Angiotensin-Converting Enzyme Inhibitors Improve Coronary Flow Reserve in Dilated Cardiomyopathy by a Bradykinin-Mediated, Nitric Oxide-Dependent Mechanism

Lazaros A. Nikolaidis, MD; Aaron Doverspike, MS; Rhonda Huerbin, LVT; Teresa Hentosz, BS; Richard P. Shannon, MD

Background—ACE inhibitors have been used extensively in heart failure, where they induce systemic vasodilatation. ACE inhibitors have also been shown to reduce ischemic events after myocardial infarction, although their mechanisms of action on the coronary circulation are less well understood. The purpose of the present study was to determine the effects and the mechanism of action of the ACE inhibitor enalaprilat and the AT1 antagonist losartan on regional myocardial perfusion and coronary flow and vasodilator reserve in conscious dogs with pacing-induced dilated cardiomyopathy (DCM).

Methods and Results—Twenty-seven conscious, chronically instrumented dogs were studied during advanced stages of dilated cardiomyopathy, which was induced by rapid pacing. Enalaprilat (1.25 mg IV) improved transmural distribution (endocardial/epicardial ratio) at rest (baseline, 0.91 ± 0.11; enalaprilat, 1.02 ± 0.07 mL/min per g; P<0.05) and during atrial pacing (baseline, 0.82 ± 0.11; enalaprilat, 0.98 ± 0.07; P<0.05). Enalaprilat also restored subendocardial coronary flow reserve (CFR) (baseline CFR, 1.89 ± 0.11; enalaprilat CFR, 2.74 ± 0.33; P<0.05) in DCM. These effects were abolished by pretreatment with the NO synthase inhibitor nitro-L-arginine. The effects were recapitulated by the bradykinin receptor agonist cereport but not by the AT1 antagonist losartan.

Conclusions—The ACE inhibitor enalaprilat improves transmural myocardial perfusion at rest and after chronotropic stress and restores impaired subendocardial coronary flow and vasodilator reserve in DCM. The effects of enalaprilat were bradykinin mediated and NO dependent and were not recapitulated by losartan. These data suggest beneficial effects of ACE inhibitors on the coronary circulation in DCM that are not shared by AT1 receptor antagonists. (Circulation. 2002;105:2785-2790.)

Key Words: angiotensin ■ bradykinin ■ nitric oxide ■ cardiomyopathy ■ microcirculation

Angiotensin-converting enzyme (ACE) inhibitors (ACEIs) have been used extensively in the treatment of congestive heart failure (CHF), with resultant improvements in morbidity and mortality.1–4 Surprisingly, recent evidence from clinical trials has suggested that either the substitution of or the addition of angiotensin receptor (AT1) blockers (ARBs) offers little clinical benefit over ACEIs alone.5–7 These data suggest that the effects of ACEIs may not be mediated exclusively through angiotensin II inhibition. In addition to effects on angiotensin II generation, ACEIs attenuate the breakdown of bradykinin, which potentiates their vasodilating properties.8–10 The latter mechanism is thought to contribute importantly to the sustained vasodilating effects of ACEIs after the conversion of angiotensin I to angiotensin II via non-ACE–dependent pathways.11

Although the effects of ACEIs and ARBs on systemic hemodynamics have been studied extensively,12,13 less is known about their effects on the coronary circulation, particularly in heart failure. ACEIs have been shown to reduce ischemic events in CHF,14–16 but the mechanism is not well understood. There is evidence from human studies17–19 and experimental animal models20–22 that altered myocardial perfusion contributes to the progression of cardiomyopathy, even in the absence of epicardial coronary artery disease. Whether and to what extent ACEIs and ARBs restore impaired coronary blood flow reserve (CFR) in advanced CHF is unknown. Whether there is a differential effect of these 2 classes of angiotensin II inhibitors on the coronary circulation is also controversial. It is conceivable that ACEIs may have a preferential salutary effect on the coronary circulation, given their ability to enhance the effects of endogenous bradykinin, a known potent coronary vasodilator.

