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Probucol Trial for Secondary Prevention of Atherosclerotic Events in Patients with Coronary Heart Disease (PROSPECTIVE)

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Aims: Although intensive statin therapy reduced cardiovascular risks, cardiovascular events have not been completely prevented. Probucol is a potent antioxidant and reduces tendon xanthomas in familial hypercholesterolemia patients despite reduction of high-density lipoprotein (HDL)-cholesterol (HDL-C). We investigated whether probucol can reduce cardiovascular events on top of conventional lipid-lowering therapy in patients with coronary heart disease (CHD).

Methods: PROSPECTIVE is a multicenter, randomized, prospective study that recruited 876 Japanese patients with CHD and dyslipidemia with a low-density lipoprotein (LDL)-cholesterol (LDL-C) level of ≥ 140 mg/dL without medication or those treated with lipid-lowering drugs. Lipid-lowering agents were administered during the study period in the control group (n=438), and probucol 500 mg/day was added to lipid-lowering therapy in the probucol group (n=438). Patients were randomly assigned to two treatment groups by adjusting the LDL-C level and presence of diabetes and hypertension and followed up for more than 3 years. The primary end point was a composite of cerebrovascular and cardiovascular events (cardiovascular disease death including sudden death, nonfatal myocardial infarction, nonfatal stroke, hospitalization for unstable angina, hospitalization for heart failure, or coronary revascularization). The secondary end point was carotid intima–media thickness in a subset of patients.

Results: The incidence of the primary end point showed a trend to be lower in the probucol group compared with that in the control group despite reduced HDL-C without serious adverse events. Anti-atherogenic effects of probucol may be attributed to its potent antioxidative function and enhancement of reverse cholesterol transport.

Conclusion: Since there was no statistical significance between the probucol and control groups despite a marked reduction of HDL-C, further studies on the clinical outcomes of probucol on top of conventional therapy may be necessary in the future (UMIN000003307).

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Key words: Probucol, Coronary heart disease, Prevention, Reverse cholesterol transport, Antioxidants

Introduction

Although intensive low-density lipoprotein (LDL)-lowering therapy using statins[1], in combination with an intestinal cholesterol transporter inhibitor (ezetimibe)[2] and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors such as evolocumab[3] and alirocumab[4], could significantly reduce the event rate of atherosclerotic cardiovascular diseases, these events have not been completely prevented. Therefore, additional effective pharmacological intervention is necessary to mitigate "residual risks" in combination with statins.

Probucol is a diphenolic compound with potent antioxidant and anti-inflammatory properties[5, 6]. Probucol was developed to reduce LDL-cholesterol (LDL-C) levels before the development of statins[7-10] and has been used for hypercholesterolemic patients in Japan, especially those with familial hypercholesterolemia (FH) and Achilles tendon xanthomas[11].

Probucol reduces LDL-C levels even in LDL receptor-deficient Watanabe heritable hyperlipidemic (WHHL) rabbits[12] and in patients with homozygotes and heterozygotes of FH who are deficient in LDL receptor[13-14]. Thus, the LDL-C reduction by probucol was attributed to enhanced catabolism of LDL independent of an LDL receptor[14] and increased excretion of cholesterol into bile[15]. Furthermore, probucol has a potent antioxidative effect, inhibits LDL oxidation[16], and reduces skin and tendon xanthomas in patients with FH[11] despite a marked reduction in HDL-C due to enhancement of cholesteryl ester transfer protein activity[17] and hepatic expression of scavenger receptor class B type I (SR-BI)[18]. Probucol attenuated atherosclerosis in WHHL rabbits independent of its lipid-lowering effect[19]. Probucol, but not α-tocopherol or ubiquinone-10, protected WHHL rabbits against atherosclerosis[20].

Probucol Quantitative Regression Swedish Trial (PQRST)[21], a randomized controlled trial, was carried out in Sweden to elucidate whether 3 years' administration of probucol to hypercholesterolemic patients may reduce femoral atherosclerosis. Treatment of patients with probucol lowered LDL-C by 12% and HDL-C by 24%; however, lumen volume did not show a significant increase in the probucol-treated group. Thus, most of the Western countries quitted using probucol after the launch of statins. Probucol was withdrawn partly because of the reduction of serum HDL-C levels and possible QT interval prolongation, which might increase the possibility of ventricular arrhythmias.

Probucol Observational Study Illuminating Therapeutic Impact on Vascular Events (POSITIVE)[22] evaluated the long-term effects of probucol treatment on the risk of cardiovascular events in 410 patients with heterozygous FH. The primary outcome was the time to the first cardiovascular event involving hospitalization, and multivariate Cox regression analysis demonstrated that the hazard ratio of probucol use was 0.13 (95% confidence interval 0.05 to 0.34; $p<0.001$) in patients for secondary prevention. Although the hazard ratio was not significant in patients for primary prevention because significantly higher LDL-C levels were in the probucol group than in the non-probucol group, this retrospective study has strongly suggested that long-term treatment with probucol may prevent the secondary cardiovascular events in a very high-risk population such as FH. Thus, a current multicenter, randomized, prospective study has been designed to test the hypothesis that the addition of probucol to other lipid-lowering drugs prevents cerebrovascular and cardiovascular events in Japanese patients with coronary heart disease (CHD) and high LDL-C levels.

Patients and Protocol

Study Design and Recruiting Patients

The current PROSPECTIVE study is a randomized (1:1), prospective, open-label, multicenter clinical trial conducted in patients with hyper-LDL-cholesterolemia with a prior history of coronary events as CHD. The rationale and design of this study were already published elsewhere[23]. The two arms of the trial are a control group of patients on conventional lipid-lowering therapy (LLT) and a test group of patients on conventional LLT plus probucol treatment. The protocol was initially reviewed and approved by the institutional review board (IRB) of Osaka University Hospital and thereafter by the IRBs of the participating institutions. Investigators obtained IRB approval and permission from the head of each institution before conducting the study. This study is registered with UMIN (UMIN000003307, March 3, 2010). Participating facilities and study organization are described in detail in the Appendix.

The current study enrolled male and female

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patients older than 20 years with all of the following seven clinical statuses: 1) diagnosis of dyslipidemia with high LDL-C level (≧140 mg/dL) without any medication or those treated with any lipid-lowering drugs including statins before providing informed consent; 2) serum LDL-C level less than 200 mg/dL within 8 weeks before providing informed consent, as calculated by Friedewald's formula (LDL-C=total cholesterol – HDL-cholesterol – triglycerides (TG)/5); 3) history of acute myocardial infarction or angina pectoris more than 3 months before providing informed consent, old myocardial infarction, coronary artery bypass grafting (CABG) more than 3 months earlier, percutaneous coronary intervention (PCI) more than 9 months earlier, or PCI with no restenosis that was diagnosed by follow-up coronary angiography at 6–9 months after PCI; 4) normal cardiac function, mild or moderate heart failure (NYHA classification I or II); 5) older than 20 years at the time of informed consent; 6) no severe hepatic and renal dysfunction (AST <100 IU/L, ALT <100 IU/L, serum creatinine <1.5 mg/dL) within 4 weeks before providing informed consent; and 7) signed written informed consent for participation in the current study. The exclusion criteria were the presence of the following clinical statuses at the time of informed consent: 1) ongoing treatment with probucol within 6 months before the time of informed consent; 2) ongoing treatment with cyclosporine; 3) history of hypersensitivity reactions to probucol; 4) diagnosis of FH based on the NICE Clinical Guideline 71 [24]; 5) very high TG level (>400 mg/dL) within 8 weeks before providing informed consent; 6) markedly high HbA1c level (≧8%) on the most recent blood test; 7) frequent multifocal ventricular arrhythmia; 8) atrial fibrillation (Af) including paroxysmal Af; 9) long QTc interval on a resting electrocardiogram (>450 ms in males or >470 ms in females); 10) congestive heart failure (NYHA III or IV) or unstable angina; 11) participation in other clinical trials; 12) women who are pregnant, are lactating, might become pregnant, or wish to become pregnant within the study period; or 13) inappropriate candidate for participation as assessed by the doctors in the current study.

