EXPERIMENTAL STUDIES

Differential Effects of the Angiotensin-Converting Enzyme Inhibitor Lisinopril Versus the Beta-Adrenergic Receptor Blocker Atenolol on Hemodynamics and Left Ventricular Contractile Function in Experimental Mitral Regurgitation

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OBJECTIVES

The goal of this study was to determine the therapeutic efficacy of angiotensin-converting enzyme (ACE) inhibitors and beta-adrenergic receptor blockers in experimental chronic mitral regurgitation (MR), gaining knowledge using methods difficult to apply in humans.

BACKGROUND

Both ACE inhibitors and beta-blockers are cornerstones in the treatment of human congestive heart failure. However, the roles of these treatments for chronic MR is unclear.

METHODS

Mitral regurgitation was created in 11 closed-chest dogs. Three months after the creation, the ACE inhibitor lisinopril 20 mg was given orally daily. After three months of lisinopril therapy, the beta-blocker atenolol was added to lisinopril for another three months. Atenolol was begun at a dose of 12.5 mg daily and increased gradually to 100 mg daily. Hemodynamics and left ventricular (LV) function were assessed throughout the study.

RESULTS

Regurgitant fraction was consistently >50% over the course of this study. Pulmonary capillary wedge pressure and LV end-diastolic pressure were significantly increased after three months of MR and decreased during both lisinopril and the combined therapy in which it was not different from baseline. Left ventricular contractility measured by the end-systolic stiffness constant was depressed from 3.66 ± 0.20 to 2.65 ± 0.12 (p < 0.05) at three months of MR and rose insignificantly after lisinopril treatment (2.99 ± 0.17). When atenolol was added, it rose significantly and returned to normal (3.50 ± 0.22, p < 0.05).

CONCLUSIONS

Although lisinopril significantly reduced preload, its effect on LV contractility was insignificant in experimental MR. Conversely, atenolol, when added to lisinopril, achieved maximum hemodynamic benefit and also restored LV contractility. (J Am Coll Cardiol 2002;40:149–54) © 2002 by the American College of Cardiology Foundation

Angiotensin-converting enzyme (ACE) inhibitors have been conclusively shown to improve long-term prognosis in human heart failure trials (1,2). Although the exact mechanism of the beneficial effects of ACE inhibitors is still unclear, it has become clear that ACE inhibitors improve hemodynamics, block the production of angiotensin II and increase bradykinin levels (3). Furthermore, ACE inhibitors block maladaptive growth by mitogenic signaling (4–6). However, the effects of ACE inhibitors in human mitral regurgitation (MR) have been controversial and not well studied. In some studies ACE inhibitors improved left ventricular (LV) function with or without decreased regurgitation (7,8). In other studies, captopril depressed LV performance possibly by a negative inotropic effect (9–11). Therefore, the first goal of this study was to define the role of ACE inhibitors in the treatment of chronic MR in a canine model, where the consequences of the disease untreated with surgery could be ethically observed, while at the same time enabling measurements of mechanics and contractile function that are difficult to make in sick human subjects.

Beta-adrenergic receptor blockers have caused striking long-term improvement in LV function as well as in mortality in human chronic heart failure (12,13). The benefits of beta-adrenergic receptor blockers are probably a class effect (14), and they reduce mortality in patients who were already receiving ACE inhibitors (15,16). Although the exact mechanisms by which beta-blockers improve survival in heart failure patients are not yet established, we found that beta-blockers ameliorate LV contractile dysfunction in experimental MR by restoring cellular contractile elements (17), which is possibly established by energy-sparing effects through slowing heart rate (18). Accordingly, the second purpose of this study was to determine whether there were additional benefits to the addition of beta-
blockers to ACE inhibitors in chronic experimental MR and to define specific mechanisms by which each drug might be beneficial.

METHODS

Study design. As shown in Figure 1, 11 previously unreported adult male mongrel dogs weighing 18.6 to 26.8 kg were studied longitudinally when they were normal through nine months of chronic MR. At three months of MR, all animals were given lisinopril 20 mg orally daily. After three months of ACE inhibition, beta-blockade was gradually induced with atenolol at a starting dose of 12.5 mg daily to a total of 100 mg daily as previously described (17,18). The animals then were followed for another three months. Because all dogs were followed longitudinally from when they were normal, they served as their own controls. At the nine-month study, the animals were humanely euthanized under deep anesthesia. The experimental protocol was approved by the Animal Subjects Committee of the Medical University of South Carolina.

