

Simvastatin Preserves the Ischemic-Reperfused Myocardium in Normocholesterolemic Rat Hearts

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Background—Ischemia followed by reperfusion in the presence of polymorphonuclear leukocytes (PMNs) results in cardiac contractile dysfunction as well as cardiomyocyte injury. These deleterious effects are due in large part to endothelial dysfunction leading to the upregulation of cell adhesion molecules and subsequent neutrophil-endothelium interaction. At clinically relevant doses, simvastatin, an HMG-CoA reductase inhibitor, has been shown to lower serum cholesterol levels and normalize endothelial cell function. We wanted to test the effects of simvastatin on neutrophil-mediated cardiac dysfunction in a controlled model of myocardial ischemia-reperfusion.

Methods and Results—This study examines the effects of simvastatin in a neutrophil-dependent isolated perfused rat heart model of ischemia (I) (20 minutes) and reperfusion (R) (45 minutes) injury. Administration of simvastatin 25 $\mu\text{g}/\text{rat}$ improved coronary flow and preserved left ventricular developed pressure (LVDP) and dP/dt_{max} , indexes of cardiac contractile function. Final LVDP was 95 ± 5 mm Hg in I/R hearts perfused with PMNs and simvastatin, compared with 49 ± 4 mm Hg in PMN-perfused I/R hearts receiving only vehicle ($P < 0.001$). In addition, simvastatin significantly reduced PMN accumulation in the ischemic myocardium ($P < 0.01$). In PMN-perfused rat hearts after I/R, simvastatin also significantly attenuated P-selectin expression, CD18 upregulation in rat PMNs, and PMN adherence to rat vascular endothelium. Significant, although less potent, effects were obtained with pravastatin.

Conclusions—These results provide evidence that HMG-CoA reductase inhibitors are potent and effective cardioprotective agents that inhibit leukocyte-endothelial cell interactions and preserve cardiac contractile function and coronary perfusion after myocardial ischemia and reperfusion. Moreover, these effects are unrelated to the cholesterol-lowering action of this agent and appear to be mediated by enhanced endothelial release of NO. (*Circulation*. 1999;100:178-184.)

Key Words: simvastatin ■ ischemia ■ reperfusion ■ myocardium ■ leukocytes

Prompt and effective reperfusion of the ischemic myocardium plays an important role in minimizing cardiomyocyte injury associated with acute myocardial ischemia. However, myocardial reperfusion itself results in enhanced myocardial injury.^{1,2} The process of reperfusion injury is characterized by an inflammatory response in which polymorphonuclear leukocytes (PMNs) play an important role.^{2,3} On reperfusion, many activated PMNs accumulate in the coronary microvasculature and infiltrate into the myocardium.^{4,5} Activated PMNs can stimulate tissue injury by the release of a variety of cytotoxic substances, including oxygen-derived free radicals, inflammatory cytokines, and proteases.⁶ Many of these substances also mediate vascular endothelial dysfunction as well as myocardial cell injury.⁷ This process is also consistent with evidence that either decreasing the number of circulating PMNs or administration of monoclonal antibodies directed against neutrophil- or endothelial cell-derived adhesion molecules leads to signifi-

cant cardioprotection in the setting of reperfusion injury.⁸⁻¹¹ At present, there is no standard therapy for reperfusion injury that is widely accepted in the field. Moreover, myocardial ischemia in humans is often brought about by the long-term consequences of hypercholesterolemia (ie, endothelial dysfunction, plaque formation, fissures in arterial plaques, etc).¹² Against this setting, cholesterol-lowering agents have been widely used to reduce cardiovascular risk.¹³⁻¹⁵ The major action of the HMG-CoA reductase inhibitors (eg, simvastatin, pravastatin, etc), collectively known as the "statins," is inhibition of cholesterol synthesis in the liver.¹⁶ Recently, however, there has been a suggestion that these statins may exert effects separate from their cholesterol-lowering actions, including promotion of endothelial NO synthesis.¹⁷ We therefore tested the hypothesis that a clinically relevant dose of a widely used statin could exert an ameliorating effect on reperfusion injury in a neutrophil-dependent model of myocardial ischemia-reperfusion.

