Contribution of Vascular NAD(P)H Oxidase to Endothelial Dysfunction in Heart Failure and the Therapeutic Effects of HMG-CoA Reductase Inhibitor

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Background The vascular NAD(P)H oxidase-derived superoxide anion (O₂⁻) plays a crucial role in the pathological progression of hypertension and atherosclerosis, and HMG-CoA reductase inhibitors (statins) have vascular antioxidant effects. However, it is unclear whether the vascular NAD(P)H oxidase is involved in the endothelial dysfunction of congestive heart failure (CHF) and whether HMG-CoA reductase inhibitors (statins) exert their vasoprotective effects in CHF. The present study examined both the involvement of vascular NAD(P)H oxidase in endothelial dysfunction in dogs with tachycardia-induced CHF and the therapeutic effect of a statin (pitavastatin).

Methods and Results Femoral blood flow (FBF) responses to acetylcholine was significantly impaired in the CHF group, but were improved by pitavastatin. Vascular O₂⁻ production, NAD(P)H oxidase activity and Nox4 and p47phox expression were significantly elevated in CHF compared with the normal group. The elevated O₂⁻ production in the CHF group was suppressed by the NAD(P)H oxidase inhibitor, apocynin, to the normal level. In contrast, neither the gene expression nor the activity of endothelial nitric oxide synthase (eNOS) differed significantly between the normal and CHF groups. However, pitavastatin significantly suppressed O₂⁻ production, NAD(P)H oxidase activity and Nox4 and p47phox expression and increased eNOS expression and activity compared with the CHF group.

Conclusions The activated vascular NAD(P)H oxidase contributes to endothelial dysfunction in CHF, which was partly improved by pitavastatin via its inhibition of NAD(P)H oxidase. (Circ J 2004; 68: 1067–1075)

Key Words: Congestive heart failure; Endothelial dysfunction; NAD(P)H oxidase; Pitavastatin

In patients with congestive heart failure (CHF), endothelium-dependent vasodilation is significantly attenuated in both the large arteries and the microvessels of the peripheral circulation, which limits skeletal muscle blood flow and correlates with the severity of symptoms, and improvement of the endothelial dysfunction is an important target in the treatment of CHF. The main characteristic of vascular endothelial dysfunction is reduced bioavailability of nitric oxide (NO); however, even if the expression of both endothelial nitric oxide synthase (eNOS) mRNA and soluble guanylate cyclase is relatively increased in CHF, endothelium-dependent vasorelaxation is diminished as a result of the significant elevation of superoxide anion (O₂⁻) production because O₂⁻ rapidly scavenges NO and reduces the bioavailability of NO. Oxidative stress plays a pivotal role in the alteration of endothelial function in CHF.

There are several sources of O₂⁻ production within vessels, but NAD(P)H oxidase is present in vascular smooth muscle cells and endothelial cells and this enzyme generates O₂⁻ through the assembly of a multi-subunit protein complex. Angiotensin II stimulates O₂⁻ production via NAD(P)H oxidase located within the vascular wall and it has been shown that increased enzyme activity is involved in the endothelial dysfunction in angiotensin II-induced hypertensive rats. Because the renin–angiotensin system is activated in CHF, it is suggested that vascular NAD(P)H oxidase may be a major source of increased O₂⁻ formation and cause the endothelial dysfunction that occurs with the progression of CHF. However, there have been few studies of the involvement of vascular NADPH oxidase in CHF.

It is thought that the 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) reductase inhibitors (statins), which are widely used as cholesterol-lowering agents, improve endothelial dysfunction in atherosclerosis before significantly reducing the serum cholesterol concentration. In spontaneously hypertensive rats, atorvastatin partly improved endothelial dysfunction by decreasing the oxidative stress caused by the NAD(P)H oxidase subunit, p22phox. However, it has not been fully elucidated whether statins improve endothelial dysfunction by reducing O₂⁻ production via inhibition of NAD(P)H oxidase in the setting of normcholesterolemic CHF.

Therefore, we examined (1) whether increased O₂⁻ production via the vascular NAD(P)H oxidase is involved in the endothelial dysfunction in tachycardia-induced CHF dogs and (2) whether long-term treatment with pitavastatin, a HMG-CoA reductase inhibitor, improves the endothelial dysfunction.
dysfunction by inhibiting NAD(P)H oxidase in CHF.

