Reduction in Serum Cholesterol With Pravastatin Improves Endothelium-Dependent Coronary Vasomotion in Patients With Hypercholesterolemia

Kensuke Egashira, MD; Yoshitaka Hirooka, MD; Hisashi Kai, MD; Masaru Sugimachi, MD; Satoshi Suzuki, MD; Tetsuzi Inou, MD; Akira Takeshita, MD

Background This study aimed to determine if cholesterol-lowering therapy improves endothelium-dependent coronary vasomotion in patients with hypercholesterolemia.

Methods and Results Nine patients with hypercholesterolemia were studied before and after cholesterol-lowering therapy with pravastatin (an inhibitor of HMG-CoA reductase) for 6±3 months, which lowered serum cholesterol from 272±8 to 187±16 mg/dL (P<.01). Control patients with serum cholesterol of 218±23 mg/dL also were studied twice in a similar interval (8±2 months) with no cholesterol-lowering drugs. Acetylcholine (the endothelium-dependent vasodilator) and papaverine and nitrate (endothelium-independent vasodilators) were infused into the study coronary artery. Changes in the diameter of the epicardial coronary artery and coronary blood flow were assessed by quantitative coronary arteriography and an intracoronary Doppler catheter. In patients with hypercholesterolemia, acetylcholine-induced vasoconstriction of the epicardial artery was less (P<.05) and the acetylcholine-induced increases in coronary blood flow were greater (P<.001) after than before pravastatin. In control patients, responses of the epicardial coronary artery and coronary blood flow to acetylcholine did not change over the follow-up period. The vasomotor responses to papaverine or nitrate were similar between the two groups, and no interval changes in their responses were noted in either group.

Conclusions These results suggest that cholesterol-lowering therapy with pravastatin may improve endothelium-dependent coronary vasomotion, which may possibly contribute to the improvement of myocardial perfusion as well as the regression of coronary atherosclerosis. (Circulation. 1994;89:2519-2524.)

Key Words • hypercholesterolemia • cholesterol • endothelium-dependent relaxing factor • pravastatin

It is recognized that endothelial dysfunction is a major factor that contributes to the atherogenic process. Hypercholesterolemia is an important risk factor for coronary atherosclerosis, and it impairs endothelium-dependent vasorelaxation of the large coronary artery before the formation of atherosclerotic lesions. In addition, hypercholesterolemia is associated with blunted endothelium-dependent vasodilation of the coronary microcirculation in animals and humans, which may impair myocardial perfusion under hypercholesterolemia.

It has been shown that lowering serum cholesterol and regression of atherosclerosis improves defective endothelium-dependent vasodilation of atherosclerotic vessels in animals. Recently, Leung et al have shown that defective endothelium-dependent vasodilation of the large coronary artery evoked with acetylcholine is improved by cholesterol-lowering therapy with cholestyramine. However, it is not known whether lowering serum cholesterol restores endothelial function in coronary microcirculation in humans. This study attempted to determine if reduction of serum cholesterol with pravastatin, an inhibitor of HMG-CoA reductase, is associated with an improvement of endothelium-dependent coronary vasomotion in humans.

Methods

Patient Selection and Study Design

Nine consecutive patients with hypercholesterolemia and seven control patients who had single-vessel coronary artery stenosis >75% in one coronary artery and mild stenosis <40% in the other coronary artery branches were enrolled in this study. Patients with stenotic lesions in two or more coronary artery branches were excluded. All patients underwent percutaneous transluminal coronary angioplasty for effort angina pectoris. In each patient, either the left anterior or circumflex coronary artery was selected as the study artery, in which the angioplasty was not performed. Before they were enrolled in this study, no patient had received any cholesterol-lowering drugs. No patient had myocardial infarction, congestive heart failure, or left ventricular hypertrophy. All patients were treated with various anginal drugs. Serum total and high-density lipoprotein (HDL) cholesterol and triglycerides were determined by the enzyme assay method twice within 2 weeks. Low-density lipoprotein (LDL) cholesterol was calculated as [total cholesterol−HDL cholesterol−(triglyceride/5)].

After the baseline study was performed, nine patients with hypercholesterolemia (total cholesterol level ≥240 mg/dL) were assigned to receive 10 mg/d of pravastatin. If total cholesterol did not fall below 200 mg/dL after 2 weeks, the dose of the drug was increased to 20 mg/d. Seven control patients with serum cholesterol level <240 mg/dL were not treated with any cholesterol-lowering drug. Patients were allowed to take other antihypertensive or antianginal drugs,
which were not changed during the follow-up period. When clinical evidence suggesting restenosis such as angina-like chest pain occurred during the follow-up period, the patient was readmitted to our institution for coronary arteriography. Patients who did not have clinical evidence of restenosis were asked to be readmitted to our institution 6 to 12 months after follow-up for coronary arteriography.

