Simvastatin restores endothelial NO-mediated vasorelaxation in large arteries after myocardial infarction

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Bates, Kathryn, Charles E. Ruggeroli, Steven Goldman, and Mohamed A. Gaballa. Simvastatin restores endothelial NO-mediated vasorelaxation in large arteries after myocardial infarction. Am J Physiol Heart Circ Physiol 283: H768–H775, 2002. First published April 11, 2002; 10.1152/ajpheart.00826.2001.—Congestive heart failure (CHF) after myocardial infarction is associated with diminished endothelial nitric oxide (NO)-mediated vasorelaxation. The 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors have been shown to modulate vascular tone independent of the effects on lipid lowering. We hypothesized that simvastatin restores NO-dependent vasorelaxation with CHF. We found that incubation of the normal rat aorta with 0.1 mM simvastatin for 24 h enhanced ACh-mediated vasorelaxation (P < 0.05). Moreover, simvastatin increased (P < 0.05) endothelial NO synthase (eNOS) protein content by >200% (82.0 ± 14.0 vs. 21.6 ± 7.9% II/µg). In cultured endothelial cells, simvastatin (10 and 20 µM) increased eNOS levels by 114.7 ± 39.9 and 212.0 ± 75.0% II/µg protein, respectively (both P < 0.05; n = 8). In the rat coronary artery ligation model, oral gavage with 20 mg·kg⁻¹·day⁻¹ simvastatin for 3 wk decreased (P < 0.05) mean arterial pressure (121 ± 20 vs. 96.5 ± 10.8 mmHg) and left ventricular change in pressure with time (4,500 ± 700 vs. 4,091 ± 1,064 mmHg/s, n = 6). Simvastatin reduced (P < 0.05) basal vasoconstriction and improved ACh-mediated vasorelaxation in CHF arterial rings. Inhibition of NO generation by N⁵-nitro-L-arginine methyl ester (100 µM) abolished the ACh-induced vasorelaxation in all rats. In conclusion, chronic treatment of CHF with simvastatin restores endothelial NO-dependent dysfunction and upregulates eNOS protein content in arterial tissue.

congestive heart failure; arterial relaxation; endothelial nitric oxide synthase; 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors

CONGESTIVE HEART FAILURE (CHF) after myocardial infarction (MI) results in endothelial dysfunction, which contributes to increased vasoconstrictor tone in both large conduit and resistance arteries. Importantly, studies using the rat coronary artery ligation model of CHF have demonstrated nitric oxide (NO)-mediated endothelial dysfunction in the vasculature, initially in the aorta and pulmonary artery (24) and later in the hindlimb (3, 7). The mechanism thought to be responsible for this NO-mediated endothelial dysfunction is decreased NO production that is due to decreases in endothelial NO synthase (eNOS) protein levels, which can be reversed with overexpression of eNOS transgene (7).

The 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors or statins are classically thought of as inhibitors of cholesterol synthesis (29, 30). Recent work has challenged this notion and suggested that lowering of cholesterol may not be the lone benefit of statin therapy (22, 28). Statins also have extrahepatic effects, including restoration of diminished endothelial function and blood flow (31), platelet aggregatability and atherosclerotic plaque stabilization in acute coronary syndromes, possible anti-inflammatory effects and reducing rates of acute MI and stroke (20, 23, 24, 26, 27).

The effects of statin treatment on modulation of NO release have been demonstrated in many laboratories. These enhanced effects were originally shown by Liao and colleagues (21) and were later confirmed in bovine aortic endothelial cells, human cells, mice, and rats (11, 18). Moreover, simvastatin and lovastatin induce a transcriptional activation of the NOS gene in human endothelial cells in vitro, and these effects are reversed by hypoxia and oxidized low-density lipoprotein under cholesterol-clamped conditions (17). Recently, atorvastatin was shown to promote NO production via agents controlled by exogenous and endogenous cholesterol production. Statins may also improve NO-dependent vasodilatation through attenuation of superoxide anion formation in the endothelium (33).

