated and total ERK), rabbit anti-P38 polyclonal IgG (phosphorylated and total p38), rabbit anti-JNK polyclonal IgG (total JNK), mouse anti-JNK monoclonal IgG (phosphorylated JNK), and goat polyclonal anti-Ang II type 1 (AT₁) and type 2 (AT₂) receptor. The anti-phosphorylated MAPK antibodies detect only kinases catalytically activated by threonine or tyrosine phosphorylation. Tissue Ang II concentration was measured by ELISA. Protein samples (500 μg) were analyzed in a microtitre plate with an anti–Ang II antibody (Peninsula) and biotinylated Ang II. The microplate was washed 5 times with TTBS and treated with streptavidin–horseradish peroxidase. The color reaction was developed with 100 μL tetramethylbenzidine substrate and terminated with 2N HCl. Absorbance at 450 nm was recorded, and concentration was calculated from a standard curve generated for each experiment.

**Statistical Analysis**

Multiple group means were compared by ANOVA, followed by Bonferroni-corrected t tests. Wilcoxon’s signed-rank test was used to compare AF durations between groups. Results are mean ± SEM, and a 2-tailed value of P < 0.05 was considered statistically significant.

**Results**

**Changes in Hemodynamic Indices**

Overall group characteristics and hemodynamic data are provided in Table 1. Left ventricular diastolic and atrial pressures increased in 5-week VTP dogs. The enalapril-treated VTP dogs demonstrated improved hemodynamics compared with placebo-treated VTP animals.

**VTP Increases Ang II and MAPK Expression**

Atrial Ang II concentration increased almost 3-fold within 1 day of VTP and remained elevated thereafter (Figure 1A). VTP significantly increased the expression of all MAPKs. Phosphorylated ERK increased within 1 day of VTP (Figure 1B), peaked within a week, and declined thereafter. Phosphorylated JNK (Figure 1C) and p38 (Figure 1D) peaked within 24 hours of VTP and then declined. Changes in total ERK qualitatively paralleled the phosphorylated form but were smaller, and significant increases were noted only at 1 week. In contrast, changes in total JNK and p38 quantitatively paralleled the phosphorylated forms. Increases in phosphorylated ERK correlated with tissue Ang II concent-
tration ($r=0.56, n=60, P<0.0001$), whereas changes in JNK and p38 did not ($r=0.19$ and $0.12$, respectively, $P=NS$).

**Effects of Long-Term Enalapril on Ang II and MAPKs**

Enalapril reduced tissue Ang II concentrations (Figure 2A) and significantly reduced phosphorylated ERK expression (Figure 2C). Enalapril-induced changes in total ERK were small and statistically nonsignificant (Figure 2D). When values of phosphorylated ERK with and without enalapril therapy were related to values of Ang II, there was a clear correlation ($r=0.58, n=84, P<0.0001$, Figure 2B). Enalapril had no effect on pacing-induced increases in phosphorylated (Figure 3A) or total (Figure 3B) JNK or on phosphorylated (Figure 3C) or total (Figure 3D) p38.

VTP increased $\text{AT}_1$ receptor expression, which peaked at $340\pm18\%$ of control at 24 hours and then returned toward control values ($133\pm8\%$ at 5 weeks). $\text{AT}_2$ receptor expression

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<th>TABLE 1. General and Hemodynamic Measurements</th>
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LVSP indicates left ventricular systolic pressure; LVEDP, LV end-diastolic pressure; LAP, mean LA pressure; and RAP, mean RA pressure.

* $P<0.05$; † $P<0.01$ vs control; ‡ $P<0.05$; § $P<0.01$ vs 5-week tachypacing.

Figure 1. A, Atrial tissue Ang II concentration in control (CTL) dogs and in 1-day (1D) and 1- (1W), 2- (2W), and 5-week (5W) tachypaced dogs. B through D, Time course of tachypacing-induced changes in tissue ERK (B), JNK (C), and p38 (D) expression. Top, Representative gels with immunoblots for phosphorylated (P) and total (T) MAPKs. Bottom, Mean±SEM values (n=12 hearts per point). Because changes in 42- and 44-kDa isoforms (for ERK) and 46- and 54-kDa isoforms (for JNK) were not significantly different from each other, they were averaged to provide mean ERK or JNK values for each determination. * $P<0.05$, ** $P<0.01$ vs control.
showed similar changes, averaging 307±18% of control at 24 hours and 120±6% at 5 weeks. Enalapril attenuated the rapid rise in AT1 receptor expression, with AT1 receptor expression at 24 hours averaging 235±18% of control (P<0.01 versus VTP only). Enalapril had smaller and statistically nonsignificant effects on AT2 receptor overexpression, with a mean value of 263±20% at 24 hours (P=0.06 versus VTP only) in VTP-enalapril dogs.