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Methods

Isolated Rat Heart Preparation

Male Sprague-Dawley rats (270 to 320 g) were anesthetized with 60 mg/kg sodium pentobarbital, and sodium heparin 1000 U IP was administered (Elkins Sinn). Hearts were rapidly excised, the ascending aorta was cannulated, and retrograde perfusion of the nonworking heart was initiated with a modified Krebs buffer maintained at 37°C and at a constant pressure of 80 mm Hg. The Krebs buffer had the following composition (in mmol/L): glucose 17, NaCl 120, NaHCO₃ 25, CaCl₂ 2.5, EDTA 0.5, KCl 5.9, and MgCl₂ 1.2. The perfusate was aerated with 95% O₂/5% CO₂, which was equilibrated at a pH of 7.3 to 7.4. Two side arms of the apparatus in the perfusion line just proximal to the heart inflow cannula allowed the infusion of PMNs and plasma directly into the coronary inflow line. To assess cardiac contractile function, a 2.5F microtip catheter transducer (Millar Instruments) was inserted directly into the left ventricular cavity, as previously described.^{18,19} The left ventricular pressures, the maximal rate of development of left ventricular developed pressure (+dP/dt_{max}), and coronary flow were all recorded with a MacLab data acquisition system (ADI Diagnostics) in conjunction with a Power Macintosh 7600 computer (Apple Computers). All of these data were stored and analyzed at the end of each experiment.

After the first 15 minutes of perfusion, baseline left ventricular developed pressure (LVDP), +dP/dt_{max}, and coronary flow were measured every 5 minutes for 15 minutes to ensure complete equilibration of the hearts. LVDP was defined as left ventricular end-systolic minus left ventricular end-diastolic pressure. In all of the hearts, at both the initial and final readings, end-diastolic pressure was 4 to 8 mm Hg. The first derivative of left ventricular pressure (+dP/dt_{max}) was recorded from instantaneous left ventricular pressure. Flow of the Krebs buffer was then reduced to zero, creating a state of total global ischemia. This ischemia was maintained for 20 minutes. The flow was then allowed to return to values near control levels by reestablishment of coronary perfusion pressure at 80 mm Hg. At reperfusion, 100×10⁶ PMNs and 5 mL of plasma were infused directly into the coronary circulation over a period of 5 minutes via a set of side ports situated just proximal to the heart in the perfusion line. The PMNs were suspended in 5.0 mL of Krebs buffer in a syringe. Rat plasma was also placed into a different 5.0-mL syringe located just proximal to the inflow port to the coronary circulation. The hearts were allowed to reperfuse for a total of 45 minutes, during which time the data were collected every 5 minutes. Rats were given 25 µg simvastatin or pravastatin IP in 0.9% NaCl or saline alone 18 hours before isolation of the hearts. Simvastatin and pravastatin were prepared fresh from powder, dissolved in 0.9% NaCl, and stirred for 4 hours before injection to facilitate solubilization. In some experiments, hearts were isolated from statin-treated rats and perfused with normal nontreated PMNs, and in other experiments, PMNs were isolated from statin-treated rats and perfused into normal nontreated hearts.

Isolation of PMNs and Plasma

Sprague-Dawley rats (350 to 400 g) were anesthetized with pentobarbital sodium 60 mg/kg IP. PMNs were isolated from rat blood by the method of Williams et al²⁰ using the hetastarch exchange transfusion method. This method yielded 110×10⁶ to 130×10⁶ PMNs per rat, which were >95% pure and >95% viable. These PMNs were washed 5 or 6 times to remove the hetastarch before perfusion into rat hearts according to previously described procedures.²⁰

Blood from nontreated rats was centrifuged immediately in a refrigerated centrifuge at 2000g for 10 minutes, and the plasma was decanted. The plasma was infused along with the PMNs to more closely simulate the conditions present in vivo. In several rats, plasma was used for total cholesterol determination spectrophotometrically with Sigma Diagnostics reagents.

Determination of Neutrophil Infiltration of Cardiac Tissue

In several additional hearts, 6 ischemic-vehicle-reperfused and 6 ischemic hearts isolated from rats given 25 µg simvastatin at

reperfusion, histological sections were made according to previously described techniques for the counting of infiltrating PMNs.¹⁹ All neutrophil counts were determined without prior knowledge of the group from which each sample originated.

