Inhibition of matrix metalloproteinase activity by ACE inhibitors prevents left ventricular remodeling in a rat model of heart failure

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Brower GL, Levick SP, Janicki JS. Inhibition of matrix metalloproteinase activity by ACE inhibitors prevents left ventricular remodeling in a rat model of heart failure. Am J Physiol Heart Circ Physiol 292: H3057–H3064, 2007. First published February 16, 2007; doi:10.1152/ajpheart.00447.2006.—Angiotensin-converting enzyme (ACE) inhibitors represent the front-line pharmacological treatment of heart failure, which is characterized by left ventricular (LV) dilatation and inappropriate hypertrophy. The mechanism of action of ACE inhibitors is still unclear, but evidence suggests that they may act by influencing matrix metalloproteinase (MMP) activity. This study sought to determine whether ACE inhibitors can directly regulate MMP activity and whether this results in positive structural and functional adaptations to the heart. To this end, MMP-2 activity in LV tissue extracted from rats with an aortocaval (AV) fistula was assessed by in vitro incubation as well as in vivo treatment with captopril, lisinopril, or quinapril. Furthermore, LV size and function were determined in untreated AV fistula rats, AV fistula rats treated with lisinopril (3, 5, and 8 wk), and age-matched sham-operated controls. In vitro incubation with captopril, lisinopril, or quinapril significantly reduced MMP-2 activity, as did in vivo treatment. This occurred without a reduction in the available pool of MMP-2 protein. Long-term in vivo administration of lisinopril also prevented LV dilatation, attenuated myocardial hypertrophy, and prevented changes in myocardial compliance and contractility. The results herein demonstrate that ACE inhibitors prevent MMP-2 activity and, in so doing, represent a mechanism responsible for preventing the negative structural and functional changes that occur in the rat AV fistula model of heart failure.

ventricular function; ventricular dilatation; aortocaval fistula; remodeling; matrix metalloproteinase inhibition

Heart failure is characterized, in large part, by marked ventricular dilatation (35). Numerous studies have suggested that degradation of the interstitial collagen matrix by matrix metalloproteinases (MMPs) is a necessary prerequisite for dilatation to occur (8, 11, 17, 20, 30). MMP-2 is of importance in this adverse myocardial remodeling (2, 7) and is activated by a cascade of events culminating in the cleavage of pro-MMP-2 to the active form (6, 12, 16, 21, 33, 39).

Angiotensin-converting enzyme (ACE) inhibitors have become an integral component of the treatment of heart failure (13, 14) and are recommended as the standard treatment modality preferred over angiotensin II type 1 (AT1) receptor antagonists (13). Modulation of angiotensin II does not appear to be their only mechanism of action, since AT1 receptor antagonism did not alter ventricular remodeling in rats with ascending aortic stenosis (38). Additionally, Spinale et al. (31) found that whereas the ACE inhibitor, fosinopril, reduced left ventricular (LV) dilatation and improved myocyte function in dogs undergoing chronic rapid pacing, AT1 receptor antagonism with irbesartan failed to prevent the development of ventricular dilatation and dysfunction.

Although the mechanism by which ACE inhibitors prevent LV dilatation is not completely understood, it is thought that they can directly inhibit MMP activity, and this may represent one of the primary mechanisms responsible for their beneficial effects in patients with heart failure. Accordingly, we sought to test the hypothesis that ACE inhibitors directly inhibit myocardial MMP activity. In addition, the long-term effects of ACE inhibitor treatment on LV remodeling and function were also investigated in the rat aortocaval (AV) fistula model of heart failure.

Methods

All experiments were performed using 8-wk-old, adult male Sprague-Dawley (Hsd:SD) rats housed under standard environmental conditions and maintained on commercial rat chow and tap water ad libitum. All studies conformed to the principles of the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. Additionally, the protocol was approved by the University’s Animal Care and Use Committee. Anesthesia for surgical procedures and subsequent euthanasia at the experimental end point was affected by pentobarbital sodium (50 mg/kg ip). Postoperative analgesia was achieved by administration of buprenorphine–HCl (0.025 mg/kg sc).

