

Cerivastatin, a Hydroxymethylglutaryl Coenzyme A Reductase Inhibitor, Improves Endothelial Function in Elderly Diabetic Patients Within 3 Days

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Background—The short-term effects of hydroxymethylglutaryl coenzyme A reductase inhibitors (statins) on endothelial function at doses that do not affect plasma lipid levels are not known.

Methods and Results—We investigated the short-term effects of cerivastatin, a hydroxymethylglutaryl coenzyme A reductase inhibitor, on endothelial function and endothelium-related products in elderly diabetic patients. Twenty-seven elderly diabetic patients (aged 69.3 ± 3.4 years), with or without mild hypercholesterolemia, were enrolled in this study, which tested cerivastatin treatment (0.15 mg/d) for 3 days. Endothelium-dependent flow-mediated dilatation, endothelium-independent dilatation by nitroglycerin in the brachial artery, nitric oxide-related products (nitrite/nitrate and cGMP), endothelium-related products (von Willebrand Factor, soluble vascular cell adhesion molecule-1, and soluble intercellular adhesion molecule-1), and a marker of oxidant stress (8-isoprostane) were assessed. Levels of plasma lipids were not changed before and after treatment with cerivastatin. Flow-mediated dilatation was significantly increased by cerivastatin treatment, as were plasma nitrite/nitrate levels (from 16.9 ± 3.4 to 22.0 ± 3.7 $\mu\text{mol/L}$, $P < 0.05$) and cGMP values. The percent of nitroglycerin-induced dilatation was not changed. Plasma concentrations of 8-isoprostane decreased, and levels of soluble vascular cell adhesion molecule also tended to decrease with cerivastatin.

Conclusions—Improvement of endothelial function was in line with antiatherosclerotic effects. Cerivastatin improved impaired endothelial function in the short-term without affecting lipid profiles in elderly diabetic patients. This effect may be partly due to upregulation of endothelial nitric oxide synthase. (*Circulation*. 2001;104:376-379.)

Key Words: diabetes mellitus ■ aging ■ endothelium ■ nitric oxide ■ hyperlipidemia

It is well known that lipid-lowering therapy, especially using hydroxymethylglutaryl coenzyme A reductase inhibitors (statins), decreases the risk of coronary events in both primary and secondary prevention.^{1,2} The antiatherosclerotic effects of statins are thought to be attributable to changes in plasma lipid levels (ie, the decrease in LDL cholesterol and increase in HDL cholesterol).³ Recently, statins were reported to upregulate endothelial nitric oxide synthase (eNOS) in cultured endothelium.⁴ The direct action of statins on the atherosclerotic arteries from rabbits, without plasma lipid-lowering, has also been studied.⁵ However, there is no study regarding this direct action in humans to our knowledge. The present study focuses on the effect of statins on endothelial function, independent of lipid-lowering, in humans. We selected cerivastatin, which is thought to have a long and strong tissue affinity.⁶

Methods

Patients

We assessed endothelial function in 27 diabetic elderly patients (aged 69.3 ± 3.4 years), with or without mild hypercholesterolemia (total cholesterol, 200 to 260 mg/dL). They were ambulatory and presented to our geriatric clinic (Nagoya University Hospital). We treated consecutive patients who were randomized to either the cerivastatin group (0.15 mg/d of cerivastatin for 3 days; $n = 14$, 6 men) or to the control group (no treatment; $n = 13$, 6 men). Half of the cerivastatin group was continued on the prescription for 3 months ($n = 8$). All patients provided informed consent, agreed to the protocols, and were willing to participate in the study. They had no history of manifest ischemic vascular diseases, and the profile of their diabetes mellitus was as follows: glycosylated hemoglobin (HbA_{1c}), $7.3 \pm 0.7\%$; fasting plasma glucose (sugar), 141.1 ± 17.8 mg/dL; and suffering period, 11.5 ± 3.4 years. Patients were randomly divided into 2 groups. Ineligible patients included those who had ever taken any diuretics or estrogen for > 2 weeks.