Quantification of CD18 Expression on Rat Isolated Neutrophils

Flow cytometric analysis of CD18 expression on the cell surface of freshly isolated rat neutrophils was performed according to standard procedures.²¹ Rat neutrophils were freshly isolated from rat whole blood according to the method of Williams et al.²⁰ Isolated neutrophils were washed twice in calcium-free Tyrode's solution containing 0.2% BSA and suspended in PBS. Neutrophils (5×10⁵ cells/tube) were incubated with anti-CD18 antibody (Wt. 3, Endogen) (4°C for 60 minutes). Excess primary antibody was then removed by washing of neutrophils in D-PBS. A goat anti-human IgG F(ab')₂ FITC-conjugated antibody was used as the secondary antibody at a 1:100 dilution (4°C for 30 minutes). The stained neutrophils were washed twice with DPBS and finally fixed in 1% paraformaldehyde, and then analyzed by flow cytometry (FACScan, Becton-Dickinson).

Immunohistochemistry of P-Selectin Expression

Immunohistochemistry for P-selectin was performed on tissue sections embedded in plastic (Immunobed; Polysciences Inc) according to previously described techniques.²² The basic method used was the avidin-biotin immunoperoxidase technique with monoclonal antibody PB1.3 as the monoclonal antibody directed against P-selectin. Positive staining was defined as a coronary microvessel displaying brown reaction product on >50% of the circumference of its endothelium. Fifty vessels per tissue sample were examined in each of 3 hearts per group.

Neutrophil Adherence to Superior Mesenteric Artery Endothelium

Rat neutrophils were isolated as reported above. Segments of the superior mesenteric artery (SMA) were removed from control rats and simvastatin-treated rats, sectioned into 2- to 3-mm rings, opened, and placed into wells containing 2 mL Krebs-Henseleit (K-H)+2% BSA solution. The SMA tissue was challenged with 2 U/mL thrombin (Sigma Immunochemicals) to induce P-selectin surface expression on the endothelium and coincubated with labeled PMNs (10⁶), as previously described.¹⁹ The number of adherent PMNs was counted by epifluorescence microscopy. Five different fields of each endothelial surface were counted, and the results are expressed as adherent PMNs/mm² of endothelium.

Effect of Simvastatin on NO Release From Isolated Rat Aortic Segments

We used freshly isolated rat aortic rings as the source of primary endothelial cells. Thoracic aortas isolated from control rats and simvastatin-treated rats were immersed in warm oxygenated K-H solution and cleaned of adherent fat and connective tissue. Aortic rings 6 to 7 mm long were carefully cut, opened, and fixed by small pins with the endothelial surface up in culture dishes containing 1 mL K-H solution. After equilibration at 37°C, NO released into the K-H solution was measured with a polarographic NO electrode (Iso-NO, World Precision Instruments, Inc) according to the method of Guo et al.²³ Calibration of the NO electrode was performed daily before each experimental protocol. A standard calibration curve was obtained by graded concentrations of KNO₂ at 0, 5, 10, 25, 50, 100, 250, and 500 nmol/L (final concentrations) into a calibration solution containing 0.1 mol/L KI and 0.1 mol/L H₂SO₄.

Statistical Analysis

All data in the text and figures are presented as mean±SEM. The data on left ventricular function and coronary flow were analyzed by ANOVA incorporating repeated measures. Other data were compared by an ANOVA using post hoc analysis with Fisher's corrected

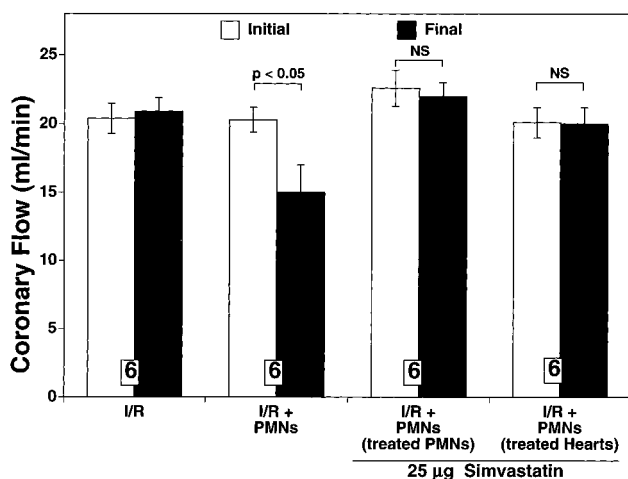


Figure 1. Initial and final coronary flow expressed in mL/min in isolated perfused rat hearts subjected to either global ischemia (I) or sham ischemia for 20 minutes and 45 minutes of reperfusion (R). Hearts were perfused in presence or absence of PMNs. All values are expressed as mean \pm SEM. Numbers at bottom of bars represent number of hearts studied.

t test. Probability values of ≤ 0.05 were considered to be statistically significant.