Although the endothelial effects of statins have been previously documented by our laboratory and others, there are no data available on the mechanism of statin-induced changes in NO release in CHF. Protein expression of eNOS was shown by our laboratory to be diminished in CHF (7), but the effects of chronic treatment with statins on arterial vasorelaxation have not been evaluated in CHF. We speculated that statins restore endothelial function via upregulation of eNOS gene expression. Therefore, we set out to explore the role of statins on endothelial NO-mediated vasorelaxation in
CHF by examining the effects of simvastatin on hemodynamic parameters in normal and CHF rats and on NO-mediated vasorelaxation and eNOS production in arterial tissues from normal and CHF rats.

METHODS

Studies were performed using bovine pulmonary artery endothelial cells (BPAECs) and six groups of Sprague-Dawley rats weighing 335–412 g: 1) normal untreated rats, 2) normal treated rats, 3) sham untreated rats, 4) sham treated rats, 5) untreated CHF rats, and 6) treated CHF rats. Normal rat aortic tissue was treated with simvastatin (0.1 mM, Merck) for 24 h. For the heart failure study, rats assigned to simvastatin received a daily dose by oral gavage of 20 mg·kg⁻¹·day⁻¹ for 3 wk. Rats assigned to placebo received a daily dose by oral gavage of water solution.

Preparation of arterial organ culture. Thoracic aortic segments were dissected from Sprague-Dawley rats using standard techniques developed in our laboratory (7). The arterial segments were incubated at 37°C in the presence of 7% CO₂ in air and 100% humidity in DMEM (Sigma Chemical; St. Louis, MO) that contained 10% fetal calf serum (PCS, HyClone; Logan, UT), penicillin (100 U/ml), streptomycin (0.1 mg/ml), and gentamicin (50 µg/ml; Sigma Chemical). Simvastatin was chemically activated by alkaline hydrolysis for use in the tissue culture as previously described (10).

Endothelial cell culture. Confluent BPAECs were used between passages 10 and 16 (27). These cells tested negative for Mycoplasma infection using the MycoFluor Mycoplasma detection kit (Molecular Probes; Eugene, OR). Cells were grown in an incubator at 37°C in the presence of 7% CO₂ in air and 100% humidity. When endothelial cells exhibited a cobblestone shape, the cells were divided into two groups. One group of cells had simvastatin added at a 0.1 µM concentration and the other group of cells was maintained in cell-culture media (DMEM) alone and used as the control group in the subsequent determination of eNOS.

Determination of eNOS protein levels. Protein levels were measured using immunoblot techniques as previously described (7). In brief, thoracic aortic segments were homogenized in ice-cold buffer [5 mM HEPES, pH 7.9, 26% glycerol (vol/vol), 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM dithiothreitol (DTT), and 0.5 mM phenylmethylsulfonyl fluoride (PMSF)] with NaCl (300 mM final concentration) and incubated on ice for 30 min. Mixtures were centrifuged at 100,000 g at 4°C for 20 min. The supernatant was fractionated using 8% SDS-PAGE after mixing with an equal volume of 2% SDS-1% β-mercaptoethanol. Proteins were transferred to nitrocellulose membranes. After the membranes were blocked for a period of 1 h at room temperature with 5% nonfat dry milk and 0.1% Tween 20, the membranes were incubated with a mouse anti-eNOS IgG antibody (1:1,000 dilution, Transduction Laboratories). eNOS was detected with horseradish peroxidase-labeled rabbit anti-mouse IgG secondary antibody (1:2,000 dilution). Sections were visualized using chemiluminescence.