**Methods**

**Animal Preparation**

All animal experiments were conducted according to the Guidelines for Animal Experimentation of the Animal Research Committee of Shiga University of Medical Science. CHF was induced by rapid right ventricular pacing in young adult beagle dogs, 10–12 months of age, as previously described. After the dogs had recovered from the surgery for at least 14 days, the pacemaker was programmed for 240 beats/min and pacing was continued for 29 days.

**Experimental Protocol 1**

**Effects of Pitavastatin on Cardiohemodynamics and Endothelium-Dependent/-Independent Vasodilation in CHF**

The dogs were randomly divided into 3 groups: (1) pitavastatin group (n=6) received pitavastatin (0.3 mg/kg per day, orally once a day), (2) CHF group (n=6) received only placebo and constituted time controls and (3) normal group (n=6) underwent sham operation without pacing. Pitavastatin is a potent and long-acting HMG-CoA reductase inhibitor and we selected a dose comparable with that used in humans (2–4 mg/day). Drug treatment commenced on the first day of pacing and continued for 4 weeks. On the 29th day after the initiation of pacing, the pacemaker was deactivated and arterial blood pressure and echocardiographic measurements were subsequently performed with the dogs in the conscious state as described previously. Blood was collected for plasma total cholesterol and neurohumoral assays of plasma angiotensin II concentrations and plasma renin activity (PRA) as previously described.

After the echocardiogram, to evaluate endothelium-dependent and -independent vasodilation, all dogs were given thiopental sodium to provide conscious sedation and allowed to breathe spontaneously during the procedure. A Doppler flow probe was placed on the femoral artery to measure femoral blood flow (FBF) with the VF-1 Pulsed Doppler Flow System (Crystal Biotech Inc, USA) as previously described. A polyethylene tube was inserted into the femoral artery immediately distal to the flow probe for drug infusions, and saline containing heparin (10 U/ml) was constantly infused at 0.6 ml/min. The partial pressure of oxygen (pO2) and pH in arterial blood were measured with a gas analyzer (Ciba-corning model 280).

After the surgical preparation was completed, indomethacin (5 mg/kg) was administered intravenously to block the cyclooxygenase pathway. At 30 min after the administration of indomethacin, the endothelium-dependent vasodilator acetylcholine (ACh) at graded doses (0.001, 0.01, 0.1 and 1 μg·kg⁻¹·min⁻¹) was infused (each for 3 min cumulatively, at 0.6 ml/min) into the femoral artery. FBF, aortic pressure (AoP), and heart rate (HR) were monitored continuously and recorded. After 15 min, all variables returned to baseline and the endothelium-independent vasodilator nitroglycerin (NTG; 0.1, 1 and 10 μg·kg⁻¹·min⁻¹) was then administered. Peak responses of FBF to ACh and NTG were used for analysis.

**Experimental Protocol 2**

**Effects of L-NMMA on Endothelium-Dependent Vasodilation in CHF Dogs Receiving Pitavastatin**

To clarify the relationship between endothelium-derived NO and ACh-induced vasodilation in the effect of treatment with pitavastatin, we prepared 4 CHF dogs that received pitavastatin in the first study and we then evaluated the ACh-induced vasodilation before and after the infusion of N⁶- monoethyl-L-arginine (L-NMMA; 1 mg/kg) into the femoral artery.

**Preparation of Aortic Tissues**

After the in vivo measurements were completed, the dogs were anesthetized with a large dose of pentobarbital sodium and killed by exsanguination from both carotid arteries. The abdominal aortas were isolated and carefully cleaned of excessive adventitial tissue to preserve the endothelium. Segments of aorta (5 mm) were placed into chilled modified Krebs/HEPES buffer (composition in mmol/L: NaCl 118, KCl 4.7, CaCl₂ 1.5, MgSO₄ 1.1, KH₂PO₄ 1.2, NaHCO₃ 25, Na-HEPES 10 and D(+)-glucose 5.5; pH 7.4) and some were immediately frozen in liquid nitrogen and stored at −80°C for the quantification of N0x4, gp91phox, p47phox, p22phox and endothelial NO synthase (eNOS) mRNA levels and NOS activity.

