Improvement of Endothelial Function by Chronic Angiotensin-Converting Enzyme Inhibition in Heart Failure
Role of Nitric Oxide, Prostanoids, Oxidant Stress, and Bradykinin

Rémi Varin, BS; Paul Mulder, PhD; Fabienne Tamion, MD; Vincent Richard, PhD; Jean-Paul Henry, BS; Françoise Lallemand, BS; Guy Lerebours, MD, PhD; Christian Thuillez, MD, PhD

Background—Chronic heart failure (CHF) impairs the endothelium-dependent, flow-mediated dilation (FMD) of small arteries. However, whether chronic angiotensin-converting enzyme (ACE) inhibition affects the impairment of FMD in CHF is unknown. We investigated the effects of long-term ACE inhibition on the FMD of peripheral arteries in rats with CHF and the mechanism(s) involved.

Methods and Results—FMD was assessed in isolated, perfused gracilis muscle arteries from sham-operated, and untreated or ACE inhibitor-treated (perindopril 2 mg·kg⁻¹·day⁻¹ for 10 weeks) rats with CHF (coronary artery ligation). The role of nitric oxide (NO), prostaglandins, and free radicals was assessed by pretreating the vessels with the NO synthase inhibitor L-arginine, the cyclooxygenase inhibitor diclofenac, or the free radical scavenger N-2-mercapto-propionyl-glycine (MPG). Endothelial NO synthase mRNA expression was determined by reverse transcriptase polymerase chain reaction. In animals with hemodynamic and echographic signs of CHF, FMD was converted into vasoconstriction, and this was prevented by ACE inhibition. FMD of arteries from sham-operated or ACE inhibitor–treated CHF rats was abolished by L-arginine. In untreated CHF rats, FMD was increased by diclofenac and MPG. In contrast, in arteries from ACE inhibitor–treated rats, neither diclofenac nor MPG affected FMD. In parallel, ACE inhibition prevented the reduction of endothelial NO synthase mRNA by CHF.

Conclusions—In CHF, ACE inhibition normalized NO-dependent dilatation and suppressed the production of vasoconstrictor prostanoid(s), resulting in improved FMD. The improvement of FMD might contribute to the beneficial effects of ACE inhibition during CHF. (Circulation. 2000;102:351-356.)

Key Words: angiotensin-converting enzyme inhibitors ▪ dilatation ▪ heart failure ▪ muscle, skeletal ▪ prostaglandins ▪ nitric oxide synthase ▪ oxidative stress

One of the main consequences of chronic heart failure (CHF) is abnormal peripheral arterial resistance,¹ both at rest and after exercise. The vascular endothelium is involved in this modification of vasomotor tone through the release of vasoactive substances such as nitric oxide (NO), prostaglandins, endothelin, or superoxide anions. Indeed, the endothelium-dependent responses to acetylcholine are impaired in humans with CHF² and in experimental models of CHF.³–⁵ Recent clinical studies have documented an impaired endothelium-dependent flow-mediated dilation (FMD) of conduit arteries in human CHF.⁶ More recently, we have shown that CHF abolishes the FMD of small muscular arteries, but it only moderately impairs the response to acetylcholine,⁷ suggesting that the response to flow is a more sensitive marker of endothelial dysfunction than the response to acetylcholine. Such an impaired response may be caused by a decrease in eNOS mRNA expression, resulting in reduced NO synthesis. Alternatively, this impaired FMD may be due in part to an increased production of vasoconstrictors, such as prostanoids, or an increased production of oxygen-derived free radicals.⁸

Angiotensin-converting enzyme (ACE) inhibitors have beneficial effects on ventricular enlargement⁹ and survival in CHF.¹⁰ This is related, in part, to the reduction in peripheral resistance they induce. Although ACE inhibitors normalize the dilator response to acetylcholine in CHF,⁵ it is unknown whether they prevent the impaired FMD seen in CHF, an effect that could contribute to the reduction in peripheral resistance. The present experiments were designed to investigate, in a rat model of CHF, the effect of long-term ACE inhibition with perindopril on the CHF-induced impairment of FMD and to assess the role of
NO, prostaglandins, oxidative stress, and bradykinin in these effects.

