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Ventricular remodeling: insights from pharmacologic interventions with angiotensin-converting enzyme inhibitors

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Abstract

Structural remodeling of the left ventricular (LV) myocardium develops in a time-dependent fashion following acute myocardial infarction and may be an integral component in the transition toward overt heart failure. Globally, the remodeling process is characterized by progressive LV enlargement and increased chamber sphericity. At the cellular level, the remodeling process is associated with myocyte slippage, hypertrophy, and accumulation of collagen in the interstitial compartment. In the present study, we examined the effects of early, long-term monotherapy with the angiotensin converting enzyme (ACE) inhibitor, enalapril, on the progression of LV remodeling in dogs with LV dysfunction (ejection fractions 30–40%) produced by multiple sequential intracoronary microembolizations. Dogs were randomized to 3 months oral therapy with enalapril (n = 7) or to no treatment (n = 7). In untreated dogs, LV end-systolic volume index (ESVI), end-diastolic volume index (EDVI) and chamber sphericity increased significantly during the 3 months follow-up period. In contrast, in dogs treated with enalapril ESVI, EDVI and chamber sphericity remained essentially unchanged. Treatment with enalapril attenuated myocyte hypertrophy and the accumulation of interstitial collagen in comparison to untreated dogs. These data indicate that early treatment with ACE inhibitors can prevent the progression of LV remodeling in dogs with LV dysfunction. Afterload reduction, inhibition of direct action of angiotensin-II and possibly the decrease in bradykinin degradation elicited by ACE inhibition may act in concert in preventing the progression LV chamber remodeling. (Mol Cell Biochem **147**: 51–55, 1995)

Key words: ventricular enlargement, myocyte hypertrophy, interstitial fibrosis, angiotensin converting enzyme inhibitors

Introduction

Structural and topographical remodeling of the left ventricle (LV) has long been recognized to develop following acute myocardial infarction. This remodeling process is progressive in nature in that it develops over a period of months or even years after the acute event. The factors that dictate the rate at which this process develops are not clear but are likely related to the extent of loss of viable myocardium. Thus, a larger infarction is likely to elicit a faster progression of LV remodeling in comparison to a smaller infarction. The term 'ventricular remodeling' includes several structural and topographical adaptations and/or maladaptations that occur in response to myocardial injury. Globally, these changes include LV chamber dilation and increased chamber sphericity [1]. At the cellular level, alterations in both the myocyte and non-myocyte compartment occur and include an increase in myocyte size and accumulation of collagen in the interstitium (reactive interstitial fibrosis) [1]. There is little doubt that this process of LV remodeling, if left unchecked, can lead to progressive LV dysfunction and ultimately to the syndrome of congestive heart failure. Despite the ominous association of LV remodeling with poor long-term prognosis, little is known about this process and the factors that dictate its development and progression. Our approach to probing the underlying factors that promote progressive LV remodeling has centered on the use of pharmacologic agents in a canine model of chronic heart failure which manifests progressive

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LV remodeling [2, 3]. In the present study, we examined the effects of early, long-term, therapy with the angiotensin converting enzyme (ACE) inhibitor, enalapril, on the progression of LV remodeling in dogs with moderate heart failure. Specifically, we examined the effects of therapy with enalapril on LV chamber enlargement, LV chamber sphericity, cardiocyte hypertrophy and interstitial fibrosis. The cohort of animals used in this study represents a subset of a larger study which examined the hemodynamic effects of other pharmacologic agents including beta-blockers and digitalis preparations [4].