**Measurement of Ex Vivo Vascular Superoxide Anion (O₂⁻) Formation**

The O₂⁻ production was measured in the aortic segments was used using the lucigenin-enhanced chemiluminescence method of Ohara et al with some modifications. Briefly, after preparation, aortic segments were placed in Krebs/HEPES buffer aerated with 95% O₂ and 5% CO₂ and equilibrated for 30 min at 37°C. The segments were placed in a polypropylene tube containing 1 ml Krebs/HEPES buffer and lucigenin (5 μmol/L). (It has been shown recently that 5 μmol/L of lucigenin correlates well with electron spin resonance as a quantitative measurement of superoxide production.) The tube was placed in an autolumicounter (Nichion, NU-1422E) that reported the relative light units (RLU) emitted, which were integrated over 1 min intervals for 10 min. We found that counts did not significantly increase with longer periods of measurement. To assess NAD(P)H oxidase activity, NADH (0.1 mmol/L) or NADPH (0.1 mmol/L) was added to the Krebs/HEPES-lucigenin solution containing the aortic segment, and the O₂⁻ concentration was measured. Other segments were preincubated for 30 min with 1 mmol/L apocynin (an inhibitor of the activity assembly of the components of NAD(P)H oxidase) or 10 μmol/L diphenyleneiodonium (DPI: an inhibitor of flavin-containing enzymes) to determine the inhibitory effects on NADPH-stimulated O₂⁻ formation. The aortic segments were then dried and their dry weights were determined. Superoxide release is expressed as the RLU above background per milligram of dry tissue.

**Quantification of N0x4, gp91phox, p47phox and p22phox (NADPH Oxidase Subunits) and eNOS Gene Expression in the Aorta by Real-Time Polymerase Chain Reaction (PCR)**

For the analysis of canine aortic N0x4, gp91phox, p47phox, p22phox and eNOS gene expression, total RNA was extracted from the frozen aorta by the acid guanidinium thiocyanate-chloroform method as previously reported. Real-time PCR was performed with a LightCycler (Roche Molecular Biochemicals, Mannheim, Germany) using a commercially available mix containing Taq DNA polymerase, SYBR-Green I, and deoxyribonucleoside triphosphates (FastStart DNA Master SYBR Green I kit; Roche). Canine
glutaraldehyde-3-phosphate dehydrogenase (GAPDH) and eNOS specific primer pairs were synthesized according to the published data.21 Putative canine PCR primers for Nox4, gp91phox, p47phox and p22phox were designed based on homology with the highly conserved regions of the coding sequence of the human genes (gene bank: AF254621, BC032720, AF330627, NM000010). The canine Nox4, gp91phox, p47phox and p22phox-specific primer pairs were as follows.

- Nox4 F: 5'-TTAAAATAGTAGGA GACTGGGAC-3' (PCR product length 416bp)
- gp91phox F: 5'-TGATAAGCAGGAGTTTCAAGATGC-3'
- p47phox F: 5'-AGCTCGAGGTCTTCTCGAGGTC-3'
- p22phox F: 5'-GGGCTGCCGGCTGATCCTC-3'

Samples were analyzed in 6 independent runs. Serial dilutions of cDNA from the total RNA were performed for each target gene and served as standard curves for quantitative analysis. After the addition of primers (final concentrations 0.5μmol/L), MgCl2 (4 mmol/L) and template DNA to the master mix, 45 cycles of denaturation (95°C, 15 s), annealing (60–65°C, 10 s) and extension (72°C, 10–18 s) were performed. Detection of the fluorescent products was performed at the end of the 72°C extension period. To confirm amplification specificity, the PCR products were subjected to a melting curve analysis and subsequent cation-exchange chromatography using equilibrated resin (cation-exchange resin) and quantified by liquid-scintillation counting (TRI-CARB1500, Packard Instrument, Meriden, CT, USA). Total NOS and Ca2+-dependent activity were each determined by subtracting counts in the absence of NADPH from the counts obtained in the presence or absence of calcium, respectively. Ca2+-dependent activity was calculated as total NOS activity minus Ca2+-independent activity. Citrulline production was normalized to the protein concentration of the homogenates and the protein content was determined using the BCA protein assay kit (Fierce, ID, USA). NOS activity was expressed as picomoles per minute per milligram protein.