Randomization and Treatment

After the inclusion and exclusion criteria were screened and patient's consent was gained, a Web-based central registration system automatically and randomly assigned patients to either the control group (conventional LLT continued) or the test group (LLT with probucol) with 1:1 allocation rate based on the registered patient's data. In randomization, LDL-C level (140 mg/dL and more vs. less than 140 mg/dL), diabetes (with vs. without), and hypertension (with vs. without) were dynamically balanced between the two groups as adjusted allocation factors. As a protocol treatment, each of the lipid-lowering agents is administered continuously during the study period in the control group, and probucol (500 mg/day, 250 mg twice daily after breakfast and dinner) was added to the LLT in the test group within 6 weeks after the registration.

End Points

The primary efficacy end point was the presence and the time from registration until the first occurrence of cerebrovascular and cardiovascular events. Cerebrovascular and cardiovascular events were recognized as follows: 1) cardiovascular death including cardiac sudden death; 2) nonfatal myocardial infarction; 3) nonfatal cerebral stroke excluding transient ischemic attack; 4) hospital admission due to unstable angina, 5) hospital admission due to heart failure; and 6) all coronary revascularizations with either PCI or CABG.

The secondary efficacy and safety end points were as follows: 1) all death; 2) all cerebrovascular and cardiovascular disease; 3) event-free survival time; 4) levels of the mean intima–media thickness (IMT) of carotid arteries and their changes; 5) levels of max IMT in common or internal carotid arteries and their changes; and 6) severe adverse events and their frequency.

Measurements and Reporting Serious Adverse Events (SAEs)

All data were input to the case report after the protocol treatment was started. During the study period, patient's background, all cerebrovascular and cardiovascular events, all adverse events, and the confirmation of being alive were determined before the protocol treatment and at 3 months; at 1, 2, and 3 years after registration; and at the time of termination and the simultaneous outcome. At the same time points, the levels of total cholesterol, TG, and HDL-C were determined by the measurement method of each facility, and the data were input to the system. High-sensitivity CRP (hsCRP) and adiponectin levels were measured at the center laboratory (SRL Inc. Tokyo, Japan) using the specimen from the facility where it was available. In maximum (max)/minimum (min) IMT of the carotid arteries, doctors and clinical technologists at each facility used the same protocol to depict the same site as much as possible using echography, submitted the images to the Imaging Evaluation Committee, which evaluated their values before the protocol treatment and at 1, 2, and 3 years after

registration.

SAEs were graded according to NCI-CTCAE by each doctor. SAEs included death, events that could lead to death, events that require hospital admission or extended hospitalization, disorders, events that could lead to disorders, others that are serious as well as the former adverse events, or congenital disease or abnormality in post-generations. The efficacy and safety evaluation committee evaluated these to make recommendation of early termination or study changes to the principal investigator.

Statistical Design and Analyses

There have been no secondary prevention studies of probucol in dyslipidemic patients in Japan except for a retrospective study of patients with FH[22]. On sample size consideration in the study, we assumed that the morbidity of cerebrovascular and cardiovascular events in the control group would be 10.7% on the basis of the results from the statin-treated group of the Japan EPA Lipid Intervention Study (JELIS)[25]. In the test group, we assumed that the rate would be 5.4%, almost half of that in the statin-treated group, because the addition of probucol to conventional LLT might improve the morbidity of cardiovascular events as well as the primary prevention shown in the Fukuoka Atherosclerosis Trial (FAST)[26]. Sample size per group was calculated as 408 patients with a two-tailed significance level of 0.05 and a power of 0.8 in 4 years' accrual period and 3 years' follow-up. Considering that several patients will be dropped out from the analysis, we decided that the target number of patients in one group would be 430 and total target number of patients in the current study would be 860.

The patient population in statistical analysis was a full analysis set based on the intention-to-treat principle. The characteristics of patients' demographics and baseline values were compared between the two groups. For the primary efficacy end point, the presence of and time from registration until the first cerebrovascular and cardiovascular events were analyzed. By estimating event-free survival curves using the Kaplan–Meier method and the 95% confidence interval (CI) at 1, 2, and 3 years, event-free survival curves for the two groups were compared using the log-rank test stratified with random allocation factors: LDL-C level (140 mg/dL and more vs. less than 140 mg/dL), diabetes (with vs. without), and hypertension (with vs. without). The secondary end points of the period until all death and period until all cardiovascular and cerebrovascular disease and event-free survival time were analyzed in the same way as the primary end point. Levels of the mean and max IMT of carotid arteries from baseline to 1, 2, and 3 years were analyzed by a mixed-effects model with repeated measurements with baseline value (included only if the change from baseline was the response variable), allocation group, visit, and interaction between allocation group and visit as fixed effects and patient as a random effect. Comparison of groups was performed. The frequency of SAEs was summarized by preferred term and intensity, and compared using Fisher's exact test. The two-tailed significant level in statistical analysis was 0.05. All statistical analyses were done with SAS version 9.3.

Results

Patients

A total of 876 patients with CHD underwent randomization at 82 sites in Japan from June 2010 through February 2014, and after evaluations for safety and full analysis sets, 831 of 876 patients were assigned to either the control group (conventional LLT continued, 431 patients) or the test group (LLT plus probucol 500 mg/day, 400 patients) (**Supplemental Fig. 1**). The mean age of the patients was 70 years, and 26.6% of the patients were women; 35.9% of the patients had a history of myocardial infarction, 63.1% angina pectoris, 6.3% cerebrovascular disease, and 3.7% peripheral artery disease. Most of the patients (97.0%) qualified with an LDL-C level of less than 140 mg/dL. Of the patients, 80.3% were accompanied by hypertension and 42.0% by diabetes mellitus. The rate of use of secondary preventive therapies was high, at the trial entry, with 99.0% (unknown eight patients were treated as not used), 93.5%, 82.3%, and 34.4% of the patients taking lipid-lowering, antiplatelet, antihypertensive, and antidiabetic agents, respectively. Lipid-lowering agents included statins (92.7%) and resins (1.4%). After randomization using the LDL-C levels and the presence or absence of diabetes or hypertension, there were no significant differences in the background characteristics between the control group and the test group except for the NYHA classification ($p=0.0433$) and smoking status ($p=0.0119$). However, both NYHA classification and smoking status were not prognostic factors. The baseline characteristics of the patients in the two groups were well balanced except for the NYHA classification and smoking status, and these are shown in **Tables 1 and 2**. Lipid-lowering agents other than probucol included statins, an intestinal cholesterol transporter inhibitor, and resins. Lipid-lowering agents were continuously administrated during the study period in the control group, and probucol (500 mg/day, 250 mg twice daily) was added to LLT in the test group.

Table 1. Baseline demographics and other characteristics (qualitative variable)

Characteristics		Control group (N=431)	Test group (N=400)	P value
		No. of patients (%)		
Gender	Male	319 (74.0)	291 (72.8)	0.695
	Female	112 (26.0)	109 (27.3)	
NYHA classification	I	418 (97.0)	376 (94.0)	0.043*
	II	13 (3.0)	24 (6.0)	
LDL-C	< 140 mg/dL	418 (97.0)	388 (97.0)	1.000
	≥ 140 mg/dL	13 (3.0)	12 (3.0)	
Diabetes mellitus	No	250 (58.0)	232 (58.0)	1.000
	Yes	181 (42.0)	168 (42.0)	
Hypertension	No	89 (20.6)	75 (18.8)	0.542
	Yes	342 (79.4)	325 (81.3)	
Cerebrovascular disease	No	404 (93.7)	375 (93.8)	1.000
	Yes	27 (6.3)	25 (6.3)	
Peripheral artery disease	No	417 (96.8)	385 (96.3)	0.710
	Yes	14 (3.2)	15 (3.8)	
Smoking status	Current smoker	34 (7.9)	15 (3.8)	0.012*
	Ex-smoker	177 (41.1)	193 (48.3)	
	Never	219 (50.8)	192 (48.0)	
	Unknown	1 (0.2)	0 (0.0)	
Coronary heart disease	Myocardial infarction	160 (37.1)	138 (34.5)	0.728
	Angina pectoris	266 (61.7)	258 (64.5)	
	Others	5 (1.2)	4 (1.0)	
	Unknown	0 (0.0)	0 (0.0)	
Lipid-lowering agents	No	2 (0.5)	0 (0.0)	0.500
	Yes	428 (99.3)	393 (98.3)	
	Statin	404 (93.7)	366 (91.5)	
	Fibrate	18 (4.2)	13 (3.3)	
	Nicotinic acid and derivatives	2 (0.5)	1 (0.3)	
	Anion exchange resin	2 (0.5)	4 (1.0)	
	Others	56 (13.0)	48 (12.0)	
	Unknown	1 (0.2)	7 (1.8)	
Antihypertensive agents	No	77 (17.9)	69 (17.3)	0.855
	Yes	353 (81.9)	331 (82.8)	
	Ca channel blockers	208 (48.3)	183 (45.8)	
	ARB	186 (43.2)	207 (51.8)	
	Diuretics	47 (10.9)	51 (12.8)	
	ACE inhibitors	68 (15.8)	58 (14.5)	
	β-blockers	135 (31.3)	141 (35.3)	
	Others	28 (6.5)	33 (8.3)	
	Unknown	1 (0.2)	0 (0.0)	