Methods

The animal model

Fourteen healthy mongrel dogs weighing between 18 and 31 kg were used in the study. Chronic LV dysfunction was produced by multiple sequential intracoronary embolizations with polystyrene latex microspheres (77–102 μm in diameter) as previously described [2]. Coronary microembolizations were performed during sequential cardiac catheterizations under general anesthesia and sterile conditions. Anesthesia consisted of intravenous injections of oxymorphone hydrochloride (0.22 mg/kg), diazepam (0.17 mg/kg) and sodium pentobarbital (150–250 mg to effect). In all dogs, coronary microembolizations were discontinued when LV ejection fraction, determined angiographically, was between 30–40%. To achieve this target ejection fraction, dogs underwent an average of five microembolization procedures performed 1–3 weeks apart. Three weeks after the last embolization procedure, all dogs underwent a pre-randomization left and right heart catheterization. One day after cardiac catheterization dogs were randomized to 3 months oral monotherapy with enalapril (10 mg twice daily, n = 7) or to no treatment at all (control, n = 7). Angiographic measurements were made at baseline, prior to any embolizations, and were repeated one day prior to randomization and initiation of therapy. Dogs were sacrificed after the final hemodynamic study namely, 3 months after initiating therapy and the hearts were removed and prepared for histologic evaluation. The study was approved by the Henry Ford Hospital Care of Experimental Animals Committee and conformed to the guiding principles of the American Physiological Society.

Ventriculographic measurements

Left ventriculograms were obtained during cardiac catheterization with the dog placed on its right side and were recorded on 35 mm cine at 30 frames/sec during the injection of 20 ml of contrast material (Hypaque meglumine 60%, Withrop Pharmaceuticals). Correction for image magnification was made with a radiopaque calibrated grid placed at the level of the LV. LV end-systolic and end-diastolic volumes were calculated from ventricular silhouettes using the area-length method [5] and were corrected for body surface area (end-diastolic volume index = EDVI and end-systolic volume index = ESVI). Global indexes of LV shape were used to quantitate changes in LV chamber sphericity. Left ventricular shape was quantified from angiographic silhouettes based upon the ratio of the major-to-minor axis at end-systole (ESR) and end-diastole (EDR) [6]. As these ratios decrease (approach unity), the shape of the LV deviates from that of a typical ellipsoid to one which approaches that of a sphere.

Immunohistochemical and morphometric assessments

From each dog, transmural tissue blocks were obtained from the LV free wall at the mid-ventricular level and rapidly frozen in isopentane cooled to −160°C in liquid nitrogen. Cryostat sections, 8–10 μm thick, were prepared and incubated at 4°C overnight in rabbit anti-human collagen type III polyclonal antibody (Chemicon International, Inc.). Sections were then stained with dichlorotriazinyl amino fluorescence (DTAF)-conjugated goat anti-rabbit IgG (Chemicon International, Inc.) to visualize interstitial collagen. Immunofluorescent staining was evaluated with an epifluorescent microscope optimized for DTAF. The same sections were used for quantitating myocyte size and volume fraction of interstitial collagen. From each section, five microscopic fields, each containing a minimum of 100 cardiocytes were selected at random for analysis. For each dog, the average myocyte cross sectional area (radial sections only) was calculated using computer-assisted planimetry (SigmaScan, Jandell Scientific). The volume fraction (%) of interstitial collagen, area occupied by collagen as a percent of total surface area, was quantified using computer-assisted videodensitometry (JAVA Video Analysis Software, Jandell Scientific). LV tissue specimen obtained from 5 normal dogs was processed in the same manner and used for comparison.

Data analysis

Comparisons of angiographic variable within each group were examined between measurements obtained just prior to the initiation of therapy and measurements made after completion of 3 months of therapy. For these comparisons, a Students paired t-test was used and a probability of 0.05 or less was considered significant. To ensure that angiographic parameters prior to randomization and initiation of therapy were similar between the untreated group and the enalapril treated

group, comparisons were made using a *t*-statistic for two means. For this test, a probability of 0.05 or less was considered significant. Differences in morphometric measures between the control (untreated group) and the enalapril treated group were also examined using a *t*-statistic for two means. A probability of 0.05 or less was considered significant. All data are reported as the mean ± standard error of the mean.