Experimental design. Three sets of experiments were designed to investigate the aforementioned aims. The first experiment was conducted in vitro to determine whether ACE inhibitors are capable of directly inhibiting MMP activity in LV tissue, independent of effects on the renin-angiotensin system. Rats with an AV fistula (n = 10) were euthanized at 24-h postfistula, and LV tissue extract from each heart was subsequently incubated with substrate buffer only (control), captopril (Squibb), lisinopril (Merck), or quinapril (Warner-Lambert) to measure MMP-2 activity by zymography. In this way, LV extract from each of the 10 hearts was analyzed using all four treatment conditions. The time point at 24-h postfistula was chosen since it corresponds to peak MMP-2 activity in the AV fistula model (2). Selection of this time point, therefore, allowed for the determination of maximal ACE inhibitor effectiveness in inhibiting MMP-2 activity.

The second set of experiments aimed to determine whether ACE inhibitor treatment prevented MMP-2 activity in vivo. Before surgery, rats were randomly divided into sham-operated (n = 11), untreated AV fistula (n = 16), AV fistula + captopril (n = 14), AV fistula + lisinopril (n = 11), and AV fistula + quinapril (n = 14). Captopril and lisinopril were administered in the drinking water at doses of 200 and 10 mg·kg⁻¹·day⁻¹, respectively. Quinapril was administered by oral gavage (10 mg·kg⁻¹·day⁻¹). Treatment was initiated 24 h before fistula surgery, with the rats euthanized 24 h after the creation of the AV fistula.

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The third set of experiments, involving in vivo treatment and ex vivo experimentation, sought to determine the long-term structural and functional adaptations that occur in the heart postfistula in rats treated with lisinopril. Before surgery, rats were randomly divided into sham-operated \( (n = 27) \), 3-wk untreated AV fistula \( (n = 16) \), 5-wk untreated AV fistula \( (n = 16) \), 8-wk untreated AV fistula \( (n = 16) \), 3-wk AV fistula + lisinopril \( (n = 9) \), 5-wk AV fistula + lisinopril \( (n = 9) \), and 8-wk AV fistula + lisinopril \( (n = 9) \). Lisinopril was administered in the drinking water at a dose of 10 mg·kg\(^{-1}\)·day\(^{-1}\) beginning 24 h before fistula surgery.

**Surgical preparation.** An AV fistula was created as previously described (3). Briefly, the aorta and caudal vena cava were exposed via a ventral abdominal laparotomy. The vessels were temporarily occluded both proximal and distal to the puncture site, and an 18-gauge needle was inserted into the abdominal aorta and advanced through the medial wall into the vena cava to create an AV fistula. The needle was withdrawn, the ventral aortic puncture was sealed with cyanoacrylate, and the flow was restored. The successful creation of an AV fistula was confirmed by the observation of the pulsatile flow of oxygenated blood into the vena cava. Abdominal musculature and skin were closed with absorbable sutures and autoclips, respectively.

**MMP activity.** The initial steps for measurement of in vitro and in vivo MMP-2 activity by zymography were performed as previously described (4). Briefly, extracts from each LV were loaded into separate lanes of a single gel using an SDS-PAGE matrix containing gelatin (1 mg/ml). For the in vitro MMP studies, the gel was cut into two-lane strips following electrophoresis, and each strip was incubated on a shaker for 24 h in substrate buffer containing either low or high concentrations of captopril, lisinopril, or quinapril, and MMP-2 activity was measured (Table 1 and Fig. 1). When compared with samples incubated in substrate buffer devoid of an ACE inhibitor (control), low-dose captopril significantly decreased MMP-2 activity by 17.4% \((P < 0.01)\). Similarly, MMP-2 activity was significantly reduced by 22.2% and 16% with low-dose lisinopril and quinapril incubation, respectively, \((P < 0.01)\). For all treatment groups, incubation with high doses of each drug produced a significantly greater degree of inhibition of MMP-2 activity than did the corresponding low-dose incubation.

**In vivo MMP activity.** The in vivo effect of ACE inhibitor treatment on LV MMP-2 activity was also assessed in rats at 24-h postfistula. As can be seen from Table 2 and Fig. 2, MMP-2 activity was significantly increased in the LV of AV fistula rats relative to controls. However, ACE inhibitor treatment with captopril, lisinopril, or quinapril prevented this increase in MMP-2 activity, maintaining MMP activity at control levels.