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Biochemical Profile

	Control at 3 Days		Cerivastatin at 3 Days		Cerivastatin at 3 Months	
	Before	After	Before	After	Before	After
Total cholesterol, mg/dL	236.0±11.3	223.0±12.4	230.6±6.6	223.8±6.4	235.6±8.5	203.6±16.3*
Triglycerides, mg/dL	128.8±23.9	126.9±20.7	125.9±14.3	119.8±13.1	125.6±14.3	120.6±14.4
HDL cholesterol, mg/dL	57.0±9.0	54.7±7.9	58.1±4.9	54.9±4.0	58.6±7.8	59.2±10.3
sVCAM-1, ng · mL ⁻¹ · mg protein ⁻¹	11.29±1.89	11.54±1.93	12.79±1.03	12.49±0.89	11.73±1.04	10.18±0.92*
Thrombomodulin, ng · mL ⁻¹ · mg protein ⁻¹	29.3±7.7	30.1±7.9	28.0±3.5	29.5±3.6	25.5±5.0	28.3±3.7
8-Isoprostane, ng · mL ⁻¹ · mg protein ⁻¹	1110.4±62.6	1122.2±80.8	1034.5±61.9	978.4±54.9*	1170.6±28.3	992.5±121.6*
CRP, ng/mL	2626.5±632.1	2598.2±529.7	2966.5±567.2	2581.2±564.3	2948.5±1029.1	1971.5±315.4*
slCAM-1, ng · mL ⁻¹ · mg protein ⁻¹	6.40±0.83	6.39±0.59	6.16±0.99	5.78±2.23	6.44±0.91	6.14±0.81
von Willebrand factor, IU · mL ⁻¹ · mg protein ⁻¹	1.76±0.47	1.79±0.46	1.93±0.49	1.99±0.37	1.97±0.46	2.22±0.47

Values are mean±SD. slCAM-1 indicates soluble intercellular adhesion molecule 1.

**P*<0.05 vs before.

Vascular Function

Flow-mediated dilatation (FMD) and dilatation by nitroglycerin were determined according to a method described previously.⁷ In brief, the diameter of the right brachial artery was measured by a high-resolution ultrasound cardiograph (SONOS 2000, Hewlett Packard).⁸ Blood pressure was monitored every 2 minutes. To produce reactive hyperemia, blood flow to the forearm was prevented by inflation of the cuff on the arm to 250 mm Hg for 5 minutes. The diameter was measured from the anterior to the posterior interface between the media and adventitia, and it was calculated from 3 cardiac cycles synchronized with the R-wave peaks on ECG. Measurement at 60 seconds after cuff release showed the maximal dilatation. The diameter change was expressed as the percent change relative to diameter during the initial resting scan (%FMD). Fifteen minutes later, a resting scan was recorded and a sublingual nitroglycerin spray (300 µg, Toa Eiyō Co) was administered. Three minutes later, the last scan was performed. The diameter change was expressed as percent dilatation by nitroglycerin.

Blood Sampling

Blood sampling was performed on the morning of the ultrasound examination. Serum total cholesterol, triglyceride, and HDL cholesterol concentrations were measured.⁹ Plasma nitrite and nitrate levels (NO₂⁻ and NO₃⁻) were measured with an automated NO detector/high-performance liquid chromatography system (ENO10, Eicom Co), as previously reported.¹⁰ In brief, nitrite and nitrate levels in the dialysates were separated by a reverse-phase separation column, and nitrate was reduced to nitrite in a reduction column. Nitrite was mixed with a Griess reagent, and the absorbance at 540 nm was measured by a flow-through spectrophotometer. The concentration of cGMP was assayed using a cGMP radioimmunoassay kit. von Willebrand factor, soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble intercellular adhesion molecule 1, thrombomodulin, and 8-isoprostane (8-epi-prostaglandin F2α) were assessed by ELISA kits (Cytoscreen Immunoassay Kit, Bioxytech 8-isoprostane assay kit, Oxis international Inc). High-sensitivity serum C-reactive protein (CRP) levels were determined by turbidimetric immunoassay.

Safety Measures

All adverse events were recorded at each examination. Physical examinations, hematology, and serum and urine chemistry assays (liver and renal function and creatine phosphokinase) were conducted throughout the study.

Statistical Analysis

Two unpaired *t* tests were performed to compare the mean±SD values of each group of patients. Pearson's correlation coefficient was calculated to test the association between 2 variables with a normal distribution. In the case of a non-normal distribution, Spearman's rank correlation coefficient was used.

