Increased inactivation of nitric oxide is involved in impaired coronary flow reserve in heart failure

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Nakamura, Ryo, Kensuke Egashira, Kenichi Arimura, Youji Machida, Tomomi Ide, Hiroyuki Tsutsui, Hiroaki Shimokawa, and Akira Takeshita. Increased inactivation of nitric oxide is involved in impaired coronary flow reserve in heart failure. Am J Physiol Heart Circ Physiol 281: H2619–H2625, 2001.—Recent evidence suggests that increased inactivation of endothelium-derived nitric oxide (NO) by oxygen free radical (OFR) formation is involved in the pathogenesis of endothelial dysfunction in heart failure (HF). However, it is unclear whether increased OFR limits coronary flow reserve in HF. To test this hypothesis, we examined the effects of antioxidant therapy on coronary flow reserve in a canine model of tachycardia-induced HF. The flow reserve (percent increase in coronary blood flow) to adenosine or to 20-s ischemia was less and OFR formation was greater in HF dogs than in controls. Immunohistochemical staining of 4-hydroxy-2-nonenal, an OFR-induced lipid peroxide, was detected in coronary microvessels of HF dogs. Intracoronary infusion of a cell-permeable OFR scavenger, tiron, suppressed OFR formation and improved the vasodilating capacity to adenosine or brief ischemia in HF dogs but not in controls. A NO synthesis inhibitor, N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA), diminished the beneficial effects of tiron in HF dogs. Vasodilation to sodium nitroprusside was similar between control and HF dogs, and no change in its response was noted with tiron or tiron + L-NMMA in either group. In summary, antioxidant treatment with tiron improved coronary flow reserve by increasing NO bioactivity in HF dogs. Thus increased OFR formation may impair coronary flow reserve in HF by reducing NO bioactivity.

MAXIMAL CORONARY VASODILATING CAPACITY (coronary flow reserve) in response to a brief period of ischemia (reactive hyperemia) and pharmacological vasodilator, such as adenosine, is attenuated in an animal model of heart failure (HF) (28). Limited vasodilator capacity to dipyridamole has also been demonstrated in patients with HF. Although the vasodilator response to a brief period of ischemia must be more complex than the response with drugs like adenosine and dipyridamole, prior studies (5, 13, 24–26) imply impaired coronary flow reserve in both animal and human models of HF. Elevated filling pressure, increased heart rate (HR), and decreased coronary perfusion pressure have been attributed to the impairment of coronary vasodilating capacity. However, intrinsic microvascular dysfunction may also contribute to such abnormal vasodilating capacity in HF, because abnormal coronary blood flow (CBF) responses to pacing tachycardia (metabolic coronary vasodilation) and to dipyridamole infusion (pharmacological coronary flow reserve) occur in patients with cardiomyopathy before development of overt HF (24). Recent clinical and experimental studies (6, 17, 19, 31, 34) have documented impaired endothelium-dependent nitric oxide (NO)-mediated relaxation of coronary and peripheral arteries in HF. There is growing evidence that HF is associated with increased oxygen free radicals (OFR) (3, 4, 22). Recently, our laboratory (2, 11) reported increased formation of OFR from vascular and myocardial tissues of tachycardia-induced HF dogs. Moreover, we and others have shown that endothelium-dependent coronary and peripheral vasodilation evoked by acetylcholine is attenuated in human and animal models of HF, which was restored by antioxidant therapy with vitamin C (10) or with the cell-permeable OFR scavenger sodium dihydroxybenzene disulfonate (tiron) (2). OFR, especially superoxide anion, reacts with NO to form peroxynitrite, which in turn inactivates NO. Overall, enhanced inactivation of NO by OFR may be involved in endothelial dysfunction in HF. However, it is still not known whether such OFR-induced reduction in NO bioavailability limits coronary flow reserve (maximal vasodilating capacity to adenosine or brief ischemia) in HF. Accordingly, the aim of the present study was to test the hypothesis that increased inactivation of endothelium-derived NO by OFR is involved in the mechanism of impaired coronary flow reserve in a canine model of tachycardia-induced HF.