Results

Angiographic findings

There were no significant differences in any of the pre-randomization angiographic parameters between dogs that were subsequently randomized to no treatment and dogs randomized to active treatment with enalapril. In dogs randomized to no treatment, LV ESVI was 44 ± 5 ml/m^2 and increased to 64 ± 7 ml/m^2 at the end of 3 months of follow-up compared to pre-randomization (71 ± 7 vs. 86 ± 9 ml/m^2, p = 0.007). During the 3 months follow-up period, in this untreated group of dogs, there was also associated significant reduction in ESR (1.56 ± 0.04 vs. 1.42 ± 0.04, p = 0.03) and EDR (1.43 ± 0.04 vs. 1.29 ± 0.05, p = 0.02) indicating increased LV chamber sphericity. In contrast, in dogs treated with enalapril, all angiographic parameters were not significantly different at the end of 3 months therapy compared to values obtained before randomization. In this cohort of treated dogs, ESVI was similar before and after therapy (44 ± 5 vs. 44 ± 5 ml/m^2, p = 0.94), as was EDVI (67 ± 8 vs. 72 ± 7 ml/m^2, p = 0.24), ESR (1.51 ± 0.08 vs. 1.46 ± 0.10, p = 0.67) and EDR (1.36 ± 0.07 vs. 1.27 ± 0.08, p = 0.036).

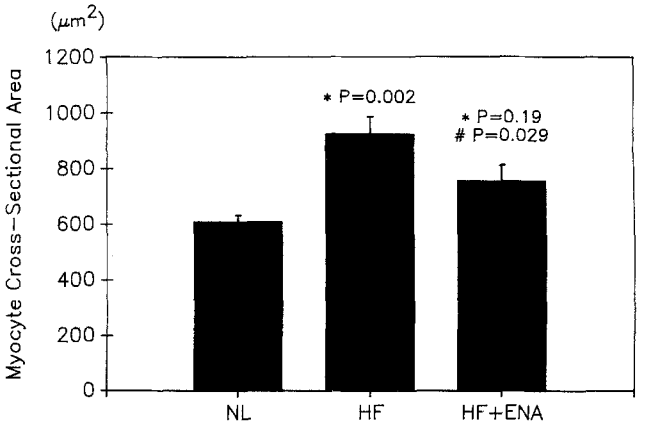

Fig. 1. Bar graph depicting values (mean ± SEM) of average myocyte cross-sectional area in normal dogs (NL), dogs with moderate heart failure that are untreated (HF) and dogs with moderate HF treated with enalapril (HF + ENA). * = p-value relative to NL; # = p-value relative to HF.

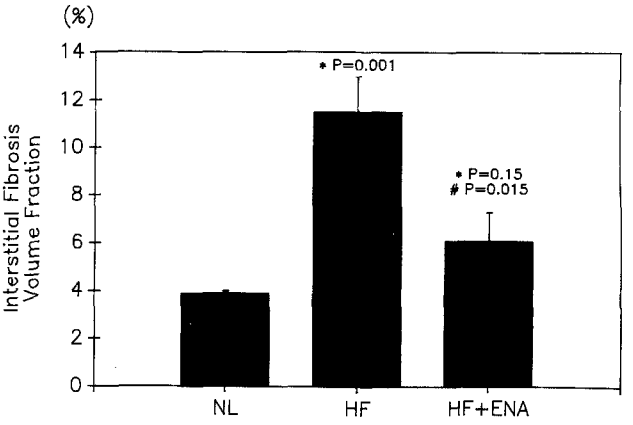

Fig. 2. Bar graph depicting values (mean ± SEM) of volume fraction of interstitial collagen in normal dogs (NL), dogs with moderate heart failure that are untreated (HF) and dogs with moderate HF treated with enalapril (HF + ENA). * = p-value relative to NL; # = p-value relative to HF.

Changes in myocyte size and volume fraction of interstitial collagen

In untreated dogs (control arm), the average LV myocyte cross-sectional area was substantially greater than in the LV of normal dogs (924 ± 63 vs. 608 ± 25 μm^2) (p = 0.002). In dogs treated with enalapril, the average LV myocyte cross-sectional area (711 ± 58 μm^2) was significantly smaller than untreated dogs (p = 0.029) and not significantly different than normal dogs (p = 0.19) (Fig. 1). In untreated dogs, the volume fraction of interstitial collagen was nearly 4-fold greater than that observed in normal dogs (11.5 ± 1.5 vs. 3.9 ± 0.1%) (p = 0.001). In dogs treated with enalapril, the volume fraction of interstitial collagen (6.1 ± 1.2%) was significantly lower than in untreated dogs (p = 0.015) and not significantly different than normal dogs (p = 0.15) (Fig. 2).

