Original Article

Effects of Combination Therapy with Cilostazol and Probucol versus Monotherapy with Cilostazol on Coronary Plaque, Lipid and Biomarkers: SECURE Study, a Double-Blind Randomized Controlled Clinical Trial

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***Aim*: The study aim is to investigate synergistic effects of cilostazol and probucol combination therapy on coronary plaque volume and composition.**

***Methods*: A total of 119 patients undergoing coronary stenting were treated with probucol and cilostazol combination therapy (group Ⅰ) or with cilostazol monotherapy (group Ⅱ) in a double-blind, randomized multicenter trial, and evaluated by virtual histology intravascular ultrasound (VH-IVUS) at baseline and 9-month follow-up for changes in coronary plaque volume and composition at an index intermediate lesion with luminal narrowing ≥30% and <70% and for neointimal hyperplasia at the stented segment. In all patients simvastatin 20 mg was started with enrollment.**

***Results*: Qualifying VH-IVUS data from 91 patients were included in the final analysis. There were no significant differences between group Ⅰ and Ⅱ with respect to the primary endpoint, nominal change in normalized total atheroma volume (TAV) of the index intermediate coronary lesion (Δ −12.6±17.7 vs. −14.2±20.2 mm^3, $p=0.691$), or plaque composition. Plaque regression was observed in more than 70% of patients in both groups. Diabetes was the only significant independent determinant of changes in TAV ($\beta=0.22$, $p=0.037$). There were greater decreases in total cholesterol (Δ −51.8±33.0 vs. −25.4±39.1 mg/dL, $p<0.001$) and LDL (Δ −33.5±30.5 vs. −20.3±30.8 mg/dL, $p=0.044$) levels in group Ⅰ than in group Ⅱ. However, HDL cholesterol (Δ −11.2±8.5 vs. 2.7±7.7 mg/dL, $p<0.001$) and apoA1 (Δ −18.2±21.4 vs. 10.0±16.5 mg/dL, $p<0.001$) levels were also significantly decreased in group Ⅰ compared with group Ⅱ.**

***Conclusions*: There were no significant differences in changes in plaque volume or composition between the cilostazol and probucol combination therapy and cilostazol monotherapy group despite different impacts of the treatments on lipid biomarkers.**

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Key words: Atherosclerosis, Coronary artery disease, Probucol, Cilostazol

Introduction

Probucol, a mild cholesterol-lowering agent with antioxidant and anti-inflammatory properties, has been shown to reduce atherosclerosis in previous clinical trials[1-3]. However, its action mechanisms remain unclear. In addition to inhibiting oxidative modification of LDL cholesterol, enhancing reverse cholesterol

transport (RCT) by activating cholesteryl ester transfer protein (CETP) and scavenger reverse cholesterol class B type 1 (SR-B1) has been suggested as an important contributing mechanism to the antiatherogenic effect of probucol[4-6].

Cilostazol is an inhibitor of type 3 phosphodiesterase with antiplatelet activity that acts through suppression of cyclic adenosine monophosphate degradation. Cilostazol is also known to have antiatherogenic properties[7]. Studies have shown that cilostazol improves endothelial function by increasing nitric oxide production, promoting scavenging of free radicals, and inhibiting foam cell formation and smooth muscle cell proliferation[8-11]. Furthermore, cilostazol increases high-density lipoprotein (HDL) cholesterol and decreases triglyceride levels, possibly by increasing the activity of lipoprotein lipase[12, 13].

Recent preclinical studies have suggested synergistic inhibitory effects of combined treatment with cilostazol and probucol on the atherosclerotic process[14, 15].

Aim

The aim of the present clinical study was to investigate possible synergistic actions of cilostazol and probucol combination therapy on coronary plaque volume and composition compared with cilostazol monotherapy using virtual histology intravascular ultrasound (VH-IVUS).