The research proposal was approved by the institutional review committee for clinical research. Written informed consent was obtained from each patient after the study protocol was explained.

**Quantitative Coronary Arteriography**

Quantitative coronary arteriography was performed as we described previously.10,15,16 Angiograms were recorded on 35-mm cinefilm (60 frames/s) using a cineangiographic system (Siemens). The diameter of the segment of interest (an angiographically normal segment in the study artery 3 mm distal to the tip of the Doppler catheter) was measured blindly using a validated videodensitometric analysis system (Kontron Instruments). The diameter of the Judkins catheter was used for calibrating the arterial diameter in millimeters.

**Measurements of Coronary Blood Flow Velocity and Blood Flow**

A 3F Doppler flow velocity catheter (model DC-201, Millar Instruments) was introduced into the study coronary artery through a guiding catheter.15,16 The catheter tip was placed at the same site in the baseline and follow-up studies. Mean and phasic blood flow velocity signals were obtained by the use of a Millar DC-101 velocimeter. Coronary blood flow was estimated from the product of the mean coronary blood flow velocity and the cross-sectional area of the arterial segment. The papaverine-induced increase in coronary blood flow was assessed from the mean blood flow velocity and the baseline cross-sectional area.

**Study Protocol**

The study was performed by the percutaneous femoral approach in the fasting state. All antianginal medications were discontinued 12 hours before the study.

After completion of the diagnostic cardiac catheterization, the following studies were performed during continuous intravenous infusion of isosorbide dinitrate at 20 to 40 μg/min as described.15 First, papaverine (10 mg/5 mL) was administered through the guiding catheter; second, saline (0.5 mL/min for 2 minutes) through the Doppler catheter; third, acetylcholine (0.5 mL/min) at doses of 1, 3, 10, and 30 μg/min (for 2 minutes at each dose) through the Doppler catheter; and finally, isosorbide dinitrate 2 mg (2 mg/4 mL) through the guiding catheter in 1 minute.

The coronary blood flow velocity, arterial pressure, heart rate, and ECG were continuously monitored and recorded. Values during a steady-state condition were used for analysis.

**Statistical Analysis**

Coronary blood flow responses to acetylcholine were assessed in two ways: percent changes from the baseline level and slope of the dose-flow relation. The latter index was estimated using a linear regression analysis by plotting the percent changes in coronary blood flow (y axis) against four doses of acetylcholine in each patient (x axis: log[μg/min]).

Data are expressed as mean±SD. When serial changes in hemodynamic variables were compared within a group, one-way ANOVA for repeated measures was used. Comparisons of serial changes of these variables between the groups were done using two-way ANOVA. Student's t tests were used for comparisons of paired or unpaired data. A probability level of <.05 was considered significant.

**Results**

**Clinical Characteristics and Hemodynamic Variables**

Clinical characteristics are shown in the Table, all of which were comparable between the patients with hypercholesterolemia and control patients. One control patient and two hypercholesterolemic patients with smoking habits reported that they had quit smoking after their angioplasty; the proportion of patients who quit smoking did not differ between the two groups. The follow-up period varied from 3 to 12 months among patients, but the mean follow-up interval did not significantly differ between patients with hypercholesterolemia and control patients. Four patients with hypercholesterolemia and two control patients who developed angina as a result of restenosis were readmitted to our institution 3 to 6 months after angioplasty and underwent coronary angioplasty again; the second study was performed before the second angioplasty procedure in these patients. The other five patients with hypercholesterolemia and five control patients did not have evidence of restenosis and thus were readmitted at 6 to 12 months after angioplasty; the second study was performed at the follow-up arteriography.

Baseline mean arterial pressure and heart rate in patients with hypercholesterolemia (93±12 mm Hg, 66±11 beats per minute) did not differ from those in control patients (89±5 mm Hg, 72±13 beats per minute). There were no significant interval changes in these variables between baseline and follow-up studies in either group. Intracoronary administration of acetylcholine, papaverine, and isosorbide dinitrate did not alter mean arterial pressure and heart rate.

