Effects of Probucol and Pravastatin on Common Carotid Atherosclerosis in Patients With Asymptomatic Hypercholesterolemia

Fukuoka Atherosclerosis Trial (FAST)

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OBJECTIVES
This study investigated the effect of reducing serum lipids on carotid artery intima-media thickness (IMT) in asymptomatic patients with hypercholesterolemia from Fukuoka, Japan.

BACKGROUND
Carotid atherosclerosis is a strong, independent predictor of morbidity and mortality in patients with coronary heart disease (CHD).

METHODS
A total of 246 asymptomatic hypercholesterolemic patients (mean age 66 years) were randomized to receive either probucol (500 mg/day, n = 82) or pravastatin (10 mg/day, n = 83) or to enter a control group (diet alone, n = 81); they were followed for two years. The change in IMT in the common carotid artery was the primary end point measure, and the incidence of major cardiovascular events was the secondary measure.

RESULTS
Over the two-year period, serum low-density lipoprotein (LDL) cholesterol was significantly reduced in the pravastatin group (36%), the probucol group (29%) and the control group (12%) (p < 0.0001, p < 0.0001 and p < 0.05, respectively). After two years, the probucol and pravastatin groups showed a significant reduction in IMT (−13.9% and p < 0.01 and p < 0.01, respectively), but there was significant IMT thickening (23.2%; p < 0.05) in the control group. Probucol reduced the rate of IMT increase, independently of its reduction of LDL or high-density lipoprotein cholesterol. Moreover, there was a significantly lower incidence of cardiac events in the probucol group (2.4%) than in the control group (13.6%) (p = 0.0136).

CONCLUSIONS
Probucol reduced cholesterol levels and stabilized plaque, leading to a lower incidence of cardiac events in these hypercholesterolemic patients. (J Am Coll Cardiol 2002;39:610–6)

Overwhelming evidence from epidemiologic and clinical studies (1) has demonstrated that low-density lipoprotein (LDL) cholesterol is a key element in the development of atherosclerosis, and that reducing LDL cholesterol levels leads to a lowered risk of coronary heart disease (CHD). Carotid atherosclerosis is a strong independent predictor of morbidity and mortality in patients with CHD (2). High plasma total LDL cholesterol concentrations are associated with an increased prevalence of carotid atherosclerosis, and drug therapy to reduce LDL cholesterol has been shown to decrease the extent of carotid atherosclerosis (3).

Measurement of the carotid artery intima-media thickness (IMT) by high-resolution B-mode ultrasonography has previously been used for the noninvasive detection of early carotid atherosclerosis. The IMT is also a reliable end point for intervention trials assessing disease progression. Furthermore, ultrasonography can directly quantify early atherosclerotic changes and the response to risk factor modification. In addition, the measurement of carotid artery IMT by high-resolution ultrasound shows less variability than that with angiographic measurement of the carotid arteries (4), so a smaller sample size is required to determine the benefits of treatment or to accurately assess the presence of early atherosclerosis.

Development of the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) has led to the widespread use of lipid-lowering drugs. The clinical benefit of statins for primary and secondary prevention of the cardiovascular complications of atherosclerosis, especially in association with coronary artery disease, has been investigated in several large clinical trials (5). The Kuopio Atherosclerosis Prevention Study (6) and the Carotid Atherosclerosis Italian Ultrasound Study (7) have recently evaluated the effect of pravastatin on carotid atherosclerosis, as measured by B-mode ultrasonography. Both studies showed a significant reduction in the rate of carotid artery IMT progression (6,7).

Probucol, 4,4′-isopropylidenedithio bis(2,6-di-tert-butylphenol), has been reported to prevent atherogenesis by acting as an antioxidant and suppressing the oxidative modification of LDL, in addition to its recognized action in lowering cholesterol levels (8). It has been shown to cause marked regression of cutaneous (9) and tendon (10) xan-
thomas in familial hypercholesterolemia. Retardation of the progression of coronary (11) and femoral (12) atherosclerosis has been related to a decrease of LDL cholesterol and an increase of high-density lipoprotein (HDL) cholesterol levels, in agreement with the general concept of the role of HDL in reverse cholesterol transport (13). Because probucol is known to decrease HDL cholesterol, it seems important to study the effect of this drug on atherosclerosis.