Results

Serum lipids concentrations (total cholesterol, triglycerides, and HDL cholesterol) were unchanged in all patients in response to 3 days of treatment with cerivastatin; however, total cholesterol decreased at 3 months in the cerivastatin group (Table). The %FMD in those receiving cerivastatin significantly increased at 3 days (Figure 1A). No difference in the response to nitroglycerin was demonstrated between the 2 groups before or after treatment (Figure 1B). The level of %FMD after 3 months of treatment with cerivastatin was comparable to that in patients receiving it for 3 days (Figure 1A). Plasma nitrite/nitrate and cGMP levels were also higher in patients receiving cerivastatin (Figure 2). Plasma concentrations of 8-isoprostane decreased, and those of CRP and sVCAM-1 tended to decrease after 3 days of treatment of cerivastatin (Table). CRP and sVCAM-1 levels decreased significantly, soluble intercellular adhesion molecule 1 levels tended to decrease, and von Willebrand factor levels tended to rise with 3 months of treatment (Table). No abnormal data were noted in the other biochemical data, including creatine phosphokinase levels, throughout the treatment term in either group (data not shown).

Discussion

Various mechanisms other than lipid lowering have been proposed to explain the antiatherosclerotic effects of statins, including antioxidant activity and enhanced NO activity.^{6,11} Statins reportedly increase eNOS activity in vitro.^{4,12} Because NO shows many antiatherosclerotic effects, such as inhibiting monocytes migration and smooth muscle cell proliferation, the increase in the activity of eNOS in response to statins may partially explain their antiatherosclerotic effects. FMD has been studied extensively in recent years, and it is believed to

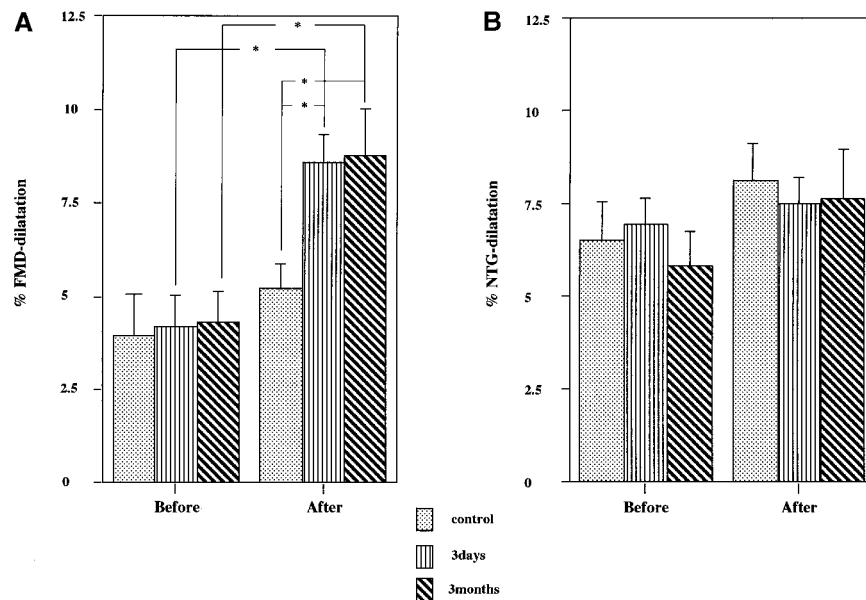


Figure 1. A, Endothelial function assessed by measuring dilatation of the brachial artery using high-resolution vascular ultrasound in response to reactive hyperemia (endothelial-dependent FMD). Percent increase in vessel diameter induced by FMD (% FMD) is shown. * $P < 0.05$. B, Endothelial-independent function assessed by measuring dilatation in response to sublingual nitroglycerin (NTG) infusion. Percent increase in diameter induced by nitroglycerin is shown. No significant differences were observed with or without cerivastatin treatment. Data are expressed as mean \pm SD.

constitute NO function in vessels.¹³ The impairment of FMD has been reported to precede coronary artery disease.¹⁴ Further, the sum of coronary risk factors, such as hyperlipidemia and diabetes mellitus, is related to the severity of impairment of FMD.¹⁵

In this study, age and diabetes, with or without hypercholesterolemia, had additive effects, and FMD was quite low. The endothelial function measured by FMD improved in diabetic elderly patients receiving cerivastatin for only 3 days, and their lipid profile did not change. The increases in plasma nitrite/nitrate and cGMP levels support the hypothesis that cerivastatin improves NO function. These increases might also be in line with the antiatherosclerotic effects of statins via the improvement of endothelial function. Because the FMD, nitrite/nitrate, and cGMP levels in the group receiving cerivastatin for 3 months were compatible to those in the group receiving cerivastatin for 3 days, a large part of

the effect of cerivastatin on endothelial NO may be due to a direct effect of the statin on vessels and not by an indirect effect through plasma lipid-lowering. Although statin treatment in humans has been reported to improve endothelial function in as short as 4 weeks,¹⁶ a short-term effect independent of plasma lipid levels, such as that in the present study, has not been reported previously.