METHODS

Induction of Pacing-Induced HF

This study was approved by the Committee on the Ethics of Animal Experiments, Faculty of Medicine, Kyushu Uni-

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Coronary flow reserve in failing heart

Surgical Preparation and Measurements

After echocardiographic examination, the animals were sedated with intravenous diazepam (10 mg), intubated after intravenous administration of pentobarbital sodium (25 mg/kg), and ventilated with a respirator. Dogs were then anesthetized with intravenous infusion of α-chloralose (3.75 mg/min). A thoracotomy was performed in the left fourth intercostal space, and the heart was suspended in a pericardial cradle. A heating pad was used to maintain the rectal temperature of animals within the range of 36.0–37.0°C.

A 7-Fr catheter was inserted into the aortic arch through the left carotid artery for measurement of aortic pressure (AoP), and a 7-Fr catheter-tipped pressure transducer was inserted into the aortic arch through the left carotid artery for measurement of aortic pressure (AoP).

AoP was measured using a strain-gauge transducer (TP-400T, Nihon-Kohden). A cardiograph triggered by AoP pulses was used to monitor HR. LVP was measured with a catheter-tipped transducer (PC-350, Millar Instruments; Houston, TX), and the positive first derivative of LVP (LV dP/dt) was obtained by electronic differentiation. A transit-time flow probe was placed at the midportion of the left anterior descending (LAD) coronary artery (16), and CBF was measured with an ultrasonic flowmeter (T201, Transonic Systems; Ithaca, NY). Peak responses of CBF to drugs were used for analysis. All variables were continuously monitored and recorded using a polygraph system (RM-6000, Nihon-Kohden).

Heparin-filled tubing (2-Fr size) was inserted into the LAD immediately distal to the flow probe for drug infusion. A 3-Fr catheter was inserted into the great cardiac vein and was advanced into the anterior interventricular vein for venous blood sampling. The hemoglobin content in each venous blood sample was also measured.

Myocardial oxygen consumption (MV O2) was calculated from the following formula: MV O2 (in ml/min) = CBF (in ml/min) × 0.0136 × Hb (in g/dl) × (SaO2 (in percent) − SvO2 (in percent))/100, where Hb is hemoglobin content and SaO2 and SvO2 represent oxygen saturation of the coronary arterial and venous blood, respectively.

Drugs

Tiron was dissolved in normal saline and neutralized by addition of equimolar NaOH. Indomethacin was diluted with sodium carbonate. Adenosine, sodium nitroprusside (SNP), and N⁴-monomethyl-l-arginine (l-NMMMA) were dissolved in normal saline. All drugs were obtained from Sigma (St. Louis, MO).

Experimental Protocols

After the surgical preparation was completed, indomethacin (5 mg/kg) was administered intravenously to block the cyclooxygenase pathway. The animals were studied 30 min after the administration of indomethacin, when all of the hemodynamic parameters had stabilized.

Protocol 1: effects of tiron and tiron + l-NMMA on CBF response to adenosine, reactive hyperemia, and SNP. Six control dogs and nine HF dogs were used. Adenosine at graded doses (3, 10, and 30 μM) was infused into the LAD for 1 min while CBF at the LAD, AoP, LVP, LV dP/dt, and HR were monitored continuously and recorded. Adenosine at the dose of 30 μM/min was chosen because this dose induced maximal coronary vasodilation (percent increase in CBF by nearly 400%) in both control and HF dogs without affecting other hemodynamic variables. The percentage of coronary vasodilation by adenosine at 30 μM/min was comparable with that by brief coronary occlusion (Fig. 1 and Table 1). After all of the variables returned to baseline, reactive hyperemia was produced by LAD occlusion just proximal to the flow probe for 20 s and complete reperfusion. After 5 min, all
of the variables returned to baseline, and the endothelium-independent vasodilator SNP (30 μg/min) was then administered. After return to baseline, tiron was infused at 7 mmol/l per CBF (in ml/min) into the LAD (2). This dose of tiron was selected because this dose scavenged OFR in both intracellular and extracellular environment in vitro (20, 23). Ten minutes after the beginning of tiron infusion, the infusions of adenosine and SNP and reactive hyperemia were repeated during tiron infusion. After the subsequent return to baseline, tiron + the NO synthase inhibitor L-NMMA (1 mg/kg) were infused into the LAD. We recently showed that L-NMMA at this dose significantly inhibited the CBF response to acetylcholine (3 μg/min) in both control and HF dogs (P < 0.01) without affecting other hemodynamic variables or myocardial metabolism (2). Adenosine and SNP injection and reactive hyperemia were repeated.