(Cont. Table 1)

Characteristics		Control group (*N*=431)	Test group (*N*=400)	*P* value
		No. of patients (%)		
Antidiabetic agents	No	33 (7.7)	32 (8.0)	0.891
	Yes	149 (34.6)	137 (34.3)	
	Sulfonylurea	44 (10.2)	56 (14.0)	
	Insulin preparations	16 (3.7)	20 (5.0)	
	Insulin resistance improving agents	53 (12.3)	43 (10.8)	
	α-Glucosidase inhibitors	39 (9.0)	34 (8.5)	
	Others	95 (22.0)	77 (19.3)	
	Unknown	249 (57.8)	231 (57.8)	
Antiplatelet agents	No	30 (7.0)	23 (5.8)	0.482
	Yes	400 (92.8)	377 (94.3)	
	Cilostazol	30 (7.0)	21 (5.3)	
	Ticlopidine	35 (8.1)	38 (9.5)	
	Dipyridamole	5 (1.2)	2 (0.5)	
	Aspirin	350 (81.2)	334 (83.5)	
	Sarpogrelate	9 (2.1)	8 (2.0)	
	Clopidogrel	124 (28.8)	115 (28.8)	
	Others	25 (5.8)	25 (6.3)	
	Unknown	1 (0.2)	0 (0.0)	

In case of "yes", multiple choices were allowed.
Fisher's exact test was used to evaluate the significance of differences between groups.
*: The level of significance was set at $p<0.05$ (2-sided).
ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blockers; LDL-C, low-density lipoprotein cholesterol; NYHA, New York Heart Association

Table 2. Baseline demographics and other characteristics (quantitative variable)

Characteristics	Control group		Test group		*P* value
	N	mean ± SD or median (IQR)	*N*	mean ± SD or median (IQR)	
Age (years)	431	70.0 ± 9.4	400	70.0 ± 9.0	0.750
Height (cm)	431	160.2 ± 8.5	400	160.5 ± 8.8	0.561
Weight (kg)	431	63.2 ± 11.4	400	63.7 ± 12.0	0.635
TC (mg/dL)	431	169.5 ± 28.7	400	169.0 ± 28.6	0.591
HDL-C (mg/dL)	431	54.4 ± 14.4	400	53.6 ± 13.6	0.510
LDL-C (mg/dL)	431	90.4 ± 23.5	400	89.6 ± 22.7	0.511
TG (mg/dL)	431	107 (80 - 154)	400	114 (83 - 158)	0.302
hs-CRP (ng/mL)	69	570 (216 - 1640)	56	698 (409 - 1585)	0.268
Adiponectin (μg/mL)	69	8.60 (6.00 - 11.10)	56	8.00 (6.05 - 11.55)	0.880
AST (IU/L)	431	22 (18 - 28)	400	23 (19 - 27)	0.345
ALT (IU/L)	431	19 (15 - 26)	400	19 (15 - 27)	0.473
Serum creatinine (mg/dL)	431	0.87 ± 0.20	400	0.86 ± 0.21	0.711
HbA1c (%)	431	5.70 (5.30 - 6.20)	400	5.70 (5.35 - 6.20)	0.651
No. of cigarettes smoked on average daily (only current smokers)	34	20 (10 - 20)	15	10 (7 - 20)	0.327
Time from enrollment until the first onset of CAD (months)	429	48 (20 - 92)	399	50 (20 - 96)	0.461

Two-sample Wilcoxon test was used to evaluate the significance of differences between groups.
AST, aspartate transaminase; ALT, alanine transaminase; CAD: coronary artery disease; hs-CRP, high sensitive C-reactive protein; Hb, hemoglobin; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride

Withdrawal of consent occurred in 3 and 12 patients in the control and test groups, respectively. Premature permanent discontinuation of the study regimen (probucol) occurred in 21 patients in the test group. The durations of follow-up were 3.90 (3.12–4.77) (median [IQR]) years and 3.82 (3.15–4.79) years in the control group and the test group, respectively.

Lipid Data

The LDL-C levels at baseline were 90.4 ± 23.5 (mean ± standard deviation) mg/dL and 89.6 ± 22.7 mg/dL in the control group and the test group, respectively (**Table 2**). At 3 months, the absolute reduction of least square mean in LDL-C levels in the test group, as compared with that in the control group, was 7.3 mg/dL (95% CI, 4.7 to 9.9; $p < 0.0001$). The reduction in LDL-C levels was maintained over time (**Fig. 1a**). At 36 months, the absolute reduction of least square mean in LDL-C levels in the test group, as compared with that in the control group, was 8.5 mg/dL (95% CI, 5.4 to 11.7; $p < 0.0001$).

The baseline levels in the control group and the test group were 169.5 ± 28.7 and 169.0 ± 28.6 mg/dL for total cholesterol, 54.4 ± 14.4 and 53.6 ± 13.6 mg/dL for HDL-C, and 107 (80–154) and 114 (83–158) mg/dL for TG. Total cholesterol, HDL-C, or triglycerides levels in the test group at 3 and 48 months were consistently and significantly lower than those in the control group, except for the TG levels at 3 months (no difference between the two groups) (**Fig. 1b, c, and d**). At 3 months, the absolute reductions of least square means in total cholesterol, HDL-C, and TG levels in the test group, as compared with those in the control group, were 22.9 mg/dL (95% CI, 19.7 to 26.2; $p < 0.0001$), 14.9 mg/dL (95% CI, 13.7 to 16.2; $p < 0.0001$), and 2.6 mg/dL (95% CI, 6.2 to 11.5; $p = 0.5601$), respectively. At 36 months, the absolute reductions of least square means in total cholesterol, HDL-C, and TG levels in the test group, as compared with those in the control group, were 27.5 mg/dL (95% CI, 23.8 to 31.2; $p < 0.0001$), 16.3 mg/dL (95% CI, 14.8 to 17.7; $p < 0.0001$), and 10.8 mg/dL (95% CI, 2.3 to 19.3; $p = 0.0126$), respectively.

Efficacy End Points

The primary end point occurred in 44 patients (10.2%) in the control group and in 31 patients (7.8%) in the test group (adjusted hazard ratio, 0.746; 95% CI, 0.471 to 1.182; stratified log-rank test, $p = 0.1839$) (**Table 3 and Fig. 2**). We observed no significant difference between the control group and the test group in the incidence of primary endpoint of cerebrovascular and cardiovascular events. Additionally, there were no significant differences in the incidence of the key composites of the primary end point of cerebrovascular and cardiovascular events; death because of cardiovascular diseases including sudden death, nonfatal myocardial infarction, nonfatal stroke excluding transient ischemic attack, hospitalization for unstable angina, and hospitalization for heart failure or coronary revascularization such as PCI or CABG after at least 3 years of follow-up. As a note, although the differences did not reach statistical significance, the numbers of patients (%) tended to be decreased in the test group, compared with those in the control group, in the incidence of the key composites of the primary end point of cerebrovascular and cardiovascular events except nonfatal stroke excluding transient ischemic attack; death because of cardiovascular diseases including sudden death (0.8 vs. 1.2), nonfatal myocardial infarction (0.3 vs. 0.9), nonfatal stroke excluding transient ischemic attack (1.3 vs. 0.9), hospitalization for unstable angina (0.8 vs. 1.4), and hospitalization for heart failure (1.3 vs. 1.6) or coronary revascularization such as PCI or CABG after at least 3 years of follow-up (4.0 vs. 5.6).