Results

To determine whether clinically relevant doses of simvastatin can attenuate leukocyte-endothelium interactions and improve cardiac contractile function in acute myocardial ischemia-reperfusion, we perfused nonischemic control rat hearts for 80 minutes at 80 mm Hg at a control flow of 20 to 25 mL/min or for 15 minutes at control flow followed by 20 minutes of total global ischemia and 45 minutes of reperfusion at control flows either with or without PMNs and plasma. Perfusion of additional simvastatin-treated hearts was also performed at a perfusion pressure of 80 mm Hg for 80 minutes. Perfusion of simvastatin-treated hearts at 80 mm Hg during sham ischemia or during ischemia-reperfusion without PMNs did not result in any change in coronary flow, LVDP, or dP/dt_{max} at the end of the observation period, indicating that simvastatin did not exert any direct cardiodynamic effect. Furthermore, perfusion of untreated nonischemic hearts with PMNs did not alter any of the cardiac function variables measured, indicating that PMNs did not provoke cardiac dysfunction in normal nonischemic hearts. Only ischemic-reperfused rat hearts perfused with PMNs experienced a marked reduction in cardiac contractile function and coronary flow.

Ischemic-reperfused hearts perfused with PMNs and plasma exhibited significant cardiac dysfunction ($P < 0.01$). Thus, coronary flow was decreased $27 \pm 3\%$ ($P < 0.05$) from initial values (Figure 1), LVDP decreased $51 \pm 4\%$ from initial values ($P < 0.01$) (Figure 2), and dP/dt_{max} decreased $58 \pm 4\%$ from initial values ($P < 0.01$) (Figure 3). This decrease in coronary flow and marked reduction in cardiac contractility can be attributed largely to the presence of PMNs at the time of reperfusion. This cardiac dysfunction is therefore due to the interaction of PMNs with the coronary microvascular endothelium. Against this background, simva-

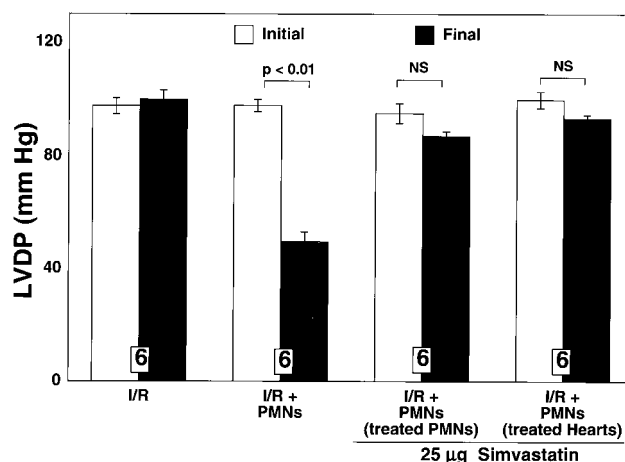


Figure 2. Initial and final LVDP expressed in mm Hg in isolated perfused rat hearts before ischemia (I) and after reperfusion (R). Hearts were perfused in presence or absence of PMNs. PMNs induced a marked contractile dysfunction, which was attenuated by simvastatin. All values are expressed as mean \pm SEM. Numbers at bottom of bars represent number of hearts studied.

statin, given to rats 18 hours before isolation of the heart and subsequent induction of ischemia-reperfusion, markedly attenuated all 3 components of the cardiac dysfunction. Thus, simvastatin prevented the coronary flow deficit and enhanced LVDP and dP/dt_{max} at the end of the reperfusion period whether hearts or only PMNs were used from simvastatin-treated rats (Figures 1, 2, and 3). Because no increase in coronary flow, LVDP, or dP/dt_{max} occurred in nonischemic controls in the presence or absence of PMNs, these cardioprotective effects are not due to direct cardiodynamic effects of the simvastatin. Moreover, these salutary effects are unrelated to the cholesterol-lowering effect of simvastatin, because plasma cholesterol was 38 ± 5 mg/dL in 5 control rats, 44 ± 3 mg/dL in 5 simvastatin-treated rats, and 47 ± 5 mg/dL in 5 pravastatin-treated rats.