Protocol 2: reproducibility of vasodilatory response to adenosine, reactive hyperemia, and SNP. Five control and five HF dogs were used. The vasodilatory responses to adenosine, reactive hyperemia, and SNP were repeated three times at 30-min intervals without any treatment.

Protocol 3: measurement of OFR formation in myocardial tissues. Ten control dogs with (n = 5) or without (n = 5) intracoronary administration of tiron (7 mmol/l·1−1·min−1) for 10 min and 10 HF dogs with (n = 5) or without (n = 5) intracoronary tiron were used. To confirm OFR formation, electron-spin resonance (ESR) spectroscopy was used (11). ESR measurements were performed at room temperature using an X-band (9.45 GHz) ESR spectrometer (JES-RE-1X, Jeol). Freeze-clamped myocardial samples weighing 100 mg each were excised from the LV free wall and homogenized in 50 mmol/l sodium phosphate buffer containing protease inhibitors. The homogenates were immediately reacted with 4-hydroxy-2,2,6,6-tetramethyl-piperidine-N-oxyl (0.1 mmol/l), and the ESR spectra were recorded. We (11) previously reported that the increase in the ESR signal decay in HF dogs was scavenged by tiron or catalase, indicating that superoxide is produced in myocardium from HF dogs.

Protocol 4: immunohistochemistry of 4-hydroxy-2-nonenal-modified protein. To assess the cellular localization of lipid peroxidation by histochemical analysis, sections of LV myocardium were immunolabeled with an antibody raised against 4-hydroxy-2-nonal (HNE)-modified protein, an aldehydic byproduct of lipid peroxidation (32, 38). Paraffin-embedded tissue sections (5 μm thick) were deparaffinized with xylene, refixed with Bouin’s solution for 20 min, immersed in PBS, and incubated with 0.3% H2O2 in methanol for 30 min. The sections were further incubated with polyclonal antiserum raised against a HNE-modified histidyl peptide (Gly3-His-Gly3; 4 or 8 μg/ml, NN2050–70, Funakoshi). After the sections were rinsed with 0.01 mol/l PBS, they were incubated with biotin-labeled goat anti-rabbit IgG (0.1 mmol/ l), and the ESR spectra were recorded. We (11) previously reported that the increase in the ESR signal decay in HF dogs but not in controls (Table 1). Values are means ± SE; n = 6 control dogs and 9 heart failure (HF) dogs. SNP, sodium nitroprusside; CBF, coronary blood flow; L-NMMA, N(G)-monomethyl-l-arginine. *P < 0.05 vs. no treatment; †P < 0.01 vs. corresponding control dogs; ‡P < 0.05 vs. no treatment and tiron treatment; §P < 0.05 vs. tiron treatment.

Statistical Analysis

Data are presented as means ± SE. Differences between two experiments were compared using Student’s t-tests. Differences among three or experiments were determined using two-way analysis of variance and a Bonferroni’s multiple comparison test. A P value of 0.05 or less was considered statistically significant.

RESULTS

Echocardiographic examination revealed that long-term pacing tachycardia caused a significant decrease in LV ejection fraction (71 ± 2% and 32 ± 3%, respectively, in control and HF dogs) and an increase in LV end-diastolic dimensions (33 ± 1 and 45 ± 1 mm, respectively, in control and HF dogs). Hemodynamic parameters, which were measured under anesthesia, are shown in Table 1. Mean AoP and LV dP/dt were less and LV end-diastolic pressure (LVEDP) was greater in HF dogs than in controls (P < 0.01). There was no significant difference between the two groups in CBF and HR.