**Methods**

**Animals and Treatment**

Myocardial infarction was produced in 11-week-old male Wistar rats (Charles River, Saint Aubin Les Elbeuf, France) by left coronary artery ligation using the method of Pfeffer et al., as modified in our laboratory. With this method, the 24-hour mortality rate was \(\approx 22\%\). Seven days after ligation, rats with CHF were randomized into the following 2 groups: untreated or ACE inhibitor–treated (perindopril 2 mg \(\cdot\) kg \(^{-1}\) \(\cdot\) day \(^{-1}\) in drinking water). Sham-operated rats were also evaluated.

**Hemodynamic Parameters Assessed in Anesthetized Rats**

After 10 weeks, rats were anesthetized with pentobarbital (50 mg/kg IP). The right carotid artery and the right external jugular vein were cannulated with a micromanometer-tipped catheter (SPR 407, Millar Instruments) and advanced into the aorta and thoracic vena cava, respectively, to record arterial pressure and central venous pressure (CVP). The aortic catheter was then advanced into the left ventricle (LV) to record LV pressure and its maximal rate of rise (dP/dt\(_{max}\)).

**Echocardiographic Studies**

Transthoracic Doppler echocardiographic studies were performed after 10 weeks of treatment in rats anesthetized with metohexital (50 mg/kg IP) using an echocardiographic system equipped with a 7-MHz transducer (Acuson 128 XP/10C), as previously described.

**In Vitro Vascular Studies**

After assessment of the hemodynamic parameters, an artery of the gracilis muscle was carefully isolated under a dissecting microscope and transferred to an arteriograph. Arteries were preconstricted by the addition of phenylephrine, after which cumulative concentrations of acetylcholine (10\(^{-5}\) to 10\(^{-4}\) mol/L) were added under zero-flow conditions. The vessels were then washed and again preconstricted with phenylephrine; basal FMD was then assessed. For this purpose, perfusate flow rate was increased from 0 to 370 \(\mu\)L/min in a stepwise manner.

Four series of experiments were performed on arteries obtained from different rats; in these experiments, the role of NO, prostaglandins, free radicals, and bradykinin were assessed using the NO synthase inhibitor \(\text{N}^\text{W}\)-nitro-L-arginine (L-NA; 10\(^{-5}\) mol/L), the cyclooxgenase inhibitor diclofenac (10\(^{-3}\) mol/L), the free radical scavenger N-2-mercaptopropionyl-glycine (MPG; 10\(^{-2}\) mol/L), and the bradykinin B\(_2\) antagonist S16118 \(\cdot\) 2 \(\cdot\) 14 (10\(^{-2}\) mol/L), respectively. In each vessel, FMD was assessed before (basal values) and after incubation (20 minutes) with an inhibitor, scavenger, or antagonist. At the end of each experiment, maximal vasodilatation was assessed by the response to sodium nitroprusside (10\(^{-4}\) mol/L) under zero-flow conditions.

**Endothelial NO Synthase mRNA Expression**

Endothelial NO synthase (eNOS) mRNA expression was assessed at the level of the gracilis muscle, as described previously.

**Cardiac Morphometry**

Infarct size and cardiac collagen density were assessed using an image analyzer (Cyberview, Cervus), as described previously.

**Statistical Analysis**

All reported values are given as means \(\pm\) SEM. The responses to acetylcholine and sodium nitroprusside and the responses to flow to are expressed as a percentage of the reversal of the phenylephrine-induced constriction. These responses, as well as the hemodynamic, echocardiographic, and morphometric parameters in the sham-operated, untreated CHF, and ACE inhibitor–treated CHF rats were compared by \(t\) test or by ANOVA followed by a Tukey test for multiple comparisons. Differences were considered significant at \(P<0.05\).

**Results**

**Mortality and Exclusions**

A total of 110 rats were included in the study (sham-operated, \(n=33\); untreated, \(n=44\); ACE inhibitor–treated, \(n=33\)). During the treatment period, 11 untreated and 3 treated CHF rats died. Furthermore, 13 animals were excluded from the study because of technical difficulties during dissection.

**Hemodynamic and Echocardiographic Studies**

Table 1 illustrates the cardiac hemodynamics, CVP, and echocardiographic parameters measured in anesthetized animals after 10 weeks of CHF. Compared with sham-operated animals, CHF decreased LV systolic pressure and LV dP/dt\(_{max}\) and increased LV end-diastolic pressure and CVP. ACE inhibition reduced LV systolic pressure compared with untreated rats and normalized both LV end-diastolic pressure and CVP without affecting LV dP/dt\(_{max}\).