All the animals in this study received humane care in compliance with the animal use principles of the American Physiological Society and the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guidelines for the Care and Use of Laboratory Animals prepared by the National Institutes of Health (19). At the initial study, catheter access was established over the right cervical vessels and over either right or left femoral vessels at the subsequent studies. Two pigtail catheters were introduced into the LV and the ascending aorta via the carotid or the femoral artery and were connected to mercury-calibrated water-filled transducers. A Swan–Ganz catheter was advanced into the pulmonary artery via the jugular or the femoral vein to obtain pulmonary capillary wedge pressure (PCWP) and cardiac output by the thermodilution method. A 25-mm balloon catheter was advanced into the inferior vena cava through the same vein to alter LV volume and pressure during ventriculography by inflating and deflating the balloon. After baseline measurements, a standard left ventriculogram using nonionic contrast (iohexol) was performed. After a 10-min equilibration, a second ventriculogram was then taken and timed to coincide with balloon deflation such that beat-by-beat increases in both LV pressure and volume were recorded simultaneously. These procedures were repeated at each observation period shown in Figure 1. The pressure-volume data produced from these alterations would be used to construct the index of LV contractile function discussed below.

Creation of MR. Mitral regurgitation was created by the technique we have previously described (17–23). Briefly, after baseline measurements were made in the initial study, a urologic calculus retrieving forceps was advanced to the mitral valve apparatus via a 7F sheath introduced into LV retrograde through the right carotid artery and was used to rupture chordae tendinae. When PCWP rose to 20 mm Hg and forward stroke volume was reduced to 50% of its baseline value, a ventriculogram was taken to confirm angiographically that severe MR had been created and to calculate regurgitant fraction to quantify the amount of MR.

Assessment of in vivo LV contractile function. In vivo contractile function was assessed in this experiment using the end-systolic stiffness constant derived from end-systolic stress-(ESS)-strain relation analysis (21). Because strain is a dimensionless property, this index is independent of LV chamber size. It has correlated well with changes in contractile function of isolated cardiac myocytes in our previous studies in which sarcomere contractility served as an independent standard of contractile function (17,18,20,23). At all times, studies were made during acute beta-blockade induced by the infusion of esmolol given intravenously with

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Creation of MR</th>
<th>3 months measurements</th>
<th>6 months measurements</th>
<th>9 months measurements</th>
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<td></td>
<td></td>
<td></td>
<td>Lisinopril 20 mg/day</td>
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<td></td>
<td></td>
<td></td>
<td>Atenolol 12.5 to 100 mg/day</td>
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Figure 1. Study protocol. MR = mitral regurgitation.
a loading dose of 1.5 mg/kg/min followed by a constant infusion of 300 μg/kg/min. Acute beta-blockade was used to prevent adrenergic reflexes from confounding measurements of intrinsic contractility and provide consistency of beta-adrenergic effects at four experimental observations (23).

**Calculations.** Left ventricular volumes were determined by the area-length method. This method has previously been validated as providing an accurate measurement of LV volume and mass in dogs with MR in studies in our laboratory (19). The regurgitant fraction (RF) (%) was calculated as: RF (%) = [(SVa - SVf) / SVa] × 100, where SVa is angiographically measured stroke volume (end-diastolic volume - end-systolic volume) and SVf is forward stroke volume (actual cardiac output measured by thermodilution/heart rate). Wall stress was calculated by Mirsky's formula (24). The end-systolic stiffness constant (K-index) was determined by fitting ESS and end-systolic wall thickness data to the curvilinear equation as: $\sigma = C e^{\kappa ln(1/H)}$, where $\sigma$ is end-systolic wall stress, C is a constant, $\kappa$ is the end-systolic stiffness constant and ln(1/H) is the natural logarithm of the reciprocal of wall thickness. A detailed discussion of mathematical derivation of this index was previously described (21).

**Statistics.** All data are expressed as mean ± 1 SEM. Comparisons made regarding given parameters over the course of this study represented multiple repeated comparisons. Therefore, we used two-way analysis of variance (ANOVA) to test statistical differences followed by a Newman-Keuls test if ANOVA testing demonstrated that significant differences were present. A value of p < 0.05 was considered statistically significant.

**RESULTS**

Figure 2 demonstrates that, although the combined therapy of lisinopril and atenolol reduced RF slightly, RF did not vary significantly over the course of this study and consistently was >50%. As shown in Table 1, peak LV pressure remained significantly lower than baseline after MR was created as did end-systolic stress (ESS). Left ventricular volume normalized for body weight (BW) significantly decreased over the course of this study represented multiple repeated comparisons.