There were no significant differences between the control group and the test group in the secondary efficacy and safety end points either; all death, all cerebrovascular and cardiovascular disease, event-free-survival time, levels of the mean IMT of carotid arteries and their changes, or levels of max IMT in common or internal carotid arteries and their changes (**Supplemental Fig. 2a–d**). In the current study, the number of patients for whom ultrasonography of carotid arteries was performed was small. There may be a limitation in the accurate measurement of mean and max IMT at the same point in each patient. Therefore, the tendency of reduction of IMT in the control group and slight increase of IMT in the probucol-treated group may reflect these limitations. Furthermore, the changes of IMT may not be related to the cerebrovascular and cardiovascular event rate.

In a limited number of patients, the serum concentrations of hsCRP and adiponectin were measured and compared between the control group and the test group. There were no significant differences in the changes of serum hsCRP and adiponectin concentrations between the control group and the test group (data not shown).

Safety

The SAEs occurred in 14 patients (3.2%; 95% CI, 1.8 to 5.3) in the control group and in 12 patients (3.0%; 95% CI, 1.6 to 5.2) in the test group (**Table 4**). There was no significant difference in the frequencies of SAEs between the two groups ($p = 1.0000$).

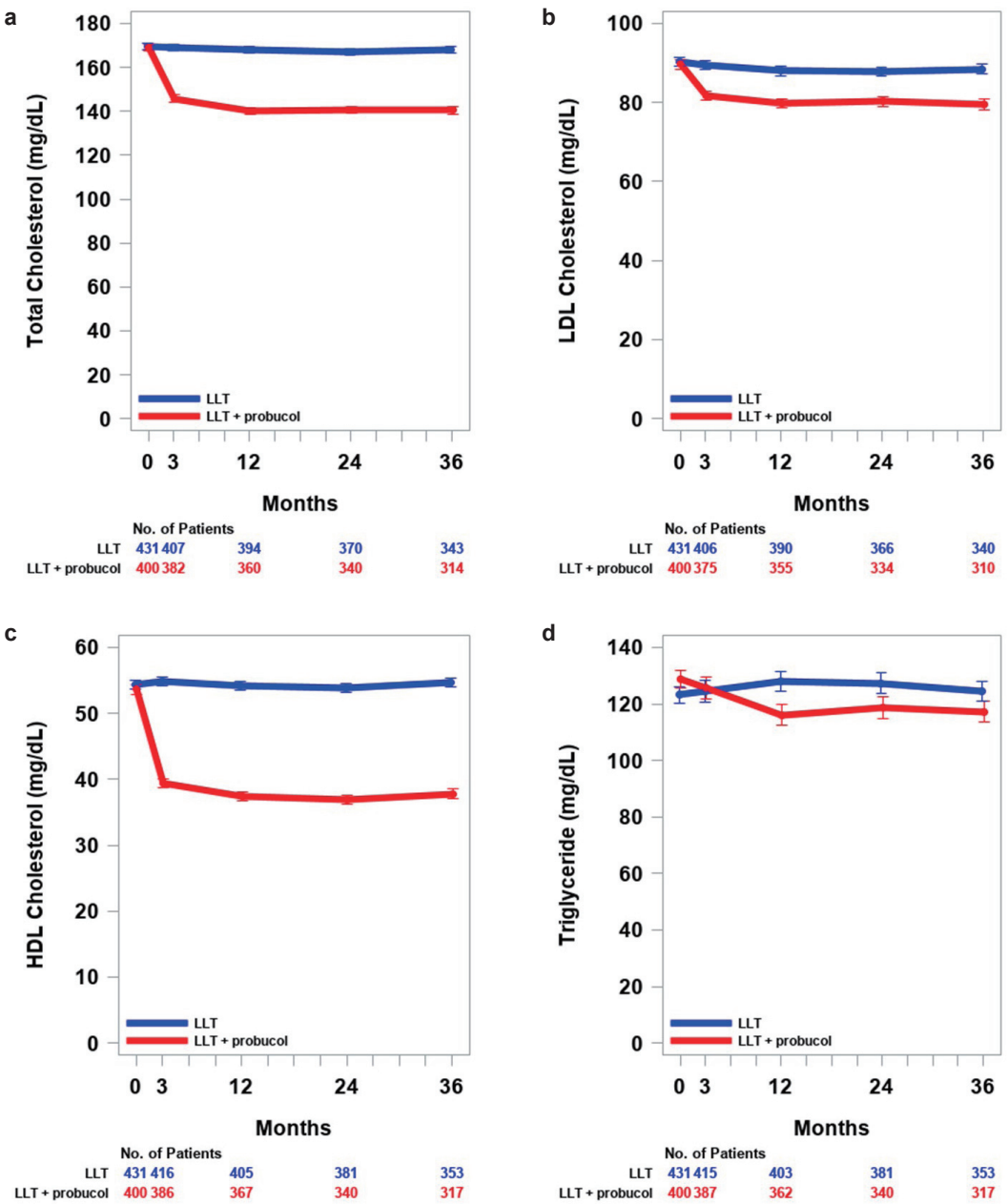

Fig. 1. Total cholesterol (a), LDL-cholesterol (b), HDL-cholesterol (c), and triglycerides levels (d) over time by treatment group (FAS)

Measured values at each visit were shown as least square mean with bars of standard error and analyzed by mixed-effects models with repeated measures (MMRM) with allocation group, visit (at baseline and 3, 12, 24, and 36 months), and interaction between allocation group and visit as fixed effects and patient as a random effect. Comparisons between treatment groups at each visit were made using the least square mean of each group. The MMRM analyses were performed using the Kenward–Roger method for adjusting degrees of freedom in an unstructured (UN) covariance structure. Blue and red circles with bars and lines show the control group (conventional LLT continued) and the test group (LLT plus probucol), respectively.

The incidence of any adverse events was significantly higher in the test group than in the control group (55.9% vs 46.7%; $p=0.0085$): note that the frequencies of ventricular arrhythmia and each composite of laboratory examination (other than increase in serum creatine phosphokinase) tended to be high in both groups, because all reports of no examination with the grade level of unknown were evaluated as

Table 3. The incidence of cerebro- and cardiovascular events

	Control group (*N*=431)	Test group (*N*=400)	*P* value*
	No. of patients (%)		
Cerebro- and cardiovascular events	44 (10.2)	31 (7.8)	0.228
Cardiovascular death including sudden cardiac death	5 (1.2)	3 (0.8)	0.727
Nonfatal myocardial infarction	4 (0.9)	1 (0.3)	0.375
Nonfatal stroke excluding TIA	4 (0.9)	5 (1.3)	0.745
Unstable angina requiring hospitalization	6 (1.4)	3 (0.8)	0.508
Heart failure requiring hospitalization	7 (1.6)	5 (1.3)	0.775
Coronary revascularization (PCI and CABG)	24 (5.6)	16 (4.0)	0.332

Fisher's exact test was used to evaluate the significance of differences between groups.
*: The level of significance was set at $p<0.05$ (2-sided).