Experimental MI. Heart failure was created in rats using standard techniques developed in our laboratory (8). Using these techniques, there was 50% mortality within the first 3 wk. In brief, rats were anesthetized with ketamine (50 mg/kg ip) and acepromazine (50 mg/kg ip), and a left thoracotomy was performed. The heart was expressed from the thorax, and a ligature was placed around the proximal left coronary artery. The heart was returned to the chest, and the thorax was closed. The rats were maintained on standard rat chow and water ad libitum with acetaominophen in drinking water (67 mg/l) for postoperative analgesia. Our laboratory has shown that rats undergoing this procedure have large MI that average 40% of the left ventricle (8). After 3 wk, rats were anesthetized with methoxyflurane and a nine-lead electrocardiogram was performed for screening purposes. The presence of Q waves (>1 mV) in limb lead I or lead aVL and a sum of the R waves in the precordial leads (<10 mV) correlated well with the presence of a large left ventricular (LV) MI. However, MI was definitively determined 3 wk later by hemodynamics [LV end-diastolic pressure (EDP) > 16 mmHg] and the presence of a myocardial scar on the free wall of the left ventricle. Rats with hemodynamic evidence for large MI were used in the heart failure group. Animals that underwent thoracotomy but did not have the coronary artery ligated were designated as sham-operated controls. Randomization to treatment was assigned 3 wk postinfarction. The animals were studied up to 6 wk after surgery.

LV hemodynamics. For sham and CHF rats 3 wk postinfarction, the hemodynamic measurements were obtained using methods reported previously by our laboratory (8). In brief, after anesthesia and tracheal intubation with subsequent connection to a rodent ventilator (Harvard Instruments), a 2-Fr catheter with two pressure sensors was inserted through the left carotid artery such that one sensor was located in the left ventricle and the second sensor was located in the ascending aorta. We measured heart rate, aortic pressure, LVEDP, and the rate of LV pressure change (LV dp/dt) by using a two-sensor pressure transducer (Millar). These studies were repeated after treatment with simvastatin for 3 wk.

Vasorelaxation in large arteries. Measuring vasorelaxation in an aortic segment is standard in our laboratory (7). In brief, a 3.0- to 3.5-mm section of the ascending thoracic aorta was mounted to a ring apparatus. The segment of artery was attached to stainless steel wire stirrups with one wire fixed in place and the other attached to the transducer. The tissue was suspended in a 37°C bath of Krebs-Henseleit (KH) solution sulfused with 95% O₂-5% CO₂. The aortic ring segment was stretched to a resting tension of 1 g and allowed to equilibrate for 45 min, precontracted with 60 mM KCl for 30 min, and then returned to KH solution and allowed to equilibrate again for 45 min. Because the intrinsic vascular tone was usually insufficient to allow measurement of vascular relaxation, it was standard procedure to preconstrict the artery before applying escalating doses of relaxant agonist (6).

Therefore, in this study, the artery was preconstricted with phenylephrine (PE, 3 µM) under constant flow conditions until a steady-state constriction was observed. To study the role of receptor-mediated NO release in vasorelaxation, vasorelaxation induced by increasing concentrations of ACh (10⁻⁹ to 10⁻⁴ M) was measured. Percent relaxation was calculated based on the maximal response to PE such that the ACh dose that completely abolished the PE-induced pre-constriction corresponded to 100% relaxation. To establish that these responses were NO mediated, the ACh-stimulated dose-response studies were repeated in the presence of the NOS inhibitor N⁵-nitro-L-arginine methyl ester (L-NAME, 200 µM) in both sham control and CHF rats. To show that the observed effects of simvastatin treatment were due to endothelially mediated NO, the vasorelaxation responses to the endothelium-independent, NO donor sodium nitroprusside (SNP, 10⁻⁹ to 10⁻⁴ M) dose responses were measured.

Statistics. Data are expressed as means ± SD. Vasorelaxation was calculated as the percentage of the contractile response to PE. Two-way ANOVA with repeated measures was used to compare ACh dose-response data between dis-
ease and L-NAME treatment. Student’s t-test was used to compare 24-h treated normal arteries versus culture media-
treated arteries. The effect of simvastatin in CHF was exam-
ined using a two-way ANOVA (CHF/sham, treated/untreat-
ed). When the effect of treatment or disease was significant,
a Newman-Keuls analysis was performed. \( P < 0.05 \) indicated statistical significance.