Table 1: Effects of Pitavastatin on the Hemodynamic Parameters and Blood Analysis

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=6)</th>
<th>CHF (n=6)</th>
<th>Statin (n=6)</th>
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</thead>
<tbody>
<tr>
<td>FBF, ml/min</td>
<td>47±2.6</td>
<td>33±2.4</td>
<td>37±1.0</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>123±9</td>
<td>167±11</td>
<td>162±9</td>
</tr>
<tr>
<td>Mean AoP, mmHg</td>
<td>137±2.9</td>
<td>109.7±6.1</td>
<td>114.3±5.2</td>
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<tr>
<td>LVDd, mm</td>
<td>20.3±0.4</td>
<td>40±2.4</td>
<td>38±0.6</td>
</tr>
<tr>
<td>%FS</td>
<td>29.4±1.0</td>
<td>10.2±0.9</td>
<td>12.8±0.7</td>
</tr>
<tr>
<td>pO2, mmHg</td>
<td>96.5±1.9</td>
<td>90.2±4</td>
<td>92.0±3.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.4±0.02</td>
<td>7.3±0.03</td>
<td>7.3±0.02</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>140±8</td>
<td>124±10</td>
<td>119±2</td>
</tr>
<tr>
<td>Plasma angiotensin II, pg/ml</td>
<td>398±8</td>
<td>188±42</td>
<td>166±28</td>
</tr>
<tr>
<td>Plasma renin activity, ng·ml⁻¹·h⁻¹</td>
<td>1.1±0.2</td>
<td>7.2±1.2</td>
<td>7.8±2.0</td>
</tr>
</tbody>
</table>

Values are means±SEM. FBF, femoral blood flow; HR, heart rate; AoP, aortic pressure; LVDd, left ventricular end-diastolic dimension; %FS, % fractional shortening. *p<0.05, †p<0.01 vs normal.

Results

As shown in Table 1, after 4 weeks of pacing, the mean AoP decreased and HR increased in the CHF group compared with the normal group and those values did not differ significantly between the CHF group and the pitavastatin group. The left ventricular (LV) end-diastolic dimension increased significantly and LV percent fractional shortening decreased after chronic tachycardia. There were no significant differences in these LV parameters between the CHF and pitavastatin groups. Plasma angiotensin II concentrations and plasma renin activity were significantly increased in both paced groups compared with the normal group. However, those plasma concentrations did not differ significantly between the CHF and pitavastatin groups.
There was no significant difference in the total plasma cholesterol concentrations among all groups.

**Effect of Pitavastatin on FBF Response to Acetylcholine and Nitroglycerin in CHF**

Baseline FBF was significantly decreased in both the CHF and pitavastatin groups compared with the normals, as shown in Table 1. The pO\textsubscript{2} and pH in arterial blood did not differ among the 3 groups. The percent ratio of the ACh-induced FBF to baseline FBF was significantly impaired in the CHF group at doses of 0.01 and 0.1 \text{lg·kg}^{-1}·\text{min}^{-1}, compared with the normal group (Fig 1A), but the ratio was significantly enhanced by pitavastatin when compared with the CHF group (Fig 1A). The percent ratio of the NTG-induced FBF to baseline FBF did not differ among the 3 groups (Fig 1B). As shown in Fig 2, the infusion of L-NMMA markedly reduced the ACh-induced increase in FBF in the CHF dogs receiving pitavastatin.

**Effects of Pitavastatin on Vascular O\textsubscript{2}\textsuperscript{−} Formation and NAD(P)H Oxidase Activity in CHF**

As shown in Fig 3, O\textsubscript{2}\textsuperscript{−} formation in the aorta was greater in the CHF group than in the normal group, and pitavastatin suppressed O\textsubscript{2}\textsuperscript{−} formation to normal levels. Apocynin
and DPI treatment in the CHF group also significantly decreased O$_2^-$ formation in the aorta to the normal levels. As shown in Fig 4, NADH and NADPH oxidase activities in the aorta were also greater in the CHF group than in the normal group and pitavastatin decreased both those activities to normal levels. Apocynin treatment normalized NADPH oxidase activity in the aorta in the CHF group whereas DPI treatment significantly decreased NADPH oxidase activity in the aorta in all groups.

**Effect of Pitavastatin on Vascular Nox4, gp91phox, p47phox and p22phox Expression in CHF**

As shown in Figs 5 and 6, the mRNA levels of Nox4 and p47phox in the aorta were increased in the CHF group compared with the normal group and pitavastatin significantly suppressed the levels of expression. However, the mRNA levels of gp91phox and p22phox in the aorta did not significantly differ among all groups.