One other finding in the present study was the decrease in 8-isoprostane, an oxidant marker, which was measured in plasma. In atherosclerotic arteries, the increase of oxygen radicals and their decrease by statin treatment is well known; however, this is the first report showing a short-term effect independent of lipid metabolism.¹⁷ We think this decrease is due to the direct effect of statins on vessels, because we observed that statins could decrease O_2^- release from rabbit aortas when rabbits were fed regular chow.¹⁸ However, the effects of statins on vessels must be further elucidated,

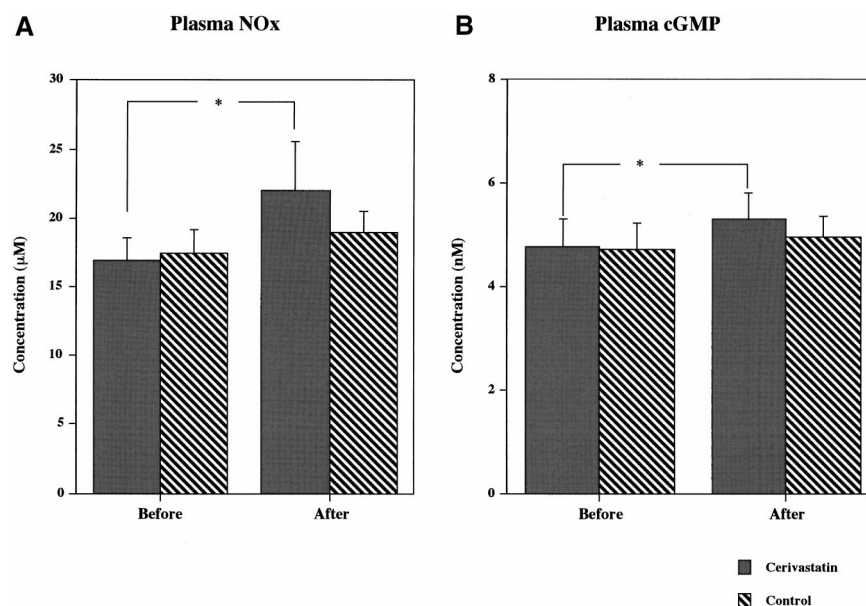


Figure 2. Plasma nitrite/nitrate (NOx; A) and cGMP levels (B) before and after cerivastatin treatment. Data are expressed as mean \pm SD. * $P < 0.05$.

because it is possible that an increase of NO downregulates an O_2^- releasing enzyme such as NADPH oxidase. Decrease of O_2^- releasing enzyme may contribute to the prolongation of NO lifespan and indirectly improve endothelial function.

8-Isoprostane levels at 3 months were comparable to those at 3 days. The difference in average levels of 8-isoprostane before and after 3 months of statin treatment was larger than that before and after 3 days of treatment; however, this difference did not achieve statistical significance. The difference may mean that statin treatment decreases oxidative stress with time. The decrease of CRP also supports cerivastatin's antiatherosclerotic effects, especially in the stabilization of atheroma. The levels of adhesion molecules and the markers of endothelial function tended to change in line with the antiatherosclerotic effect of statin treatment, although some changes did not attain statistical significance. CRP and sVCAM-1 levels after 3 months of cerivastatin treatment were substantially lower than those at 3 days, which may suggest that cerivastatin improves inflammation and endothelial cell activation with time. FMD levels after 3 months of cerivastatin treatment were not improved compared with those after 3 days. There may be a limitation of vascular dilatation due to atherosclerosis in elderly diabetic patients, because the amount of dilatation to nitroglycerin was not greater than FMD levels after statin treatment. Dilatation to nitroglycerin in this study occurred at levels lower than those measured in younger patients.^{15,19}

In conclusion, in elderly diabetic people, 3 days of treatment with cerivastatin may improve endothelial function without changing the lipid profile.

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