Protocol 1: Effects of Tiron and Tiron + L-NMMA on CBF Response to Adenosine, Reactive Hyperemia, and SNP

In control and HF dogs, treatment with tiron did not affect basal CBF, other hemodynamic parameters, or myocardial metabolic states (Table 2). The increase in CBF evoked by adenosine at 30 μM/min was significantly impaired in HF dogs compared with controls (Fig. 1). Treatment with tiron significantly enhanced the adenosine-induced increase in CBF in HF dogs but not in controls (Fig. 1). Peak reactive hyperemia after 20-s ischemia was also significantly impaired in HF dogs compared with controls. Tiron treatment significantly enhanced the peak reactive hyperemia in HF dogs but not in controls (Table 1). The repayment (area

<table>
<thead>
<tr>
<th>SNP</th>
<th>% Increase in CBF by SNP</th>
<th>Control Dogs</th>
<th>HF Dogs</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Before tiron</td>
<td>After tiron</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 ± 15</td>
<td>101 ± 15</td>
<td>108 ± 13</td>
</tr>
<tr>
<td></td>
<td>98 ± 15</td>
<td>91 ± 6</td>
<td></td>
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<tr>
<td>Reactive hyperemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak reactive hyperemia</td>
<td>(increase in CBF)</td>
<td>377 ± 15</td>
<td>367 ± 22</td>
</tr>
<tr>
<td>Mean reactive hyperemia, ml</td>
<td></td>
<td>24 ± 3</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>Repayment-to-debt ratio, %</td>
<td></td>
<td>416 ± 36</td>
<td>415 ± 19</td>
</tr>
<tr>
<td>Duration of reactive hyperemia, s</td>
<td></td>
<td>62 ± 9</td>
<td>66 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 control dogs and 9 heart failure (HF) dogs. SNP, sodium nitroprusside; CBF, coronary blood flow; L-NMMA, N(G)-monomethyl-l-arginine. *P < 0.05 vs. no treatment; †P < 0.01 vs. corresponding control dogs; ‡P < 0.05 vs. no treatment and tiron treatment; §P < 0.05 vs. tiron treatment.

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of reactive hyperemia after reperfusion (area of debt during LAD occlusion) ratio was similar between the two groups. Tiron treatment had no effects on the repayment-to-debt ratio in both groups (Table 1). After treatment with tiron, the increase in CBF by adenosine at 30 μM/min and peak reactive hyperemia did not significantly differ between the two groups (Fig. 1 and Table 1). Treatment with tiron + L-NMMA abolished the beneficial effect of tiron in adenosine-induced increases in CBF and peak reactive hyperemia in HF groups. SNP-induced increases in CBF were similar between the two groups and no changes in its response were noted with tiron or tiron similar between the two groups and no changes in its induced increases in CBF and peak reactive hyperemia abolished the beneficial effect of tiron in adenosine-

**Table 2. Effect of tiron and tiron + L-NMMA on hemodynamic parameters**

<table>
<thead>
<tr>
<th></th>
<th>Control Dogs</th>
<th>HF Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before tiron</td>
<td>After tiron</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>157 ± 0</td>
<td>158 ± 1</td>
</tr>
<tr>
<td>CBF, ml/min</td>
<td>24 ± 2</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Mean AoP, mmHg</td>
<td>118 ± 4</td>
<td>120 ± 3</td>
</tr>
<tr>
<td>dP/dt, mmHg/s</td>
<td>2,044 ± 112</td>
<td>1,944 ± 112</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>9 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>SvO2, %</td>
<td>97.7 ± 0.6</td>
<td>98.3 ± 0.4</td>
</tr>
<tr>
<td>MVO2, ml/min</td>
<td>4.1 ± 2.4</td>
<td>4.1 ± 3.6</td>
</tr>
<tr>
<td>MVO2, ml/min</td>
<td>2.3 ± 0.3</td>
<td>2.3 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 control dogs and 9 HF dogs. HR, heart rate; AoP, aortic pressure; dP/dt, first derivative of left ventricular (LV) pressure; LVEDP, LV end-diastolic pressure; SvO2, and SvO2, oxygen saturation in coronary arterial and venous blood, respectively; MVO2, myocardial oxygen consumption. *P < 0.01 vs. corresponding control dogs.