Methods

Study Design

The SECURE (Synergistic Effect of Combination Therapy with Cilostazol and Probucol on Plaque Stabilization and Lesion Regression) study was designed as a randomized, double-blind, placebo-controlled, multicenter clinical trial[16]. Patients were randomized (1:1) to either the combination therapy group (cilostazol 100 mg and probucol 250 mg twice a day) or cilostazol monotherapy (cilostazol 100 mg and placebo one tablet twice a day) group using a web-based randomization system. The randomization was stratified according to enrolling site and the presence of diabetes mellitus. All study drugs including cilostazol, probucol, and placebo were provided by Korea Otska Pharmaceutical Co. Ltd (Seoul, Korea). The trial protocol was approved by the local institutional review boards of each patient-enrolling center, and has been registered at www.clinicaltrials.gov (NCT01031667). All patients provided written informed consent. The primary end point was the nominal change in the normalized total atheroma volume (TAV) of index intermediate lesions from baseline to 9-month follow-up. The secondary end points include change in percent atheroma volume (PAV) and plaque composition by VH IVUS, clinical outcomes, neointimal volume obstruction at index PCI target lesions, change in blood levels of lipid components and biomarkers [hsCRP, VCAM-1, oxidized LDL, Lp(a), vWF].

Study Population

Inclusion criteria were age over 20 years, one intermediate lesion on native coronary arteries with luminal narrowing ≥30% and <70% by visual estimation, and presence of at least one percutaneous intervention (PCI) target lesion (reference diameter, 2.5-4 mm; lesion length, ≤26 mm) with >50% diameter stenosis that could be covered with a single stent. Exclusion criteria were intermediate lesions that might provide difficult for IVUS evaluation because of heavy calcification or severe tortuosity; previous coronary artery bypass graft; cardiogenic shock; inability to take antiplatelet therapy (aspirin and clopidogrel); known hypersensitivity or contraindication to medications such as heparin, aspirin, clopidogrel, cilostazol, probucol, statin, and contrast media; history of severe ventricular arrhythmia; significant QTc prolongation (≥470 ms); congestive heart failure (New York Heart Association class Ⅲ/Ⅳ) or left ventricular ejection fraction ≤35%; familial hypercholesterolemia or uncontrolled hypertriglyceridemia (>400 mg/dL); chronic renal failure (serum creatinine, ≥2 mg/dL); severe liver disease; and women who were pregnant or of childbearing age. Acute coronary syndrome (ACS) is defined as symptoms related to acute myocardial ischemia and includes ST-segment elevation myocardial infarction (STEMI), non-ST elevation myocardial infarction (NSTEMI), and unstable angina[17]. In this study, we also excluded patients with STEMI undergoing primary or rescue PCI. All patients received a starting dose of 20 mg simvastatin. The dose of simvastatin was adjusted to reach a target LDL level <100 mg/dL. Concomitant use of other drugs that interfere with serum lipid levels, such as niacin, fenofibrate or omega-3, was not allowed.

Percutaneous Coronary Intervention

All patients received aspirin (250 mg) and clopi-

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dogrel (300-600 mg) loading at least 12 hours before PCI, unless they had been on aspirin (100-325 mg/d) and clopidogrel (75 mg/d) for ≥5 days. Unfractionated heparin was administered to maintain an active clotting time >250 seconds. The criteria for an index PCI target lesion were a coronary segment with a stenosis diameter >50% and lesion length ≤26 mm that could be covered by a single stent. All PCI target lesions, including additional lesions not established as index lesions, were treated with zotarolimus-eluting stents (Endeavor Sprint; Medtronic Vascular Inc., Santa Rosa, CA, USA). After implantation of stents, aspirin (100 mg/d) and clopidogrel (75 mg/d) were administered during the period of study.

IVUS Imaging and VH Reconstruction

In all patients, one index intermediate lesion and one index PCI target lesion were evaluated by serial VH IVUS at baseline and 9-month follow-up. The index intermediate lesion was defined as a *de novo*, native coronary artery lesion with luminal narrowing ≥30% and <70% by visual estimation which did not require PCI. All index intermediate lesions were located in major coronary arteries other than PCI target vessels. All IVUS imaging was performed using a 20-MHz 2.9F, phased-array IVUS catheter (Eagle Eye; Volcano Therapeutics, Rancho Cordova, CA, USA) after intracoronary injection of nitroglycerin (200 μg). The IVUS catheter was placed distal to the target lesion and then pulled back at 0.5 mm/s using a motorized pullback system. During pullback, gray-scale IVUS was recorded and raw radiofrequency data were captured at the top of the R-wave for reconstruction of the color-coded map by a VH-IVUS data recorder (Volcano Therapeutics). For evaluation of the index intermediate lesion, a segment at least 30 mm in length was evaluated with IVUS and VH at baseline and 9-month follow-up.