RESULTS

Determination of simvastatin operating dose. To de-
fine the operating dose of simvastatin, we obtained
dose-response data with concentrations from 0.01 to 7 mM added to the bath and aortic ring measurements
obtained at 5-min intervals (Fig. 1). Doses \( \leq 0.1 \) mM
showed no effect on vascular tone, which suggests that
at these concentrations, simvastatin does not act as an endothelial agonist. Therefore, in the remainder of the
study, the aortic rings were incubated at a dose of 0.1
mM. At doses \( > 0.1 \) mM, simvastatin induces vasorelaxation in the aortic rings, a response that appears to
be endothelium independent. Thus the dose used in the
current study ensured that the vasorelaxation re-
sponse measured was due to an endothelium-depen-
dent effect of simvastatin.

Effects of 24-h treatment with simvastatin on NO-
mediated arterial vasorelaxation in normal rats. To
determine the effect of simvastatin on arterial vasore-
lation, arterial rings were incubated for 24 h with simvastatin in an organ-culture system. Compared
with untreated aortic rings, simvastatin did not change
the response to PE (data not shown). In contrast, com-
pared with arterial tissue incubated with culture me-
dium only, incubation with 0.1 mM simvastatin for
24 h enhanced vasorelaxation \( (P < 0.05) \) at all concen-
trations of \( \text{ACH} > 10^{-7} \) M, with peak vasorelaxation
observed at \( 10^{-4} \) M (60 ± 11% in simvastatin treated
vs. 33.7 ± 7.8% in controls, \( n = 6; P < 0.05 \); Fig. 2). The
data in Fig. 2 are presented as percent vasorelaxation,
which is the relaxation induced by ACh normalized to
the vasoconstriction induced by PE. The addition of
L-NAME abolished the ACh-mediated response in both
the control and simvastatin-treated vessels (data not
shown). Furthermore, there were no differences in the
response to SNP between the simvastatin and control
groups (Fig. 3).

Effects of 24-h treatment with simvastatin on eNOS
content in arterial tissue from normal rats. To deter-
mine whether the enhancement in vasorelaxation in-
duced by simvastatin was due to upregulation of eNOS,
we measured eNOS protein levels in arterial tissue
treated with simvastatin (0.1 mM) for 24 h. Incubation
for 24 h of arterial tissue with simvastatin increased
eNOS protein content by 10.220.32.247 on April 7, 2017 http://ajpheart.physiology.org/ Downloaded from
endothelial cell line treated with varying concentrations of simvastatin. As shown, endothelial cells transfected with the eNOS transgene were used as a positive control for the immunoblot (Fig. 5A). These positive control cells were produced by incubating endothelial cells with $5 \times 10^6$ plaque-forming units of adenoviral construct that carries cDNA for eNOS and is driven by SV40 promoter as described previously by our laboratory (6).

As displayed in Fig. 5B, simvastatin increased eNOS protein levels in a dose-dependent manner. Treatment with 10 and 20 $\mu$M simvastatin increased eNOS levels by $114.7 \pm 39.9$ and $212.0 \pm 75.0\%$ II/µg protein, $n = 8$; both $P < 0.05$, respectively, compared with control.

Effects of simvastatin on hemodynamics in CHF.