The aim of the present study was to determine whether treatment of hypercholesterolemic patients with probucol or pravastatin could retard the progression and promote the regression of carotid atherosclerosis, by measuring the effect of two years of treatment with probucol or pravastatin on carotid artery IMT in hypercholesterolemic patients. To the best of our knowledge, this is the first study to use B-mode ultrasonography to compare the effects of probucol and pravastatin on carotid atherosclerosis.

METHODS

Study group. Between February 1996 and February 2000, asymptomatic hypercholesterolemic patients who met the following criteria were eligible to be enrolled in the study: 1) primary hypercholesterolemia (defined as a serum total cholesterol level of at least 220 mg/dl); and 2) treatment with either probucol or pravastatin. A total of 246 Japanese patients between the ages of 30 and 89 years were enrolled.

Exclusion criteria included a serum triglyceride level ≥350 mg/dl; uncontrolled heart failure; recent (<6 months) myocardial infarction (MI); severe or unstable angina pectoris; hypothyroidism/hyperthyroidism or other endocrine diseases; secondary hyperlipidemia; uncontrolled diabetes mellitus; uncontrolled hypertension; heavy drinking; obese patients on weight reduction programs; diseases that might interfere with drug absorption; any severe illness; and treatment with certain drugs, including corticosteroids, other lipid-lowering agents or antacids containing aluminum salts. Written, informed consent was obtained from each patient, and the trial was approved by the Ethics Committee of Kyushu University Hospital.

Study design. Patients were randomly assigned to one of the following three groups: 1) a probucol group (n = 82, age 41 to 80 years) that received probucol at 500 mg twice daily after meals; 2) a pravastatin group (n = 83, age 41 to 89 years) that received pravastatin at 10 mg/day after the evening meal; and 3) a control group (n = 81, age 30 to 89 years) that was on diet alone. Randomization was done by the minimization method, controlling for the following four factors: total cholesterol level, age, gender and IMT. Hospital visits for monitoring were scheduled after two weeks and every four weeks thereafter. At each visit, a brief physical examination was done, and the number of tablets was counted to assess compliance. Measurement of lipids, lipoproteins and safety laboratory variables was also done at each visit. Thirty-four (21%) of the 165 patients in the intent-to-treat population did not complete the study.

DEFINITION OF END POINTS. The primary end point was the rate of progression of carotid atherosclerosis, which was measured as the slope of the change in the mean IMT of six carotid segments (3 sites each in the left and right common carotid arteries, which were 2, 2.5 and 3 cm proximal to the carotid bifurcation) on ultrasound examination. An increase in IMT over two years was defined as “progression” and a decrease in IMT over the same period was defined as “regression.” Ultrasonography was performed at enrollment and then every six months for the next 24 months. The secondary end point was the incidence of major atherosclerotic events, as effected by each treatment.

CLINICAL EVENTS. Clinical cardiac, cerebrovascular and peripheral vascular events, as well as deaths, were monitored. The time until the first cardiac event (coronary angioplasty, coronary artery bypass graft surgery, definite or probable MI or unstable angina pectoris requiring hospital admission) and the time until death from any cause were determined.

B-MODE ULTRASONOGRAPHY. The procedure used for measuring carotid artery IMT, as well as its reproducibility, has been described elsewhere (14). In brief, ultrasonography was performed with the patient in the supine position, using an Aloka SSD-2000 system (Aloka, Tokyo, Japan) with a 7.5-MHz transducer. All examinations were performed by one trained physician who had no knowledge of the clinical history and risk factor profile of the subjects. The IMT of the far wall of the carotid artery was measured at 2, 2.5 and 3 cm proximal to the carotid bifurcation in each of the right and left common carotid arteries. Measurements were made on longitudinal scans obtained in the anterior oblique, lateral and posterior oblique views. The IMT was defined as the distance between two echogenic lines separated by a hypoechoic or anechoic space, with the outer line corresponding to the media-adventitia border and the inner line representing the lumen-intima border. The mean IMT was calculated as the average value of the IMT measurements for the six sites in the carotid arteries. Stenosis was defined as plaque (a site where IMT was ≥1.10 mm) occupying more than half of the luminal circumference of the artery on a transverse scan. A three-lead electrocardiogram was recorded simultaneously, and videotape recording of the examination was done continuously. Images of each of the IMT measurement sites were recorded on super VHS videotape for subsequent off-line analysis.
Laboratory tests. Blood samples were collected between 8 and 9 AM after a 12-h fast. Serum cholesterol and triglyceride levels were measured enzymatically. High-density lipoprotein cholesterol was measured in the supernatant containing lipoprotein B–containing lipoproteins, according to the calcium heparin method, and LDL cholesterol was computed using Friedewald’s formula (15). All measurements were made on the day of blood collection, in which case the blood was stored for no longer than three days at −4°C until assay.