Discussion

Results of the present study indicate that in the absence of any drug interventions, dogs with moderate heart failure manifest considerable LV remodeling evidenced by progressive chamber enlargement, progressive chamber sphericity, increased cross-sectional area (hypertrophy) of residual cardiocytes and excessive accumulation of interstitial collagen. Long-term treatment with the ACE inhibitor, enalapril, on the other hand prevented or markedly attenuated all of these features of LV remodeling. Enalapril therapy prevented progressive LV dilation and attenuated the increase in chamber sphericity, myocyte size and interstitial fibrosis.

The observation that early long-term therapy with enalapril prevents progressive LV enlargement supports the conclusions of several recent clinical trials [7–9]. In the prevention arm of the SOLVD trial (Studies of Left Ventricular Dysfunction), early treatment with enalapril in asymptomatic patients with reduced LV ejection fraction was shown to reduce the incidence of congestive heart failure compared to patients randomized to placebo [7]. In a subset of patients with mild to moderate heart failure and reduced LV ejection fraction enrolled in the treatment arm of the SOLVD trial, long-term treatment with enalapril was also shown to prevent progressive LV enlargement compared to patients randomized to placebo [9]. In patients with a first anterior myocardial infarction and reduced LV ejection fraction, early therapy with the ACE inhibitor, captopril, was also shown to attenuate LV dilation [8]. Treatment with captopril in the first year after an anterior myocardial infarction was also shown to attenuate the increase in LV chamber sphericity [10]. The effects of other prototypical drugs used in the treatment of heart failure have also been examined in terms of their efficacy in preventing progressive LV chamber dilation. In dogs with moderate heart failure produced by multiple sequential intracoronary microembolizations, we showed that early, long-term therapy with the beta-blocker, metoprolol, can also prevent progressive LV dilation [4]. In the same dog model, however, early, long-term treatment with digoxin failed to prevent progressive LV dilation [4]. Consistent with these findings, studies in patients with dilated cardiomyopathy showed that long-term therapy with metoprolol was also effective in reducing LV chamber dimensions [11]. In patients with anterior myocardial infarction, treatment with digoxin initiated 7 to 10 days after the onset of symptoms failed to prevent the progressive increase in LV end-systolic and end-diastolic volume indexes after one year of therapy [12].

Although clear evidence exist to indicate that interference with the renin-angiotensin system in the form of ACE inhibition can modulate LV chamber enlargement in patients with chronic LV dysfunction and some evidence to suggest that this form of therapy can prevent progressive LV shape changes, there is no direct studies in patients which implicate ACE inhibition in the prevention or regression of myocyte hypertrophy or in the prevention of interstitial fibrosis. There are studies in animal models, however, that support the concept that ACE inhibitors can have a direct effect on myocyte hypertrophy and on the accumulation of interstitial collagen. In spontaneously hypertensive rats, for instance, treatment with captopril resulted in regression of LV hypertrophy [13]. In a rat model of renovascular hypertension, pretreatment with captopril was also shown to largely prevent the appearance of myocardial interstitial and perivascular fibrosis [14].

The mechanisms through which ACE inhibition and for that matter beta-adrenergic blockade elicit a beneficial effect on LV remodeling remain unclear. Certainly modulation of afterload must be taken into account when considering potential mechanisms of action of both ACE inhibitors and beta-blockers. Studies performed in this laboratory in dogs with moderate heart failure, from which the present animal cohort was selected, showed that therapy with either enalapril or metoprolol attentuated the progressive rise in systemic vascular resistance seen in untreated dogs; whereas monotherapy with digoxin did not [4]. These data provide compelling evidence that afterload reduction can prevent progressive LV enlargement in both patients and animals with chronic LV dysfunction. In spontaneously hypertensive rats, blood pressure reduction after therapy with captopril was also shown to be associated with a significant reduction in LV weight and with a significant reduction in total myocardial collagen content [15].