Changes in body weight, systolic and diastolic pressures, LV dP/dt, and LVEDP after MI are listed in Table 1. The important changes in heart failure after MI were decreased LV dP/dt and increased LVEDP. Right ventricular (RV) weight was increased, although LV weight-to-body weight was unchanged. These data are consistent with previous reports from our laboratory in the same model (8). In normal rats, simvastatin treatment decreased both mean arterial pressure (MAP) and LV dP/dt ($116 \pm 4$ vs. $105 \pm 6.7$ mmHg and $7,938 \pm 703$ vs. $4,289 \pm 673$ mmHg/s, respectively, $n = 6$; both $P < 0.05$). In CHF rats, simvastatin treatment resulted in a decrease in both MAP ($121 \pm 20$ vs. $96.5 \pm 10.8$ mmHg, $n = 6$; $P < 0.05$) and LV dP/dt ($4,500 \pm 700$ vs. $4,091 \pm 1,064$ mmHg, $n = 6$; $P < 0.05$).

In contrast, LVEDP did not change in CHF rats treated with simvastatin ($27 \pm 8$ vs. $21.2 \pm 6.21$ mmHg, $n = 6$) compared with untreated CHF rats.

Effects of simvastatin on arterial basal tone in CHF.

No significant difference was found in the PE-induced vasoconstrictor response in arteries from sham untreated rats and sham rats treated with simvastatin, which suggests that simvastatin did not change the basal vascular tone in sham rats (Fig. 6). Compared with sham rats, the vasoconstrictor response to 3 $\mu$M PE was increased ($P < 0.05$) in CHF rat arteries after MI. Moreover, there was a decrease in the vasoconstrictor response to PE in CHF rat arteries treated with simvastatin compared with untreated CHF rats.

![Fig. 4. A: typical endothelial nitric oxide synthase (eNOS) immunoblot for arterial tissue treated with (0.1 mM) simvastatin. Lane 1, bovine endothelial cells (BEC) transfected with eNOS transgene (BEC as a positive control); lane 2, arterial tissue treated for 24 h with simvastatin; lane 3, arterial tissue treated with culture medium only (Cont). B: eNOS protein content in control and 24-h simvastatin (0.1 mM)-treated thoracic aortas. Data are means $\pm$ SD; $n$ = 6 rats/group. *$P < 0.05$.](http://ajpheart.physiology.org/)

![Fig. 5. A: typical eNOS immunoblot for endothelial cells treated with simvastatin (10 and 20 $\mu$M). Lane 1, BEC treated with culture medium only (Control); lane 2, endothelial cells treated with 10 $\mu$M simvastatin; lane 3, endothelial cells treated with 20 $\mu$M simvastatin; lane 4, endothelial cells transfected with eNOS transgene (BEC as a positive control). These cells are endothelial cells transfected with adenoviral vector that carries the eNOS transgene driven by Rous sarcoma virus (RSV) promoter (6). B: eNOS protein content in endothelial cells treated in culture with simvastatin at concentrations of 10 and 20 $\mu$M. Data are presented as a percent change from control. Controls in these experiments are endothelial cells treated with culture medium only for 24 h. As a positive control for the immunoblot, BEC-eNOS cells were used. Data are means $\pm$ SD; $n$ = 6 rats/group. *$P < 0.05$.](http://ajpheart.physiology.org/)
Inhibition of NO generation using L-NAME abolished simvastatin increased eNOS protein levels in vivo, CHF. To determine whether chronic treatment with (data not shown). The addition of 1 mM L-arginine resulted in a decrease in the vasoconstrictor response to PE in all groups of rats (data not shown).

Effects of simvastatin in NO-mediated vasorelaxation in CHF. Because the PE-induced vasoconstriction is different in sham, CHF, and treated CHF rats, ACh-induced vasorelaxation was reported as a ratio of vasorelaxation to the corresponding PE contractile response in the four groups of rats (untreated and treated sham and CHF). Similarly, the vasorelaxation responses to ACh after the addition of L-NAME were also reported as a ratio of vasorelaxation to the corresponding baseline values. There was a dose-dependent increase in arterial vasorelaxation in response to ACh in all rat groups. Arteries from heart failure rats demonstrated a markedly depressed ACh response compared with sham control rats ($P < 0.05$; Fig. 7). However, simvastatin improved ACh-mediated vasorelaxation in CHF rats compared with untreated CHF rats at doses of $10^{-7}$ to $10^{-4}$ M with a maximal response of $66 \pm 5.7$ versus $32.6 \pm 6.8\%$, $n = 7$, respectively ($P < 0.05$). Inhibition of NO generation using L-NAME abolished the ACh-induced vasorelaxation in all four rat groups (data not shown).

