Treatment of Congestive Heart Failure
Interfering the Aldosterone-Cardiac Extracellular Matrix Relationship

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Abstract—Cardiac extracellular matrix undergoes extensive and continuous turnover involved in the lesion-reparation process, such as in cardiac remodeling, in hypertensive cardiac hypertrophy, in dilated cardiomyopathy, after myocardial infarction in the transition to heart failure, and during the progression of left ventricular dysfunction. Cardiac fibrosis is a major determinant of diastolic dysfunction and pumping capacity, and it may provide the structural substrate for arrhythmogenicity, thus potentially contributing to the progression of heart failure and sudden death. Aldosterone was shown to promote cardiac fibrosis in various experimental models. It was demonstrated that spironolactone may oppose the effect of aldosterone in promoting cardiac fibrosis. Measurement of cardiac collagen turnover by use of serological markers is a useful tool for monitoring cardiac tissue repair and fibrosis in experimental models or clinical conditions. We found that high serum levels of a marker of collagen turnover (procollagen type III N-terminal peptide) in patients with chronic heart failure receiving conventional therapy, including ACE inhibitors, was associated with high mortality and hospitalization rates. In RALES (Randomized Aldactone Evaluation Study), in patients randomized to placebo, markers continued to increase or remained unchanged after 6-month follow-up. On the contrary, adding spironolactone 25 mg daily significantly decreased the levels of these serum markers during the same period. Most importantly, the spironolactone-related morbidity and mortality benefit was most predominant in subgroups with highest baseline levels of serum markers. These results suggest that limitation of the aldosterone-related excessive extracellular matrix turnover may be one of the various extrarenal mechanisms contributing to the beneficial effect of spironolactone in patients with chronic heart failure. (Hypertension. 2001;38:1227-1232.)

Key Words: aldosterone ■ renin-angiotensin system ■ collagen ■ extracellular matrix ■ heart failure

In the myocardium, the role of collagen fibers was thought to be limited to providing a supporting framework for myocytes and blood vessels and acting as lateral connections between muscle bundles. Far from being an inert collection of macromolecules that serves as a scaffold for cells, cardiac extracellular matrix (ECM) undergoes extensive and continuous turnover involved in cardiac remodeling, in hypertensive cardiac hypertrophy, in dilated cardiomyopathy, and after myocardial infarction, as well as in the transition to heart failure and during the progression of left ventricular dysfunction. ECM controls numerous cellular functions and serves as a substrate for cell adhesion, a source of anti-apoptotic signals, a reservoir for growth factors, and a determinant of tissue mechanics.

Collagen biosynthesis is regulated at different levels of transcription and translation. Type I and type III are the 2 major types of collagen present in the myocardium in both normal and diseased myocardial tissue. Fibrillar collagens within the myocardium, such as collagen type I and type III, are important substrates for matrix metalloproteinases (MMPs). The actual activity of MMPs, a tightly regulated process, depends on the rate of synthesis, activation, and the balance between active enzyme and inhibitors.

Butt et al1 observed that collagen production was stimulated by some of the growth factors tested (in order of potency, transforming growth factor [TGF]-1 > platelet-derived growth factor > insulinlike growth factor > basic fibroblast growth factor). Recently, evidence has been presented that defines a role of angiotensin (Ang) II, a potent vasoconstrictor, in stimulation of collagen gene expression and protein turnover in cultured cardiac fibroblasts.

Ang II stimulates collagen synthesis2 and regulates collagen degradation by attenuating interstitial MMP-1 activity by enhancing tissue inhibitor of MMP-1 (TIMP-1) production in endothelial cells.2–4

Fibroblasts constitute the vast majority (>90%) of non-myocyte cells in the heart. Cardiac fibroblasts increase the production of fibronectin and collagen when the heart is exposed to a variety of injuries, such as myocardial infarction, pressure overload, and myocarditis. Proliferation of cardiac fibroblasts and increased content of ECM proteins during cardiac remodeling is one the major causes of cardiac
Cardiac fibrosis is a major determinant of diastolic dysfunction and pumping capacity, and it may provide the structural substrate for arrhythmogenicity, thus potentially contributing to the progression of heart failure and to sudden death.

The molecular mechanism for this abnormal collagen formation during the chronic phase is not clearly understood.

**Pharmacological Modulation of ECM**

Inhibitors of the renin-angiotensin system, such as ACE inhibitors and Ang II receptor antagonists, have been shown to affect collagen turnover in experimental and clinical settings. All ACE inhibitors are well known to reduce fibrosis in hypertensive cardiac hypertrophy and after myocardial infarction, possibly crucial components of their clinical benefit. Other therapeutic agents, such as digoxin, β-blockers, tumor necrosis factor-α receptor antagonists, and endothelin antagonists, may exert part of their effects through ECM turnover modulation.