Five additional hearts treated with 25 μ g pravastatin and 5 with saline were subjected to the same ischemia-reperfusion

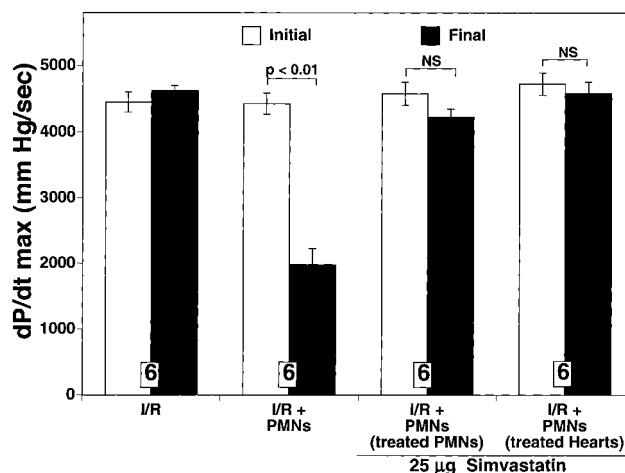


Figure 3. Initial and final $+dP/dt_{max}$ expressed in mm Hg/s in rat hearts subjected to ischemia and reperfusion. Ischemic hearts were perfused in presence or absence of PMNs. PMNs produced a significant impairment, which was eliminated by simvastatin. All values are expressed as mean \pm SEM. Numbers at bottom of bars represent number of hearts.

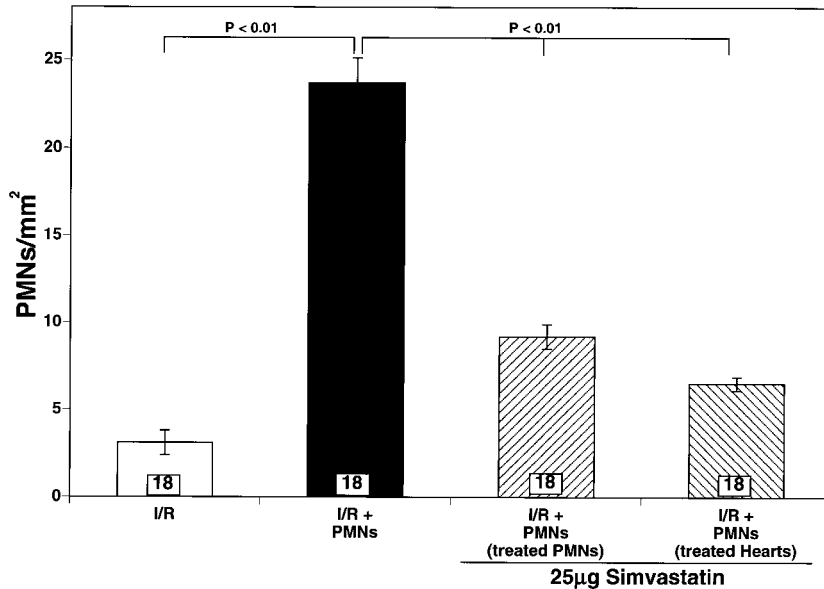


Figure 4. Histological assessment of extravascular infiltrated PMNs in isolated perfused rat heart samples taken from 6 rats per group and 3 sections per heart. All values are mean numbers of PMNs/mm² of heart area \pm SEM. I/R indicates ischemia-reperfusion.

protocol with PMNs as was the simvastatin group. The pravastatin-treated hearts responded significantly, although somewhat less than the simvastatin-treated hearts. Thus, final LVDP was 52 ± 3 versus 88 ± 3 mm Hg ($P < 0.01$) in saline- versus pravastatin-treated hearts, compared with 102 ± 4 mm Hg initially. This corresponds to an 86% recovery versus a 97% recovery in simvastatin-treated hearts. Similarly, final $+dP/dt_{max}$ was 2188 ± 184 versus 3428 ± 177 in saline- versus pravastatin-treated hearts ($P < 0.02$), compared with 4464 ± 215 initially. This corresponds to a 77% recovery compared with a 97% recovery in simvastatin-treated hearts.