The purpose of this study was to determine the effects of ACEIs and ARBs on baseline myocardial flow and regional myocardial flow reserve in conscious dogs with pacing-induced DCM. A second goal was to determine whether the
effects of ACEIs on CFR were mediated by blockade of angiotensin II production or bradykinin. A third goal was to determine whether the benefits of these agents on CFR were mediated by direct effects on the coronary circulation or by systemic hemodynamic effects resulting in reduced extravascular compressive forces.

**Methods**

Twenty-four mongrel dogs (Martin Creek Animals, Williford, Ark) were sedated with 10 mg/kg xylazine and anesthetized with halothane (1 to 1.5 vol%). Through a left thoracotomy, catheters were placed in the descending thoracic aorta, left atrium, and coronary sinus. A solid-state pressure transducer (Konigsberg Instruments) was implanted in the left ventricle (LV) through an apical approach that facilitated high-fidelity LV pressure recordings. Transonic flow probes were placed on the proximal left circumflex coronary artery (LCX) and on the ascending aorta for continuous measurement of coronary and aortic blood flow. A bipolar pacing lead was sutured to the left atrial appendage to control heart rate under defined circumstances. All animals received analgesics for 72 hours and cephalexin (1 g IV) for 7 days postoperatively. The dogs were allowed to recover from the surgical procedure for 2 weeks. Animals were maintained in accordance with the guidelines of the Animal Committee of Allegheny General Hospital and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (DHHS publication No. NIH 85-23, revised 1996). Hemodynamic data were recorded as previously described.20

Regional myocardial blood flow was measured using neutron-activated microspheres (BioPal) administered as described previously from our laboratory.20 Eight microspheres were available, including samarium, lanthanum, antimony, ytterbium, europium, rhenium, iridium, and gold. After intravenous administration of near-maximal doses of adenosine (4.7 μmol/kg per min), CFR and vasodilator reserve were measured. The mean arterial pressure minus the left ventricular end-diastolic pressure (MAP–LVEDP) was used as a measure of coronary perfusion pressure and the respective blood flows. The minimum CVR after adenosine administration (CVR_{min}) was used as a measure of coronary vasodilator reserve.

CFR and CVR_{min} were assessed in 5 normal dogs and in 15 dogs with pacing-induced dilated cardiomyopathy (DCM) at an average of 26 days of pacing and reassessed in 9 dogs with advanced DCM after intravenous administration of enalaprilat (1.25 mg). Measurements were made when systemic hemodynamics had reached steady state (20 to 30 minutes after administration). Transmural myocardial flow was assessed during atrial pacing at 180 minutes in the presence and absence of enalaprilat to determine the impact on subendocardial flow during chronotropic stress in 6 of the 9 dogs. In 5 dogs with advanced DCM, the effects of enalaprilat on CFR and CVR_{min} were assessed after pretreatment with the NO synthase inhibitor nitro-L-arginine (NL-A, 30 mg/kg).

Transmural myocardial perfusion, CFR, and CVR_{min} were assessed in 5 dogs with advanced DCM after intravenous administration of the AT1 receptor antagonist losartan (2.5 mg/kg, then 0.1 mg/kg per min). The dose was chosen to result in a similar decrease in MAP to that observed with enalaprilat after dose-response experiments (Figure 1). Measurements were made at 15 to 20 minutes after initiation of the infusion.

The same parameters were assessed in 8 additional dogs with advanced DCM after intravenous infusion of the bradykinin (B_{2}) agonist cereport (0.01 μg/kg per min). Measurements were made at 15 to 20 minutes after initiation of infusion when systemic hemodynamics had reached steady state. The dose was chosen to result in similar decrease in MAP to that observed with enalaprilat after dose-response experiments (Figure 2). Measurements were made at 15 to 20 minutes after initiation of the infusion.