**MMP-2 protein levels.** Western blot assessment of MMP-2 protein levels for 24-h sham-operated, untreated fistula, and fistula + lisinopril rats are depicted in Fig. 3. No differences in the level of MMP-2 protein were found between the groups.

### Results

**In vitro MMP activity.** To investigate the direct effect of ACE inhibitors on MMP activity, in vitro experiments were conducted on LV tissue obtained from rats 24 h after creating an AV fistula. Extracts from each LV were incubated with low and high concentrations of captopril, lisinopril, or quinapril, and MMP-2 activity was measured (Table 1 and Fig. 1). For all treatment groups, incubation with high doses of each drug produced a significantly greater degree of inhibition of MMP-2 activity than did the corresponding low-dose incubation.

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<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Control, %</th>
<th>Low Dose, %</th>
<th>High Dose, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Captopril</td>
<td>10</td>
<td>100 (SD 4.6)</td>
<td>82.6 (SD 7.3)*</td>
<td>48.6 (SD 8.9)**</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>10</td>
<td>100 (SD 5.6)</td>
<td>77.8 (SD 9.0)*</td>
<td>47.6 (SD 8.7)**</td>
</tr>
<tr>
<td>Quinapril</td>
<td>10</td>
<td>100 (SD 2.9)</td>
<td>84.0 (SD 6.8)*</td>
<td>63.9 (SD 6.0)**</td>
</tr>
</tbody>
</table>

Values are means (SD); \( n \); number of rats. Matrix metalloproteinase (MMP) activity of control extract was arbitrarily set to 100% with the mean activities of treatment groups expressed as a percentage of the control. Control dose is 0.0 mM; low dose is 0.25 mM (captopril), 0.035 mM (lisinopril), and 0.1 mM (quinapril); and high dose is 2.5 mM (captopril), 3.5 mM (lisinopril), and 1.5 mM (quinapril). LV, left ventricular. \(* P < 0.01\) compared to control; \(† P < 0.05\) compared to corresponding low-dose group.
**Long-term effect of lisinopril treatment on ventricular morphology and function.**

Average LV, RV, lung, and body weights are presented in Table 3. There was a significant increase in LV and RV weights in the untreated fistula groups relative to age-matched controls at all time points. In contrast, the increase in LV weight in lisinopril-treated rats only reached significance at 8-wk postfistula, whereas RV weight was significantly increased ($P < 0.01$) at both 5- and 8-wk postfistula, relative to control. Lisinopril treatment also prevented the significant increase in average lung weight seen in the 8-wk untreated fistula group relative to control, indicating lisinopril-mediated prevention of pulmonary edema. A similar increase in body weight occurred in the untreated fistula rats at 8-wk postfistula, also indicative of edema. In contrast, body weight was significantly lower in lisinopril rats at all time points relative to controls. Accordingly, heart weight indexed to body weight was not reported because of the concurrent presence of systemic edema in the untreated AV fistula group and significant reductions in body weight in the lisinopril-treated groups.

The in vitro LV volumes over the EDP range of 0–25 mmHg are presented in Table 4, with the average LV EDP/end diastolic volume (EDV) curves shown in Fig. 4. There was a significant, time-dependent rightward shift in the LV EDP/EDV relationship for untreated AV fistula rats relative to controls. This shift in the pressure-volume (P-V) relationship was due to both structural ventricular dilatation and an overall increase in ventricular compliance. An assessment of ventricular dilatation was made by comparing the values for $V_0$ (LV volume at an EDP of 0 mmHg), whereas an indication of ventricular compliance was ascertained from the volume required to increase EDP from 0 to 25 mmHg ($\Delta V_{0-25}$; as is illustrated in Fig. 5 by the plotting of EDV normalized for $V_0$ over the EDP range of 0–25 mmHg). Lisinopril prevented the changes in ventricular size and compliance at all time points with the exception of a modest nonparallel leftward shift of the EDP/EDV relationship in the 3-wk lisinopril-treated group. Measures of intrinsic systolic contractility are given in Table 5. The relationship between peak isovolumetric pressure and EDV was highly linear, as evidenced by the range of correlation coefficients. The slope for the $P_{\text{max}}$-V relationship was significantly decreased in the 8-wk untreated fistula, indicating decreased intrinsic myocardial contractility. This depression in contractility was prevented by lisinopril treatment at all time points. Given the effects of lisinopril treatment on hypertrophy and P-V relationships, the LV mass-to-EDV ratio (Fig. 6) was calculated as a measure of the adequacy of the ventricular hypertrophic response. This demonstrates that the average LV mass-to-EDV ratio was decreased in the 8-wk AV fistula group, indicative of an inappropriate hypertrophic response. However, the 8-wk AV fistula group treated with lisinopril developed an increased LV mass-to-EDV ratio relative to control, indicating successful compensation.