**Table 3. Time course of CBF response to adenosine, reactive hyperemia, or SNP in control and HF dogs**

<table>
<thead>
<tr>
<th></th>
<th>Control Dogs</th>
<th></th>
<th>HF Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenosine, μM/min</td>
<td>Reactive hyperemia (20 s)</td>
<td>SNP (30 μg/min)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>First experiment</td>
<td>130 ± 19</td>
<td>237 ± 29</td>
<td>367 ± 15</td>
</tr>
<tr>
<td>Second experiment</td>
<td>135 ± 15</td>
<td>261 ± 31</td>
<td>384 ± 26</td>
</tr>
<tr>
<td>Third experiment</td>
<td>114 ± 17</td>
<td>224 ± 33</td>
<td>365 ± 36</td>
</tr>
</tbody>
</table>

Values are means ± SE and reflect the percent increase in CBF by adenosine, reactive hyperemia, or SNP; n = 5 control dogs and 5 HF dogs. *P < 0.01 vs. corresponding control dogs.

**DISCUSSION**

The major finding of the present study was that antioxidant therapy with tiron improved coronary flow reserve evoked by adenosine or brief ischemia in dogs with pacing-induced HF. In contrast, tiron did not affect coronary flow reserve in healthy control dogs. Furthermore, after treatment with tiron + L-NMMA, the beneficial effects of tiron on coronary flow reserve were not noted. These findings suggest that antioxidant therapy with tiron increased the bioavailability of endothelium-derived NO and thereby improved the coronary flow reserve in HF dogs.

We and other investigators (2, 3, 11, 22) have reported an increase in OFR formation in cardiovascular tissues of HF dogs compared with controls. In this study, immunohistochemical staining of HNE-modified protein was performed in five control and five HF dogs. Lipid peroxides were positively stained in many coronary microvessels (small arteries, arterioles, and venules) in all five HF dogs without in vivo treatment with tiron (Fig. 3). Large epicardial arteries and myocardial myocytes were weakly stained in HF dogs. In contrast, no labeling was observed in coronary vessels or myocardium in control dogs. No immunoactivity was noted when the antibody against HNE-modified protein was replaced with nonimmune IgG (negative control).
suggesting that tiron indeed acted as the antioxidant in our dogs with HF. Because OFR inactivates NO (19), the bioactivity of endothelium-derived NO would be impaired in HF, which in turn decreases endothelium-dependent vasodilation (2, 3, 31, 34). Therefore, our present observations extend our previous observations (2) and suggest that an increased inactivation of NO was involved in the impaired coronary flow reserve in the animal model of HF. It is unlikely that tiron increased the vasodilatory capacity of smooth muscle cells in coronary vessels, because tiron or tiron + L-NMMA treatment did not affect the SNP-induced dilation in both control and HF dogs.

We considered the possibility that the beneficial effect of tiron on coronary flow reserve was attributable to changes in the severity of HF or in hemodynamic parameters. It has been suggested that the increase in extravascular compressive force such as elevated LVEDP may be major mechanism of reduced coronary flow reserve in HF (7, 13, 26, 28). Shannon et al. (28) reported that acute reduction in LVEDP resulted in partial improvement of subendocardial coronary flow reserve in response to adenosine in a dog model of pacing-induced HF. In contrast, others (18, 24, 25) argued that the increased LVEDP may not totally explain the mechanism of reduced coronary flow reserve in HF by demonstrating that coronary flow reserve is reduced in animal and patients with HF in the absence of increased LVEDP. In the present study, treatment with tiron had no effect on hemodynamic parameters and myocardial metabolic state (Table 2). Thus, although we did not examine the pressure-flow relationship over a range of perfusion pressure during coronary vasodilation, our present results suggest that the beneficial effects of tiron seen in HF dogs are not attributable solely to changes in hemodynamic parameters. This study suggests that reduced bioavailability of NO by increased OFR may be a major cause of reduced coronary flow reserve in HF. Because we did

Fig. 2. Oxygen free radical formation in myocardial tissues as assessed by electron-spin resonance (ESR) spectroscopy. The ESR rate of signal decay in myocardial tissues with and without in vivo tiron treatment are presented. *P < 0.01 vs. control dogs; †P < 0.01 vs. no treatment.