Ten weeks after surgery, LV end-diastolic and systolic diameters were significantly increased in infarcted animals, and LV fractional shortening was significantly reduced (Table 1). Simultaneously, both LV posterior wall thickness and wall thickening were decreased in CHF rats. Compared with untreated CHF animals, ACE inhibition significantly reduced LV end-diastolic and systolic diameters and increased LV posterior wall thickness. ACE inhibition did not significantly modify LV fractional shortening, but a trend existed toward improved LV posterior wall thickening.

Ten weeks after surgery, cardiac output and stroke volume were significantly reduced in infarcted animals compared with sham-operated rats. Compared with untreated CHF animals, ACE inhibition augmented both cardiac output and stroke volume.
TABLE 2. Histomorphometric Parameters

<table>
<thead>
<tr>
<th></th>
<th>Sham-Operated</th>
<th>Untreated CHF</th>
<th>CHF+ACE Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infarct size, % of LV</td>
<td>...</td>
<td>36±3</td>
<td>32±2</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>524±22</td>
<td>539±11</td>
<td>529±12</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.32±0.05</td>
<td>1.67±0.06*</td>
<td>1.34±0.05†</td>
</tr>
<tr>
<td>Heart weight/body weight, mg/g</td>
<td>2.55±0.07</td>
<td>3.10±0.13*</td>
<td>2.55±0.10†</td>
</tr>
<tr>
<td>LV collagen density, %</td>
<td>2.32±0.11</td>
<td>3.22±0.19*</td>
<td>2.70±0.11†</td>
</tr>
</tbody>
</table>

n=25 to 27 rats per group. *P<0.05 vs sham-operated rats; † P<0.05 vs untreated CHF rats.

Cardiac Histomorphometry

Table 2 shows the infarct size, heart weight, body weight, heart weight to body weight ratio, and LV collagen density in the 3 groups of rats. Infarct size of animals killed after 10 weeks was identical in untreated and ACE inhibitor–treated CHF rats (36±3% and 32±2%, respectively). Compared with sham-operated animals, CHF induced significant increases in heart weight, heart weight to body weight ratio, and LV collagen density. ACE inhibition decreased heart weight and heart weight to body weight ratio, and it normalized LV collagen density.

In Vitro Vascular Studies

The absolute values of arterial diameters at baseline (ie, before phenylephrine administration) and after preconstriction by phenylephrine under zero-flow conditions were similar in the 3 groups (internal diameter after phenylephrine: 104±5, 105±6, and 108±6 μm in sham-operated, untreated CHF, and ACE inhibitor–treated CHF rats, respectively).

Acetylcholine-Mediated Dilatation

Figure 1 illustrates the responses to acetylcholine obtained in phenylephrine-constricted femoral arteries from the 3 groups. Compared with sham-operated animals, CHF induced a moderate but significant impairment of the vasodilator response to acetylcholine (responses at 10⁻⁴ mol/L: 80±2% and 61±3% in sham-operated rats and untreated CHF rats, respectively; P<0.05). ACE inhibition normalized the dilator response to acetylcholine. Indeed, the response to 10⁻⁴ mol/L acetylcholine in the arteries isolated from ACE inhibitor–treated CHF rats (81±3%) was identical to that observed in sham animals.

Neither CHF nor ACE inhibition affected the response to the NO donor sodium nitroprusside (data not shown).

FMD

Figure 2 illustrates the changes in arterial diameter in response to stepwise increases in intraluminal flow at baseline. In arteries isolated from sham animals, increases in flow induced a progressive dilatation with a maximum dilatation of 40±4% at 370 μL/min. CHF abolished FMD and converted it into vasoconstriction (FMD at 370 mL/min: −4±2%). ACE inhibition prevented this impairment of FMD (FMD at 370 mL/min: 38±4%).

NO and Flow-Mediated Vasodilation

Figure 3 illustrates the effects of the NO synthase inhibitor L-NA on FMD in the 3 groups. In arteries isolated from sham-operated animals, FMD was abolished by L-NA. Indeed, at the highest value of flow, L-NA reduced FMD from the basal value of 35±8% to 2±8% (P<0.05). In untreated CHF rats, FMD was not affected by L-NA (FMD at 370 μL/min before and after L-NA, −3±3% and 1±2%, respectively). Similar to sham animals, the FMD of arteries from ACE inhibitor–treated CHF rats was abolished by L-NA. Indeed, at the highest value of flow tested, L-NA abolished FMD (2±1%; P<0.05) in arteries taken from ACE inhibitor–treated animals.

