Original Article

Long-Term Probucol Treatment Prevents Secondary Cardiovascular Events: a Cohort Study of Patients with Heterozygous Familial Hypercholesterolemia in Japan

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Aim: The POSITIVE study assessed whether long-term treatment with probucol, a potent anti-oxidant and cholesteryl ester transfer protein (CETP) activator, is associated with a lowered risk of cardiovascular events in a very high-risk population: familial hypercholesterolemia (FH).

Methods: The study cohort included 410 patients with heterozygous FH, diagnosed between 1984 and 1999 by cardiovascular and metabolic experts at fifteen centers. Traceable patients were screened using predefined eligibility criteria. The primary outcome measure for comparison between probucol exposure and non-exposure was the time to the first cardiovascular event involving hospitalization.

Results: Analysis revealed significant differences in baseline characteristics and follow-up treatment between exposure and non-exposure. An observed indication bias was the use of probucol in more severe FH at diagnosis, both for primary and secondary prevention. When the multivariate Cox regression procedure was used after adjustment for possible confounding factors, probucol lowered the risk (hazard ratio [HR], 0.13; 95% confidence interval [CI], 0.05–0.34) in secondary prevention ($n=74$) and was statistically significant ($p<0.001$), although not significant (HR, 1.5; 95% CI, 0.48–4.67; $p=0.49$) in primary prevention ($n=233$). Safety assessment found no specific difference between exposure and non-exposure.

Conclusion: Long-term probucol treatment may prevent secondary attack in a higher cardiovascular risk population of heterozygous FH.

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Key words; Atherosclerosis, Antioxidants, CETP activator, Dyslipidemia

Introduction

Cardiovascular (CV) diseases, including coronary

heart disease and stroke, are the leading cause of death in Japan. Prevention of fatal CV events is therefore the final goal as well as the rationale of cholesterol-lowering therapy.

Probucol, a conventional cholesterol-lowering drug, originated with the report by Barnhart in 1970¹⁾. The drug has been used clinically in Japan since 1985. Nearly 60,000 Japanese patients still take probucol; western countries discontinued probucol use after

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the original manufacturer's withdrawal notice to the United States FDA in 1995 after 18 year's use of the drug. Probucol's cholesterol-lowering mechanism has not yet been clearly established, but it is thought to increase catabolic excretion of cholesterol into bile²⁾. Later studies³⁻⁵⁾ have described new mechanisms of probucol, including anti-atherogenic and anti-oxidant actions. Another controversial and anti-atherogenic feature of probucol is its paradoxical effect of lowering high-density lipoprotein cholesterol (HDL-C). This action reflects, most likely, its molecular mechanisms: promoting cholesterol efflux, and enhancing reverse cholesterol transport by activation of cholesteryl ester transfer protein (CETP)⁶⁻⁸⁾ and class B type 1 scavenger receptor^{9, 10)}. Matsuzawa and his colleagues reported an observed close correlation between the extent of regression in Achilles' tendon xanthoma and probucol-induced decrease in HDL-C levels in patients with familial hypercholesterolemia (FH)¹¹⁾.

No large-scale, randomized, double blind comparative study has been conducted to justify the use of probucol in the prevention of CV events or diseases. However, clinical studies as well as pre-clinical data have been accumulating evidence of the clinical worth of probucol in arteriosclerotic diseases. Numerous clinical results, including a reduction in Achilles' tendon xanthoma thickness after long-term treatment for FH^{12, 13)}, reduced rates of restenosis after angioplasty¹⁴⁻¹⁶⁾, and a decrease in carotid artery intima-media thickness^{17, 18)} support the therapeutic and preventative effects of probucol on arteriosclerotic lesions and plaque. To evaluate the risk and benefit of long-term probucol treatment, we conducted a cohort study to determine whether probucol treatment is associated with the risk reduction of CV events in patients with heterozygous FH, a very high-risk population.