Statistical analysis. All data were recorded on standardized forms and were entered into a data base. Results are expressed as a percentage or mean value ± SD. Differences in frequencies were compared using the chi-square test, as appropriate. Comparison of median values between more than two groups was done by the nonparametric Kruskal-Wallis test. Differences between serum lipid levels at baseline and after 6, 12, 18 and 24 months were compared using Friedman’s test, with the Bonferroni correction for multiple comparisons. A p value <0.05 was considered statistically significant in all analyses. All data were analyzed on an intent-to-treat basis.

RESULTS

Baseline characteristics. The baseline characteristics of the subjects are summarized in Table 1. The mean age of the patients was 66.1 years, and 31.3% were men. Average systolic and diastolic blood pressures were 130.8 and 77.1 mm Hg, respectively. Of the 246 patients, 146 (59.3%) were recent or former smokers, 102 (41.5%) had hypertension and 56 (22.8%) had diabetes mellitus. Baseline serum total cholesterol and LDL cholesterol levels were 253.0 and 166.1 mg/dl, respectively. The HDL cholesterol and serum triglyceride levels were 57.0 and 149.2 mg/dl, respectively. The mean IMT was 1.308 mm. There were no statistically significant differences in any of these baseline characteristics among the three groups.

Drug treatment and serum lipids. The changes in total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides are shown in Figure 1.

TOTAL CHOLESTEROL. There was a significant decrease in the serum total cholesterol level after 12 months of therapy—by 20.0% in the probucol group and by 20.1% in the pravastatin group (both p < 0.001 by Friedman’s test). After 24 months of therapy, there was a further decrease in both groups—24.1% and 23.0% total reductions, respectively, when compared with baseline (both p < 0.001; Friedman’s test). In the control group, the total cholesterol

<table>
<thead>
<tr>
<th>Table 1. Baseline Characteristics of the Study Group</th>
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<tr>
<td>Total (n = 246)</td>
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<td>---------------------------------------------------</td>
</tr>
<tr>
<td>Age (years)</td>
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<tr>
<td>Men (%)</td>
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<tr>
<td>Blood pressure (mm Hg)</td>
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<tr>
<td>Systolic</td>
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<tr>
<td>Diastolic</td>
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<tr>
<td>Body mass index (kg/m²)</td>
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<tr>
<td>Smoking (%)</td>
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<tr>
<td>Recent or former</td>
</tr>
<tr>
<td>History (%)</td>
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<tr>
<td>Hypertension</td>
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<tr>
<td>Diabetes</td>
</tr>
<tr>
<td>Cerebral vessel disease</td>
</tr>
<tr>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
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<tr>
<td>High-density lipoprotein</td>
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<tr>
<td>Serum triglycerides (mg/dl)</td>
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<tr>
<td>Intima-media thickness (mm)</td>
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</table>

Data are presented as the mean value ± SD or number (%) of patients.
level was reduced at the end of the study, but the difference was not significant.

LOW-DENSITY LIPOPROTEIN CHOLESTEROL. Serum LDL cholesterol levels were also reduced after 12 months, falling by 24.2% in the probucol group (p < 0.001 by Friedman’s test), by 32.7% in the pravastatin group (p < 0.001 by Friedman’s test) and by 5.1% in the control group. After 24 months, there was a further decrease in all three groups, with a decline of 28.6%, 35.9% and 8.5%, respectively, when compared with baseline (p < 0.001, p < 0.001 and p < 0.05, respectively, by Friedman’s test).

HIGH-DENSITY LIPOPROTEIN CHOLESTEROL. The serum HDL cholesterol level of the pravastatin group was increased significantly (by 6.4%) after 24 months (p < 0.05 by Friedman’s test). In the control group, HDL cholesterol also increased between baseline and 24 months (by 5.4%), but the change was not significant. In contrast, the HDL cholesterol level of the probucol group was significantly reduced after 6 months (21.8%; p < 0.001 by Friedman’s test) and after 24 months (20.7%; p < 0.05 by Friedman’s test).

TRIGLYCERIDES. Triglyceride levels showed no significant changes throughout the course of the study.