**DISCUSSION**

Cardiac remodeling secondary to chronic volume overload is characterized by progressive ventricular dilatation, inappropriate hypertrophy, and ultimately heart failure (3, 4). Although ACE inhibitors modulate myocardial remodeling (18, 20, 24, 28, 31), the underlying mechanism by which ACE inhibitors mediate this cardioprotection is still poorly understood. However, several lines of evidence suggest that their effects on ACE do not mediate the attenuation of myocardial remodeling in volume overload (23, 31, 32). An alternative mechanism explaining the efficacy of ACE inhibitors may be the regulation of MMPs.

It is often overlooked that ACE is a zinc metalloproteinase (34). Thus compounds that inhibit ACE may also inhibit other metalloproteinases such as MMPs, which have been implicated in ventricular dilatation (8, 9, 11, 17, 20, 26, 28, 30). The AV fistula model of congestive heart failure has significant increases in MMP-2 activity initially, which return to normal by 2-wk postfistula and remains so until the heart decompensates. In contrast, consistent elevations in MMP-9 activity have not been observed. Nevertheless, there is significant collagen degradation by day 3, as measured by a reduction in collagen volume fraction, which rebounded to normal levels by 2-wk postfistula (2). Therefore, it appears that the underlying mechanisms responsible for LV dilatation are established early and occur in advance of the actual development of dilatation. With this in mind, we studied the in vitro effect of captopril,
lisinopril, and quinapril on MMP-2 activity in LV tissue obtained from AV fistula rats at 24-h postfistula. Incubation of these LV extracts with each ACE inhibitor resulted in significant reductions in the activity of MMP-2. Since this was an in vitro incubation with captopril, lisinopril, or quinapril, these findings confirm a direct effect of ACE inhibitors on MMP-2 activity independent of the renin-angiotensin system. This was also a concentration-dependent class effect, since higher concentrations of each drug elicited a proportionally greater reduction in MMP-2 activity. Furthermore, quinapril, a pro-drug, was equally as effective at inhibiting MMP activity as the active compounds captopril and lisinopril, providing further evidence that inhibition of MMP activity by ACE inhibitors is a direct effect.

Complimenting these in vitro observations, lisinopril treatment was highly effective at inhibiting MMP activation in vivo. This ACE inhibitor-mediated prevention of MMP activation in the initial stage of volume overload results in maintenance of a chronically compensated state. This is reflected in Figs. 4 and 5, where marked LV dilatation and increased compliance are present in the untreated rats at 8-wk postfistula. The unstressed LV volume ($V_0$), which provides an indication of the extent of LV dilatation independent of increases in compliance, was significantly increased at all time points in the untreated AV fistula rat. In contrast, lisinopril effectively prevented the significant increases in $V_0$ at all time points, indicative of its ability to prevent MMP-mediated structural dilatation. These findings are similar to those of Ruzicka and Leenen (27) who demonstrated that quinapril (200 mg/l in drinking water) attenuated LV dilatation and hypertrophy in the AV fistula rat. Although there was a trend for ventricular chamber stiffness to be increased in the 3-wk lisinopril-treated group in the current study, this was most likely a transient overcorrection, subsequently compensated for by non-MMP-mediated myocardial remodeling represented by a slight increase in $V_0$. However, chronic treatment with lisinopril clearly prevented the adverse remodeling normally mediated by MMPs, maintaining a normal P-V relationship, while preserving normal LV compliance and diastolic function. In addition to preventing adverse chamber remodeling, lisinopril prevented the deterioration of intrinsic contractility observed in untreated rats at 8-wk postfistula. It is likely that systolic function is maintained by lisinopril treatment simply as a by-product of preventing the initial remodeling of the LV.