Because the cardiac dysfunction appeared to be due to the cardiodepressant actions of neutrophils on a sensitized ischemic-reperfused coronary microvasculature, we studied this interaction further. The first questions to be asked were: Do neutrophils infiltrate into the ischemic-reperfused rat heart? and Does simvastatin have any effect on this neutrophil infiltration? Histological counting of PMNs was used to assess PMN infiltration. Figure 4 summarizes the PMN infiltration data in these perfused rat hearts. None of the nonischemic control rat hearts or any of the ischemic-reperfused rat hearts perfused without PMNs exhibited any significant cardiac PMN infiltration. In contrast, ischemic-reperfused hearts perfused with PMNs exhibited an 8-fold increase in PMNs/mm² of heart area ($P < 0.01$), indicative of significant PMN infiltration into these hearts. However, when either PMNs or hearts were isolated from simvastatin-treated rats and subjected to ischemia-reperfusion with PMNs, significantly lower numbers of infiltrated PMNs were observed.

To determine whether the endothelium of simvastatin-treated rats exhibited any changes in adhesion molecule expression that could account for the low degree of PMN involvement, we performed immunohistochemical analysis of P-selectin expression on the rat coronary microvascular endothelium. Figure 5 summarizes these results. Nonischemic controls exhibited very low P-selectin surface expression on the coronary endothelium. However, untreated rat hearts

subjected to ischemia and reperfused with PMNs developed a 6-fold increase in P-selectin surface expression. This was markedly attenuated ($P < 0.01$) in ischemic-reperfused rat hearts whether or not the hearts or the PMNs were isolated from simvastatin-treated rats.

To determine whether simvastatin can moderate P-selectin-mediated PMN adherence to the endothelium, we studied PMN adherence to isolated vascular segments obtained from control or simvastatin-treated rats. Figure 6 summarizes these results. Thrombin was used to upregulate P-selectin expression on the endothelium. Vascular segments stimulated with thrombin 2 U/mL exhibited a 9-fold increase in PMN adherence ($P < 0.001$). This was totally blocked by a monoclonal antibody that neutralized P-selectin. Moreover, in experiments using either blood vessels or PMNs isolated

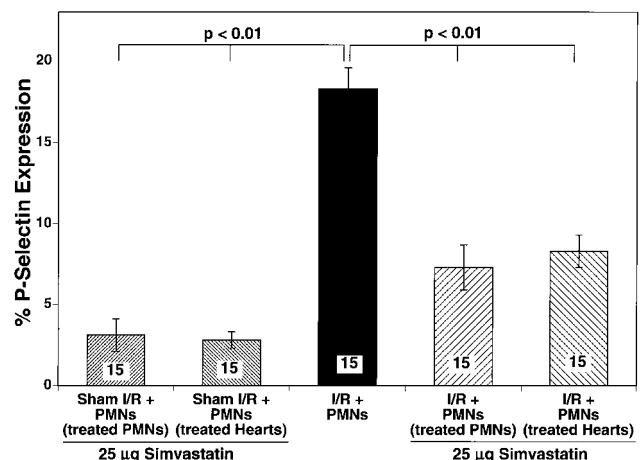


Figure 5. Immunohistological analysis of P-selectin expression in rat hearts subjected to ischemia (I) (20 minutes) and reperfusion (R) (45 minutes). Ischemic hearts were reperfused in presence or absence of PMNs. Presence of P-selectin-positive staining was then analyzed. All values are expressed as mean number of positive vessels/mm² heart area \pm SEM. Numbers at bottom of bars represent number of fields counted in 3 hearts per group.

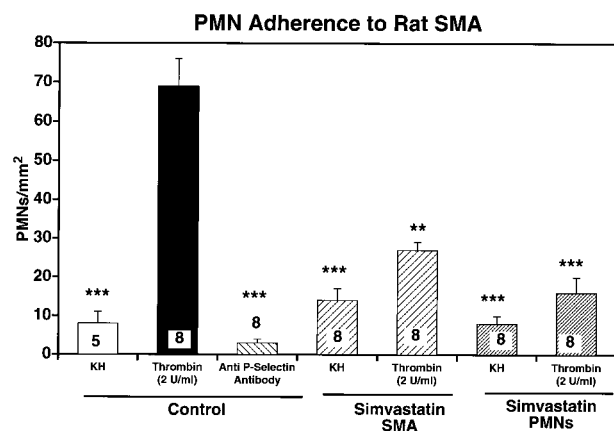


Figure 6. Adherence of rat PMNs to rat SMA endothelium is expressed as PMNs/mm². Rat SMAs were isolated from rats given simvastatin or vehicle and were either nonstimulated or stimulated with thrombin 2 U/mL. PMNs that adhered to endothelium were counted and analyzed. All values are expressed as mean \pm SEM. Numbers at bottom of bars indicate numbers of SMA segments analyzed. ** $P<0.01$ vs thrombin; *** $P<0.001$ vs thrombin.

from simvastatin-treated rats, adherence was significantly attenuated. Thus, simvastatin exerts an antiadherence effect both on PMNs and on the endothelium. This antiadherence effect could be a major factor contributing to the cardioprotection observed in simvastatin-treated hearts.