To assess the direct effects of vasodilators on regional myocardial perfusion, 3 additional dogs were instrumented with Tygon catheters placed in the proximal LCX. The effects on regional myocardial perfusion were assessed on separate days with the use of microsphere techniques.

**Statistics**

Differences between baseline transmural myocardial perfusion and response to vasodilators were compared using paired Student’s t test. CFR responses were compared by means of repeated-measures ANOVA.

**Results**

Table 1 illustrates the hemodynamic alterations associated with the development of DCM after 4 weeks of rapid RV pacing. Figure 2 illustrates the selective impairment in
subendocardial CFR in dogs with DCM compared with controls.

Hemodynamic effects of enalaprilat in 9 dogs with advanced DCM included significant reductions in systolic LVP (−6 ± 1 mm Hg), LVEDP (−5 ± 1 mm Hg), and MAP (−11 ± 3 mm Hg) and significant increases in cardiac output (+0.9 ± 0.3 L/min) compared with the same 9 dogs with DCM before enalaprilat. The average transmural blood flow was not significantly different after enalaprilat (Table 2). However, subendocardial blood flow and the endocardial/epicardial (endo/epi) ratio were increased and CVRmin was reduced. To determine whether the effects of enalaprilat were dependent on systemic vascular effects, we examined the effects of intracoronary enalaprilat (0.015 mg) on regional myocardial perfusion in 3 dogs with DCM instrumented with chronic indwelling coronary catheters. Intracoronary enalaprilat increased both subendocardial coronary blood flow (0.89 ± 0.11 to 1.34 ± 0.15 mL/min per g) and the endo/epi ratio (0.95 ± 0.04 to 1.13 ± 0.07) to levels observed in normal dogs.

Enalaprilat was associated with significant \((P<0.05)\) improvement in subendocardial CFR compared with the limited CFR noted in DCM (Figure 3A). There was also significant improvement in subendocardial \(\text{CVR}_{\text{min}}\) (DCM, 24 ± 3 mm Hg · mL\(^{-1}\) · min\(^{-1}\) per g; DCM + enalaprilat, 15 ± 3 mm Hg · mL\(^{-1}\) · min\(^{-1}\) per g; \(P<0.05\)). Notably, these improvements were not attributable to significant alterations in extravascular compressive forces, because the decline in LVEDP was modest (31 ± 2 to 26 ± 4 mm Hg). Subsequently, we examined the effects of enalaprilat in the presence and absence of NO synthase (NOS) inhibition with N-LA in 5 dogs with DCM. Pretreatment with N-LA attenuated the improvement in subendocardial CFR and \(\text{CVR}_{\text{min}}\) observed after enalaprilat (Figure 3B).

We examined the transmural distribution of blood flow during rapid atrial pacing (180 minutes\(^{-1}\)) before and after administration of enalaprilat in 6 dogs to determine whether the improvement in CFR and \(\text{CVR}_{\text{min}}\) was physiologically relevant. Dogs with DCM demonstrated limited subendocardial blood flow response to pacing (0.94 ± 0.10 to 1.36 ± 0.13 mL/min per g; \(P<0.11\)) compared with the same dogs subjected to a similar pacing stress after enalaprilat administration (1.79 ± 0.11 mL/min per g, \(P<0.05\)). The endo/epi blood flow ratio decreased with pacing in DCM from 0.98 ± 0.05 to 0.88 ± 0.04 but increased significantly \((P<0.05)\) when pacing was conducted in the presence of enalaprilat (1.10 ± 0.05).