Effects of simvastatin on eNOS protein expression in CHF. To determine whether chronic treatment with simvastatin increased eNOS protein levels in vivo, immunoblot analysis was performed on arterial tissues from untreated CHF and CHF rats treated with simvastatin for 3 wk. Compared with sham rats, there was a trend for eNOS protein content to decrease in CHF (25 ± 16 vs. 33 ± 6% II/μg protein; Fig. 8, A and B). More importantly, data in Fig. 8, A and B, show that simvastatin treatment of CHF rats increased eNOS protein levels in vivo compared with untreated CHF rats (36 ± 10 vs. 25 ± 16% II/μg; $P < 0.05$; $n = 3$).

**DISCUSSION**

The most important finding in this study is that in large arteries from rats with heart failure, chronic treatment for 3 wk with the HMG-CoA reductase inhibitor simvastatin restored endothelial NO-dependent dysfunction by increasing eNOS protein content in the endothelium. This conclusion is based on the observations that simvastatin increased eNOS protein expression in endothelial cells in culture and in large artery tissue from normal rats treated with simvastatin for 24 h and in arterial tissue from CHF rats treated with simvastatin for 3 wk. Our data show that simvastatin directly modulates endothelial cell function in vivo probably by modifying either the metabolism and/or the stabilization of eNOS, which ultimately increases the bioavailability of endothelial dependent NO. To our knowledge, this is the first report of statin treatment improving endothelial NO-mediated vasorelaxation in CHF.
We know that NO bioavailability in the vasculature plays an important role by regulating vasculature tone. In diseases such as atherosclerosis, diabetes, hypertension, and heart failure, NO bioavailability is reduced, which results in enhanced vasoconstriction (12). Reversing this vasoconstriction and thus increasing vasorelaxation is a major goal in the treatment of heart failure. We previously have shown (7) that this is possible by increasing eNOS content in the vasculature of heart failure rats via gene transfer of eNOS.

In the present work, we found that in thoracic aortic segments incubated in an organ culture system for 24 h with simvastatin, there was no change in basal vasorelaxation; however, the vasorelaxation response to ACh was enhanced. This improved vasorelaxation was associated with higher eNOS protein levels, which suggests that the endothelium is the site of action for simvastatin. The concept that statins can increase eNOS protein levels in the endothelium have been previously identified by other laboratories (20, 23). For example, previous work by Laufs and colleagues (17) demonstrated that statins had the ability to produce NO in the presence of normal cholesteroleric cells after short exposure by increasing the half-life of the mRNA for eNOS from 13 to 38 h. This occurred only after 48 h of treatment with 1 μM of statin. Other investigators (4, 19) demonstrated that inhibiting mevalonate synthesis, the rate-limiting step in cholesterol synthesis, led to decreased production of downstream isoprenoid geranylgeranylypyrophosphate and ultimately promoted NO effects. Additionally, work in a bovine endothelial cell culture system showed that the presence of a statin results in increases in eNOS mRNA at 24 h with peak levels at 72 h (18). Moreover, it has been shown that withdrawal of statin treatment in vitro suppresses NO production by negative-feedback mechanisms without altering mRNA stability (16). Therefore, it appears that the potential mechanism for endothelial function restoration in response to acute treatment with statins may be related to stabilization of eNOS gene expression (19). Our data in normal aortic tissue and endothelial cell culture support this work and demonstrate the important physiological effects of this increase in eNOS content. The possibility that statins act as direct NO agonists such as ACh or serotonin as a potential mechanism is also supported by studies in aortic rings, where enhanced relaxation in response to both pravastatin and simvastatin was observed after exposure for 10 min (14). This was observed in our study with doses in excess of 0.1 mM, where within minutes simvastatin increased vasorelaxation in an endothelium-independent fashion (see Fig. 1). However, the immediate vasorelaxation response observed would be difficult to attribute to an upregulation of eNOS gene expression but does support the possibility that statins may act also as NO agonists.