Because the adherence studies suggested that at least part of the simvastatin effect was on neutrophils, we studied further the effect of simvastatin on PMN expression of the neutrophil surface adhesion molecule CD18 via flow cytometry. Figure 7 illustrates a representative flow cytometry histogram. Unstimulated PMNs taken from control untreated rats exhibited a normal distribution. Stimulation of control rat PMNs with 100 nmol/L leukotriene B₄ (LTB₄) induced a

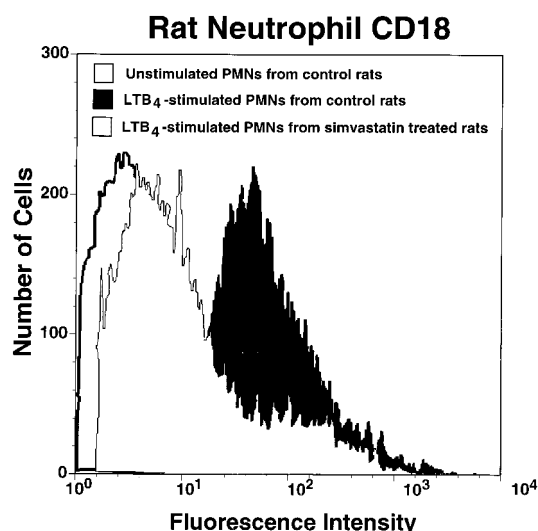


Figure 7. Flow cytometry histogram of log fluorescence intensity vs number of cells (ie, PMNs). Control unstimulated PMNs (□), LTB₄-stimulated PMNs from control rats (■), and LTB₄-stimulated PMNs from simvastatin-treated rats (▨) were incubated with anti-CD18 monoclonal antibody and labeled with a secondary antibody. Simvastatin clearly attenuated LTB₄ upregulation of CD18 in rat PMNs.

Basal NO Release From Rat Vascular Endothelium

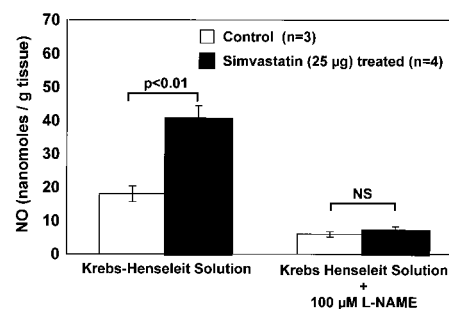


Figure 8. Basal release of NO expressed as nmol/g tissue. NO release was measured in isolated rat aortic rings obtained from control rats given vehicle and rats injected with 25 μg simvastatin. Bar heights are means; brackets are \pm SEM. High micromolar concentration of NOS inhibitor *N*^ω-nitro-L-arginine methyl ester inhibited basal release of NO in all experimental groups of rats.

marked shift to the right of the PMNs characteristic of activation. However, PMNs isolated from simvastatin-treated rats given LTB₄ remained largely unactivated, suggesting that simvastatin also exerts an antineutrophil effect that may be important in curtailing PMN-endothelium interaction.

To test whether the cardioprotective effects of simvastatin were due to increased bioavailability of NO, we measured NO release from aortic segments isolated from both vehicle- and simvastatin-treated rats. We detected a small basal level of NO release in the range of 20 \pm 4 nmol/g tissue in aortic rings isolated from vehicle-injected rats (Figure 8). However, 18 hours after rats were given 25 μg simvastatin, the basal release of NO measured in aortic rings doubled ($P<0.01$). Moreover, the addition of an NO synthase (NOS)-inhibiting concentration of *N*^ω-nitro-L-arginine methyl ester (ie, 100 μmol/L) inhibited NO release in aortic rings obtained from both vehicle-treated and simvastatin-treated rats (Figure 8). Therefore, systemic administration of simvastatin to the rats significantly increases endothelium-derived NO.