**Serum Lipid Data**

Serum total cholesterol and LDL cholesterol levels in the baseline study were significantly higher in patients with hypercholesterolemia (272±8 and 195±25 mg/dL, respectively) than in control patients (218±23 and 145±35 mg/dL, P<.05 for each). After follow-up, in patients with hypercholesterolemia, total and LDL cholesterol levels significantly decreased to 187±16 and 120±12 mg/dL (P<.01 for each versus before the follow-up), respectively, whereas in control patients, those levels did not change.

**Changes in Diameter of the Study Coronary Artery**

Baseline diameters of the large epicardial coronary artery under study before and after follow-up were 3.1±0.8 and 3.0±0.7 mm, respectively, in control patients and 3.0±1.1 and 3.1±1.2 mm in patients with hypercholesterolemia (NS between groups).

Intracoronary infusion of saline did not change the arterial diameter. In the baseline study, intracoronary infusion of acetylcholine decreased the coronary artery diameter in a dose-dependent manner. The percent decreases in arterial diameter evoked with acetylcholine were greater (P<.05) in patients with hypercholesterolemia than in control patients (Fig 1). After follow-up, the percent decreases in diameter evoked with acetylcholine were significantly attenuated in patients with hypercholesterolemia (P<.05 versus before treatment), whereas the response did not change in control patients. The percent increases in diameter evoked with isosor-
Clinical Characteristics of Control Patients and Patients With Hypercholesterolemia

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<th>Patient No.</th>
<th>Age, y/Sex</th>
<th>Hypertension</th>
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PTCA indicates percutaneous transluminal coronary angioplasty; LCx, left circumflex coronary artery; RCA, right coronary artery; LAD, left anterior descending coronary artery; N, nifedipine; NC, nicorandil; I, isosorbide dinitrate; D, diltiazem; B, β-blocker (metoprolol); and A, aspirin.

*The degrees of mild stenotic lesions in the study artery did not change during the follow-up period. The location of coronary atherosclerotic lesions is denoted according to the definition of American Heart Association (AHA Committee Report. A reporting system on patients evaluated for coronary artery disease. Circulation. 1975;51:7.).

bide dinitrate in the two groups did not differ before (7±12% and 6±12%) and after follow-up (8±6% and 8±10%).

Changes in Coronary Blood Flow

Saline infusion did not alter coronary blood flow. Acetylcholine progressively increased coronary blood flow in both groups. In the baseline study, the acetylcholine-induced increases in coronary blood flow tended to be less (P=.10) but not significantly so in patients with hypercholesterolemia than in control patients (Fig 2). In patients with hypercholesterolemia, the coronary blood flow response to acetylcholine improved significantly (P<.001) after the treatment, whereas the response did not change in control patients. The extent of cholesterol lowering correlated fairly well with changes in the dose-flow relation (r=.52, P<.05) and those in peak coronary blood flow response (r=.46, P=.065) to acetylcholine. The changes in coronary blood flow response to acetylcholine were comparable between patients with restenosis and without restenosis.

The percent increases in coronary blood flow with papaverine before and after follow-up were 271±85% and 289±100% (NS), respectively, in control patients and 337±112% and 326±102% (NS) in patients with hypercholesterolemia. The percent increases in coronary blood flow with isosorbide dinitrate before and after follow-up were 101±34% and 116±35% (NS), respectively, in control patients and 141±90% and 131±49% (NS) in patients with hypercholesterolemia.

Discussion

The results of this study suggest that cholesterol-lowering therapy with pravastatin was beneficial on endothelium-dependent coronary vasomotion in patients with hypercholesterolemia.

Endothelium-Dependent Vasomotion in the Large Epicardial Coronary Artery

Coronary vasomotion in responses to acetylcholine results from the net effects of endothelium-dependent vasodilation and direct vasoconstriction.17,18 Our results of acetylcholine-induced vasoconstriction of the epicardial coronary artery are consistent with previous reports that acetylcholine caused vasoconstriction of epicardial coronary artery segments in patients with coronary risk
factors or coronary artery stenosis because our patients had various coronary risk factors and mild atherosclerotic lesions in the study artery. In the baseline study, acetylcholine-induced vasoconstriction of the epicardial coronary artery was greater in patients with hypercholesterolemia than in control patients, whereas vasodilation evoked with nitrate was similar between the two groups. These findings are consistent with the previous suggestion that hypercholesterolemia is associated with abnormal endothelium-dependent vasomotion of the epicardial coronary artery in humans.

Our results showed that vasoconstriction of the large epicardial coronary artery evoked with acetylcholine was significantly less after cholesterol-lowering therapy with pravastatin in patients with hypercholesterolemia, whereas no interval changes in the responses to acetylcholine were seen in control patients (Fig 1). Vasodilation of the large epicardial coronary artery evoked with nitrate did not differ between baseline and follow-up studies in either group. These results agree with the report of Leung et al., who showed that cholesterol-lowering therapy with cholestyramine reversed defective endothelium-dependent vasodilation of the epicardial coronary artery with acetylcholine in patients with hypercholesterolemia.