Methods

Study Cohort

We registered patients with FH who received treatment between January 1, 1984 and December 31, 1999 at 15 centers specializing in CV and metabolic diseases, including FH, nationwide. Patients were traceable by medical record and met the diagnostic criteria for heterozygous FH under the Japan Atherosclerosis Society Guidelines (2002) for the Diagnosis and Treatment of Atherosclerotic CV Diseases¹⁹⁾. Definite heterozygous FH was defined as having at least two of the major features: total cholesterol (TC) of 260 mg/dL and above; tendon xanthoma or xanthoma tuberosum; reduced or abnormal receptor activity noted by LDL receptor analysis. Probable heterozy-

gous FH was defined as having at least one each of the major (as above) and minor features: palpebral xanthoma; arcus juvenilis (<50 years); juvenile (<50 years) ischemic heart disease. For other eligibility criteria, we excluded patients with possible homozygous FH or with severe ventricular arrhythmias (polymorphic premature ventricular contractions). Possible homozygous FH was defined as having any one of the clinical features: defect of homozygous or hetero-polymeric LDL receptors confirmed by gene analysis; no LDLR activity observed by receptor analysis; severe elevation of plasma TC higher than 500 mg/dL; xanthoma or atherosclerotic vascular lesions including symptoms of juvenile ischemic heart disease; hypercholesterolemia confirmed in both parents; history of ischemic heart disease confirmed in both parents; or poor response to any 3-hydroxy-3methyl-glutaryl-coenzyme A reductase inhibitor (statin).

During the study period between June, 2004 and September, 2005, we collected anonymous case report forms with the patients' baseline data, including medical history, findings at clinical examination, medication data, and laboratory data. The investigators transcribed the data on to case report forms (identified by a code) from the stored medical charts of the patients. The observation period was the period for which each patient's clinical course could be traced. The longest observation period exceeded 20 years for patients on stable doses of probucol.

We required a sample size of 200 in both the probucol exposure and non-exposure groups, supposing a difference of 10% in the incidence of CV events for 5 years (15% in exposure and 25% in non-exposure). A least 400 subjects were needed to detect the difference with 80% power and a type I error of 5% at the 5% significance level with two-sided log-rank test based on normal approximation. The study protocol was approved through the process of ethics committee or institutional review board at each center.

Definitions and Endpoints

The primary outcome measure was the time to the first CV event, defined as acute myocardial infarction (MI), angina pectoris (AP), heart failure (HF), stroke, transient ischemic attack (TIA) or arteriosclerotic peripheral artery diseases (PAD) leading to hospitalization or death as well as sudden death within 24 hours of an observed intrinsic event. The obtained baseline data at the first visit of each patient included demographic characteristics: sex, date of diagnosis at the participant medical center, age, height, weight, and habits of smoking and drinking. Body mass index (BMI) was calculated as weight in kilograms divided

by the square of height in meters. The other collected characteristic factors at diagnosis were the presence of xanthoma and its location, prior CV event, onset date if any prior CV event, treatment for the event, and other possible risk factors for CV events, including the presence of hypertension, diabetes, ventricular arrhythmia, and PAD. We collected data on cholesterol-lowering therapy (with or without probucol) and other concomitant therapy with anti-platelet, antihypertensive or diabetic drugs. Dates of drug initiation, discontinuation, re-administration, and termination were entered as elemental information. Treatment period was defined as the length from initiation until medication termination, or until the occurrence of the defined CV event, whichever came first. A lipid profile of TC, triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and HDL-C, blood pressure, level of fasting blood sugar (FBS), hemoglobinA_{1c} (HbA_{1c}), and thickness of tendon xanthoma in both feet were variables of interest, seen as potential predictors of CV events. We obtained measurements of those variables on a yearly basis after each patient was diagnosed. LDL-C levels were calculated from TC and HDL-C measurements with the Friedewald formula in TG < 400 mg/dL. For TG of 400 mg/dL and more than 400 mg/dL, the expression of $0.16 \times \text{TG}$ was applied in stead of $0.2 \times \text{TG}$ ²⁰. Most patients had fasted compliantly at periodic checkups of their lipid levels. We set a follow-up period of 10 years for the measurements.

Statistical Analyses

The primary objective of analysis was a comparison between probucol exposure and non-exposure to evaluate whether treatment with probucol (500 mg to 1,000 mg daily) for FH provided CV benefits. The analysis was based on intent-to-treat principles. The secondary objective was to assess whether changes in the lipid profile after probucol treatment predicted CV events in the cohort. Event-free survival, defined as the time from diagnosis to the first CV event, was determined as a response variable. Statistical analysis was performed to evaluate clinical outcomes separately for secondary and primary prevention groups; that is, patients with or without a history of CV events at diagnosis.