At present, it would be somewhat premature to suggest that afterload reduction is the sole factor responsible for the observed changes in the global and cellular feature of the LV remodeling process. Other factors should also be taken into account particularly with respect to therapy with ACE inhibitors. Angiotensin-II may have a direct effect on interstitial fibrosis through its action on fibroblasts which normally reside in the myocardium. Myocardial fibroblasts possess antiotensin-II receptor sites [16] and contain the mRNA which is responsible for gene expression of type-I and type-III collagens [17], the major fibrillar collagens of the myocardium [18]. Angiotensin-II formed locally, as a result of activation of a local renin-angiotensin system [19], may be a direct stimulus for cell growth [20] independent of its effect on afterload augmentation and may also be mitogenic to fibroblasts [16, 21]. Recent studies in rats with LV hypertrophy produced by aortic banding have suggested that specific blockade of angiotensin AT-1 receptors with LA sartan (Dup 753) was less effective than ACE inhibition in attenuating myocardial hypertrophy despite lowering of systemic blood pressure [22]. This observation would suggest that factors other than prevention of angiotensin-II formation and afterload reduction may contribute to the beneficial effects elicited by ACE inhibition. In addition to preventing the formation of angiotensin-II, ACE inhibitors also reduce the degradation of bradykinin. Potentiation of kinins following ACE inhibition can lead to increased release of nitric oxide and prostacyclin [23] both of which are thought to be antimitogenic [24]. In a recent study in rats with LV hypertrophy produced by aortic banding, Linz and Scholkens demonstrated that the beneficial antihypertrophic effect of the ACE inhibitor, ramipril, can be prevented by administration of a specific B-2 bradykinin receptor antagonist (HOE 140) in the absence of any reductions of blood pressure [25]. This observation, although unconfirmed, provides some evidence that ACE inhibition induced potentiation of bradykinin may contribute to the beneficial effects of ACE inhibitors on certain components of the LV remodeling process.

In conclusion, the results of the present study indicate that in dogs with moderate heart failure, early long-term monotherapy with enalapril prevents progressive LV remodeling as evidenced by prevention or attenuation of LV enlargement, LV chamber sphericity, myocyte hypertrophy and interstitial fibrosis. The exact mechanisms of this beneficial effect of ACE inhibitors remains uncertain. Additional studies are needed which probe the effects of angiotensin-II and bradykinin receptor blockade on LV remodeling in the setting of heart failure. In the absence of such studies, one can only conclude that the benefit of ACE inhibition on LV remodeling is likely derived from a combination of afterload reduction, direct inhibition of angiotensin-II formation and possibly from reduced degradation of bradykinin.