Prostaglandins and Flow-Mediated Vasodilation

Figure 4 illustrates the effects of the cyclooxygenase inhibitor diclofenac on FMD in the 3 groups. In sham-operated
Oxygen Free Radicals and Flow-Mediated Vasodilatation

The effects of MPG on FMD are shown in Figure 5. MPG did not affect the responses obtained in sham-operated rats. In untreated CHF rats, MPG abolished flow-induced dilatation (FMD at 370 μL/min before and after MPG: −4±2% and 19±5%, respectively; P<0.05). Similar to sham animals, the FMD of arteries from ACE inhibitor–treated CHF rats was not modified (FMD at 370 μL/min before and after MPG: 45±10% and 43±11%, respectively; P=NS).

Bradykinin and Flow-Mediated Vasodilatation

The effects of the bradykinin B2 receptor antagonist S16118–2 on FMD are shown in Figure 6. S16118–2 did not affect the responses obtained in sham-operated, untreated, or ACE inhibitor–treated rats.

eNOS mRNA Expression

eNOS mRNA expression was significantly reduced in the rats with CHF, and this was normalized by ACE inhibition.

Indeed, the eNOS-to-GADPH ratio was 32±3 in sham-operated, 10±4 in untreated CHF (P<0.05 versus sham), and 30±2 arbitrary units in ACE inhibitor–treated CHF rats (P=NS versus sham, P<0.05 versus untreated CHF rats).

Discussion

The present study was performed using the rat model of CHF induced by coronary artery ligation, and it shows that ACE inhibition prevents endothelial dysfunction, as evidenced by restored FMD. This effect of ACE inhibition seems to involve a normalization of NO bioavailability and an abolition of the release and/or the effect of vasoconstrictor prostanoids. In contrast, the stimulation of bradykinin B2 receptors did not seem to be involved in our experimental conditions. Finally, the restoration of NO bioavailability may be partly related to a normalization of eNOS expression, together with a reduced degradation of NO by oxidative stress. To the best of our knowledge, this is the first study in which the effect of long-term ACE inhibition on the FMD of resistance arteries has been studied, although other reports have discussed the effect of acute ACE inhibition on large arteries, especially in humans.

In a context of severe LV dysfunction, the well-known systemic and cardiac hemodynamic effects of ACE inhibitors were accompanied by a marked effect on endothelium-dependent vascular responses. Indeed, ACE inhibition not only prevented the reduced response to acetylcholine, but it also normalized the FMD of the gracilis artery. The fact that the NO synthase inhibitor L-NA abolished FMD in arteries from ACE inhibitor–treated rats suggests that the normalization of FMD is due, at least in part, to a restoration of the bioavailability of NO. Indeed, the reduced eNOS mRNA expression in CHF animals was completely prevented by ACE inhibition. Although we did not measure the activity or the absolute concentration of the enzyme eNOS, our results support the hypothesis that ACE inhibition prevented the decrease in eNOS.

We can only speculate about the mechanism(s) implicated in the modifications of eNOS RNA expression by ACE inhibitors. One possibility is that the chronic increase in flow, secondary to the peripheral vasodilatory effect of the ACE inhibitor, may by itself be responsible for the increase in eNOS expression. Indeed, an increase in flow induced by an
arteriovenous fistula increases endothelium-dependent relaxations to acetylcholine and aortic eNOS RNA expression in rats. Furthermore, shear stress also enhances eNOS expression and NO release in cultured endothelial cells. Alternatively, the exaggerated vasoconstriction induced by CHF at the level of the femoral muscular bed may be associated with local muscular tissue hypoxia, which reduces eNOS expression. Because of preferential vasodilation of vessels in the muscular territories, ACE inhibitors may prevent local hypoxia and thus indirectly prevent the reduction of eNOS mRNA expression. Again, this suggests that the chronic increase in muscular flow after ACE inhibition is a major contributor to the increased eNOS expression.