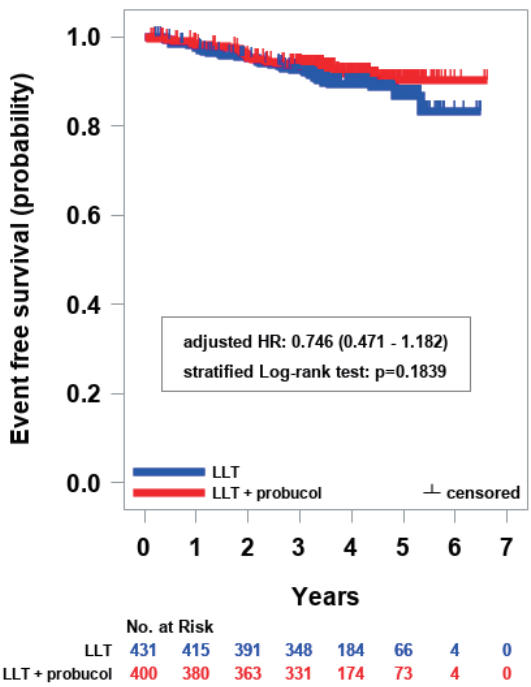

Fig. 2. Kaplan–Meier curves for the primary efficacy end point

Survival curves were estimated using the Kaplan–Meier method, and the stratified log-rank test was performed to compare the survival rates, using levels of LDL-C, presence or absence of diabetes, and presence or absence of hypertension as the stratified factors. The level of significance was set at $p<0.05$ (two-sided). The hazard ratio (HR) and 95% CI were estimated using the Cox proportional hazards model adjusted by allocation factors (levels of LDL-C, presence or absence of diabetes, and presence or absence of hypertension). Blue and red markers with bars and lines show the control group (conventional LLT continued) and the test group (LLT plus probucol), respectively.

adverse events. The incidence of ventricular arrhythmia, gastrointestinal disturbances, infectious or parasite diseases, metabolic or nutritional disturbances, benign or malignant neoplasms, mental disorders, and vascular diseases did not differ significantly between the two groups. However, the incidence of QT elongation on the electrocardiogram, a composite of laboratory examinations, was more frequent in the test group than the control group (35.9% vs. 25.3%; $p=0.0009$).

Discussion

Intensive therapies with statins and PCSK9 inhibitors have been shown to significantly reduce atherosclerotic cardiovascular events; however, these events have not completely been prevented even if LDL-C levels were lowered to less than 50 mg/dL. Therefore, additional effective pharmacological therapy intervention may be necessary to mitigate "residual risks" in combination with statins. The residual risks related to dyslipidemia include increased remnant lipoproteins and reduced HDL-C levels as well as oxidative stress and inflammation associated with dyslipidemia. In the current PROSPECTIVE study, we could not demonstrate that probucol can significantly reduce cardiovascular events in CHD patients treated with statins. However, we also showed that probucol treatment in the high-risk patients treated with statins was well tolerated and safe despite reduction of serum HDL-C. Probucol reduced LDL-C by 7.3 mg/dL at 3 months compared with control therapy, which was maintained over time (**Fig. 1**). Therefore, there might be a possibility that the reduction of LDL-C by probucol may be one of the reasons for a tendency to lower cardiovascular events.

Although we determined the sample size of this randomized controlled trial based on our retrospective

Table 4. Adverse events

Adverse events	Control group (*N*=435)	Test group (*N*=401)	*P* value
	No. of patients (%)		
Any adverse events	203 (46.7)	224 (55.9)	0.009*
Serious adverse events	14 (3.2)	12 (3.0)	1.000
Aortic valve stenosis	0 (0.0)	1 (0.2)	0.480
Haematemesis	1 (0.2)	0 (0.0)	1.000
Salivary gland calculus	0 (0.0)	1 (0.2)	0.480
Large intestine polyp	1 (0.2)	0 (0.0)	1.000
Death	2 (0.5)	1 (0.2)	1.000
Cholecystitis acute	0 (0.0)	1 (0.2)	0.480
Disseminated tuberculosis	1 (0.2)	0 (0.0)	1.000
Septic shock	0 (0.0)	1 (0.2)	0.480
Tibia fracture	1 (0.2)	0 (0.0)	1.000
Lumbar spinal stenosis	1 (0.2)	0 (0.0)	1.000
Bladder cancer	0 (0.0)	1 (0.2)	0.480
Breast cancer female	0 (0.0)	1 (0.2)	0.480
Gastric cancer	1 (0.2)	2 (0.5)	0.610
Lung cancer	1 (0.2)	1 (0.2)	1.000
Malignant pleural effusion	0 (0.0)	1 (0.2)	0.480
Pancreatic cancer	1 (0.2)	1 (0.2)	1.000
Renal cancer	1 (0.2)	0 (0.0)	1.000
Tongue neoplasm malignant stage unspecified	1 (0.2)	0 (0.0)	1.000
Hepatocellular carcinoma	1 (0.2)	1 (0.2)	1.000
Cerebral hemorrhage	1 (0.2)	0 (0.0)	1.000
Depression	1 (0.2)	0 (0.0)	1.000
Hypoxia	1 (0.2)	0 (0.0)	1.000
Aspiration pneumonia	0 (0.0)	1 (0.2)	0.480
Blue toe syndrome	1 (0.2)	0 (0.0)	1.000

Fisher's exact test was used to evaluate the significance of differences between groups.
*: The level of significance was set at $p<0.05$ (2-sided).

observational study, the study results may indicate that the sample size is underpowered to address the hypothesis. Other large-scale clinical studies that tried to show non-statin benefits, such as IMPROVE-IT[2], FOURIER[3], and ODYSSEY OUTCOMES[4], have recruited more than 10,000 patients. Therefore, we might need another trial to show the benefit of probucol in high-risk patients for cardiovascular disease with a larger sample size. Indeed, Kang *et al.* have also tried to investigate the effect of probucol on carotid IMT and the rate of cerebrovascular and cardiovascular events in Korea and China (IMPACT Trial) with a similar regimen[27]. We are planning to conduct a meta-analysis by combining the data in these three countries.

Probucol was withdrawn from the market because of its HDL-C lowering effect and QT elongation. Many CETP inhibitors have been developed to increase serum HDL-C levels as well as to decrease serum LDL-C levels. However, most of CETP inhibitors such as torcetrapib[28], dalcetrapib[29], and evacetrapib[30] that can increase serum HDL-C levels have failed to show cardiovascular risk reduction in patients treated with statins. Although anacetrapib has shown some benefits on top of statins[31], the pharmaceutical company (Merck) has quitted its development possibly because of its prolonged accumulation in adipose tissues[32] and relatively small event risk reduction. The failure of CETP inhibitors may be attributed partly to their strategy to increase serum HDL-C levels by inhibiting the transfer of cholesteryl ester from HDL particles, which resulted in the formation of very large cholesteryl ester-rich HDL particles with impaired antiatherogenic functions[33]. Thus, the strategies for

increasing serum HDL-C levels have not been shown to be effective to reduce the cardiovascular risk in statin-treated patients.

In contrast to CETP inhibitors, probucol has been shown to decrease serum HDL-C levels through enhancement of plasma CETP activity[17, 34] and hepatic expression of SR-BI[18, 35]. Probucol was shown to enhance hepatic SR-BI protein expression, possibly through species-specific stabilization of the protein[18]. The reduction of HDL-C by probucol may be explained by both an enhanced CETP-mediated transfer of cholesteryl esters from HDL to apolipoprotein B-containing lipoproteins and an increased hepatic selective uptake of cholesteryl esters from HDL via SR-BI. Probucol also enhanced the formation of lipid-poor prebeta1-HDL involved in cellular cholesterol efflux[36]. The reduction of serum HDL-C may reflect the enhancement of the reverse cholesterol transport[37, 38]. The HDL particles from probucol-treated patients are small and poor in cholesteryl ester and were shown to possess a more potent antiatherogenic activity against foam cell formation[39] and a more marked antioxidative activity partly due to an increase in antioxidative paraoxonase 1 (PON1) activity[40]. The LDL particles from probucol-treated patients were strongly protected from oxidation[16]. Probucol was also demonstrated to have anti-inflammatory effects[41, 42] and enhance xanthoma regression in patients with FH despite reduction in serum HDL-C levels along with the attenuated progression of atherosclerosis in animal models[43, 44].

Furthermore, clinical studies on the effects of probucol on xanthomas, restenosis after PCI, and atherosclerosis extensively reviewed in Yamashita *et al.*[10] demonstrated antiatherogenic effects of probucol in clinical settings. As described above, a retrospective POSITIVE study[22] in patients with heterozygous FH has implicated that long-term probucol treatment may reduce secondary cerebrovascular and cardiovascular events in a very high-risk population such as FH. The effect of probucol therapy on long-term survival following complete revascularization was also reported in 1,694 patients after complete revascularization with PCI and/or CABG by a propensity score analysis[45], in which the use of probucol was associated with a markedly significant decrease in all-cause death in a propensity score-adjusted model (hazard ratio=0.57; p=0.008). In post-match patients, the risk of all-cause mortality was markedly lower in the probucol group than in the non-probucol group (hazard ratio=0.45; p=0.002). Although probucol use was not an independent predictor of long-term survival from cardiac death, cardiac death tended to be lower, whereas non-cardiac death was significantly reduced in the probucol group than in the non-probucol group. Thus, probucol was demonstrated to significantly reduce all-cause mortality in CHD patients following complete revascularization.

