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

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**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 bradykinin2 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. 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. 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. 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.

Although the effects of ACEIs and ARBs on systemic hemodynamics have been studied extensively, less is known about their effects on the coronary circulation, particularly in heart failure. ACEIs have been shown to reduce ischemic events in CHF, but the mechanism is not well understood. There is evidence from human studies and experimental animal models 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...
epicardial coronary resistance ($CVR$) was calculated as the quotient of mean, endocardial, and myocardium. Average transmural myocardial blood flow was re- ported as the average of flow in the 3 layers. Mean arterial pressure ($MAP$) was used as a measure of coronary perfusion pressure minus the left ventricular end-diastolic pressure ($LVEDP$) was used as a measure of coronary vasodilator reserve.

CVR and $CVR_{\text{min}}$ 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 $CVR_{\text{min}}$ were assessed after pretreatment with the NO synthase inhibitor nitro-L-arginine (NL-A, 30 mg/kg).

Transmural myocardial perfusion, CFR, and $CVR_{\text{min}}$ were assessed in 5 dogs with advanced DCM after intravenous administration of the AT$_1$ 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 ceroxip (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. In 5 dogs, the effects of ceroxip were assessed after pretreatment with NL-A.

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 CVR\textsubscript{min} 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 CVR\textsubscript{min} (DCM, 24±3 mm Hg·mL\textsuperscript{-1}·min\textsuperscript{-1} per g; DCM+enalaprilat, 15±3 mm Hg·mL\textsuperscript{-1}·min\textsuperscript{-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±1 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 CVR\textsubscript{min} observed after enalaprilat (Figure 3B).

We examined the transmural distribution of blood flow during rapid atrial pacing (180 minutes\textsuperscript{-1}) before and after administration of enalaprilat in 6 dogs to determine whether the improvement in CFR and CVR\textsubscript{min} was physiologically relevant. Dogs with DCM demonstrated limited subendocardial coronary 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\textsubscript{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 CVR\textsubscript{min} (DCM, 24±4 mm Hg·mL\textsuperscript{-1}·min\textsuperscript{-1} per g; DCM+losartan, 26±4 mm Hg·mL\textsuperscript{-1}·min\textsuperscript{-1} per g). Intra-coronary 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
LVEDP was of a magnitude similar to that observed with enalaprilat (30 ± 3 to 24 ± 3 mm Hg).

Similarly, the bradykinin (B₂) receptor agonist cereport 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 cereport. The systemic hemodynamic effects of cereport were comparable to those of enalaprilat and losartan. Taking 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 + Cereport</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 CVRₘᵦ, 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.

**Figure 3.** Effects of enalaprilat on regional CFR in conscious dogs with pacing-induced cardiomyopathy (A, n=9). The beneficial effects of enalaprilat on subendocardial CFR were attenuated after pretreatment with N-LA (B, n=5).

**Figure 4.** Effects of losartan on regional and average transmural CFR in conscious dogs with advanced cardiomyopathy (n=5). In contrast to enalaprilat, there was no significant improvement in regional or average transmural CBF response.
perfusion in the face of chronotropic stress. Prior studies from
our laboratory,20,23 and other studies,21,22 have revealed de-
creased resting transmural myocardial perfusion and signifi-
cant 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 ceroport but not
losartan. Selective impairment in regional myocardial perfu-
sion may be masked when only average transmural perfusion
is assessed. It is important to note that diminished suben-
docardial CFR contributes disproportionately to regional
contractile dysfunction as the subendocardium contributes pref-
entially 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
CFR 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 suben-
docardial 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 infu-
sion were preserved,34 in contrast to other NO-dependent
vasodilators. In the present study, we observed that admin-
istration of the B2 receptor agonist ceroport at a dose designed
to reduce MAP by 10 mm Hg was associated with increased
selective perfusion and normalization of CFR in the suben-
docardium. The magnitude of the benefit was comparable
with that observed with enalaprilat and was similarly attenu-
ated with N-LA, consistent with NO dependence of the
vascular effects of bradykinin. Prior studies have demon-
strated 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 stud-
ies33–35 have demonstrated that bradykinin antagonists attenu-
ate the systemic and coronary vascular effects of enalapril.
Our study extends these observations by demonstrating that
the bradykinin agonist ceroport 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 vascu-
lar 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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