Analysis of IVUS and VH Data

IVUS and VH reconstructions images at baseline and follow-up were displayed side-by-side and analyzed for plaque volume and plaque composition (fibrotic, fibrofatty, dense calcium, and necrotic core components) by experienced personnel of an independent core laboratory (Cardiac Core Analysis Laboratory, Stanford University Medical Center, Stanford, CA, USA) blinded to treatment assignment. Vessel (external elastic membrane) area and lumen area were manually traced at 1-mm intervals, and plaque area was defined as vessel area minus lumen area. TAV was calculated by summation of plaque area from each measured image as TAV = Σ (vessel area − lumen area). Normalized TAV was calculated as normalized TAV = [Σ (vessel area − lumen area)/number of images in pullback] × median number of images in a cohort[18]. PAV was determined using the formula: PAV = 100 × [Σ (vessel area − lumen area)/Σ (vessel area)]. The tissue composition of the plaque area was characterized automatically using custom-built software (IVUS Lab software; Volcano Therapeutics) based on mathematical autoregressive spectral analysis of IVUS backscattered data. The absolute values of each plaque component were calculated automatically by the software. The volume of each tissue component was determined by summation of the tissue component area, and the percentage of the volume comprised by each tissue component was calculated as tissue component volume divided by TAV.

Index PCI target lesions were analyzed for neointimal growth at 9-month follow-up with gray-scale IVUS. Vessel, stent, and lumen areas were traced manually at 1-mm intervals in stented segments and in adjacent reference segments (5-mm long). Vessel, stent, lumen, and neointimal (stent minus lumen) volumes were obtained by summation of each area. Percent neointimal volume obstruction was calculated as neointimal volume divided by stent volume. Neointimal volume index was defined as neointimal volume divided by stent length.

Laboratory Assessment

Blood levels of lipid composition and biomarkers were evaluated at baseline and 9 months. Blood samples were obtained after overnight fasting. Serum was separated by centrifugation and stored at −80℃ until assayed. Serum lipids and apolipoproteins were measured using standard enzymatic methods. High sensitivity C-reactive protein (hsCRP; Nitobo Inc., Tokyo, Japan), lipoprotein(a) [Lp(a); Daiichi Pure Chemicals, Tokyo, Japan], oxidized LDL (Mercodia AB, Uppsala, Sweden), VCAM-1 (R&D Systems, Minneapolis, USA), and von Willebrand factor (vWF; R&D Systems, Minneapolis, USA) levels were measured using commercially available enzyme-linked immunosorbent assay kits. All measurements were performed at a central clinical laboratory (Seoul Clinical Laboratories, Seoul, Korea). The particle-size distribution of LDL, isolated by sequential flotation ultracentrifugation, was analyzed using a pore-gradient lipoprotein system (CBS Scientific Inc., Solana Beach, CA, USA). Circulating HDL subfractions were quantified using a Pore Gradient Lipoprotein electrophoresis system (CSI Scientific Electrophoresis LPE-4003; CBS Scientific Inc.) after micro-ultracentrifugation of serum.