Table 3. Morphometric parameters

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Body Weight, g</th>
<th>LV Weight, mg</th>
<th>RV Weight, mg</th>
<th>Lung Weight, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>421 (SD 21)</td>
<td>943 (SD 90)</td>
<td>272 (SD 35)</td>
<td>1,778 (SD 174)</td>
</tr>
<tr>
<td>AV fistula</td>
<td>16</td>
<td>429 (SD 78)</td>
<td>1,254 (SD 190)*</td>
<td>373 (SD 68)*</td>
<td>2,375 (SD 563)</td>
</tr>
<tr>
<td>Fistula + lisinopril</td>
<td>9</td>
<td>339 (SD 16)†</td>
<td>999 (SD 131)</td>
<td>304 (SD 44)</td>
<td>1,789 (SD 262)</td>
</tr>
<tr>
<td>5 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>454 (SD 22)</td>
<td>928 (SD 169)</td>
<td>231 (SD 27)</td>
<td>1,630 (SD 205)</td>
</tr>
<tr>
<td>AV fistula</td>
<td>16</td>
<td>450 (SD 52)</td>
<td>1,359 (SD 131)*</td>
<td>440 (SD 71)†</td>
<td>2,368 (SD 552)</td>
</tr>
<tr>
<td>Fistula + lisinopril</td>
<td>9</td>
<td>368 (SD 24)†</td>
<td>992 (SD 114)</td>
<td>325 (SD 39)†</td>
<td>1,902 (SD 216)</td>
</tr>
<tr>
<td>8 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>484 (SD 33)</td>
<td>935 (SD 98)</td>
<td>241 (SD 15)</td>
<td>1,948 (SD 28)</td>
</tr>
<tr>
<td>AV fistula</td>
<td>16</td>
<td>547 (SD 107)*</td>
<td>1,667 (SD 266)†</td>
<td>537 (SD 95)†</td>
<td>2,593 (SD 620)*</td>
</tr>
<tr>
<td>Fistula + lisinopril</td>
<td>9</td>
<td>391 (SD 37)†</td>
<td>1,292 (SD 325)*</td>
<td>420 (SD 122)†</td>
<td>1,804 (SD 204)</td>
</tr>
</tbody>
</table>

Values are means (SD); n, number of rats. RV, right ventricular. *P < 0.05 compared with age-matched control; †P < 0.01 compared with age-matched control.
Despite the efficacious prevention of LV dilatation, lisinopril did not eliminate hypertrophy at 8-wk postfistula, although the marked attenuation of the hypertrophic response was comparable with that of a 3- to 5-wk untreated AV fistula rat. Furthermore, the extent of hypertrophy seen with ACE inhibition was similar to that observed in previous studies with mast cell stabilization (5) and MMP inhibition (8). Nevertheless, the ability of this lesser hypertrophy to normalize wall stress can be appreciated by examining the LV mass-to-volume ratio (Fig. 6). The LV mass-to-volume ratio is depressed relative to control animals in the untreated AV fistula group at 8-wk (Fig. 6). The LV mass-to-volume ratio is depressed relative to control animals in the untreated AV fistula group at 8-wk postfistula. This reflects an insufficient hypertrophic response relative to the extent of ventricular dilatation. Conversely, in rats treated with lisinopril until 8-wk postfistula, the LV mass-to-volume ratio is actually higher than in controls, indicating a reduction in myocardial stress that enabled the heart to achieve a compensated state. This adaptive response to lisinopril treatment resembles that achieved with both the mast cell membrane-stabilizing drug, nedocromil (5), and the MMP inhibitor, PD-166793 (8). Stabilization of mast cells prevented MMP activation (2) and attenuated myocardial remodeling and function (5) in a manner consistent with the findings of the current study. Together, the remarkable similarity of the findings relative to the extent of ventricular dilatation. Conversely, in rats treated with lisinopril until 8-wk postfistula, the LV mass-to-volume ratio is actually higher than in controls, indicating a reduction in myocardial stress that enabled the heart to achieve a compensated state. This adaptive response to lisinopril treatment resembles that achieved with both the mast cell membrane-stabilizing drug, nedocromil (5), and the MMP inhibitor, PD-166793 (8). Stabilization of mast cells prevented MMP activation (2) and attenuated myocardial remodeling and function (5) in a manner consistent with the findings of the current study. Together, the remarkable similarity of the findings