Effects of Enalapril on the AF Substrate

In unpaced dogs (Figure 4A), atria appeared grossly normal. In 5-week tachypaced dogs (Figure 4B), bundles of myofibers were separated by thick layers of fibrous tissue. In enalapril-treated dogs (Figure 4C), interstitial fibrosis appeared attenuated. Overall, the percentage of fibrous tissue was increased 10-fold in placebo dogs, with fibrosis significantly attenuated by enalapril (Figure 4D).

Figure 2. A, Ang II concentration in control, VTP alone, and tachypacing with enalapril (E) dogs (mean±SEM, n=12, 6 for untreated and treated dogs, respectively, at each time point). B, Phosphorylated ERK (P-ERK) as function of Ang II concentration (best-fit regression line shown). Time course of phosphorylated (C) and total (D) ERK in tachypaced dogs with and without enalapril. Top, One original gel for each with 1 sample per lane at each time point studied. Bottom, Mean±SEM expression (normalized to control values) for all dogs; n=12, 6 hearts per point for without and with enalapril. **P<0.01 vs control; #P<0.05, ##P<0.01 for results with vs without enalapril (A, B, D). Abbreviations as in Figure 1.

VTP increased AF duration from 17±8 to 65±164 seconds (P<0.01, Table 2). Mean AF duration in enalapril-VTP dogs was reduced significantly, to 219±75 seconds (P<0.05 versus VTP only).

Figure 3. JNK and p38 expression in tachypacing-alone and tachypacing with enalapril dogs (mean±SEM, n=12 controls per determination, n=6 enalapril-treated dogs per determination). Abbreviations as in previous figures.

Figure 4. Masson’s trichrome-stained light micrographs (×500) of transverse sections of LA from a representative control (CTL) dog (A), a 5-week tachypacing (5W) dog (B), and a 5-week tachypacing, enalapril-treated (5W+E) dog (C). D, Mean±SEM percent connective tissue in CTL, 5W, 5W+E, and hydralazine/isosorbide-treated (5W+HI) VTP dogs.
Hydralazine/Isosorbide Dogs
VTP dogs treated with hydralazine/isosorbide had left ventricular diastolic and atrial pressures similar to those of enalapril dogs and lower than those of VTP-only dogs (Table 1). Despite this hemodynamic improvement, mean AF duration was similar to VTP-only dogs, and 4 of 5 dogs (80%) had at least one episode of persistent AF. Hydralazine/isosorbide did not affect CHF-induced fibrosis (Figure 4D), which averaged $11.7 \pm 1.5\%$ in hydralazine/isosorbide-VTP dogs ($P_{\text{NS}}$ versus VTP only).

Discussion
We found that VTP increased atrial Ang II concentration and phosphorylated MAPK. Enalapril attenuated VTP-induced changes in Ang II and ERK, without affecting JNK and p38, and reduced atrial arrhythmogenic remodeling (fibrosis, conduction abnormalities, and AF duration changes). Vasodilator therapy with hydralazine/isosorbide did not affect VTP-induced atrial fibrosis or AF promotion.

Atrial Structural Remodeling, Signal Transduction Systems, and AF
Interstitial fibrosis is common in patients with AF. The likelihood of AF increases with increasing extent of fibrosis. Prominent fibrosis is also a feature in animal models of AF associated with heart disease. Electrically maintained AF in normal goats causes cellular ultrastructural remodeling, without apoptotic or fibrotic changes. In dogs with atrial remodeling caused by atrial tachypacing (640 bpm) and mitral regurgitation, a combination of electrical and structural remodeling increases vulnerability to AF induction.

Little is known about the potential signal transduction systems leading to the structural remodeling associated with AF. Goette et al. reported that AT$_1$ receptors are downregulated and AT$_2$ receptors upregulated in patients with AF. The same group observed increased expression of ACE and ERK in atria of AF patients.