Baseline characteristics of each group were explored to detect risk factors for CV events because potential confounders, including indication bias, were anticipated. For baseline comparison, Wilcoxon's rank sum test and Fisher's exact test were used for continuous variables and categorical variables respectively. For detection of risk factors, univariate Cox proportional

hazards regression with a baseline variable as covariate was used as a screening step to determine the relationship with CV events. Variables that achieved significance at the level of 20% in univariate analysis were subsequently included in a multivariate Cox proportional hazards regression using backward variable selection. Variables proving significant at the 10% significance level were selected as risk factors to be adjusted. Consequently, probucol treatment effect was evaluated using the multivariate Cox model with adjustment for the selected baseline variables. Finally, the other observed treatment factors: cholesterol-lowering drugs other than probucol, LDL-apheresis, anti-platelet drugs, anti-hypertensive drugs, and diabetic drugs were entered into that model to assess their effects.

For the association between changes in lipid profile after probucol treatment and the risk of CV events, pre-treatment values of TG, LDL-C, HDL-C as well as TC, and each lipid reduction ratio after treatment were used as covariates. Multivariate analyses of time from probucol start to the first CV event used multivariate Cox's proportional hazards models. Statistical analysis was performed with SAS version 8.2.

Results

Patient Characteristics

We collected data from the medical records of 541 patients, and excluded the data of 131 patients that did not meet eligibility predefined in the protocol.

The flow diagram (**Fig. 1**) gives reasons for the exclusion. A substantial fraction of probucol-exposed patients, 80.0% and 93.2%, took probucol within two years after diagnosis for in primary and secondary prevention groups, respectively. Baseline characteristics at diagnosis are given for each group (**Table 1, 2**). The secondary prevention group (**Table 2**) had prior diseases of AP, MI, stroke, HF, and TIA. This group was found to have significant higher proportions of men (60.2%, $p < 0.01$), smokers (50.0%, $p < 0.01$), hypertension (40.9%, $p < 0.001$) diabetes (15.9%, $p = 0.02$), and older median age (52 years, $p = 0.01$) than the primary prevention group. Moreover, the group tended to have hypo-HDL cholesterolemia of median 42 (20–90) mg/dL, and to receive combined treatments with anti-platelet drugs (56.8%), anti-hypertensive drugs (53.4%), and LDL-apheresis (14.8%).

Comparison between probucol-exposed and non-exposed groups revealed significant differences in some baseline characteristics and treatments, which showed a confounding indication that patients with more severe FH took probucol. For baseline characteristics, the exposed group for primary prevention had more

Fig. 1. Patient Flowchart.

We collected data from the medical records of 541 patients, and excluded the data of 131 patients who did not meet the eligibility predefined in the protocol. The flow diagram gives reasons for the exclusion.

palpebral xanthoma (13.4%, $p=0.05$), thicker median measurement of tendon xanthoma (12.5 mm, $p<0.01$), higher median HbA_{1c} (5.8%, $p=0.03$), and more use of antihypertensive drugs (25.3%, $p<0.01$). Their lipid profile was more severe with a higher median baseline TC (325 mg/dL, $p=0.001$), a higher median LDL-C level (253 mg/dL, $p<0.001$), and a lower HDL-C level (47 mg/dL, $p<0.001$) than the unexposed group. The exposed group for secondary prevention had a higher prevalence of post-MI (44.6%, $p<0.01$) than the unexposed group. Observed medications were also significantly different between the exposed and unexposed groups. The exposed group used anti-hypertensive drugs concomitantly at a higher rate (25.3% vs. 11.2%, $p<0.01$) for primary prevention.

Descriptive analysis of baseline characteristics and treatments during observation implies that in both primary and secondary prevention, the exposed groups tended to include patients with more severe FH at diagnosis. Arguably, patients considered more severe at diagnosis would receive more intensive treatment, including probucol.

Outcomes

We present the absolute number of CV events requiring hospitalization by prevention group with

details of the events (**Table 3**). The incidence of CV events without consideration of confounding factors was 11.6% in the exposed group and 4.5% in the unexposed group for primary prevention. For secondary prevention, the incidence was 27.0% in the exposed group and 64.3% in the unexposed group. The event-free survival curve of the secondary prevention group is given (**Fig. 2**).