**Effects of Pitavastatin on Vascular eNOS Expression and Ca$^{2+}$-Dependent NOS Activity in CHF**

As shown in Fig 7, the mRNA levels of eNOS and Ca$^{2+}$-dependent NOS activity in the aorta did not significantly differ between the normal and the CHF groups; however, pitavastatin significantly increased aortic eNOS gene expression and aortic Ca$^{2+}$-dependent NOS activity compared with those groups.

**Discussion**

In the present study, endothelium-dependent vasodilation in dogs with tachycardia-induced CHF was attenuated via an increase in the formation of vascular NAD(P)H oxidase-derived O$_2^-$, accompanied by upregulation of the expression of Nox4 and p47phox, which are NADPH oxidase subunits. Pitavastatin, a HMG-CoA reductase inhibitor, did not affect cardiac systolic function, but it does have antioxidant properties associated with suppression of NAD(P)H oxidase activity and the expression of vascular Nox4 and p47phox, resulting in improvement of endothelial dysfunction in normocholesterolemic CHF. In addition, pitavastatin increased both vascular eNOS expression and activity. In our experimental design, the effects of pitavastatin on vascular O$_2^-$ production and eNOS expression were found to be independent of plasma cholesterol concentrations.

**Vascular Oxidative Stress and NAD(P)H Oxidase in CHF**

The involvement of NO in endothelial dysfunction is well known, but another contributing mechanism of the endothelial dysfunction observed in CHF is the increased production of reactive oxygen species. There have been previous reports that an oxygen free radical scavenger, tiron, inhibited O$_2^-$ formation and improved coronary endothelial dysfunction and coronary flow reserve by increasing NO bioactivity in CHF; however, it is unclear exactly what is the major source of the increased production of reactive oxygen species in the peripheral vessels in CHF. NAD(P)H oxidase is an inducible electron transport system and represents the most important source of O$_2^-$ production in vascular cells. NAD(P)H oxidase consists of a membrane-integrated b-type cytochrome, cytochrome b$_{558}$, which is composed of 91 and 22kDa subunits (gp91phox and p22phox, respectively), and at least 3 cytosolic proteins (p47phox, p67phox and p21rac). Although the catalytic subunit of vascular NAD(P)H oxidase has been largely unknown for a long period of time, a family of gp91phox-like proteins, termed the non-
phagocytic NAD(P)H oxidases (Nox-1, -3, -4, -5), has been identified recently and Nox4 in particular is the predominant vascular isoform. In the present study, we demonstrated that vascular O$_2^-$ production assessed by lucigenin chemiluminescence was significantly elevated, and endothelium-dependent vasorelaxation was attenuated, in dogs with CHF and normocholesterolemia, despite no changes in either vascular eNOS expression or activity. We also observed that vascular NAD(P)H-oxidase activity was significantly elevated in the CHF dogs and the expression of vascular Nox4 and p47phox was significantly increased whereas that of vascular gp91phox and p22phox was unchanged. In CHF rats with myocardial infarction, aortic superoxide anion generation was significantly enhanced, accompanied by an elevation of the expression of the NAD(P)H oxidase subunit p47phox protein. Nox4 serves as a catalytic NAD(P)H oxidase subunit in nonphagocytic cells in contrast to the expression of gp91phox which is
mainly associated with increased macrophage content of the arteries. Different localization and cell types among each NAD(P)H oxidase subunit may be attributed to the disparities in the altered expression of the enzyme subunits in CHF. Furthermore, apocynin, which is a specific inhibitor of NAD(P)H oxidase that impedes the assembly of the p47phox subunit with the membrane complex inhibited vascular \( \text{O}_2^- \) production and NADPH oxidase activity in CHF, but had no effect in normal dogs. DPI, an inhibitor of flavin-containing enzymes, also exerted similar effects to apocynin on vascular \( \text{O}_2^- \) production in CHF, inhibiting not only NADPH oxidase but also various sources of reactive oxygen species such as xanthine oxidase, nitric oxide synthase. Because xanthine oxidase inhibition with allopurinol attenuates allatoin, a marker of oxygen free radical generation, and improves peripheral vasodilator capacity in hyperuricemic patients with CHF, xanthine oxidase may, in part, contribute to the endothelial dysfunction observed in CHF via upregulation of oxidative stress. Furthermore, when tetrahydrobiopterin, which is a critical cofactor for eNOS, is decreased, eNOS becomes uncoupled from the cofactor and produces \( \text{O}_2^- \) instead of NO. In addition, eNOS uncoupling in platelets from patients with CHF has been associated with decreased platelet-derived NO production and impaired endothelium-dependent vasodilation. However, in the present study, the changes in vascular \( \text{O}_2^- \) production after apocynin treatment were similar to those observed after DPI treatment. Moreover, the elevated NADPH oxidase activity in CHF was inhibited to almost normal levels by apocynin. Therefore, the involvement of other vascular \( \text{O}_2^- \) generating systems including xanthine oxidase and eNOS may have been minimal in the present model.