A multicenter, randomized controlled trial (PICASSO)[46] has recently been reported; it evaluated the efficacy and safety of cilostazol versus aspirin, with and without probucol, in patients with ischemic stroke with a high risk of cerebral hemorrhage. In this randomized, controlled, 2×2 factorial trial, patients with ischemic stroke with a history of or imaging findings of intracerebral hemorrhage or two or more microbleeds were enrolled in three Asian countries. Patients were randomly assigned to receive, 1) cilostazol (200 mg/day), 2) aspirin (100 mg/day), 3) cilostazol plus probucol (500 mg/day), or 4) aspirin plus probucol. The co-primary outcomes were the incidence of a composite of stroke, myocardial infarction, or vascular death and the incidence of hemorrhagic stroke. Interestingly, the incidence of vascular events was significantly lower in the probucol group than in the non-probucol group (hazard ratio 0.69; 95% CI 0.50 to 0.97; p=0.0316). In patients with ischemic stroke at high risk of cerebral hemorrhage, cilostazol was not inferior to aspirin for the prevention of cardiovascular events, but it did not reduce the risk of hemorrhagic stroke. Thus, probucol on top of aspirin or cilostazol may be beneficial to reduce the incidence of cardiovascular events in patients with ischemic stroke.

Another important finding in the current study may be that the reduction of serum HDL-C by probucol does not increase cerebrovascular and cardiovascular events, but rather it tended to decrease them. After the report of PQRST[21], the reduction of serum HDL-C by probucol was supposed to be harmful and probucol disappeared from the market in the United States and Europe. PQRST was performed in Sweden to investigate whether 3 years' treatment of hypercholesterolemic patients with probucol may affect femoral atherosclerosis. The primary end point was the change in atheroma volume estimated as a change in lumen volume of femoral artery assessed by quantitative arteriography. Probucol-treated patients showed 12% lower LDL-C and 24% lower HDL-C levels than did control subjects, but no significant change was observed in lumen volume between the probucol-treated and control groups. However, changes in atheroma volume may occur without changes in the lumen because of vessel remodeling. Therefore, we should be very careful when we evaluate the results of PQRST since probucol may retard the compensatory remodeling observed in *de novo* atherosclerosis in apolipoprotein E-deficient mice because of the inhibition of macrophage accumulation and decrease in MMP-2

and MMP-9 [47]. Furthermore, regression of xanthoma or xanthelasma may be the most well-established effect of probucol[11]. A close correlation was observed between the extent of regression in Achilles tendon xanthoma and the reduction of serum HDL-C levels in patients with heterozygous FH[48]. Probucol may also prevent lipid storage in macrophages by suppressing the uptake and stimulating the release of cholesterol and other lipids from macrophages[49, 50]. Thus, the reduction of serum HDL-C by probucol may not be harmful but might rather suggest the enhancement of reverse cholesterol transport via increased CETP and hepatic SR-BI. Although the current study only showed a trend of probucol to reduce cerebrovascular and cardiovascular events, other previous studies using probucol have already shown antiatherogenic effects of probucol in high-risk patients[9, 10, 22, 42]. Therefore, administering probucol to reduce the residual risk on top of other LLTs such as statins might be an option for such patients.

Regarding the safety issues of probucol, no significant difference was observed in the frequencies of SAEs between the probucol-treated and control groups. Additionally, our data have shown no increase in ventricular arrhythmias in probucol-treated patients in spite of the fact that probucol significantly prolonged the QT interval. Similarly, previous studies including POSITIVE[22], PICASSO[46], and a study by Kasai *et al.*[45] as well as those reviewed by Yamashita and Matsuzawa[9] and Yamashita *et al.*[10] demonstrated no significant increases in severe ventricular arrhythmia. Therefore, QT prolongation by probucol may not enhance the occurrence of lethal ventricular arrhythmias.

In conclusion, in the current PROSPECTIVE study, we could not demonstrate that probucol can significantly reduce cardiovascular events in CHD patients treated with statins, but probucol showed a tendency to lower cardiovascular events. We could also demonstrate that probucol treatment in the high-risk patients treated with statins was well tolerated and safe despite a marked reduction of serum HDL-C due to enhancement of reverse cholesterol transport. The results of IMPACT Trial[27] in patients with CHD performed in Korea and China with a similar protocol has been published in the same issue of J Atheroscler Thromb. However, the primary end point of IMPACT is the changes in carotid IMT, and the secondary end point is the rate of cerebrovascular and cardiovascular events. A meta-analysis of PROSPECTIVE and IMPACT in the future may elucidate whether probucol on top of conventional LLTs could prevent cerebrovascular and cardiovascular events in patients with CHD.

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Conflict of Interest

Dr. Yamashita reports grants and personal fees from Kowa Company, Ltd., Bayer Yakuhin, Ltd., MSD K.K., Takeda Pharmaceutical Company, Ltd., Astellas Pharma Inc., grants from Kyowa Medex Co., Ltd., Hayashibara Co., Ltd., and personal fees from Skylight Biotec, Inc., Astellas Amgen, Sanofi, Aegerion, outside the submitted work. Dr. Arai reports personal fees from MSD, Pfizer, Kowa, Daiichi Sankyo, Astellas, during the conduct of the study. Dr. Bujo has nothing to disclose. Dr. Masuda reports grants and personal fees from Nippon Boehringer Ingelheim Co., Ltd., MSD K.K., Takeda Pharmaceutical Company, Ltd., Daiichi-Sankyo Company, Ltd., Mochida Pharmaceutical Company, Ltd., Kowa Company Ltd., Kowa Company Ltd., Kissei Pharmaceuti-