**Table 1. Hemodynamic Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Baseline (No MR)</th>
<th>MR (3 Months)</th>
<th>Lisinopril MR (6 Months)</th>
<th>Lisinopril + Atenolol MR (9 Months)</th>
<th>ANOVA p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beat/min)</td>
<td>87 ± 2</td>
<td>103 ± 3*</td>
<td>86 ± 3†</td>
<td>74 ± 3‡</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LV pressure peak (mm Hg)</td>
<td>122.8 ± 3.5</td>
<td>98.3 ± 4.0*</td>
<td>89.0 ± 2.7*</td>
<td>95.2 ± 2.4†</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EDP (mm Hg)</td>
<td>9.9 ± 0.9</td>
<td>15.5 ± 1.1*</td>
<td>11.0 ± 0.9†</td>
<td>7.2 ± 0.8‡</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PCWP (mm Hg)</td>
<td>9.7 ± 0.9</td>
<td>15.0 ± 1.1*</td>
<td>11.1 ± 1.2†</td>
<td>7.9 ± 0.8‡</td>
<td>0.001</td>
</tr>
<tr>
<td>EDV/BW (ml/kg)</td>
<td>3.32 ± 0.15</td>
<td>4.58 ± 0.33*</td>
<td>4.62 ± 0.47*</td>
<td>5.00 ± 0.49*</td>
<td>0.0213</td>
</tr>
<tr>
<td>EF (%)</td>
<td>52.5 ± 1.7</td>
<td>59.6 ± 1.3*</td>
<td>60.8 ± 2.1*</td>
<td>61.1 ± 1.8*</td>
<td>0.003</td>
</tr>
<tr>
<td>ESS (kdyne/cm²)</td>
<td>177.1 ± 12.1</td>
<td>127.0 ± 5.2*</td>
<td>119.2 ± 8.7*</td>
<td>125.1 ± 5.9*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EDS (kdyne/cm²)</td>
<td>25.0 ± 2.4</td>
<td>39.9 ± 3.8*</td>
<td>29.9 ± 4.4†</td>
<td>30.3 ± 4.8†</td>
<td>0.0205</td>
</tr>
</tbody>
</table>

Mean ± SEM, *p < 0.05 vs. baseline; †p < 0.05 vs. MR (3 months); ‡p < 0.05 vs. lisinopril by Newman-Keuls test.

ANOVA = analysis of variance; Atenolol = beta-adrenergic receptor blocker; EDP = end-diastolic pressure; EDS = end-diastolic stress; EDV/BW = ratio of end-diastolic volume to body weight; EF = ejection fraction; ESS = end-systolic stress; LV = left ventricular; MR = mitral regurgitation; PCWP = pulmonary capillary wedge pressure.
increased after creation of MR and remained consistently elevated during the rest of the course of the study. Pulmonary capillary wedge pressure and LV end-diastolic pressure (EDP) were significantly increased at three months of MR. They decreased significantly during lisinopril therapy when they were not different from baseline (Table 1). Combined therapy further reduced EDP compared with lisinopril therapy alone. Along with these changes in PCWP and EDP, LV EDS increased at three months of MR then decreased significantly during lisinopril and the combined therapy. However, it remained significantly greater than baseline.

Figure 3 demonstrates LV mass normalized for body weight. It increased significantly at three months of MR. During lisinopril therapy, it decreased insignificantly, but it was no longer greater than baseline. It increased again during the combined therapy and was significantly greater than baseline. Abbreviations as in Figure 2. ANOVA = analysis of variance.

Figure 3. Left ventricular mass normalized for body weight increased significantly after three months of MR. During lisinopril therapy, it decreased insignificantly, but it was no longer greater than baseline. It increased again during the combined therapy and was significantly greater than baseline. Abbreviations as in Figure 2. ANOVA = analysis of variance.

forward stroke volume determined by thermodilution was depressed at three months of MR, as shown in Figure 4. It increased slightly but insignificantly on lisinopril. During the combined therapy, it rose significantly and returned to normal. Figure 5 demonstrates the change in end-systolic stiffness constant (K-index), a load-independent contractility index. It was depressed after three months of MR. It rose insignificantly on lisinopril therapy. It rose further on the combined

Figure 4. Forward stroke volume by thermodilution method was significantly depressed after three months of MR. Lisinopril increased it slightly. During the combined therapy, it rose significantly and returned to normal. Abbreviations as in Figures 2 and 3.
therapy and was significantly greater than untreated MR and not different from baseline.