ACE inhibition augments eNOS expression and probably also decreases the degradation of NO. Reactive oxygen species are potent inactivators of NO. In humans with CHF, an intra-arterial infusion of vitamin C improves the FMD of the brachial artery, suggesting increased oxidant stress in this setting. In our experiments, ACE inhibition reduced oxidative stress to a level that did not impair FMD, as illustrated by the fact that MPG had no effect on FMD in ACE inhibitor–treated CHF rats. However, we cannot exclude from the present experiments the possibility that MPG might act by mechanisms other than the scavenging of free radicals. In any case, although we did not measure the amount of free radicals or the oxidative state of the endothelial cells, our data indirectly suggest that ACE inhibition improves FMD partially through decreased oxidative stress.

Several mechanisms may explain the decreased oxidative stress found after ACE inhibition in CHF rats. Indeed, because NO inactivates oxygen-derived free radicals, it is possible that the normalized production of NO by ACE inhibition may by itself reduce oxidative stress. Also, it is possible that the changes in oxidant status may be secondary to the changes in flow. Indeed, in cultured endothelial cells, exposure to shear stress increases superoxide dismutase expression and activity. Thus, again, it is possible that the chronic increase in tissue flow after ACE inhibition is associated with a maintained or increased antioxidant state in endothelial cells. Alternatively, angiotensin II itself may be a trigger for oxidant stress. Indeed, angiotensin II activates free-radical generating enzymes such as NAPDH oxidase, and this is sufficient to induce severe endothelial dysfunction. By reducing local tissue angiotensin II levels, ACE inhibitors could reduce oxidative stress.

Restoration of the NO pathway is not the only mechanism by which ACE inhibition improves endothelial dysfunction. Indeed, in our experimental conditions, the cyclooxygenase inhibitor diclofenac partially restored FMD in arteries from untreated rats, but it had no effect on arteries from ACE inhibitor–treated rats. Thus, in addition to the decreased NO-mediated response, a concomitant production of a vasoconstrictor prostaglandin also contributes to the impaired FMD, and this is probably responsible for the flow-induced vasoconstriction that was observed in CHF rats. A similar increased cyclooxygenase-dependent contraction has also been observed in spontaneously hypertensive rats. The fact that the effect of diclofenac was absent in arteries isolated from ACE inhibitor–treated rats suggests that ACE inhibition also prevents the flow-mediated production of vasoconstrictor prostanooids, contributing to the normalization of FMD after ACE inhibition.

Interestingly, the contribution of a cyclooxygenase-dependent vasoconstrictor has been implicated in the alteration of vascular function seen in models of increased oxidant stress, such as the vitamin E–deprived rat. Moreover, reactive oxygen species activate the COX. Thus, the increased oxidative stress in CHF may, in turn, induce the production of a COX–dependent constricting factor. This mechanism could explain why ACE inhibition prevents the release of both reactive oxygen species and of cyclooxygenase-dependent vasoconstrictors.

In patients with CHF, the contributions of prostaglandins to the regulation of vascular tone and to the interactions between ACE inhibitors and prostaglandins are controversial, especially at the level of the skeletal muscle. Indeed, studies have suggested that aspirin and indomethacin either decrease or do not affect the response to enalapril on skeletal muscle blood flow. However, another study has shown that prostaglandins contribute to exercise-induced skeletal muscle vasodilation in patients with CHF. The difference between our results and this latter study might be due to the fact that our data were obtained in vitro in the rat femoral artery, and this might markedly differ from the human skeletal vasculature in vivo.

Surprisingly, in our experimental conditions, the bradykinin B2 receptor antagonist S16118–2 did not affect FMD. The vasodilatory effects of bradykinin are not uniform in different species and different vascular beds. Thus, the lack of effect of the B2 antagonist in our experiments may be due to the fact that the endogenous production of bradykinin is low in our experimental preparations. Alternatively, peripheral muscular arteries may not express B2 receptors on endothelial cells. This latter hypothesis is supported by the fact that the administration of bradykinin did not induce any vasodilator effect in our vascular preparations, even in the presence of flow and/or chronic ACE inhibition (data not shown).

In conclusion, our study demonstrates that chronic treatment with an ACE inhibitor prevents the CHF-induced impairment of flow-mediated vasodilatation. These beneficial effects are related to an increase in eNOS mRNA expression and are associated with a decreased release of both free radicals and vasoconstrictor prostanooids. The improvement of flow-mediated vasodilatation in small peripheral arteries by ACE inhibitors may contribute to their beneficial effects on exercise capacity in CHF.

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