Table 4. Isolated heart size and diastolic function

<table>
<thead>
<tr>
<th></th>
<th>(V_0), (\mu L)</th>
<th>(\Delta V_{0-25}), (\mu L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>215 (SD 52)</td>
<td>185 (SD 36)</td>
</tr>
<tr>
<td>3-wk fistula</td>
<td>262 (SD 59)*</td>
<td>283 (SD 142)*</td>
</tr>
<tr>
<td>5-wk fistula</td>
<td>323 (SD 58)†</td>
<td>286 (SD 142)*</td>
</tr>
<tr>
<td>8-wk fistula</td>
<td>370 (SD 105)†</td>
<td>476 (SD 264)†</td>
</tr>
<tr>
<td>3-wk fistula + lisinopril</td>
<td>224 (SD 41)</td>
<td>113 (SD 65)</td>
</tr>
<tr>
<td>5-wk fistula + lisinopril</td>
<td>248 (SD 71)</td>
<td>177 (SD 56)</td>
</tr>
<tr>
<td>8-wk fistula + lisinopril</td>
<td>281 (SD 62)</td>
<td>234 (SD 115)</td>
</tr>
</tbody>
</table>

Values are means (SD); \(n\), number of rats. \(V_0\), volume at end-diastolic pressure (EDP) of 0 mmHg; \(\Delta V_{0-25}\), change in LV volume between EDP of 0 and 25 mmHg. For simplicity, age-matched control groups were combined since no statistical differences existed between the individual groups. *\(P < 0.05\) compared with control; †\(P < 0.01\) compared with control.

Table 5. Isolated heart: intrinsic systolic contractility

<table>
<thead>
<tr>
<th></th>
<th>Slope (P_{\text{max}}-V), mmHg/(\mu L)</th>
<th>(r_{P_{\text{max}}-V}) Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.465 (SD 0.29)</td>
<td>0.95–1.00</td>
</tr>
<tr>
<td>3-wk fistula</td>
<td>0.268 (SD 0.120)</td>
<td>0.96–1.00</td>
</tr>
<tr>
<td>5-wk fistula</td>
<td>0.376 (SD 0.198)</td>
<td>0.98–0.99</td>
</tr>
<tr>
<td>8-wk fistula</td>
<td>0.207 (SD 0.136)*</td>
<td>0.76–1.00</td>
</tr>
<tr>
<td>3-wk fistula + lisinopril</td>
<td>0.657 (SD 0.35)</td>
<td>0.98–0.99</td>
</tr>
<tr>
<td>5-wk fistula + lisinopril</td>
<td>0.438 (SD 0.22)</td>
<td>0.95–1.00</td>
</tr>
<tr>
<td>8-wk fistula + lisinopril</td>
<td>0.365 (SD 0.17)</td>
<td>0.88–1.00</td>
</tr>
</tbody>
</table>

Values are means (SD); \(n\), number of rats. \(r_{P_{\text{max}}-V}\) range, range of regression coefficient of the linear maximal pressure-volume (\(P_{\text{max}}-V\)) relations. For simplicity, age-matched control groups were combined as no statistical differences existed between the individual groups. *\(P < 0.05\) compared with control.
across these studies strongly suggests MMP inhibition, whether directly or indirectly, as a primary mechanism by which lisinopril prevents myocardial collagen breakdown, thereby preventing ventricular dilatation.

Several studies focused on cancer metastasis have also demonstrated the direct inhibition of MMPs by ACE inhibitors (22, 25, 37, 40). However, these studies evaluated just captopril and, with the exception of Williams et al. (40), reported MMP-2 inhibition at significantly higher concentrations than used herein. One criticism applicable to all of these studies is that the concentrations used in vitro do not reflect dosages achievable in vivo. However, a study by Sorbi et al. (29) clearly demonstrated that the inhibition of MMP could be achieved using nanomolar concentrations of captopril in a collagen degradation assay, yet to demonstrate this effect by zymography, 20 to 40 mM concentrations of captopril were required. These findings indicate that MMP inhibition is achievable by using clinically relevant concentrations.