To identify risk factors for CV events, we determined the relationship between the incidence and every baseline variable using univariate Cox regression at a significant level of 20%. Variables proving significant at the 10% significance level in multivariate Cox regression were selected as risk factors to be adjusted. We estimated the effect of treatment after adjusting the selected risk factors. We calculated hazard ratios (HRs) with 95% confidence interval (CI) for binary variables, BMI ≥ 25 vs BMI < 25 , drinking vs no drinking, for example, and the indicated HRs corresponded to a 1 standard deviation increase for continuous variables, including TC. Estimated results are given (**Table 4**).

In the primary prevention group, significant variables were BMI ≥ 25 (HR 1.86, 95% CI 0.87–3.98; $p=0.11$), drinking (HR 2.17, 95% CI 1.02–4.63; $p=0.05$), tendon xanthoma (HR 2.17, 95% CI 0.76–6.23; $p=0.15$), prior diseases other than CV events (HR 1.87, 95% CI 0.87–3.99; $p=0.11$), PAD (HR 5.23, 95% CI 0.70–39.2; $p=0.11$), diabetes (HR 2.27, 95% CI 0.79–6.50; $p=0.13$), TC (HR 1.37, 95% CI 0.99–1.89; $p=0.06$), HDL-C (HR 0.75, 95% CI 0.50–1.12, $p=0.16$), SBP (HR 1.48, 95% CI 1.00–2.18; $p=0.05$), and the thickness of tendon xanthoma (HR 1.50, 95% CI 1.06–2.14; $p=0.02$). Three of these variables, drinking, TC, and PAD were selected for adjustment at the 10% significance level as a result of a multivariate Cox regression with backward variable selection. After adjustment for these three baseline variables, we found no significant effect by probucol at the 5% significant level. The estimated hazard ratio of probucol use for CV events was 1.50 (95% CI 0.48–4.67; $p=0.49$).

In the secondary prevention group, significance variables were drinking (HR 1.74, 95% CI 0.80–3.79; $p=0.17$), presence of palpebral xanthoma (HR 5.34, 95% CI 2.26–12.61, $p<0.001$), TIA (HR 4.16, 95% CI 0.54–32.21; $p=0.17$), history of coronary artery bypass graft (HR 0.31, 95% CI 0.11–0.90; $p=0.03$), hypertension (HR 0.58, 95% CI 0.26–1.28; $p=0.18$), diabetes (HR 2.89, 95% CI 1.30–6.42; $p<0.01$), and fasting blood sugar (HR 1.31, 95% CI 0.91–1.89; $p=0.15$). Two of these variables, palpebral xanthoma and diabetes, were selected for adjustment at the 10% sig-

Table 1. Baseline characteristics of patients in primary prevention group[†]

Characteristics	Primary prevention No. (%) of patients			<i>p</i>
	All <i>n</i> =322	Exposed <i>n</i> =233 (72.4)	Unexposed <i>n</i> =89 (27.6)	
Age, mean (range)	49 (27–74)	50 (20–74)	47 (20–72)	0.18
Men, No. (%)	134 (41.6%)	96 (41.2%)	38 (42.7%)	0.90
BMI ≥25	71 (22.5%)	49 (21.4%)	22 (25.6%)	0.45
Smoker	99 (33.2%)	74 (34.1%)	25 (30.9%)	0.68
Drinker	124 (42.2%)	93 (43.7%)	31 (38.3%)	0.43
Xanthoma	259 (80.7%)	190 (81.9%)	69 (77.5%)	0.43
Tendon xanthoma	245 (76.3%)	181 (78.0%)	64 (71.9%)	0.30
Nodular xanthoma	28 (8.7%)	22 (9.5%)	6 (6.7%)	0.51
Palpebral xanthoma	36 (11.2%)	31 (13.4%)	5 (5.6%)	0.05
PAD	4 (1.2%)	1 (0.4%)	3 (3.4%)	0.07
Hypertension	54 (16.8%)	40 (17.2%)	14 (15.7%)	0.87
Diabetes	22 (6.9%)	17 (7.3%)	5 (5.6%)	0.81
Lipid profile, mg/dL				
TC [§]	320 (188–493)	325 (188–493)	307 (194–464)	0.001
TG [§]	120 (28–1289)	121 (34–1068)	120 (28–1289)	0.96
HDL-C [§]	49 (20–108)	47 (20–90)	52 (27–108)	<0.001
LDL-C [§]	244 (45–425)	253 (98–425)	223 (45–403)	<0.001
Blood Pressure, mmHg				
SBP [§]	129 (82–190)	128 (82–190)	131 (90–190)	0.57
DBP [§]	0 (48–120)	80 (48–120)	80 (56–120)	0.91
FBS (mg/dL) [§]	95 (63–276)	94 (63–140)	95 (81–276)	0.41
HbA _{1c} (%) [§]	5.7 (4.1–12.4)	5.8 (4.1–9.7)	5.3 (4.3–12.4)	0.03
Tendon xanthoma thickness (mm) [§]	12.1 (7.5–49.0)	12.5 (7.5–49.0)	10.5 (8.0–20.0)	<0.01
Treatment				
Cholesterol-lowering drugs (non-probucol)	302 (93.8%)	219 (94.0%)	83 (93.3%)	0.80
LDL-apheresis	7 (2.2%)	6 (2.6%)	1 (1.1%)	0.68
Anti-platelet drugs	49 (15.2%)	41 (17.6%)	8 (9.0%)	0.06
Anti-hypertensive drugs	69 (21.4%)	59 (25.3%)	10 (11.2%)	<0.01
Diabetic drugs	15 (4.7%)	12 (5.2%)	3 (3.4%)	0.37