Administration of the AT\(_1\) antagonist losartan in 5 dogs with advanced DCM induced modest but significant reductions in LVP (−5 ± 1 mm Hg), LVEDP (−6 ± 1 mm Hg), and MAP (−11 ± 2 mm Hg) and significantly increased CO (+0.8 ± 0.2 L/min) compared with the same 5 dogs with DCM before losartan. The hemodynamic effects of losartan were comparable to those of enalaprilat. The average transmural blood flow was not significantly different after losartan (Table 2). However, in contrast to the response to enalaprilat, subendocardial blood flow, the endo/epi ratio, and the coronary vasodilator response were unchanged after losartan. Losartan was associated with no improvement in subendocardial CFR compared with the limited reserve noted in DCM (Figure 4). There was no improvement in subendocardial \(\text{CVR}_{\text{min}}\) (DCM, 24 ± 4 mm Hg · mL\(^{-1}\) · min\(^{-1}\) per g; DCM + losartan, 26 ± 4 mm Hg · mL\(^{-1}\) · min\(^{-1}\) per g). Intracoronary losartan had limited effects on subendocardial flow (0.78 ± 0.15 to 0.91 ± 0.21 mL/min per g). The failure of losartan to improve coronary flow and vasodilator reserve was not attributable to significant differences in the effects on extravascular compressive forces because the decline in

| TABLE 1. Systemic and Coronary Hemodynamics in Dilated Cardiomyopathy |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                                | Control        | DCM            | DCM + Enalaprilat | DCM + Losartan | DCM + Cereport  |
| LVP, mm Hg                      | 118 ± 4        | 104 ± 3²       | 101 ± 4         | 95 ± 3²        | 105 ± 4        | 108 ± 6‡       | 108 ± 4        | 101 ± 3‡  |
| LVEDP, mm Hg                    | 9 ± 1          | 31 ± 4²        | 31 ± 1          | 26 ± 4²        | 30 ± 3         | 24 ± 4‡        | 33 ± 3          | 27 ± 4‡   |
| LV dP/dt, mm Hg/s               | 2645 ± 114     | 1324 ± 213*    | 1376 ± 114      | 1576 ± 233     | 1340 ± 133     | 1489 ± 193     | 1268 ± 63       | 1608 ± 149‡ |
| Heart rate, min⁻¹               | 88 ± 6         | 117 ± 7²       | 116 ± 6         | 110 ± 7        | 89 ± 1         | 121 ± 7        | 171 ± 6         | 128 ± 7‡   |
| MAP, mm Hg                      | 95 ± 5         | 90 ± 55        | 89 ± 5          | 78 ± 5‡        | 90 ± 5         | 79 ± 5‡        | 93 ± 5          | 83 ± 5‡   |
| CO, L/min                       | 2.63 ± 0.11    | 2.09 ± 0.51‡   | 2.09 ± 0.11     | 2.63 ± 0.41†   | 2.41 ± 0.51    | 3.13 ± 0.67‡   | 2.23 ± 0.31      | 3.43 ± 0.51† |
| Systemic vascular resistance, dyn·s·cm⁻³ | 2889 ± 145 | 3445 ± 211*    | 3407 ± 213      | 2205 ± 323†    | 2988 ± 169     | 2019 ± 323‡    | 3336 ± 271      | 1988 ± 402‡ |

Coronary blood flow, mL/min 31 ± 3 29 ± 3‡ 29 ± 3 32 ± 3 29 ± 3 34 ± 5 33 ± 3 38 ± 4

*\(P<0.01\) vs control. †\(P<0.01\) vs DCM. ‡\(P<0.05\) vs control.
LVEDP was of a magnitude similar to that observed with enalaprilat (30±3 to 24±3 mm Hg).