**DISCUSSION**

There were two major findings of this study. First, lisinopril significantly reduced LV EDP, PCWP and EDS. However, the effects of lisinopril on forward stroke volume and LV contractility were insignificant. Left ventricular contractility remained depressed on lisinopril therapy. Second, only when a beta-blocker was added did forward stroke volume and cardiac contractility return to normal. Addition of atenolol to lisinopril further reduced EDP and PCWP. Heart rate was significantly reduced after the addition of atenolol among four measurements in this study. Left ventricular mass decreased insignificantly on lisinopril therapy and increased back insignificantly again during the combination therapy.

**ACE inhibitors and the MR heart.** Our canine model of pure severe volume overload significantly decreased ESS, potentially accounting for a decline in LV wall thickness (22). It also strikingly increased EDS and contributed to eccentric hypertrophy and remodeling (25). Despite contractile dysfunction, ejection fraction was preserved due to favorable loading conditions. In the current study, ACE inhibition reduced preload, but not afterload, possibly because afterload was already subnormal. On the other hand, ACE inhibitors alone had no measurable effect on LV mass or volume in this study, consistent with previous reports (26). Consistent with heart failure studies in humans, unloading achieved by ACE inhibition had no effect on LV contractility. This contrasts with single-dose studies where ACE inhibition had beneficial effects on LV function (7) or negative inotropic effects (9).

Our study used indexes of contractility difficult to apply in humans. These indexes, which found little change in contractility with ACE inhibition help, confirm the concept that ACE inhibition may produce its well-proven beneficial effects in ways other than by directly enhancing myocardial contractility.

**Beta-blockers and the MR heart.** While lisinopril failed to improve LV contractile function in our MR model, addition of the beta-blocker atenolol to lisinopril improved the LV contractility substantially. Left ventricular contractility assessed by the end-systolic stiffness constant returned to normal on combination therapy. This beneficial effect of beta-blockade on LV contractile function in this study was consistent with the findings in our previous reports in which three months' treatment of beta-blockers alone substantially ameliorated the LV contractile dysfunction and improved intrinsic contractility by restoration of contractile elements in the cardiac myocytes isolated from MR hearts (17,18). Myofibrillar density was significantly more associated with the increased cross-sectional area of cardiac myocytes in the beta-blocker treatment group than in untreated MR (17). The beneficial findings of beta-blockers were associated with significantly increased LV mass in the previous study (17). A tendency towards increased LV mass occurred after atenolol was added to lisinopril in this study.

Although precise mechanisms of the beneficial effects of beta-blockers remain unclear, we suspect that beta-blockers permitted the increase in the LV mass associated with an increase in the number of contractile elements by allowing load-induced cardiac hypertrophy again to occur under protection from catecholamine toxicity (17). As previously reported (18), the beneficial effects of beta-blockers on cellular and ventricular contractile function were associated with a reduction in heart rate. Bradycardia might lead to: 1) decreased oxygen consumption (27); 2) improved calcium

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**Figure 5.** End-systolic stiffness constant (K-index) was significantly depressed after three months of MR. It rose insignificantly on lisinopril therapy. Only when atenolol was added did contractility return to normal. Abbreviations as in Figures 2 and 3.
handling, which could reduce calcium overload causing myocardial injury; 3) improved myocardial metabolism by restoring high energy phosphate (28); and 4) by moving the ventricle to the point where contractility is maximum on its force-frequency relationship (29). Angiotensin-Converting enzyme inhibition reduced heart rate, but beta-blockade reduced heart rate still further in this study. This difference might be an explanation as to the different effects of the two drugs on LV contractility in MR.

Study limitations. Data obtained in this study are limited to an experimental model of MR, and applicability to humans is uncertain.

This report is lacking a nine-month control group of pharmacologically untreated chronic MR. However, the changes after lisinopril and then after the combination therapy would not be part of the natural history of chronic MR because our untreated chronic MR model was characterized as follows (17,18): 1) RF was unchanged over the course of MR; 2) LV EDV continuously increased after MR creation; 3) LV mass/BW increased by up to 45% at three months and plateaued after six months; and 4) K-index was severely depressed at three months, and this depression persisted for the next three months. These previous findings support that the chronic MR hearts in our model have consistent LV dysfunction with continuous LV dilation after three months of MR.

Conclusions. We conclude that ACE inhibition has beneficial effects on filling pressure in experimental MR. However, only when beta-blockade was added was there a substantial improvement in LV contractility and forward stroke volume.

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REFERENCES