[†]Continuous variables compared by Wilcoxon's rank sum test, distribution of categorical variables by Fisher's exact test. [§]Data are median (range). All data are number (%) unless otherwise indicated. Each percentage shown is related to the total number with measurement data. BMI, body mass index; PAD, peripheral artery disease; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; HbA_{1c}, hemoglobin A_{1c}. LDL-C was calculated with the Friedewald formula.

nificance level as a result of multivariate Cox regression analysis using a backward variable selection. After adjustment for these two baseline variables, the hazard ratio of probucol use for CV events was estimated to be 0.13 (95% CI 0.05–0.34) and significant ($p < 0.001$). In sensitivity analyses, we also obtained similar estimation results on probucol for various sets of baseline covariates for adjustment.

The lipid levels of TC, LDL-C and HDL-C were lowered after probucol treatment both in primary and secondary prevention. In the primary prevention

group, the median (range) levels of TC, TG, LDL-C and HDL-C closest to before treatment were respectively 305 (165–493), 119 (35–1068), 228 (107–425) and 48 (25–96) mg/dL, and those at 10-year treatment were, respectively, 222 (141–371), 94 (43–335), 157 (91–311) and 39 (17–81) mg/dL. In the secondary prevention, the median levels of TC, TG, LDL-C and HDL-C closest to before treatment were, respectively, 320 (191–469), 129 (37–636), 240 (117–381) and 44 (24–90) mg/dL, and those at 10-year treatment were, respectively, 211 (135–305), 71 (48–475),