Similarly, the bradykinin (B₂) receptor agonist cerveport in 8 dogs with DCM induced significant (P<0.05) reductions in LVP (−7±2 mm Hg), LVEDP (−6±1 mm Hg), and MAP (−10±2 mm Hg) and significant (P<0.05) increases in CO (+1.2±0.4 L/min) compared with the same 8 dogs with DCM before cerveport. The systemic hemodynamic effects of cerveport were comparable to those of enalaprilat and losartan. The average transmural blood flow was not significantly different after cerveport, comparable with the response to enalaprilat. However, subendocardial blood flow, the endo/epi ratio, and the coronary vasodilator response were increased after cerveport in the same dogs with advanced DCM. The improvement in CFR was comparable to intracoronary enalaprilat. Systemic cerveport administration in DCM was associated with significant improvement in subendocardial CFR (Figure 5A) and CVR_{min} (DCM, 23±3 mm Hg · mL⁻¹ · min⁻¹ per g; DCM + cerveport, 14±4 mm Hg · mL⁻¹ · min⁻¹ per g; P<0.05). Notably, the improvements in CFR and CVR_{min} were not attributable to significant alterations in extravascular compressive forces, because the decline in LVEDP, although significant, was modest (33±3 to 27±3 mm Hg). NOS inhibition attenuated the improvement in subendocardial CFR and CVR_{min} observed after cerveport (Figure 5B).

Discussion

In the present study, we observed that short-term parenteral administration of the ACEI enalaprilat had no significant effect on average transmural myocardial perfusion, yet it improved resting subendocardial perfusion and CFR in conscious dogs with DCM. The improvement in CFR was associated with significantly increased CBF response to chronotropic stress and was independent of differences in LV extravascular compressive forces but was attenuated by N-LA. This effect was mimicked by the B₂ agonist cerveport but not by the AT₁ antagonist losartan. Taken together, these findings suggest that ACEIs but not ARBs have an acute salutary effect on regional myocardial perfusion in DCM that is mediated primarily through bradykinin.

Studies in humans have demonstrated impaired CFR in idiopathic DCM, which contributes to limited myocardial

TABLE 2. Effects of Vasodilators on Resting Regional Myocardial Perfusion in Conscious Dogs With Pacing-Induced Heart Failure

<table>
<thead>
<tr>
<th></th>
<th>DCM</th>
<th>DCM + Enalapril</th>
<th>DCM + Cerveport</th>
<th>DCM + Losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regional CBF, mL/min per g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV endo</td>
<td>1.05±0.07</td>
<td>1.24±0.12*</td>
<td>1.26±0.07*</td>
<td>0.97±0.05</td>
</tr>
<tr>
<td>LV epi</td>
<td>1.10±0.08</td>
<td>1.06±0.13</td>
<td>1.12±0.06</td>
<td>0.93±0.09</td>
</tr>
<tr>
<td>LV transmural</td>
<td>1.09±0.08</td>
<td>1.13±0.12</td>
<td>1.12±0.07</td>
<td>0.94±0.07</td>
</tr>
<tr>
<td>LV endo/epi ratio</td>
<td>0.98±0.07</td>
<td>1.20±0.07*</td>
<td>1.13±0.02*</td>
<td>1.04±0.05</td>
</tr>
<tr>
<td>Regional CVR_{min}, mm Hg · mL⁻¹ · min⁻¹ per g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV endo</td>
<td>55±6</td>
<td>42±4*</td>
<td>44±5*</td>
<td>57±4</td>
</tr>
<tr>
<td>LV epi</td>
<td>53±6</td>
<td>49±9</td>
<td>50±7</td>
<td>59±4</td>
</tr>
<tr>
<td>LV transmural</td>
<td>55±6</td>
<td>46±10</td>
<td>50±5</td>
<td>59±6</td>
</tr>
</tbody>
</table>

*P<0.05 vs DCM.
perfusion in the face of chronotropic stress. Prior studies from our laboratory,20,23 and other studies,21,22 have revealed decreased resting transmural myocardial perfusion and significant impairment in CFR and vasodilator reserve that was selective to the subendocardium in conscious animals with pacing-induced DCM. These alterations occur in the absence of coronary atherosclerosis and predispose to regional and global left ventricular dysfunction in the face of physiological stress such as pacing20 or exercise.23 A distinguishing feature of our study has been to reveal selective improvement in subendocardial CFR after enalaprilat and cerexpur but not losartan. Selective impairment in regional myocardial perfusion may be masked when only average transmural perfusion is assessed. It is important to note that diminished subendocardial CFR contributes disproportionately to regional contractile dysfunction as the subendocardium contributes preferentially to regional wall thickening.24