The endothelium can be viewed as an independently functioning entity situated between vascular media and the lumen of the vessel thus being responsible for the local production of mediators of vasomotor tone. We found an increased basal large artery vasoconstrictor response to PE in CHF rats compared with sham rats. It has been suggested that this increase in the vasoconstrictor response in heart failure may be due to a variety of factors including but not limited to a decrease in endothelium-related vasorelaxors such as NO, an overabundance of oxygen free radicals resulting in NO degradation, an increase in released vasoconstrictors from the endothelium in CHF, or oversensitivity to vasoconstrictors. However, our study shows that the augmented PE response in heart failure rats was decreased with simvastatin treatment for 3 wk. Furthermore, we show that simvastatin improved NO-mediated vasorelaxation in CHF in vivo and that chronic simvastatin treatment restored defective levels of NO characteristically seen in heart failure. Because the vasorelaxation response was more pronounced in the CHF rats treated with simvastatin compared with untreated CHF and sham control rats, one may speculate that the availability of the NO substrate L-arginine is not the cause of attenuated NO-mediated endothelial dysfunction after MI. Therefore, our data suggest that chronic treatment with simvastatin directly affects endothelial cell function probably by modifying either the metabolism and/or the stabilization of
eNOS, which ultimately restores the bioavailability of endothelium-dependent NO.

There are several potential mechanisms for improving NO-mediated vasorelaxation in large arteries in CHF with chronic simvastatin treatment. First, the chronic effects observed in the current study may be associated with intracellular calcium handling or through activation of the phosphoinositide 3-kinase (PI3K)/Akt pathway and subsequent increases in NO availability. Statins were recently shown to stimulate the protein kinase Akt, which activates the enzymatic activity of eNOS (2, 5, 15). This may play a role in the endothelial cell dysfunction that is observed in CHF. One may speculate that statin-induced stimulation of the Akt/eNOS pathway may contribute to functional improvement in endothelial cells. Another possibility is the ability to scavenge oxygen-derived free radicals (34). Several statins (atorvastatin, pravastatin, and cerivastatin) were shown to inhibit endothelial superoxide anion formation in endothelium-intact segments of the rat aorta (33). This may be particularly important as higher levels of oxygen free radicals result in diminished levels of NO bioavailability in CHF. Data from our laboratory showed that upregulation in endothelial dependent vasorelaxation was seen with a reduction in oxygen free radical formation (13). This aspect of statin therapy may contribute to the protective effects on endothelial tissue in CHF.

In summary, the results of the present study clearly demonstrate that statins, specifically simvastatin, improve endothelial function and increase endothelial eNOS protein content with short-term treatment. Additionally, we have confirmed that there are abnormalities in NO bioavailability in heart failure that directly alter hemodynamics and vasorelaxation. More importantly, in CHF, 3 wk of simvastatin therapy resulted in augmented endothelial NO-dependent vasorelaxation in response to ACh, which was abolished by L-NAME. Finally, this improvement in vasorelaxation is associated with upregulation of eNOS protein expression in arterial tissue from CHF rats.

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REFERENCES


26. Scandinavian Study Survival Group. Scandinavian simva-
27. Schaeffer RC Jr, Gong F, and Bitrick MS. Restricted diffusion of macromolecules by endothelial monolayers and small-
28. Schwartz GG, Olsson AG, and Study Investigators MIR-
29. Shepard J, Cobbe SM, Ford I, Isles CG, Lorimer AR, Mac-
Farlane PW, McKillip JH, and Packard CJ. Prevention of coronary heart disease with pravastatin in men with hypercho-