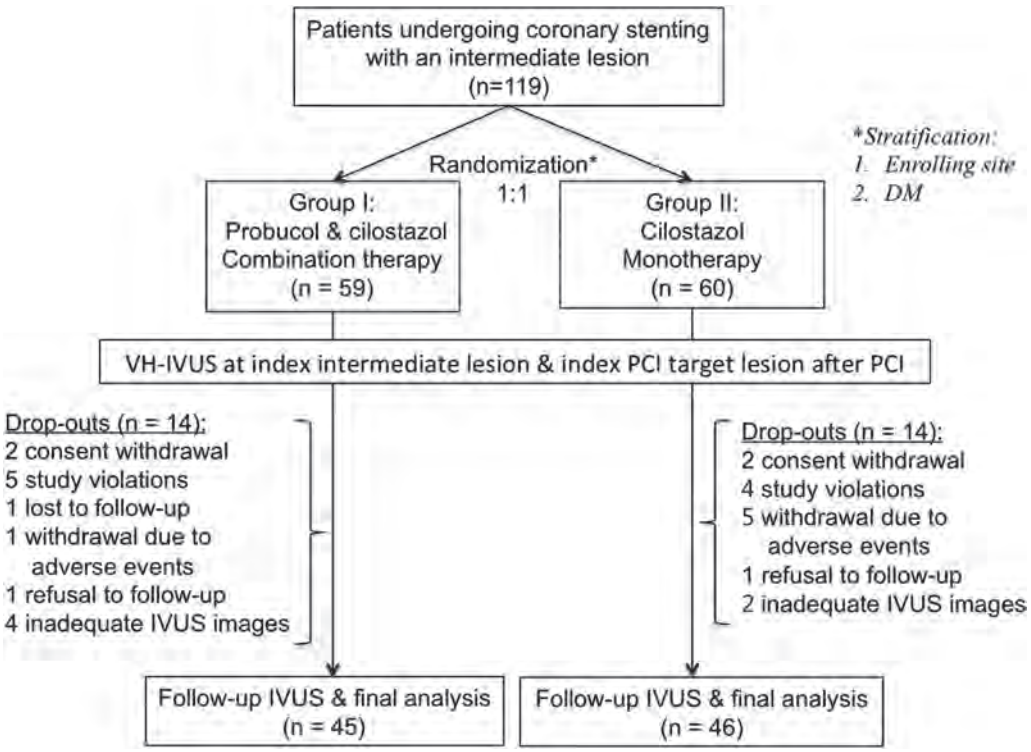

Fig. 1. Study flow chart.

Statistical Analysis

Sample size calculations have been described previously[16]. The calculation of the sample size was based on an inequality design and a two-sample, two-sided test. Assuming a standard deviation of 8%, an α-level of 0.05 and a statistical power of 80%, 41 patients are required for each group (combination therapy, cilostazol monotherapy) to demonstrate a 5% difference in the plaque volume of the index intermediate lesion. Making allowance for a drop-out rate of 30% increases the number of patients required for each group to 59.

Continuous variables with an approximately normal distribution were expressed as means ± standard deviations, and differences between the two treatment groups were compared using Student's *t*-test. Continuous variables not distributed normally were compared using the Mann-Whitney *U* test. Comparisons of continuous variables between baseline and follow-up in each group were performed by paired *t*-test for normally distributed variables and by Wilcoxon signed rank test for non-normally distributed variables. Categorical variables were described as frequency and percentage and compared using chi-squared statistics or Fisher's exact test, as appropriate. Univariate and multivariate linear regression analyses were performed to identify independent predictors of changes in normalized TAV. Variables with a *p*-value <0.15 on univariate analysis and probucol were included in multivariate analyses. A *p*-value <0.05 was considered statistically significant. All statistical analyses were conducted using IBM PASW Statistics 20.0 (IBM Corporation, New York, NY, USA).

Results

Study Subjects and Baseline Characteristics

Between February 2010 and August 2010, a total of 119 patients at five centers were randomized to combination therapy with probucol and cilostazol (group I, $n=59$) or a monotherapy of cilostazol (group II, $n=60$). The final analysis included data from 91 patients (group I, $n=45$; group II, $n=46$) who had completed evaluable IVUS investigations at baseline and after 9 months of treatment (**Fig. 1**). Of the 28 patients who were not included in the IVUS analysis, four patients (two in group I, two in group II) withdrew consent; nine patients (five in group I, four in group II) violated study protocol; one patient (group I) was lost for follow-up; six patients [one in group I (diarrhea); five in group II (liver enzyme elevation, headache, fever of unknown origin, pancreatic cancer, and hepatocellular carcinoma, respectively)] were withdrawn