Our findings are consistent with an important role for Ang II in mediating VTP-induced increased ERK phosphorylation and arrhythmogenic structural remodeling. Ang II activates ERK in cardiac fibroblasts by a tyrosine kinase–dependent mechanism. Mechanical stretch of cardiomyocytes activates both ERK and JNK, but only ERK activation depends on Ang II production. ERK activation is particularly important in the stimulation of fibroblast proliferation. Our results suggest that the changes in atrial angiotensin and ERK systems noted by Goette et al. in AF may be mechanistically related to AF-promoting structural remodeling.

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Role of the Renin-Angiotensin System and Related Pathways in Cardiac Fibrosis

Exposure of cultured fibroblasts to Ang II or aldosterone promotes collagen synthesis; in addition, Ang II reduces collagenase activity. Activation of the renin-angiotensin system leads to cardiac fibrosis in a variety of pathological conditions. In experimental hypertension, Ang II and aldosterone cause ventricular fibrosis that can be dissociated from hemodynamic load. Inhibition of ACE or AT1 receptor blockade prevents collagen accumulation in the noninfarcted myocardium after acute myocardial infarction in rats. AT1 receptor inhibition attenuates fibrotic changes in isoproterenol-induced heart failure. Overexpression of AT1 receptors in transgenic mice results in cardiac hypertrophy, interstitial fibrosis, and death due to progressive heart failure. The present study shows that inhibition of Ang II production reduces atrial fibrosis and arrhythmogenic remodeling in dogs with CHF.

The MAPKs ERK, JNK, and p38 can all be activated by Ang II. ERK is most typically stimulated via G protein–coupled receptors like the AT1 receptor. ERK is important in cell growth, proliferation, and differentiation and plays a key role in the response of fibroblasts to growth signals. We found differences in the expression pattern of the MAPKs studied. Changes in phosphorylated JNK and p38 were quantitatively similar to those of total expression (Figures 1C and 1D), consistent with an increase in JNK and p38 protein as the primary alteration caused by VTP. Conversely, increases in phosphorylated ERK were considerably greater than those of total ERK (Figure 1B), pointing to increased ERK phosphorylation as the primary mechanism for atrial ERK activation.

Novel Aspects and Potential Significance

This is the first study of the role of Ang II and MAPK expression changes in an experimental model of AF promoting atrial structural remodeling. Our findings indicate that enalapril reduces atrial fibrosis, conduction abnormalities, and AF promotion while reducing atrial Ang II concentrations and ERK activation. AF has traditionally been treated with membrane-active antiarrhythmic drugs that can prevent AF at the price of potential mortality-enhancing proarrhythmic actions. An alternative approach is to attack the arrhythmia substrate. In the present study, ACE inhibition attenuated CHF-induced atrial fibrosis that causes local conduction abnormalities, which help to sustain AF. Our findings provide mechanistic support for clinical observations pointing to efficacy against AF of ACE inhibitors in patients with CHF or left ventricular dysfunction after acute myocardial infarction.

It remains to be determined whether interfering with the renin-angiotensin system can reverse established arrhythmogenic atrial structural remodeling. Lisinopril reverses ventricular fibrosis in patients with hypertensive heart disease, but captopril failed to reverse tissue fibrosis after heart failure was established in spontaneously hypertensive rats. It also remains to be determined whether ACE inhibition can prevent atrial arrhythmogenic remodeling associated with conditions other than CHF that promote atrial fibrosis and AF, such as hypertension and senescence.

Potential Limitations

Although enalapril significantly attenuated a range of effects of CHF, the protection conferred was incomplete: atrial fibrosis still occurred, and AF duration was greater in enalapril-treated dogs than in controls. Incomplete protection may have been because enalapril attenuated, but did not eliminate, the tissue Ang II increase associated with CHF (Figure 2A). In addition, enalapril did not prevent activation of JNK and p38-MAPK. Furthermore, other profibrotic signal transduction elements may have been activated by CHF and not affected by enalapril. Further work is necessary to determine whether other agents (such as other ACE inhibitors, AT1 receptor antagonists, β-adrenoceptor blockers, or aldosterone antagonists) are effective in preventing atrial structural remodeling and to define the mechanisms involved.

Conclusions

CHF increases atrial Ang II concentrations and MAPK expression. Enalapril reduces VTP-induced Ang II concentration, ERK activation, atrial fibrosis, conduction abnormalities, and AF promotion. The inhibition of structural remodeling may be a promising new approach in the treatment of AF.

Acknowledgments

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References

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