Few studies have examined the effects of ACEIs on the coronary circulation in DCM, with conflicting results. Using intracoronary enalaprilat, Foult et al25 demonstrated increases in resting CBF and enhanced responses to pacing stress in patients with DCM. In contrast, Kiowski et al26 and Mohri et al27 observed no difference in resting CBF. In our present study, intravenous enalaprilat increased subendocardial blood flow preferentially without a significant increase in overall transmural flow. We noted that the observed improvement in CFR was associated with an improved regional CBF response to pacing stress. We also observed that the salutary effects of ACEIs were attenuated by L-NA. These findings are in keeping with the observations of Mohri et al,27 who observed that the beneficial effects of enalaprilat in the face of a pacing stress were abolished by pretreatment with LMNNA, and Zhang et al,28 who demonstrated that NO production by coronary microvessels harvested from dogs with pacing-induced DCM was enhanced in the presence of enalaprilat and abolished by NOS inhibition.

There are conflicting results as to the coronary vascular effects of ARBs in CHF. Gervais et al29 and Richer et al30 demonstrated that losartan had no effect on resting CBF or CFR in rats with postinfarction heart failure. In contrast, Schieffer et al31 demonstrated that losartan increased resting CBF and normalized impaired CFR in a similar rat model. In our study, losartan did not improve regional myocardial perfusion or the impaired subendocardial CFR in conscious dogs with DCM, despite comparable systemic hemodynamic effects to enalaprilat. These data suggest that angiotensin II does not contribute importantly to the impaired subendocardial CFR and that the improvement observed with ACEIs is largely dependent on bradykinin-mediated effects.

Prior studies have demonstrated that bradykinin levels are elevated in pacing-induced DCM in conscious dogs.32–34 Furthermore, coronary vascular responses to bradykinin infusion were preserved,34 in contrast to other NO-dependent vasodilators. In the present study, we observed that administration of the B2 receptor agonist ceroxpur at a dose designed to reduce MAP by 10 mm Hg was associated with increased selective perfusion and normalization of CFR in the subendocardium. The magnitude of the benefit was comparable with that observed with enalaprilat and was similarly attenuated with N-LA, consistent with NO dependence of the vascular effects of bradykinin. Prior studies have demonstrated that AT1 antagonism is associated with increased bradykinin activity mediated through the AT2 receptor.10 These observations have been confined largely to rodent models and have not been reported in large mammalian models.

We did not test the effects of enalaprilat in the presence of a bradykinin antagonist, because the combination of the bradykinin antagonist HOE-140, enalaprilat, and adenosine resulted in profound hypotension (MAP <45 mm Hg) and vomiting that dramatically altered the hemodynamic stability of these conscious dogs with advanced DCM. Other studies33–35 have demonstrated that bradykinin antagonists attenuate the systemic and coronary vascular effects of enalapril. Our study extends these observations by demonstrating that the bradykinin agonist ceroxpur recapitulated the effects of enalaprilat, whereas losartan did not.

Our findings are the first to examine the effects of ACE inhibition on regional myocardial perfusion, coronary flow, and vasodilator reserve in a conscious animal model of DCM. Our findings that both ACEI and bradykinin infusion but not AT1 receptor antagonism improved regional and transmural CFR suggest that the dominant effects of ACEIs in the coronary circulation are mediated by bradykinin. The extent to which these substantial differences in the coronary vascular responses to ACEIs versus ARBs contribute to limited clinical efficacy of ARBs compared with ACEIs in human heart failure remains to be determined.

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References
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