Table 1. Baseline clinical characteristics

	Group I Probucol & cilostazol ($n=45$)	Group II Cilostazol only ($n=46$)	p-value
Age (years)	62.6 ± 9.1	59.1 ± 7.5	0.050
Male	35 (77.8%)	34 (73.9%)	0.667
Diabetes mellitus	13 (28.9%)	9 (19.6%)	0.229
Hypertension	27 (60.0%)	31 (67.4%)	0.463
Hypercholesterolemia	28 (62.2%)	28 (60.9%)	0.895
Current smoker	7 (15.6%)	12 (26.1%)	0.217
Renal failure (sCr ≥ 1.5 mg/dL)	0 (0.0%)	1 (2.2%)	1.000*
LVEF < 45%	3 (6.7%)	1 (2.2%)	0.361*
Previous MI	1 (2.2%)	2 (4.3%)	1.000*
Previous stroke	2 (4.4%)	4 (8.7%)	1.000*
Previous statin	16 (35.6%)	22 (47.8%)	0.235
Multi-vessel disease	28 (62.2%)	26 (56.5%)	0.580
Clinical presentation			0.357
Chronic stable angina	24 (53.3%)	29 (63.0%)	
Unstable angina	14 (31.1%)	14 (30.4%)	
NSTEMI	7 (15.6%)	3 (6.5%)	
Concomitant medications			
Aspirin	45 (100%)	46 (100%)	1.000
Clopidogrel	45 (100%)	46 (100%)	1.000
Beta-blocker	38 (84.4%)	39 (84.8%)	0.964
ACE inhibitor/ARB	31 (68.9%)	27 (58.7%)	0.312
Statin (simvastatin)	45 (100%)	46 (100%)	1.000
Final simvastatin dose (mg)	20.4 ± 4.7	20.0 ± 5.2	0.670

Abbreviations: ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; EES, everolimus-eluting stent; SES, sirolimus-eluting stent; sCr, serum creatinine; PCI, percutaneous intervention; CABG, coronary artery bypass graft; MI, myocardial infarction; LVEF, left ventricular ejection fraction; NSTEMI, non-ST-segment elevation myocardial infarction.
*p-value determined by Fisher's exact test. P-value of 1.0 by Fisher's exact test indicates a p-value close to 1.0.

due to an adverse event; two patients (one in group I, one in group II) refused to undergo 9-month follow-up angiography and IVUS investigation; and six patients had inadequate IVUS images for quantitative analysis (four in group I, two in group II). We included only the patients who took >75% of the prescribed study drugs during the follow-up into the final analysis. All patients were on aspirin, clopidogrel, and simvastatin during the study period. There were no significant differences in baseline clinical characteristics or medication between the two treatment groups **Table 1**. However, group I showed a trend toward older age compared to group II (62.6 ± 9.1 vs. 59.1 ± 7.5 years, $p=0.050$).

Biochemical Parameters

Table 2 presents blood levels of lipid and biochemical parameters during treatment for the 91 patients who completed the trial. All patients received a stating dose of simvastatin 20 mg with enrollment. Baseline levels of the parameters did not differ significantly between the two treatment groups. However, there was a trend toward higher LDL (102.7 ± 28.9 vs. 94.2 ± 21.8 mg/dL, $p=0.118$) and oxidized LDL levels (37.9 ± 9.0 vs. 35.0 ± 8.0 U/L, $p=0.101$), and larger LDL particle size (23.8 ± 1.0 vs. 23.4 ± 1.1 nm, $p=0.086$) in group I compared to group II. At 9-month follow-up, total cholesterol (117.0 ± 30.6 vs. 140.3 ± 26.9 mg/dL, $p<0.001$), HDL (32.0 ± 9.2 vs. 46.0 ± 11.2 mg/dL, $p<0.001$), apoliprotein A1 (apoA1; 98.2 ± 22.6 vs. 131.4 ± 19.0 mg/dL, $p<0.001$), and HDL_{2b} (31.9 ± 2.5% vs. 34.1 ± 2.4%, $p<0.001$) were significantly lower in group I than in group II. HDL_{2a} (23.2 ± 1.4% vs. 22.1 ± 1.1%, $p<0.001$) and HDL_{3a} (18.2 ± 1.2% vs. 17.3 ± 1.0%, $p<0.001$) were significantly higher in group I than in group II at 9 months. LDL cholesterol, triglycerides, apoliprotein B (apoB), LDL particle size, HDL_{3b}, HDL_{3c}, Lp(a), oxidized