Mukherjee and Sen first showed the diverse effects of antihypertensive treatment on collagen metabolism. They showed that captopril causes regression of hypertrophy associated with reduced collagen and normalization of blood pressure and reversal of the alteration of collagen phenotypes. The molecular mechanism of such a process is not known. Acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP), a natural inhibitor of procollagen cleavage, has been shown to stimulate collagen I and III synthesis. Its mechanism of action is not clear; however, Ac-SDKP may be an important endogenous regulator of fibroblast proliferation and collagen synthesis in the heart. It may participate in the antiproliferative and antifibrotic effects of ACE inhibitors. The clinical relevance of these findings may be important.

In patients with hypertensive heart disease, lisinopril was shown to regress myocardial fibrosis, irrespective of left ventricular hypertrophy regression. This was accompanied by improved left ventricular diastolic function. In another study, it was shown that ACE inhibitor treatment normalizes serum markers of excess cardiac collagen synthesis in patients with hypertensive heart disease.

In a genetic rat model of hypertension, the administration of an angiotensin type 1 (AT1) receptor antagonist has been shown to stimulate extracellular collagen degradation and to reverse myocardial fibrosis. Factors produced by myocytes are necessary for upregulation of collagen genes in vitro. Fibroblast-myocyte crosstalk is required for Ang II–induced collagen upregulation. Chronic AT1 blockade with losartan resulted in inhibition of TIMP-1 expression and stimulation of collagenase activity in the left ventricle of spontaneously hypertensive rats (SHR). It is proposed that Ang II may facilitate myocardial fibrosis in SHR by depressing the collagenase-mediated extracellular degradation of collagen fibers.

Endothelin-1 appears to mediate cardiac effects of Ang II. Endothelin-1 is synthesized by cardiac myocytes and fibroblasts and has been shown to stimulate collagen I and III synthesis. In rats with chronic heart failure, blockade of endothelin receptors has been shown to decrease left ventricular collagen accumulation. It remains to be determined whether a similar mechanism is operational in the failing human heart.

**Serum Markers of ECM: Monitoring Cardiac ECM Turnover From a Distance**

Noninvasive measurement of cardiac collagen turnover by use of serological markers was shown to be a useful tool for monitoring from a distance cardiac tissue repair and fibrosis, both in experimental models and in clinical conditions.

There is accumulating evidence to show that serum levels of procollagen peptide fragments and of MMP can be used as markers of cardiac collagen turnover. Serum levels of procollagen type III N-terminal peptide (PIIINP) and other markers of collagen synthesis are raised chronically in patients with hypertensive left ventricular hypertrophy and are correlated to the extent of scar formation after acute myocardial infarction. In patients with congestive heart failure (CHF), high baseline serum levels of PIIINP and other markers are significantly associated with poor outcome.

PIIINP is the most frequently studied marker. Collagen scar formation after acute myocardial infarction causes left ventricular dysfunction, and serum markers of extracellular matrix turnover could be quantified by measurements of serum PIIINP concentrations. PIIINPs are also raised chronically in patients with hypertensive left ventricular hypertrophy and in patients with dilated cardiomyopathy. In a recent work, we found that PIIINP serum levels were on average 3.5 ± 0.31 μg/L in normal adult subjects, 4.2 ± 0.25 in hypertensive patients with diabetes (P < 0.05 versus control), and 4.5 ± 0.32 in CHF patients (P < 0.05 versus control, P < 0.05 versus hypertension with diabetes).

The validation of the studied serum markers as indicators of cardiac ECM turnover has been reported in experimental models. Procollagen type I N- (PINP) and C-terminal peptides (PICP) and PIIINP are liberated during collagen biosynthesis, it is possible to use them as markers of this process. Changes in PIIINP have been shown to be induced by acute myocardial infarction in humans and may reflect both synthesis and degradation of collagen. In hypertensive patients Quequejela et al. showed recently a strong correlation between myocardial collagen content and the serum concentration of PICP.
Prognostic Significance of Serum Levels of ECM Turnover

In patients with acute myocardial infarction, the PICP, PINP, and PIIINP serum levels were shown to correlate to infarct size, left ventricular dysfunction, and coronary occluded arteries.\(^\text{18}\) High PIIINP levels on admission and for the following few days after acute myocardial infarction predicted LV dilatation\(^\text{30}\) and poor outcome.\(^\text{20}\) In patients with CHF from idiopathic or ischemic dilated cardiomyopathy, serum PIIINP levels were independent predictors of mortality.\(^\text{21}\)