Intima-media thickness. The IMT changes in the probucol, pravastatin and control groups are shown in Figure 2. In the probucol group, IMT was significantly reduced after 12 months of therapy (8.3%; p < 0.01 by Friedman’s test). After 24 months of therapy, there was a further significant reduction, for a total reduction of 13.9%, compared with baseline (p < 0.01 by Friedman’s test). No significant reduction in IMT was found during the first 18 months of the study.
therapy in the pravastatin group, but there was a significant reduction by 13.9% after 24 months (p < 0.01 by Friedman's test). In the control group, IMT increased significantly by 23.3% after 24 months (p < 0.05 by Friedman's test). The change in IMT was significantly greater in the probucol and pravastatin groups than in the control group (both p < 0.001 by the Kruskal-Wallis test). There was no significant difference in the change in IMT between the probucol and pravastatin groups at 24 weeks after the end of treatment.

**Multiple regression analysis of the reduction in IMT.**
Forward stepwise multiple linear regression analysis was performed to assess the factors influencing IMT regression. Lipid-lowering therapy was found to be the most important independent factor associated with the rate of IMT regression (F = 13.60, p < 0.0001). The reduction of LDL cholesterol after two years of treatment was also independently associated with the rate of IMT regression (F = 12.28, p = 0.0007). In contrast, age showed an independent association with the rate of IMT progression (F = 11.53, p < 0.0009). There were no significant associations between IMT changes and any of the other variables investigated.

**Correlation between percent reduction in IMT progression and LDL cholesterol.** There was a weak but significant and positive correlation between the absolute change in IMT and the change in LDL cholesterol in the probucol group (r = 0.363, p = 0.0051 by Spearman's method), but there was no such correlation in the probucol or control group (r = 0.065, p = 0.5892 and r = 0.130, p = 0.3321, respectively, by Spearman's method). There was no correlation between the absolute change in IMT and the change in HDL cholesterol in any of the groups.

**Cumulative incidence of major cardiovascular events and mortality.** Among the 82 patients in the probucol group, two had a major cardiovascular event (2 died of CHD), compared with 4 of the 83 patients in the pravastatin group (3 died of CHD and 1 had a nonfatal MI) and 11 of the 81 patients in the control group (8 died of CHD and 3 had a nonfatal MI) (Table 2, Fig. 3). Of the 16 deaths that occurred during this study, two were in the pravastatin group, 5 were in the pravastatin group and 9 were in the control group. Among these 16 deaths, 13 were from cardiovascular causes, whereas the others were from gastrointestinal bleeding and infection. There was a significantly lower incidence of cardiac events in the pravastatin group than in the control group (p < 0.05 by the log-rank test). There was also a lower incidence of death in the pravastatin group than in the control group, but the difference was not significant.

**DISCUSSION**

The present study adds new information to the ever-growing pool of data on anti-atherogenic regimens. The Fukuoka AtheroSclerosis Trial (FAST) is the first study to demonstrate the benefit of probucol in patients with hypercholesterolemia and also to demonstrate an effect of probucol on the incidence of cardiovascular disease.

Probucol is among the most extensively used lipid-lowering drugs and has been associated with many potentially beneficial outcomes. Despite this, probucol therapy lost some credibility after the apparently negative findings of the Probucol Quantitative Regression Swedish Trial (PQRST) (16). The changes in IMT in our probucol group are in striking contrast to those reported in PQRST, in which the target vessel was the femoral artery. However, reports of IMT regression in the femoral artery are limited, and the arterial caliber monitoring method used in PQRST could potentially be influenced by arterial remodeling (17).

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**Table 2.** Serious Adverse Events Reported During the Two-Year Follow-Up Period

<table>
<thead>
<tr>
<th>Clinical Event</th>
<th>Probucol Group (n = 82)</th>
<th>Pravastatin Group (n = 83)</th>
<th>Control Group (n = 81)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cardiovascular events</td>
<td>2 (2.4%)</td>
<td>4 (4.8%)</td>
<td>11 (13.6%)</td>
</tr>
<tr>
<td>Fatal MI</td>
<td>2 (2.4%)</td>
<td>3 (3.6%)</td>
<td>8 (9.9%)</td>
</tr>
<tr>
<td>Nonfatal MI</td>
<td>0</td>
<td>1 (1.2%)</td>
<td>3 (3.7%)</td>
</tr>
<tr>
<td>PTCA/CABG</td>
<td>0</td>
<td>1 (1.2%)</td>
<td>0</td>
</tr>
<tr>
<td>Total cerebrovascular events</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total other events</td>
<td>0</td>
<td>2 (2.4%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>Total deaths</td>
<td>1 (2.4%)</td>
<td>5 (6.0%)</td>
<td>9 (11.1%)</td>
</tr>
</tbody>
</table>