Table 2. Lipid and biochemical parameters at baseline and 9-month follow-up

		Group I Probucol & cilostazol (n=45)	Group II Cilostazol only (n=46)	p-value
Total cholesterol (mg/dL)	Baseline	169.2±34.8	165.7±26.3	0.595
	Follow-up	117.0±30.6	140.3±26.9	<0.001
	Δ	−51.8±33.0†	−25.4±39.1†	0.001
LDL (mg/dL)	Baseline	102.7±28.9	94.2±21.8	0.118
	Follow-up	69.1±26.0	73.9±21.7	0.348
	Δ	−33.5±30.5†	−20.3±30.8†	0.044
HDL (mg/dL)	Baseline	42.7±11.3	43.3±8.6	0.787
	Follow-up	32.0±9.2	46.0±11.2	<0.001*
	Δ	−11.2±8.5‡	2.7±7.7‡	<0.001*
Triglycerides (mg/dL)	Baseline	120.7±53.1	134.0±53.4	0.246
	Follow-up	102.2±57.7	108.2±49.7	0.343*
	Δ	−15.9±65.0	−31.8±54.5†	0.225
ApoA1 (mg/dL)	Baseline	116.2±19.7	121.4±19.7	0.224
	Follow-up	98.2±22.6	131.4±19.0	<0.001*
	Δ	−18.2±21.4†	10.0±16.5‡	<0.001
ApoB (mg/dL)	Baseline	71.4±16.7	69.1±15.8	0.502
	Follow-up	53.5±14.4	54.4±12.4	0.762
	Δ	−17.9±15.1†	−14.7±19.3	0.196*
LDL particle size (nm)	Baseline	23.8±1.0	23.4±1.1	0.086
	Follow-up	23.7±1.0	23.6±1.0	0.968*
	Δ	−0.2±0.9	0.2±1.1	0.126
HDL_{2b} (%)	Baseline	32.4±2.6	32.8±2.4	0.444
	Follow-up	31.9±2.5	34.1±2.4	<0.001*
	Δ	−0.5±3.2	1.3±2.8‡	0.001*
HDL_{2a} (%)	Baseline	22.5±1.2	22.5±1.1	0.970
	Follow-up	23.2±1.4	22.1±1.1	<0.001*
	Δ	0.7±1.8‡	−0.4±1.8	0.006
HDL_{3a} (%)	Baseline	18.3±1.1	18.1±1.3	0.331
	Follow-up	18.2±1.2	17.3±1.0	<0.001
	Δ	−0.2±1.5	−0.8±1.6	0.064
HDL_{3b} (%)	Baseline	12.7±1.1	12.6±1.1	0.634
	Follow-up	12.4±1.2	12.2±0.9	0.181*
	Δ	−0.3±1.5	−0.4±1.1†	0.668
HDL_{3c} (%)	Baseline	14.1±2.2	13.9±2.3	0.955*
	Follow-up	14.3±1.7	14.3±2.0	0.857
	Δ	0.2±2.7	0.4±3.9	0.740
Lp(a) (mg/dL)	Baseline	17.7±16.0	18.6±20.4	0.537*
	Follow-up	17.5±16.6	21.5±25.4	0.846*
	Δ	−0.1±7.3	2.8±8.8‡	0.151*
Oxdized LDL (U/L)	Baseline	37.9±9.0	35.0±8.0	0.101
	Follow-up	28.2±7.7	29.3±8.5	0.505
	Δ	−9.8±8.2†	−5.6±8.8†	0.024

(Cont Table 2)

		Group I Probucol & cilostazol (n=45)	Group II Cilostazol only (n=46)	p-value
sVCAM-1 (ng/mL)	Baseline	597.6±151.0	552.8±117.1	0.125
	Follow-up	634.1±189.5	591.0±145.8	0.237
	Δ	36.5±97.5†	38.2±103.1†	0.937
vWF (mU/mL)	Baseline	1120.1±652.8	1094.9±679.4	0.861
	Follow-up	1241.5±795.3	1124.4±924.1	0.167*
	Δ	121.4±891.7	29.4±785.9	0.611
hsCRP (mg/L)	Baseline	1.3±1.6	2.1±4.5	0.699*
	Follow-up	2.3±4.4	1.2±2.0	0.166*
	Δ	0.0±2.0	−0.8±2.7‡	0.316*

Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein; Lp(a), lipoprotein (a); sVCAM, soluble vascular cell adhesion molecule; vWF, von Willebrand factor; hsCRP, high sensitivity C-reactive protein. *p-value determined by Mann-Whitney U test. †p-value <0.05, determined by paired t-test. ‡p-value <0.05, determined by Wilcoxon signed rank test.

LDL, sVCAM, vWF, and hsCRP levels were similar between the two groups at 9-months. There were significant decreases in blood levels of total cholesterol, LDL cholesterol, and oxidized LDL from baseline to 9-month follow-up in both groups. However, there were greater decreases in total cholesterol (Δ −51.8±33.0 vs. −25.4±39.1 mg/dL, $p<0.001$) and LDL (Δ −33.5±30.5 vs. −20.3±30.8 mg/dL, $p=0.044$) levels in group I than in group II. ApoB levels showed significant decrease during follow-up only in group I. HDL cholesterol (Δ −11.2±8.5 vs. 2.7±7.7 mg/dL, $p<0.001$) and apoA1 (Δ −18.2±21.4 vs. 10.0±16.5 mg/dL, $p<0.001$) levels were significantly decreased from baseline to follow-up in group I; however, they showed a significant increase in group II. Group II showed a shift from HDL_{2a}, HDL_{3a}, and HDL_{3b} toward the larger HDL subtype, HDL_{2b}, whereas group II exhibited an increase in the HDL_{2a} subtype. There was no change in LP(a) level in group I; however, LP(a) levels were significantly increased in group II (Δ −0.1±7.3 vs. 2.8±8.8 mg/dL, $p=0.151$). Oxidized LDL showed a greater decrease in group I than in group II at 9 months (Δ −9.8±8.2 vs. −5.6±8.8 U/L, $p=0.024$).

Plaque Volume and Composition of Index Intermediate Lesions

IVUS and VH data for index intermediate lesions at baseline and 9-month follow-up are presented in **Table 3**. Lesion location and length, and baseline TAV, normalized TAV, and PAV did not differ between the two treatment groups at baseline. There were no significant differences in the primary end-point-nominal change in normalized TAV (Δ −12.6±17.7 vs. −14.2±20.2 mm^3, $p=0.691$)- or PAV (Δ −7.1±9.8% vs. −6.6±13.5%, $p=0.855$) between group I and group II. However, in both groups, normalized TAV [nominal change −12.6±17.7 mm^3 ($p<0.001$) for group I; −14.2±20.2 mm^3 ($p<0.001$) for group II] and PAV [nominal change −3.4±4.6% ($p<0.001$) for group I; −3.2±5.8% ($p=0.001$) for group II] were significantly reduced from baseline to 9-month follow-up. Plaque regression based on normalized TAV was observed in 33 patients (73.3%) in group I and in 34 patients (73.9%) in group II ($p=0.950$).

Plaque composition, as assessed by VH, showed no significant differences in changes in percent volumes of fibrofatty and fibrous tissues, necrotic core, and dense calcium between the two groups during treatment. However, group I showed a significant decrease in the percent volume of fibrotic tissue, whereas group II exhibited a significant increase in the percent volume of fibrofatty tissue.

Independent Determinants of Plaque Regression

To investigate independent determinants of nominal change in normalized TAV of index intermediate lesions, we performed univariate and multivariate linear regression analyses using various clinical and biochemical factors **Table 4**. In univariate analysis, the presence of DM, clinical presentation of acute coronary syndrome, and change in LP(a) levels were significantly associated with a change in normalized TAV. Multivariate analysis revealed DM [$\beta=0.22$; 95% confidence interval (CI), 0.62-19.05; $p=0.037$] as the only significant independent determinant of nominal

Table 3. Baseline and 9-month follow-up gray-scale IVUS and VH data for index intermediate lesions