Table 2. Baseline characteristics of patients in secondary prevention group

Characteristics	Secondary prevention			<i>p</i>
	All <i>n</i> = 88	No. (%) of patients Exposed <i>n</i> = 74 (84.1)	Unexposed <i>n</i> = 14 (15.9)	
Age, mean (range)	52 (23–71)	51 (29–70)	53 (23–71)	0.62
Men, No. (%)	53 (60.2%)	46 (62.2%)	7 (50.0%)	0.55
BMI ≥ 25	21 (25.3%)	17 (24.3%)	4 (30.8%)	0.73
Smoker	42 (50.0%)	38 (53.5%)	4 (30.8%)	0.23
Drinker	39 (46.4%)	33 (46.5%)	6 (46.2%)	1.00
Xanthoma	75 (85.2%)	63 (85.1%)	12 (85.7%)	1.00
Tendon xanthoma	71 (80.7%)	61 (82.4%)	10 (71.4%)	0.46
Nodular xanthoma	7 (8.0%)	6 (8.1%)	1 (7.1%)	1.00
Palpebral xanthoma	8 (9.1%)	5 (6.8%)	3 (21.4%)	0.11
PAD	2 (2.3%)	2 (2.7%)	0 (0.0%)	1.00
Hypertension	36 (40.9%)	30 (40.5%)	6 (42.9%)	1.00
Diabetes	14 (15.9%)	9 (12.2%)	5 (35.7%)	0.04
Lipid profile, (mg/dL)				
TC [†]	332 (191–469)	334 (191–469)	322 (229–444)	0.41
TG [†]	128 (37–636)	128 (37–636)	136 (63–318)	0.85
HDL-C [†]	42 (20–90)	42 (20–90)	39 (26–73)	0.91
LDL-C [†]	249 (117–381)	256 (117–381)	245 (138–354)	0.57
Blood Pressure, mmHg				
SBP [†]	129 (90–180)	128 (96–180)	136 (90–166)	0.97
DBP (mmHg) [†]	80 (52–114)	80 (52–114)	78 (60–104)	0.33
FBS (mg/dL) [†]	96 (72–252)	97 (72–197)	94 (79–252)	0.96
HbA1c (%) [†]	5.8 (4.1–10.6)	5.5 (4.1–8.1)	6.4 (5.3–10.6)	0.06
Tendon xanthoma thickness (mm) [†]	14.5 (5.8–25.0)	15.0 (5.8–25.0)	10.0 (8.5–18.8)	0.09
Prior CV events				
Angina Pectoris	45 (51.1%)	36 (48.6%)	9 (64.3%)	0.39
Myocardial Infarction	34 (38.6%)	33 (44.6%)	1 (7.1%)	<0.01
Stroke	7 (8.0%)	4 (5.4%)	3 (21.4%)	0.08
Heart failure	2 (2.3%)	2 (2.7%)	0 (0.0)	1.00
TIA	2 (2.3%)	1 (1.4%)	1 (7.1%)	0.29
Treatment				0.08
Cholesterol-lowering drugs (non-probucol)	81 (92.0%)	70 (94.6%)	11 (78.6%)	
LDL-apheresis	13 (14.8%)	11 (14.9%)	2 (14.3%)	1.00
Anti-platelet drugs	50 (56.8%)	44 (59.5%)	6 (42.9%)	0.38
Anti-hypertensive drugs	47 (53.4%)	42 (56.8%)	5 (35.7%)	0.24
Diabetic drugs	6 (6.8%)	3 (4.1%)	3 (21.4%)	0.05

[†] Data are the median (range). All data are numbers (%) unless otherwise indicated. Each percentage is related to the total number with measurement data. TIA indicates transient ischemic attack.

147 (124–197) and 33 (17–70) mg/dL. Sub-analysis of changes in the lipid profile after probucol treatment detected significant three predictors of CV event risk: higher baseline TC (HR 2.74, 95% CI 1.05–7.16; $p=0.04$) in the primary prevention group; reduction in TG (HR 0.22, 95% CI 0.06–0.86; $p=0.03$); and reduction in LDL-C (HR 0.17, 95% CI 0.03–0.90; $p=0.04$) after treatment in the subset of the secondary

prevention group on stable doses of probucol. Neither TC nor HDL-C after treatment was associated with CV event risk in the probucol-exposed group, which indicates that reduction of the HDL-C level after probucol treatment is not related to CV event risk for probucol-exposed patients.

We evaluated the safety of probucol for all collected data from 541 patients, and found 56 adverse

Table 3. Incidence of cardiovascular events

		Cardiovascular Event	No event	Total	<i>p</i>
Primary prevention (<i>n</i> =322)	Exposed (<i>n</i> =233)		27 (11.6%)	206	0.058
		MI	4		
		AP	18		
		Str.	3		
		TIA	1		
	Unexposed (<i>n</i> =89)	PAD	1		
			4 (4.5%)	85	
		AP	1		
		Str.	2		
		TIA	1		
Secondary prevention (<i>n</i> =88)	Exposed (<i>n</i> =74)		20 (27.0%)	54	0.012
		MI	6		
		AP	12		
		HF	1		
		Str.	1		
	Unexposed (<i>n</i> =14)		9 (64.3%)	5	
		MI	2		
		AP	6		
		Str.	1		
				14	

MI, myocardial infarction; AP, angina pectoris; HF, heart failure; Str., stroke; TIA, transient ischemic attack; PAD, peripheral artery disease.

[†]One of the 4 patients died after 12 months of probucol termination.

events in 18 patients. Malaise, pruritus, macrocytic anemia and pain in the extremities were recorded as adverse drug reactions associated with probucol. We noted and reported gastric cancer stage III immediately to the Ministry of Health and Welfare as an unexpected serious event, because of an unknown drug relation due to many concomitant drugs, although probucol was found to be non-carcinogenic alone²¹. Six deaths were observed in the population not taking probucol or stopping probucol. There was no other difference in the incidence of adverse events, including serious events, between probucol exposure and non-exposure.