We found that high serum levels of several markers for cardiac fibrosis in patients with CHF who were receiving conventional therapy, including ACE inhibitors, were associated with high mortality and hospitalization rates. On stable drug regimen, markers continued to increase or remained unchanged after 6-month follow-up.\(^\text{31}\)

Baseline PIIINP, with the best cutoff value\(^\text{32}\) of 3.85 μg/L, had a significant independent negative relation to survival and hospital-free-survival in the placebo group. Patients with baseline PIIINP >3.85 μg/L, compared with patients with PIIINP <3.85 μg/L, had a relative risk of death of 2.36 (95% confidence interval [95% CI], 1.34 to 4.18; \(P=0.003\)) and a relative risk of death and/or hospitalization of 1.83 (95% CI, 1.18 to 2.83; \(P=0.007\)). In the 81 patients (61.8%) with baseline PIIINP levels >3.85 μg/L survival and hospital-free survival were, respectively, 69.1% and 44.3% compared with 79.6% and 63.4% in the remaining 50 patients with levels <3.85 μg/L (Figure 1).

Our results confirm the prognostic value of PIIINP serum level in a large group of patients with CHF receiving conventional therapy, including an ACE inhibitor. This marker was independently associated to an increased risk of death. Furthermore, our results are the first to describe a correlation between this marker and the risk of hospitalization. Interestingly, although we did not find a correlation between collagen serum markers and the severity of CHF as assessed by the New York Heart Association functional class or left ventricular ejection fraction, we found that patients with ischemic heart disease had higher levels of PIIINP than patients with CHF from a nonischemic etiology. This may be a further indication of the prognostic value of PIIINP, because mortality is usually higher in patients with CHF from ischemic origin.\(^\text{33}\)

Aldosterone and the Heart

There is evidence to suggest that the heart is equipped with functional aldosterone synthesis that may participate in cardiac fibrosis during cardiac remodeling and is regulated by Ang II. Production of aldosterone is activated in the failing human ventricle in proportion to heart failure severity.\(^\text{36}\) Ang II modifies cardiac levels of aldosterone, thereby effecting changes in collagen accumulation. Aldosterone production is also under the control of other factors, such as potassium and corticotrophin, independently from Ang II. This raises the possibility that local variations in aldosterone, even under conditions of renin-angiotensin system blockade may participate into the control of ECM turnover.

Aldosterone is a potent mineralocorticoid that promotes sodium retention and elevation of arterial pressure. Independent of its effect on blood pressure, aldosterone may also play a role in cardiac hypertrophy. Within the myocardium, aldosterone acts via mineralocorticoid receptors to enhance ECM and collagen deposition. Pathological patterns of left ventricle geometry have been associated with elevations of plasma aldosterone concentrations in patients with essential hypertension and the early onset of left ventricular hypertrophy has been described in patients with primary aldosteronism.

Experimental and clinical observations with spironolactone, a mineralocorticoid antagonist, highlight the potentially adverse effects of aldosterone on cardiac structure and function.

Aldosterone was shown to promote cardiac fibrosis in various experimental models.\(^\text{37,38}\) The temporal cellular response and appearance of myocardial fibrosis associated with chronic elevation of Ang II and/or aldosterone differ, indicating that separate pathogenic mechanisms are operative with these effector hormones of the renin-angiotensin-aldosterone system.\(^\text{37}\) Cardiac Ang II receptor may be a target for aldosterone, possibly leading to increased responsiveness of cardiac cells to Ang II. Ventricular density of AT\(_1\) receptors...
is increased in rats treated for 1 month with aldosterone and high-salt diet, this increase being prevented by both spironolactone and losartan.38 The regulation of the key enzyme of collagen degradation MMP-1, which has been identified in the heart, is largely unknown. MMP-1 activity in cultured cardiac fibroblast preparation was not influenced by aldosterone.39 Because collagen accumulation in the myocardium represents the balance between collagen synthesis and degradation, aldosterone results in a net accumulation of collagen.

In several reports, it was demonstrated that spironolactone may oppose the effect of aldosterone in promoting cardiac fibrosis.37–41 Interestingly, a low dose of spironolactone, with no effect on blood pressure and with no or little effect on left ventricular mass, was able to prevent selectively cardiac collagen accumulation in models of renovascular and aldosterone hypertension.40 Aldosterone-induced fibrosis appears in both ventricles and is shown to be independent from hemodynamic factors. Age-induced increase in cardiac and aortic ECM accumulation cannot be antagonized by chronic ACE inhibition but could be prevented by spironolactone treatment without affecting blood pressure, in old normotensive rats.42 The clinical relevance of these findings is currently under investigation.