**Chronic Effects of Pitavastatin on Endothelial Dysfunction in CHF**

Pitavastatin improves endothelial dysfunction in atherosclerosis before there is a significant reduction in serum cholesterol concentration, suggesting a pleiotropic effect of statins beyond the reduction in plasma cholesterol. One important action of the statins is their ability to suppress the production of oxygen-derived free radicals inhibited vascular \( \text{O}_2^- \) production and NADPH oxidase activity in CHF, but had no effect in normal dogs. DPI, an inhibitor of flavin-containing enzymes, also exerted similar effects to apocynin on vascular \( \text{O}_2^- \) production in CHF, inhibiting not only NADPH oxidase but also various sources of reactive oxygen species such as xanthine oxidase, nitric oxide synthase. Because xanthine oxidase inhibition with allopurinol attenuates allatoin, a marker of oxygen free radical generation, and improves peripheral vasodilator capacity in hyperuricemic patients with CHF, xanthine oxidase may, in part, contribute to the endothelial dysfunction observed in CHF via upregulation of oxidative stress. Furthermore, when tetrahydrobiopterin, which is a critical cofactor for eNOS, is decreased, eNOS becomes uncoupled from the cofactor and produces \( \text{O}_2^- \) instead of NO. In addition, eNOS uncoupling in platelets from patients with CHF has been associated with decreased platelet-derived NO production and impaired endothelium-dependent vasodilation. However, in the present study, the changes in vascular \( \text{O}_2^- \) production after apocynin treatment were similar to those observed after DPI treatment. Moreover, the elevated NADPH oxidase activity in CHF was inhibited to almost normal levels by apocynin. Therefore, the involvement of other vascular \( \text{O}_2^- \) generating systems including xanthine oxidase and eNOS may have been minimal in the present model.

**Study Limitations**

First, we evaluated endothelial function by measuring the ACh-induced increase in FBF, which is involved in not only conduit but also resistance arterial endothelial function. However, we examined eNOS expression and \( \text{O}_2^- \) production in only the conduit artery. Additional future studies will be needed in order to evaluate the changes in eNOS expression and \( \text{O}_2^- \) production in the resistance artery. Second, another contributing mechanism to the endothelial dysfunction in CHF may be decreased antioxidant defenses. We did not assess the effect of pitavastatin on vascular antioxidant activities in CHF; however, it has been reported that the activities of various endogenous antioxidants, such as superoxide dismutase, catalase and glutathione peroxidase in the failing heart, are not decreased in the tachycardia-induced CHF model. Further studies are also needed to clarify whether or not endogenous antioxidants contribute to endothelial dysfunction under
chronic treatment of pitavastatin in CHF. Third, statins have direct anti-inflammatory properties and reduce macrophage proliferation and activation in the vasculature. Because the expression of gp91phox mRNA was not increased in the present study, we considered that infiltrating macrophages did not contribute to the activated NADPH oxidase in the vessels. However, we did not examine this by immunohistochemistry. Additional future studies will, thus, be needed in order to evaluate the effect of statins on the vascular anti-inflammatory reaction in CHF.

Clinical Implications

Statins are known to reduce the morbidity and mortality in patients with coronary artery disease by lowering serum cholesterol concentrations. However, their therapeutic effects in non-ischemic heart failure still remain unclear. In the present study, pitavastatin improved peripheral endothelial dysfunction in CHF, so we anticipate an improvement in both exercise capacity and tissue perfusion in patients with CHF. Therefore, statins may become a widely used strategy in the treatment of CHF, independent of their cholesterol lowering effect.

Conclusions

The present findings suggest that increased O2·− production via activated vascular NAD(P)H oxidase contributes to the endothelial dysfunction in tachycardia-induced CHF dogs. Pitavastatin improved the endothelial dysfunction by modulating the NO/O2·− balance via both significant reduction via activated vascular NAD(P)H oxidase and upregulation of eNOS expression.

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References