Discussion

It is well known that hypercholesterolemia, even in the absence of plaque formation, results in endothelial dysfunction.^{24,25} This endothelial dysfunction is characterized by a reduction in biologically active NO released from the endothelium. Whether this is due to an absolute reduction in endothelium-derived NO or in part to rapid quenching of NO by superoxide radicals remains to be clarified.²⁶ In any event, moderate degrees of hypercholesterolemia do not physically injure the endothelium or induce its sloughing.^{27,28} Furthermore, increased plasma cholesterol levels in rabbits in the absence of atherosclerotic plaque formation has been shown to exacerbate reperfusion injury after myocardial ischemia.²⁹ One widely used means of controlling hypercholesterolemia is HMG-CoA reductase inhibitors, the statins. This is because they inhibit hepatic biosynthesis of cholesterol at the mevalonate step.¹⁶ This reduced cholesterol synthesis results in reduced coronary artery disease^{14,30} and stroke.¹⁵ Moreover, statins have been found to restore endothelial function toward normal in animals³¹ and humans.¹²⁻¹⁴ These effects are presumed to be due directly to reduced cholesterol synthesis,

leading to reduced cholesterol droplets in endothelial cells and therefore to preservation of endothelial function, including maintenance of NO release.

The present findings clearly point toward an action other than cholesterol lowering for the statins. In this study, either simvastatin or pravastatin given 18 hours before induction of myocardial ischemia exerted a marked cardioprotective effect of dramatically blunting reperfusion injury. The data also suggest that statins attenuated neutrophil-endothelium interaction in the coronary microvasculature. Several possibilities may explain the cardioprotective effects of statins at normal cholesterol levels. These mechanisms all relate to preservation of endothelial function, inhibition of neutrophil activation, or a combination of the two effects.

With regard to neutrophil function, it is known that lovastatin reduces monocyte CD11b expression and monocyte adhesion to the endothelium in patients³² independently of its cholesterol-lowering effect. CD11b is the α -chain of the β_2 -integrins, which participates in an important way in promoting firm adhesion of leukocytes to the endothelium. In our studies, we assessed the common β -chain of the β_2 -integrins, CD18, in rat neutrophils. Simvastatin clearly attenuated LTB₄ upregulation of CD18. Moreover, statins significantly inhibited neutrophil and monocyte chemotaxis in human blood cells.³³ This effect could partially explain the results obtained in the present study. Thus, there is evidence that the statins may exert anti-inflammatory actions on blood cells in the absence of hypercholesterolemia.

A second and perhaps related effect of the statins may be upregulation of endothelial NO synthesis³⁴ as well as inhibition of hypoxia-mediated inhibition of NOS activity.³⁵ Maintenance of endothelium-derived NO may be a very important aspect of the cardioprotective effects of simvastatin. NO exerts a variety of important homeostatic actions that could help explain these results. NO has been shown to act as a physiological inhibitor of leukocyte-endothelial cell interaction³⁶ by suppressing upregulation of several endothelial cell adhesion molecules, including P-selectin,³⁷ VCAM-1, and ICAM-1.³⁸ NO is able to inhibit neutrophil-induced cardiac dysfunction by preserving endothelial release of NO. Similar salutary effects were observed with simvastatin and lovastatin in mice subjected to cerebral ischemia-reperfusion.³⁹ These effects were dependent on enhanced NO formation, because they did not occur in endothelial constitutive NOS knockout mice,³⁹ and were unrelated to the inhibitory effect of simvastatin on cholesterol biosynthesis. Moreover, this endothelial preservation with its subsequent attenuation of neutrophil activation effectively prevents neutrophil-induced cardiac dysfunction. In this regard, we have presented new evidence showing that simvastatin significantly increases basal NO release from the vascular endothelium by a specific mechanism involving NOS. This increased bioavailability of NO is likely to attenuate P-selectin upregulation on the coronary microvascular endothelium, thus limiting neutrophil adherence to the endothelium during inflammatory conditions. These effects may relate to purported effects of the statins on remodeling of the vessel wall,¹⁷ which may be partially due to enhanced NO generation. Thus, the statins may have important biological effects that promote endothelial preservation

and vascular homeostasis independently of their cholesterol-lowering effects. These actions may have important applications in attenuating leukocyte-induced cell injury such as that occurring in reperfusion injury.

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