Discussion

Many data from large-scale randomized controlled trials have overwhelmingly demonstrated the clinical benefits of lowering cholesterol with statins^{22, 23}, yet the rapid and extensive prophylactic use of cholesterol-lowering drugs remains controversial. Few studies have addressed the clinical risks and benefits of long-term treatment of hyperlipidemia among women²⁴ or elderly patients²⁵. The safety of long-term cholesterol-lowering therapy, including the issue of associated cancer risk or benefit, remains inconclusive because of conflicting clinical evidence²⁶. More importantly,

conclusions from the results of randomized controlled trials are limited by their relatively short follow-up periods (generally less than 5 years) in the analyzed studies.

In long-term treatment for FH, probucol was used with other cholesterol lowering drugs in over 80% of the secondary prevention group—those with a more severe clinical outlook than the primary prevention group: a higher prevalence of hypertension and diabetes, significant thicker tendon xanthoma, more combined therapy with LDL-apheresis, anti-platelet drugs, and anti-hypertensive drugs. The high rate of probucol use in FH was surprising, different from expected. This might partly reflect the prescription behavior of experts with the result that intractable patients responded to the regimen.

In the secondary prevention, the higher-risk group, probucol exposure was associated with a reduction in the risk of cardiovascular events (HR 0.13; 95% CI 0.05–0.34) with high significance ($p < 0.001$), while it was not significant in the primary prevention group. This result was also contrary to our expectation that probucol exposure would likely be associated with increased event risk due to a confounding indication—that patients considered more severe at diagnosis would receive more treatment, including probucol. We did not collect the details of non-probucol drugs

Fig. 2. Kaplan-Meier Estimates of Event-free Rate.

For secondary prevention, the incidence of cardiovascular events was 27.0% in the exposed group and 64.3% in the unexposed group. An event-free survival curve for the secondary prevention group is given.

Table 4. The results of multivariate analysis using Cox regression procedure

Factor	Primary prevention			Secondary prevention		
	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
Baseline variables						
Total cholesterol	1.58	1.06–2.33	0.02	–	–	–
Drinking	2.43	1.09–5.44	0.03	–	–	–
Peripheral artery disease	5.27	0.51–54.63	0.16	–	–	–
Palpebral xanthoma	–	–	–	2.94	1.02–8.47	0.05
Diabetes	–	–	–	2.58	0.76–8.76	0.13
Treatment in follow-up						
Probucol use	1.50	0.48–4.67	0.49	0.13	0.05–0.34	<0.001
Anti-platelet drug use	–	–	–	2.48	1.00–6.17	0.05

to simplify the study procedure. However, we would likely exclude underused statins because of the reduced use of non-probucol drugs from the possible factors of the higher event rate in the unexposed group, because statins were available when all of the 9 recurrent patients (Table 3) started and the patients continued on cholesterol-lowering drugs. We suppose, therefore,

that the reasons for this unanticipated great risk reduction include some antioxidant and anti-atherogenic actions^{3, 4, 27)} of probucol. The finding in second prevention may be suggested by the report²⁷⁾ that probucol significantly decreased *in vitro* LDL oxidizability measured under typically strong oxidative conditions, and that long-term treatment with probucol had an

anti-atherogenic effect in Watanabe Heritable Hyperlipidemic rabbits. From the observation that the baseline lipid profile was not different between the two groups of exposure and non-exposure in secondary prevention, the drug might exhibit greater effectiveness in post-cardiovascular disease patients, in possibly advanced lipid accumulation and inflammation, which are associated with the circulation of oxidized LDL²⁸⁾.

In primary prevention, we observed an almost significant increase of events in the exposed group (**Table 3**), and an apparently increased risk (HR 1.5), although not statistically significant after adjustment (**Table 4**). We suppose, however, that the ideal effects of probucol might be concealed by the following factors noted in primary prevention. The exposed group had a worse lipid profile (TC, LDL-C and HDL-C levels), higher HbA_{1c}, and thus definitely a higher risk than the unexposed group. Furthermore, 8 (nearly 30%) of the 27 patients experiencing cardiovascular events in the exposed group discontinued probucol when they had events. This was consistent with the different finding between primary and secondary preventions in the exposed group: less than half of the patients (113 of 233) in primary prevention continued on probucol, while 53 (72%) of 74 patients continued in secondary prevention. This estimation might be conservative.