**Endothelium-Dependent Vasomotion of the Resistance Coronary Artery**

Our results are consistent with the previous suggestion that endothelium-dependent vasodilation of the resistance
coronary artery is impaired in patients with hypercholesterolemia.\textsuperscript{9,10} The new finding of this study is that cholesterol-lowering therapy with pravastatin improved coronary blood flow responses to acetylcholine in patients with hypercholesterolemia, whereas no changes in the responses to acetylcholine were noted in control patients (Fig 2). The extent of cholesterol lowering was correlated with improvement in acetylcholine-induced coronary blood flow responses. The coronary blood flow responses to the endothelium-independent vasodilators papaverine and nitrate did not differ between the two groups, and there were no interval changes in these responses between baseline and follow-up studies in either group. These findings strongly suggest that reduction in serum cholesterol with pravastatin improved endothelium-dependent vasodilation of the resistance coronary artery in patients with hypercholesterolemia. To our knowledge, this study is the first demonstration that cholesterol-lowering therapy improves endothelium-dependent vasodilation of the resistance coronary artery in patients with hypercholesterolemia.

Limitations of the Study

This study was not a prospective, randomized trial. Our control patients did not receive placebo. The cholesterol levels were significantly lower in control patients; nevertheless, age, sex, coronary risk factors other than hypercholesterolemia, severity of coronary artery disease, and average follow-up period were comparable between patients with hypercholesterolemia and control patients.

The studies were done under antianginal drugs, which were unchanged during the follow-up period and were comparable between the two groups. In addition, we used the continuous infusion of isosorbide dinitrate during baseline and follow-up studies because all patients had significant flow-limiting stenotic lesions, at least in the baseline study. Infusion of isosorbide dinitrate was done in the same way at baseline and follow-up studies. Although infusion of nitrate might lead to underestimation of the coronary vasodilatory responses, we believe it unlikely that the beneficial effects of cholesterol-lowering therapy on endothelium-dependent coronary vasodilation was totally modulated by infusing nitrate.

Clinical Implications

Recent clinical trials demonstrated that cholesterol-lowering therapy markedly reduced cardiovascular events associated with a modest regression of atherosclerotic stenosis.\textsuperscript{2-5} Of note was that the cholesterol-lowering therapy decreased angina symptoms within months of treatment, in which time the regression of coronary atherosclerosis is unlikely to occur. We consider the possibility that marked reduction in clinical events by lowering serum cholesterol resulted from an improvement in endothelial function rather than regression of atherosclerotic stenosis per se. In this respect, our finding of the beneficial effects of lowering cholesterol on endothelium-dependent coronary vasomotion may be relevant. Recently, it has been reported that endothelium-dependent vasodilation of the resistance coronary artery is impaired in patients with angina pectoris and normal coronary angiograms, in whom abnormal vasomotion of resistance coronary artery may contribute to myocardial ischemia.\textsuperscript{19,20} Taken together, these findings tempt us to speculate that in patients with typical effort angina pectoris with critical stenosis in the epicardial coronary artery, impaired endothelium-dependent vasomotion may be involved at least in part in modulating myocardial perfusion under hypercholesterolemia.

Conclusions

This study suggests that cholesterol-lowering therapy with pravastatin for an average of 6 months improves endothelium-dependent coronary vasomotion in patients with hypercholesterolemia, which may contribute to correction of altered myocardial perfusion as well as the regression of coronary atherosclerosis. However, we do not know whether altered acetylcholine-induced coronary vasomotion after cholesterol-lowering therapy was caused by the increased endothelium-dependent vasodilation through muscarinic receptor stimulation, by the increase in flow-mediated endothelium-dependent vasodilation, by the decreased release of endothelin-derived contracting factors, or by altered sensitivity of vascular smooth muscle to acetylcholine.

Acknowledgments

This study was supported in part by Grants-in-Aid for Scientific Research 05670619 and 08587085 from the Ministry of Education, Science, and Culture, Tokyo; a Research Development Award from the Japan Heart Foundation 1992, Tokyo; Japan Cardiovascular Research Foundation Grant 1993, Osaka, Japan; and by ONO Medical Research Foundation Grant 1993, Tokyo.

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Circulation. 1994;89:2519-2524
doi: 10.1161/01.CIR.89.6.2519

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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