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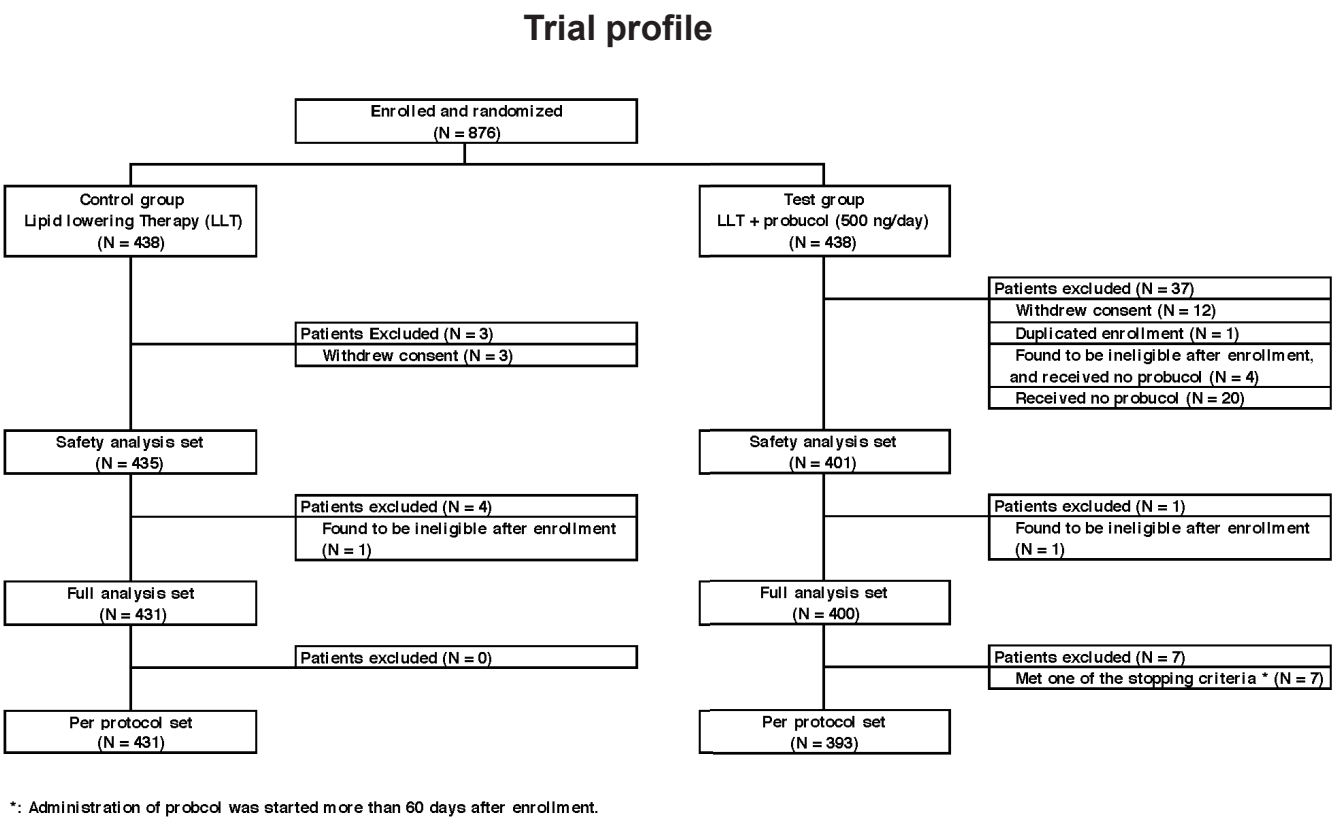
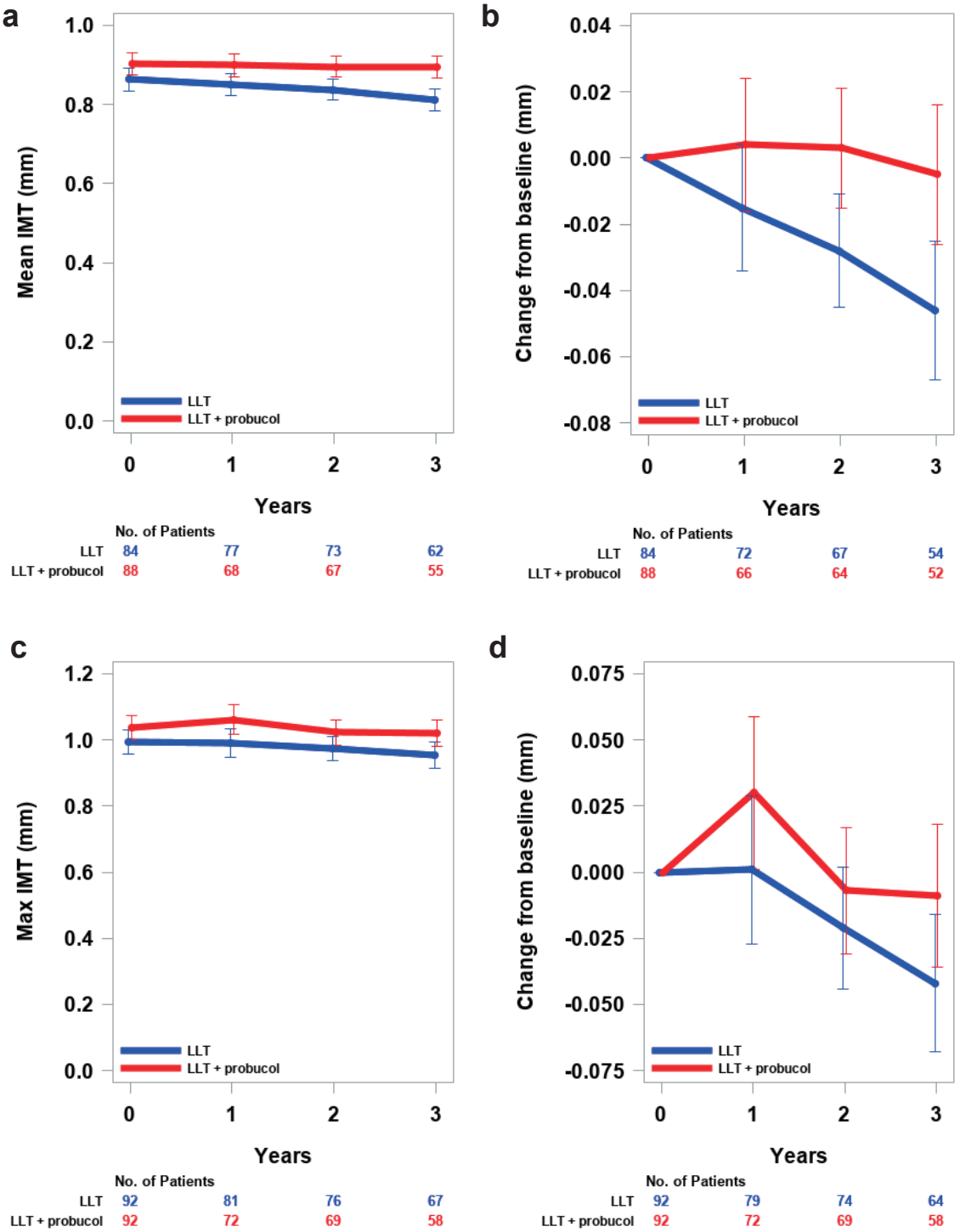


Supplemental Fig. 1. Trial profile of PROSPECTIVE



Supplemental Fig. 2. Levels in mean (a) and max (c) IMT and changes from baseline in mean (b) and max (d) IMT over time by treatment group (FAS)

Measured values at each visit were shown as least square mean with bars of standard error and analyzed using a mixed-effects models with repeated measures (MMRM) with allocation group, visit (at baseline and 12, 24, and 36 months), and interactions between allocation group and visit defined as fixed effects and patient as a random effect. Comparisons between treatment groups at each visit were made using the least square mean of each group. The MMRM analyses were performed using the Kenward–Roger method for adjusting degrees of freedom in an unstructured (UN) covariance structure.

APPENDIX

Inclusion Criteria

This study included male and female patients older than 20 years with all of the following eight clinical statuses:

1) diagnosis of dyslipidemia with high LDL-C level (≧140 mg/dL) without any medication;

2) treatment using any lipid-lowering drugs including statins for more than 8 weeks before providing informed consent;

3) serum LDL-C level less than 200 mg/dL within 8 weeks before providing informed consent, as calculated by Friedewald's formula (LDL-C = total cholesterol – HDL-cholesterol – triglycerides (TG)/5);

4) history of acute myocardial infarction or angina pectoris more than 3 months before providing informed consent, old myocardial infarction, CABG more than 3 months earlier, PCI more than 9 months earlier, or PCI with no restenosis that was diagnosed by follow-up coronary angiography at 6–9 months after PCI;

5) normal cardiac function and mild or moderate heart failure (NYHA classification I or II);

6) older than 20 years at the time of informed consent;

7) no severe hepatic and renal dysfunction (AST < 100 IU/L, ALT < 100 IU/L, and serum creatinine < 1.5 mg/dL) within 4 weeks before providing informed consent; and

8) signed written informed consent for participation in this study.

Exclusion Criteria

The exclusion criteria were the presence of the following clinical statuses at the time of informed consent:

1) ongoing treatment with probucol within 6 months before the time of informed consent;

2) ongoing treatment with cyclosporine;

3) history of hypersensitivity reactions to probucol

4) diagnosis of FH based on the NICE Clinical Guideline[1];

5) very high TG level (>400 mg/dL) within 8 weeks before providing informed consent;

6) markedly high HbA1c level (≧8%) on the most recent blood test;

7) frequent multifocal ventricular arrhythmia;

8) Af including paroxysmal Af;

9) long QTc interval on a resting electrocardiogram (>450 ms in males or >470 ms in females);

10) congestive heart failure (NYHA III or IV) or unstable angina;

11) participation in other clinical trials;

12) women who are pregnant, are lactating, might become pregnant, or wish to become pregnant within the study period; and

13) inappropriate candidate for participation as assessed by the doctors in the current study.

Serious Adverse Events (SAEs)

SAEs were designated as death, events that could lead to death, events that require hospital admission or extended hospitalization, disorders, events that could lead to disorders, others that are serious as well as the former adverse events, or congenital disease or abnormality in post-generations.