The controversial and paradoxical action of probucol—lowering HDL-C—level was not associated with the risk of CV events in the cohort, therefore, the association between low levels of HDL-C and an increased risk for CV events or death indicated by the early Framingham Heart Study²⁹⁾ may not be extrapolated to probucol-treated patients. This proposition is consistent with recent findings that a lowered HDL-C level is not always atherogenic, but that the quality or function of HDL-C is more important than the HDL-C levels³⁰⁾. In fact, increased levels of HDL-C with torcetrapib, a CETP inhibitor, were not associated with a significant clinical benefit in patients with coronary disease³¹⁾, FH³²⁾ or mixed dyslipidemia³³⁾.

We speculate that enhanced reverse cholesterol transport by CETP activation as a result of probucol treatment also contributed to the detected risk reduction in the cohort. The observed positive outcome of probucol, a CETP activator, might be a mirror image of the negative clinical trial results for the CETP inhibitor³⁴⁾. Reports^{35, 36)} of increased coronary heart disease in CETP deficiency despite increased HDL-C levels, and the molecular approach to review CETP deficiency³⁷⁾ support our hypothesis, at least in Japanese genealogy. Interestingly, a recent basic research reports

that human CETP expression enhances the mouse survival rate in an experimental systemic inflammation model³⁸⁾, indicating for the first time a role for CETP in the defense against the exacerbated production of proinflammatory mediators.

For the safety evaluation, we found no cardiotoxic adverse drug reaction including QT/QTc prolongation or torsade de pointes, in this study, although probucol can cause them^{16, 39, 40)}.

We obtained these results from an observational study with no control for inaccuracy, unexpected bias or confounding factors. We could not assure the precision of the baseline measurements due to unrecorded data. The participant centers were major hospitals for FH, but not all hospitals in Japan, because the study was conducted as part of a post-marketing study by a pharmaceutical manufacturer within the framework of the Japanese government regulations. Some restrictions on collecting data might have resulted in unexpected small numbers in the unexposed group in secondary prevention, although we think that the study cohort represents nearly a nationwide population of heterozygous FH in Japan. The results derived from patient data in Japan can not necessarily be generalized to patients in western countries.

Despite these limitations of the study, however, we could evaluate the outcome of long-term probucol treatment in the medical practice setting for FH, a high-risk population, for as long as 20 years in Japan. The significant risk reduction of CV events observed in the secondary prevention group holds clinical significance and suggests some beneficial therapeutic actions of this drug in arteriosclerotic diseases. The hypothesis from the findings warrants a randomized controlled trial for verification of the secondary prevention, and needs further research into the molecular mechanisms or roles of CETP in pathogenesis.

Author Contributions

Dr. Yamashita had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Matsuzawa, Kita, Saito, Fukushima, Matsui. Acquisition of data: Yamashita, Bujo, Arai, Harada-Shiba, Saito, Kita, Matsuzawa. Analysis and interpretation of data: Yamashita, Bujo, Arai, Harada-Shiba, Matsui, Saito, Fukushima, Kita, Matsuzawa.

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and Matsuzawa. Statistical analysis: Matsui and Fukushima. Administrative, technical, or material support: Fukushima, Matsui, Kita, Saito, and Matsuzawa. Study supervision: Yamashita, Fukushima, Matsui, Kita, Saito, and Matsuzawa.

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Disclosures

From the formerly Daiichi and Otsuka, Dr. Matsui, Dr. Fukushima, Dr. Matsuzawa, and Dr. Kita received fees and expenses for meetings related to protocol design, statistical and clinical interpretation of the data; Dr. Bujo, Dr. Arai, Dr. Harada-Shiba received honoraria and travel expenses for lectures, Dr. Yamashita, Dr. Bujo, Dr. Arai received fees and travel expenses for a meeting related to clinical interpretation of the data. Dr. Yamashita received consultancy fees from Otsuka. Dr. Matsuzawa is contracted as a short-term adviser to Otsuka in medical science. Dr. Saito received travel expenses only.

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