Observations similar to ours were reported by Reinhardt et al. (26); however, their findings were obtained from dilated explanted hearts, presumably from patients in end-stage heart failure at the time of transplantation. They also examined the in vitro inhibitory capacity of different ACE inhibitors (captopril, lisinopril, and ramipril) and emphasized the differences in MMP inhibitory capacity between compounds. The studies by Reinhardt et al. (26) and Sorbi et al. (29) both concluded that significantly greater concentrations of lisinopril were required to achieve inhibition. Interestingly, we found that at lower concentrations lisinopril was more potent than the other ACE inhibitors evaluated. Furthermore, the efficacy of lisinopril in preventing MMP activation in vivo demonstrates the feasibility of this being the mechanism by which ACE inhibitors prevent structural remodeling.

A similar conclusion was reached by Sakata et al. (28) in evaluating the effect of enalapril (5 mg·kg⁻¹·day⁻¹) on MMPs in Dahl salt-sensitive rats during heart failure. They conclude that enalapril attenuated MMP-2 and MMP-9 activity, thereby preventing LV remodeling and systolic dysfunction. However, caution needs to be exercised in the interpretation of their results since the zymograms presented corresponded to the latent form of MMP-2 and thus do not reflect a direct effect of enalapril treatment on MMP activity. Furthermore, another consideration is that ACE inhibition can modulate MMP synthesis. Previous studies by Sakata et al. (28) and Li et al. (19) found that ACE inhibitors normalized the increase in MMP transcription seen in heart failure. However, we saw no difference in MMP-2 protein levels attributable to acute ACE inhibition, although stabilization of latent MMPs might be expected to decrease the requirement for synthesis.

Thus the mechanism by which ACE inhibitors inhibit MMP activity appears to be related to the catalytic Zn²⁺ ion that is essential for the proteolytic activity of MMPs (1, 29, 34, 41). A variety of metal-binding groups has been used in synthetic inhibitors of MMPs, including hydroxamate, thiol, carboxyl, and sulfhydryl groups, all of which bind to the catalytic Zn²⁺ ion, thereby inactivating the enzyme (10, 15, 36). The ACE inhibitors used in the current study contain either sulfhydryl (captopril) or carboxyl (lisinopril and quinapril) groups capable of binding to the catalytic Zn²⁺ ion in MMPs, rendering them inactive. Although not addressed in this study, it should be emphasized that, in addition to the direct effect on MMP-2 activity reported herein, there are several upstream pathways leading to MMP-2 activation that also may be affected by ACE inhibition.

The current study together with our previous studies (5, 8) suggests that increased MMP activity produces collagen matrix degradation, subsequent dilatation of the LV, and LV dysfunction. ACE inhibitors appear to act on MMPs to inhibit their activation, thereby preventing the subsequent detrimental remodeling. Consistent with this, McElmurray et al. (20) has shown in pigs undergoing rapid atrial pacing that ACE inhibitor treatment with fosinopril (5 mg·kg⁻¹·day⁻¹) had similar effects as the MMP inhibitor PD-166793 (2 mg·kg⁻¹·day⁻¹), including prevention of increased MMP activity, reduced LV dilatation, improved fractional shortening, and decreased peak wall stress. However, in that study, rapid pacing without treatment failed to induce changes in LV compliance and, as such, does not represent a decompensated myocardium. Our study extends these findings to the decompensated heart. This study also raises the possibility that ACE inhibitors may also exert beneficial effects through other mechanisms, such as inhibiting MMP-2 activity or inhibiting the process that activates MMP-2.

In summary, although there is substantial evidence that increased MMP activity is responsible for degradation of the extracellular matrix and subsequent ventricular dilatation, this study establishes that ACE inhibitors are capable of directly inhibiting MMP-2 activation. Furthermore, treatment with an ACE inhibitor can significantly attenuate LV dilatation induced by a sustained volume overload, as well as maintain diastolic and systolic function, consistent with clinical observations. Since these findings suggest that inhibition of MMP activity by ACE inhibitors may be a mechanism responsible for the attenuation of myocardial remodeling in heart failure, it illustrates the need to revisit MMP inhibition as a therapeutic modality in preventing heart failure.
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GRANTS

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