End Points

The primary efficacy end point was the presence and the time from registration until the first occurrence of cerebrovascular and cardiovascular events, as follows:

1) cardiovascular death including cardiac sudden death;

2) nonfatal myocardial infarction;

3) nonfatal cerebral stroke excluding transient ischemic attack;

4) hospital admission due to unstable angina;

5) hospital admission due to heart failure; and

6) all coronary revascularizations with either PCI or CABG.

The secondary efficacy and safety end points are as follows:

1) all death;

2) all cerebrovascular and cardiovascular disease;

3) event-free survival time;

4) levels of the mean IMT of carotid arteries and their changes;

5) levels of max IMT in common or internal carotid arteries and their changes; and

6) severe adverse events and their frequency.

Statistical Design and Analysis

As shown in the randomized prospective study of primary prevention for common carotid atherosclerosis in Japan using probucol, the Fukuoka Atherosclerosis Trial (FAST)[2] (a randomized prospective study), the morbidity of cerebrovascular and cardiovascular events within 2 years of the observation period was 2.4% in the probucol-treated group, which was half that in the lipid-lowering agent-treated group (4.8%). At the same time, there are no secondary prevention studies in dyslipidemic patients who had a prior history of cardiovascular events in Japan, except for the report evaluating the effect of probucol on secondary

prevention in patients with FH[3]. Therefore, when we referred to the result of the Japan EPA Lipid Intervention Study (JELIS)[4] that compared the effect of eicosapentaenoic acid (EPA) in addition to statins on the secondary prevention of cardiovascular events, the morbidity of cardiovascular events in patients treated with statins (pravastatin or simvastatin) for the secondary prevention of cardiovascular diseases was 10.7% for 5 years.

On sample size consideration in the study, we assumed that the morbidity of cerebrovascular and cardiovascular events in the control group would be 10.7% on the basis of the results from the statin-treated group of the JELIS study. In the test group, we assume that the rate would be 5.4%, almost half of that in the statin-treated group because the addition of probucol to conventional LLT might improve the morbidity of cardiovascular events as well as the primary prevention shown in the FAST study[2]. On the condition, sample size per group was calculated as 408 patients with a two-tailed significance level of 0.05 and a power of 0.8 in 4 years' accrual period and 3 years' follow-up. Considering that several patients will be dropped out from the analysis, we decided that the target number of patients in one group would be 430 and total target number of patients in this study would be 860.

The patient population in statistical analysis was a full analysis set based on the intention-to-treat principle. The characteristics of patients' demographics and baseline values were compared between the two groups. For the primary efficacy end point, the presence of and the time from registration until the first cerebrovascular and cardiovascular events were analyzed. By estimating event-free survival curves using the Kaplan–Meier method and the 95% confidence interval at 1, 2, and 3 years, event-free survival curves for the two groups were compared using the log-rank test stratified with random allocation factors: LDL-C level (140 mg/dL and more vs. less than 140 mg/dL), diabetes (with vs. without), and hypertension (with vs. without). The secondary end points of the period until all death and period until all cardiovascular and cerebrovascular disease and event-free survival time were analyzed in the same way as the primary end point. Levels of the mean- and max-IMT of carotid arteries from baseline to 1, 2, and 3 years were analyzed by a mixed-effects model with repeated measurements with baseline value (included only if the change from baseline was the response variable), allocation group, visit, and interaction between allocation group and visit as fixed effects and patient as a random effect. Comparison of groups was performed. Frequency of SAEs was summarized by preferred term and intensity, and compared using Fisher's exact test. The two-tailed significant level in statistical analysis was 0.05. All statistical analyses were be done with SAS version 9.3.

Study Organization and Their Works

Executive Committee:

Principal Investigator: Shizuya Yamashita. Professor, Department of Cardiovascular Medicine, Department of Community Medicine, Osaka University Graduate School of Medicine, Osaka, Japan (Present address: Department of Cardiology, Rinku General Medical Center, Izumisano, Osaka, Japan)

Vice Principal Investigator:

Hidenori Arai. President, The National Center for Geriatrics and Gerontology, Obu, Japan.

Hideaki Bujo. Professor, Department of Clinical-Laboratory and Experimental-Research Medicine, Toho University, Sakura Medical Center, Sakura, Japan

The executive committee is working on the study design, execution, protocol amendments, and the supervision of the study. It must adjust the various problems that might occur during the execution of the study and review the process of the study at appropriate time points to maintain the safety of patients and the integrity of the study.

Project Director: Daisaku Masuda. Assistant Professor, Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Osaka, Japan (Present address: Department of Cardiology, Rinku General Medical Center, Izumisano, Osaka, Japan)

Project Manager: Tohru Ohama. Assistant Professor, Department of Dental Anesthesiology, Osaka University Graduate School of Dentistry, Suita, Japan

The project director manages the review of the protocol in the IRB of Osaka University Hospital, communicates the approved protocol to participating institutions, and addresses every unexpected complication that might occur during the execution of the study by making various adjustments for more appropriate progression.

Advisory Board:

Yuji Matsuzawa. President, Sumitomo Hospital, Osaka, Japan

Toru Kita. President, Kobe City Medical Center General Hospital, Kobe, Japan (Present address: President, Kobe City College of Nursing, Kobe, Hyogo,

Japan)

Yasushi Saito. Professor Emeritus, Graduate School of Medicine, Chiba University, Chiba, Japan

Masunori Matsuzaki. Professor Emeritus, Yamaguchi University Graduate School of Medicine, Ube, Japan

The advisory board provides advice regarding the appropriate management and the scientific significance of the study.

Protocol Committee:

Yoji Nagai. President, Translational Research Center for Medical Innovation, Foundation for Biomedical Research and Innovation at Kobe, Japan

Mariko Harada-Shiba. Director, Department of Molecular Innovation in Lipidology, National Cerebral and Cardiovascular Center Research Institute, Suita, Japan

Tohru Ohama, Assistant Professor, Department of Dental Anesthesiology, Osaka University Graduate School of Dentistry, Suita, Japan

The protocol committee creates the protocol, examines the need for the protocol revision if the probability for protocol revision appears during the execution of the study, and reports it to the principal investigator.

Imaging Evaluation Committee:

Hiroyuki Daida. Professor, Department of Cardiology, Juntendo University School of Medicine, Tokyo, Japan

Masao Moroi. Associate Professor, Department of Cardiology, Toho University Ohashi Medical Center, Tokyo, Japan

Hiroshi Matsuo. President, Matsuo Clinic, Yao, Japan

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Trial Statistician: Kenichiro Tanabe. Translational Research Center for Medical Innovation, Foundation for Biomedical Research and Innovation at Kobe, Kobe, Japan

Data Center: Translational Research Informatics Center, Foundation for Biomedical Research and Innovation at Kobe, Kobe, Japan

The study data center is independent of all committees and is planning to maintain and review all study data.

Efficacy and Safety Evaluation Committee:

Chairperson: Jun Sasaki*. Professor, Graduate School of Health and Welfare Sciences, International University of Health and Welfare Graduate School, Ohtawara, Japan

*Deceased

Seiji Umemoto. Deputy Director, Center for Clinical Research, Yamaguchi University Hospital, Ube, Japan

Shigeyuki Matsui. Professor, Department of Data Sciences, The Institute of Statistical Mathematics, Tachikawa, Japan

Hidenao Fukuyama. Professor, Human Brain Research Center, Graduate School of Medicine Kyoto University, Kyoto, Japan

The efficacy and safety evaluation committee evaluates the following reports to recommend early termination or study changes to the principal investigator: 1) the report of severe adverse events that is sent from the principal investigator at appropriate time points; 2) related reports from other studies such as papers and conference presentations at an appropriate time point; 3) the report of study progress from the data center every 3 months during the registration period or every 6 months after registration; and 4) total results of the safety information.

Cerebro- and Cardiovascular Events Evaluation Committee:

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Akira Sumitsuji. Associate Professor, Department of Advanced Cardiovascular Therapeutics, Osaka University Graduate School of Medicine, Suita, Japan

Kazuo Kitagawa. Professor, Department of Neurology, Graduate School of Medicine, Tokyo Women's Medical University, Tokyo, Japan

The cerebro- and cardiovascular events evaluation committee determines whether the reported problem corresponds to the cerebro- and cardiovascular event recognized as a primary end point in this study unless it appears to be clearly relevant.

Participating Faculties:

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