Fig. 3. Immunohistochemical micrograph of coronary microvessels (arterioles) stained for 4-hydroxy-2-nonenal (HNE)-modified histidine peptide or nonimmune IgG.
not measure the perfusion area distal to the flow probe, we cannot demonstrate CBF per gram of myocardium in the present study. However, we previously measured CBF/myocardial weight and reported that CBF per unit of myocardial mass was less in HF dogs compared with control dogs (37). Regarding LV weight, our laboratory (12) previously reported that the weight of the myocardium per body weight did not differ between control and HF dogs.

The coronary vasodilation evoked by adenosine has been shown to be attenuated after inhibition of NO synthesis (15, 29, 36) or after endothelial removal (8, 27), suggesting that adenosine-induced coronary vasodilation is mediated in part by endothelium-derived NO. Adenosine may cause coronary vasodilation by a combination of direct receptor-mediated relaxation of vascular smooth muscles, receptor-mediated release of NO from the endothelium, and secondary flow-mediated release of NO from the endothelium (9, 14, 21, 30). Reduced bioavailability of NO thereby impairs adenosine-induced coronary vasodilation, as seen in the HF dogs in the present study. We show herein reduced peak flow in HF dogs, whereas the repayment-to-debt ratio did not significantly differ between control and HF dogs. Because peak reactive hyperemia was not inhibited by a NO synthesis inhibitor in healthy normal dogs (1, 35), improvement of peak reactive hyperemia by tiron treatment cannot be explained by a mere increase in NO bioactivity. However, in the presence of adenosine receptor blockade, additional treatment with a NO synthesis inhibitor has been shown to reduce peak reactive hyperemia in healthy normal dogs (35). Therefore, the decrease in bioactive NO from the endothelium may decrease peak reactive hyperemia in HF dogs. In preliminary studies, we examined several durations of brief coronary artery occlusion (5, 10, 20, and 60 s) in control and HF dogs and found that a 20-s occlusion of the coronary artery was sufficient to achieve the maximal reactive hyperemic response in both control and HF dogs (data not shown). We then examined the effects of tiron on reactive hyperemia in response to the 20-s coronary occlusion in control and HF dogs. Thus we do not know whether or not the same conclusion can be drawn when different occlusion durations are used.

There are at least three limitations in the present study. First, coronary flow reserve and other hemodynamic parameters were measured under anesthesia. In physiological conscious conditions, resting HR must be higher in HF dogs due to increased chronotropic drive. However, this was not the case in the present study. Furthermore, increased resting HR in control dogs might drive up the basal myocardial oxygen demand, which in turn decreased coronary flow reserve. Therefore, some of the differences in coronary flow reserve might have been masked. Second, multiple interventions, such as the adenosine infusion, were not randomized. Because reproducibility of vasodilatory response was presented, it is unlikely that the observed effects of tiron and tiron + L-NMMA treatment are time-dependent nonspecific effects. Third, although we have shown here that tiron suppressed increased OFR formation in HF dogs, we cannot exclude a possibility that tiron might affect coronary flow reserve through a currently unrecognized mechanism.

In conclusion, the present study demonstrated that the antioxidant treatment with tiron is capable of improving abnormal coronary flow reserve in response to adenosine and brief ischemia in a dog model of pacing-induced HF. Our present observations suggest that increased oxidative stress in the coronary vascular bed in HF not only impairs bioactive NO and causes endothelial dysfunction but also impairs coronary flow reserve. Reduced coronary flow reserve in patients with HF has been reported to be associated with myocardial ischemia (33), suggesting that a reduced coronary flow reserve in HF may initiate myocardial hypoperfusion and thus lead to the progression of HF. If antioxidant therapy proves to ameliorate reduced perfusion of vital organs such as the heart, brain, and kidney in HF, increased OFR may be a new therapeutic target for HF.

REFERENCES