Acknowledgements

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References

1. Sabbah HN, Goldstein S: Ventricular remodeling: consequences and therapy. Europ Heart J 14: 24–29, 1993
2. Sabbah HN, Stein PD, Kono T, Gheorghiade M, Levine TB, Jafri S, Hawkins ET, Goldstein S: A canine model of chronic heart failure produced by multiple sequential coronary microembolizations. Am J Physiol 260: H1379–H1384, 1991
3. Sabbah HN, Hansen-Smith F, Sharov VG, Kono T, Lesch M, Gengo PG, Steffen RP, Levine TB, Goldstein S: Decreased proportion of type I myofibers in skeletal muscle of dogs with chronic heart failure. Circulation 87: 1729–1737, 1993
4. Sabbah HN, Shimoyama H, Kono T, Gupta RC, Sharov VG, Scicli G, Levine TB, Goldstein S: Effects of long-term monotherapy with enalapril, metoprolol, and digoxin on the progression of left ventricular dysfunction and dilation in dogs with reduced ejection fraction. Circulation 84: 2852–2859, 1994
5. Dodge HT, Sandler H, Baxley WA, Hawley RR: Usefulness and limitations of radiographic methods for determining left ventricular volume. Am J Cardiol 18: 10–24, 1966
6. Sabbah HN, Kono T, Stein PD, Mancini GBJ, Goldstein S: Left ventricular shape changes during the course of evolving heart failure. Am J Physiol 263: H266–H270, 1992
7. The SOLVD Investigators: Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. N Engl J Med 327: 685–691, 1992
8. Pfeffer MA, Lamas GA, Vaughan DE, Parisi AF, Braunwald E: Effect of captopril on progressive ventricular dilatation after anterior myocardial infarction. N Engl J Med 319: 80–86, 1988
9. Konstam MA, Rousseau MF, Kronenberg, MW, Udelson JE, Melin J, Stewart D, Dolan N, Edens TR, Ahn S, Kinan D, Howe DM, Kilcoyne L, Metherall J, Benedict C, Yusuf S, Pouleur H: Effects of the angiotensin converting enzyme inhibitor enalapril on long-term progression of left ventricular dysfunction in patients with heart failure. Circulation 86: 431–438, 1992
10. Mitchell GF, Lamas GA, Vaughan DE, Pfeffer MA: Left ventricular remodeling in the year after first anterior myocardial infarction: a quantitative analysis of contractile segment lengths and ventricular shape. J Am Coll Cardiol 19: 1136–1144, 1992
11. Waagstein F, Caidahl K, Wallentin I, Bergh C-H, Hjalmarson A: Long-term beta-blockade in dilated cardiomyopathy. Effects of short and long-term metoprolol treatment followed by withdrawal and readministration of metoprolol. Circulation 80: 551–563, 1989
12. Bonaduce D, Petretta M, Arrichiello P, Conforti G, Montemurro MV, Attisano T, Bianchi V, Morgano G: Effects of captopril treatment on left ventricular remodeling after anterior myocardial infarction: comparison with digitalis. J Am Coll Cardiol 19: 858–863, 1992
13. Pfeffer JM, Pfeffer MA, Mirsky I, Braunweld E: Regression of left ventricular hypertrophy and prevention of left ventricular dysfunction by captopril in the spontaneously hypertensive rat. Proc Natl Acad Sci 79: 3310–3314, 1982
14. Jalil E, Janicki JS, Pick R, Weber KT: Coronary vascular remodeling and myocardial fibrosis in the rat with renovascular hypertension: response to captopril. Am J Hypertens 4: 51–55, 1991
15. Mukherjee D, Sen S: Collagen phenotypes during development and regression of myocardial hypertrophy in spontaneously hypertensive rats. Circ Res 67: 1474–1480, 1990
16. Schorb W, Booz GW, Dostal DE, Conrad KM, Chang KC, Baker KM: Angiotensin II is mitogenic in neonatal rat cardiac fibroblasts. Circ Res 72: 1245–1254, 1993
17. Eghbali M, Czaja MJ, Zeydel M, Weiner FR, Seifter S, Blumenfield OO: Collagen mRNAs in isolated adult heart cells. J Mol Cell Cardiol 20: 267–276, 1988
18. Medugorac I, Jacob R: Characterization of left ventricular collagen in the rat. Cardiovasc Res 17: 15–21, 1983
19. Dzau VJ: Circulating versus local renin-angiotensin system in cardiovascular homeostasis. Circulation 77: I-4–I-13, 1988
20. Khairallah PA, Robertson AL, Davila D: Effect of angiotensin-II on DNA, RNA and protein synthesis. In: J Genest, R. Koiw (eds) Hypertension. Springer-Verlag, New York, 1972, pp 212–220
21. Ganten D, Schelling P, Flugel RM, Ganten U: Effect of angiotensin and an angiotensin antagonist on iso-renin and cell growth in 3T3 mouse cells. Int Res Commun Med Sci 3: 327–332, 1975
22. Linz W, Henning R, Scholkens BA: Role of the angiotensin II receptor antagonism and converting enzyme inhibition in the progression and regression of cardiac hypertrophy in rats. J Hypertension 8:L S400–S401, 1991
23. Wiemer G, Scholkens BA, Becker RHA, Busse R: Ramilprilat enhances endothelial autacoid formation by inhibiting breakdown of endothelium-derived bradykinin. Hypertension 18: 558–563, 1991
24. Garg UC, Hassid A: Nitric oxide-generating vasodilators and 8-bromocyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. J Clin Invest 83: 1774–1777, 1989
25. Linz W, Scholkens BA: A specific B2-bradykinin receptor antagonist HOE 140 abolishes the antihypertrophic effect of ramipril. Br J Pharmacol 105: 771–772, 1992