Data are presented as the number (%) of patients.
CABG = coronary artery bypass graft surgery; MI = myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty.
B-mode ultrasonography. The B-mode ultrasonography modality used in this study is an attractive tool for assessing the effect of drugs on atherosclerosis. The amount of data obtained by this method is growing through a number of other clinical trials now in progress. Moreover, increasingly precise estimates of the progression rate under various clinical conditions and the effect of treatment on progression have become available (16). This may be because cardiovascular events influence the vessel wall rather than the lumen and because the association between arterial IMT and the lumen diameter is imprecise (17). In support of this view, we found that the IMT progression in the common carotid artery was significantly reduced in the probucol and pravastatin groups, but not in the control group. The changes in carotid artery IMT in the pravastatin group resembled those reported previously (6,7).

Lipid-lowering effect. Evidence from epidemiologic and clinical studies has demonstrated that LDL cholesterol is a key element in the development of atherosclerosis, and that reducing LDL cholesterol levels leads to a lower risk of CHD. Recent studies using pravastatin have demonstrated a substantial reduction in cholesterol, along with a significant reduction in coronary and all-cause mortality (18). The three groups in this study showed a substantial reduction in the LDL cholesterol level. We found the reduction in LDL cholesterol to be more significant in the pravastatin than in the probucol and control groups. Although the control group showed a significant reduction of LDL cholesterol by diet alone, progression of IMT still occurred, unlike the outcome in the active treatment groups. This suggests that life-style modification alone did not adequately retard IMT progression.

Another mechanism of the anti-atherogenic effect of probucol. Our study revealed that probucol not only reduced the LDL level, but also caused a decrease in HDL cholesterol. This reduction in HDL cholesterol by probucol may reflect an increase in reverse cholesterol transport, most likely resulting from the direct induction of cholesterol ester transfer protein (CETP), consequently having an anti-atherogenic effect (19), because probucol has been reported to enhance reverse cholesterol transport from the periphery to the liver by HDL (20), and this activity appears to be dependent on increased CETP activity (19). However, probucol therapy retarded the progression of IMT, independent of its LDL or HDL cholesterol-lowering effect in the present study. Moreover, the IMT reduction occurred earlier with probucol than with pravastatin. These findings suggest that there may be another mechanism involved in the effect on IMT progression that is independent of the lipid-lowering effect of probucol.

Stabilization of plaque. Numerous pathophysiologic observations in humans and animals have led to the response-to-injury hypothesis of atherosclerosis, which emphasizes endothelial dysfunction as the first step in the atherosclerotic process. Endothelial dysfunction is possibly related to an increase of LDL and modified LDL, genetic alterations, elevation of plasma homocysteine or infection with microorganisms, or a combination of these or other factors (21). The atherosclerotic process leads to the enlargement of vulnerable plaques, which are characterized by a lipid-rich core and a thin fibrous cap. The advanced stages are associated with accumulation of monocytes and lymphocytes, platelet adhesion and apoptosis. Cardiac events, such as acute coronary syndromes (e.g., unstable angina and MI), appear to result from plaque rupture (22). Probucol has also been shown to modify vascular remodeling after balloon angioplasty, and remodeling is an important component of the restenotic process (23). In addition, probucol has a radical-scavenging effect and inhibits the production of platelet-derived growth factor and interleukin-1, giving it an anti-inflammatory effect (24). Such reports suggest that probucol may not only have a lipid-lowering effect, but may also stabilize plaque. Interestingly, the present study demonstrates that the incidence of cardiac events was lower in the probucol group than in the control or pravastatin group.

Study limitations. Because the sample size was small in the present study, a difference in the effects of probucol and pravastatin on IMT could not be demonstrated. Therefore, a large-scale investigation will be necessary to determine whether probucol and pravastatin show any differences in their effect on IMT. The lack of a placebo control group was another limitation. However, the use of quantitative B-mode ultrasonography allowed us to obtain unbiased data.

Conclusions. Probucol was effective in reducing the cholesterol level and stabilizing plaque in hypercholesterolemic patients, which probably explains a reduction in the incidence of cardiac events.

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