In a small clinical study, a high dose of spironolactone (50 to 100 mg/d) produced a significant decrease of PIIINP serum level in 21 patients with CHF.43 The cardioprotective effects of spironolactone may explain the survival benefit of anti-aldosterone therapy in patients with severe chronic heart failure evaluated in the Randomized Aldactone Evaluation Study (RALES) mortality trial. RALES, a placebo-controlled randomized trial, has demonstrated that the blockade of aldosterone receptors by spironolactone 25 mg OD in addition to standard therapy, including ACE inhibitors, substantially reduces the risk of both morbidity and death among patients with severe CHF as a result of left ventricular systolic dysfunction.44 The mechanism of these beneficial effects was not directly provided by the study. However, it was speculated that various mechanisms could have contributed. Spironolactone may be effective because it opposes the effects of aldosterone of sodium retention, loss of magnesium and potassium, sympathetic activation, parasympathetic inhibition, baroreceptor function, vascular damage, and arterial compliance.45–47 Spironolactone may also oppose the effect of aldosterone in promoting cardiac fibrosis.37

We investigated the interactions between serum markers of cardiac fibrosis and the effect of spironolactone on outcome in patients with CHF randomized in the RALES trial.31 A sample of 261 patients from the RALES trial participated in this substudy. All were on conventional therapy, and 92% were on ACE inhibitors. Patients were randomized to placebo or spironolactone (12.5 to 50 mg daily). Serum PICP, PINP, PIIINP, MMP-1, and TIMP-1 were assessed at baseline and at 6 months, using radioimmunoassay and ELISA. Mean follow-up was 24 months. At 6 months, markers decreased in the spironolactone group (PIIINP, −17%; PINP, −12%) but increased or remained unchanged in the placebo group (intergroup comparison, P<0.02). Spironolactone effect on survival and hospitalization was significant only in patients with above median baseline levels of PIIINP, PINP, and PICP (Figure 2). The reduction of risk of death among patients with spironolactone was, respectively, 0.60 (95% CI, 0.39 to 0.93, P=0.02) and 1.49 (95% CI, 0.76 to 2.93) in subgroups of PIIINP levels above and below median. Similarly, the reduction of risk of death and hospitalization was, respectively, 0.60 (95% CI, 0.41 to 0.87, P=0.007) and 0.89 (95% CI, 0.52 to 1.51). These findings were unchanged after adjustment for other clinical and laboratory variables.

Our results are the first to report a significant effect of a low dose of spironolactone (27 mg/d on average) on a number of serum markers of collagen in a large group of patients. Together with the demonstrated prognostic importance of serum levels of the collagen markers in our study and the significant decrease of these levels in the spironolactone-treated group of patients, this finding strongly suggests that, indeed, the limitation of the aldosterone-stimulated collagen synthesis is most probably a major mechanism explaining the clinical benefit of spironolactone in the RALES trial. More recently, results from a study in postmyocardial infarction patients gave credit to this hypothesis.48 Potassium canrenoate, the active metabolite of spironolactone, combined with ACE inhibitors reduced postinfarction collagen synthesis, as assessed with serum PIIINP monitoring, and limited concomitantly progressive left ventricular dilatation.

**Conclusion and Future Directions**

The importance of local humoral factors in the regulation of cardiac tissue remodeling has become increasingly evident. Further evidence is required to prove that a local aldosterone system is functional in the human myocardium.

Pharmacological interventions with aldosterone receptor antagonists have underscored the importance of aldosterone in the mediation of cardiac fibrosis in humans and animal models with heart failure. These studies suggest that circulating or locally produced aldosterone stimulates fibrillar collagen accumulation in the heart via mineralocorticoid receptors or, indirectly, interfering with AT1 receptors. Additional exploration is necessary to determine the various autocrine/paracrine mechanisms involved in ECM remodeling in the failing heart. Understanding these mechanisms represents a major challenge that will require the use of classical and more recent cellular and molecular biology...
techniques, including transgenes. Cardiac microdialysis, may afford the unique opportunity to peer into the cardiac interstitium and to analyze the complex humoral environment of the cardiac fibroblast and the interactions between aldosterone, the renin-angiotensin system and other growth factors. Ongoing clinical trials with the selective aldosterone receptor antagonist eplerenone, in hypertensive patients with cardiac hypertrophy as well as after acute myocardial infarction (the EPHEBUS trial\textsuperscript{9}), with ancillary studies using nuclear magnetic resonance for monitoring cardiac remodeling as well as of validated serum markers for monitoring cardiac ECM turnover, will help understanding the clinical relevance of the ECM turnover modulation with aldosterone antagonists for the therapeutic management of a variety of cardiovascular disease.

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