	Group I Probucol & cilostazol (n=45)	Group II Cilostazol only (n=46)	p-value
Lesion location			0.780
Left anterior descending	17 (37.8%)	17 (37.0%)	
Left circumflex	18 (40.0%)	16 (34.8%)	
Right coronary	10 (22.2%)	13 (28.3%)	
Lesion length (mm)	25.6±9.0	24.5±8.0	0.578
TAV			
Baseline (mm^3)	178.4±96.8	166.7±79.5	0.532
Follow-up (mm^3)	166.6±96.9	149.1±63.1	0.312
Nominal change (mm^3)	−11.8±20.3†	−17.6±34.6†	0.949*
Percent change (%)	−8.4±11.8	−7.1±19.2	0.739*
Normalized TAV			
Baseline (mm^3)	162.0±63.6	162.8±54.3	0.952
Follow-up (mm^3)	149.4±59.7	148.6±51.9	0.943
Nominal change (mm^3)	−12.6±17.7†	−14.2±20.2†	0.691
Percent change (%)	−7.7±10.2	−8.5±10.5	0.702
PAV			
Baseline (%)	48.6±10.6	46.0±9.2	0.183
Follow-up (%)	45.2±11.0	42.6±9.8	0.234
Nominal change (%)	−3.4±4.6†	−3.2±5.8†	0.879
Percent change (%)	−7.1±9.8	−6.6±13.5	0.855
Plaque regression			
Based on normalized TAV	33 (73.3%)	34 (73.9%)	0.950
Based on PAV	36 (80.0%)	36 (78.3%)	0.838
Percent fibrous tissue volume (%)			
Baseline	58.5±7.2	57.3±6.2	0.406
Follow-up	53.1±9.6	54.8±9.2	0.152*
Δ	−5.5±8.7‡	−2.5±8.6	0.060*
Percent fibrofatty tissue volume (%)			
Baseline	15.5±7.2	17.1±10.2	0.575*
Follow-up	19.8±15.1	19.7±12.6	0.250*
Δ	4.8±14.6	2.7±10.5‡	0.938*
Percent necrotic tissue volume (%)			
Baseline	19.6±7.8	19.1±9.0	0.781
Follow-up	19.4±8.4	17.9±7.9	0.252*
Δ	−0.5±7.9	−1.2±6.3	0.633
Percent dense calcium volume (%)			
Baseline	6.4±4.6	6.5±4.0	0.923
Follow-up	7.7±4.9	7.6±5.6	0.707*
Δ	1.1±4.4	1.1±4.7	0.658*

*p-value determined by Mann-Whitney U test. †p-value <0.05, determined by paired t-test. ‡p-value <0.05, determined by Wilcoxon signed rank test. Abbreviations: TAV, total atheroma volume; PAV, percent atheroma volume.

changes in normalized TAV. Patients with plaque regression based on normalized TAV showed a greater reduction in LDL level (−Δ31.2±30.0 vs. −17.0±26.7 mg/dL, p=0.045) and a greater increase in LP(a) level (Δ2.5±8.3 vs. −1.8±7.0 mg/dL, p=0.047) than patients without plaque regression (**Fig. 2**). Changes

Table 4. Univariate and multivariate linear regression analyses of determinants of nominal changes in normalized TAV

	Univariate analysis			Multivariate analysis		
	Beta	95% CI	*p*-value	Beta	95% CI	*p*-value
Probucol	0.04	−6.33-9.51	0.691	0.01	−7.56-8.03	0.953
Male	0.17	−1.64-6.60	0.107	0.11	−4.58-14.30	0.309
DM	0.26	2.58-20.73	0.012	0.22	0.62-19.05	0.037
ACS	−0.25	−17.48-−1.87	0.016	−0.20	−15.94-0.32	0.055
Change in Lp(a)	−0.26	−1.09-−0.13	0.014	−0.20	−0.94-0.03	0.066

Probucol use and all variables found to be associated with contrast-induced nephropathy in univariate analysis ($p < 0.15$) were included in the multivariate analysis. Abbreviations: TAV, total atheroma volume; CI, confidence interval; DM, diabetes mellitus; ACS, acute coronary syndrome; Lp(a), lipoprotein (a).

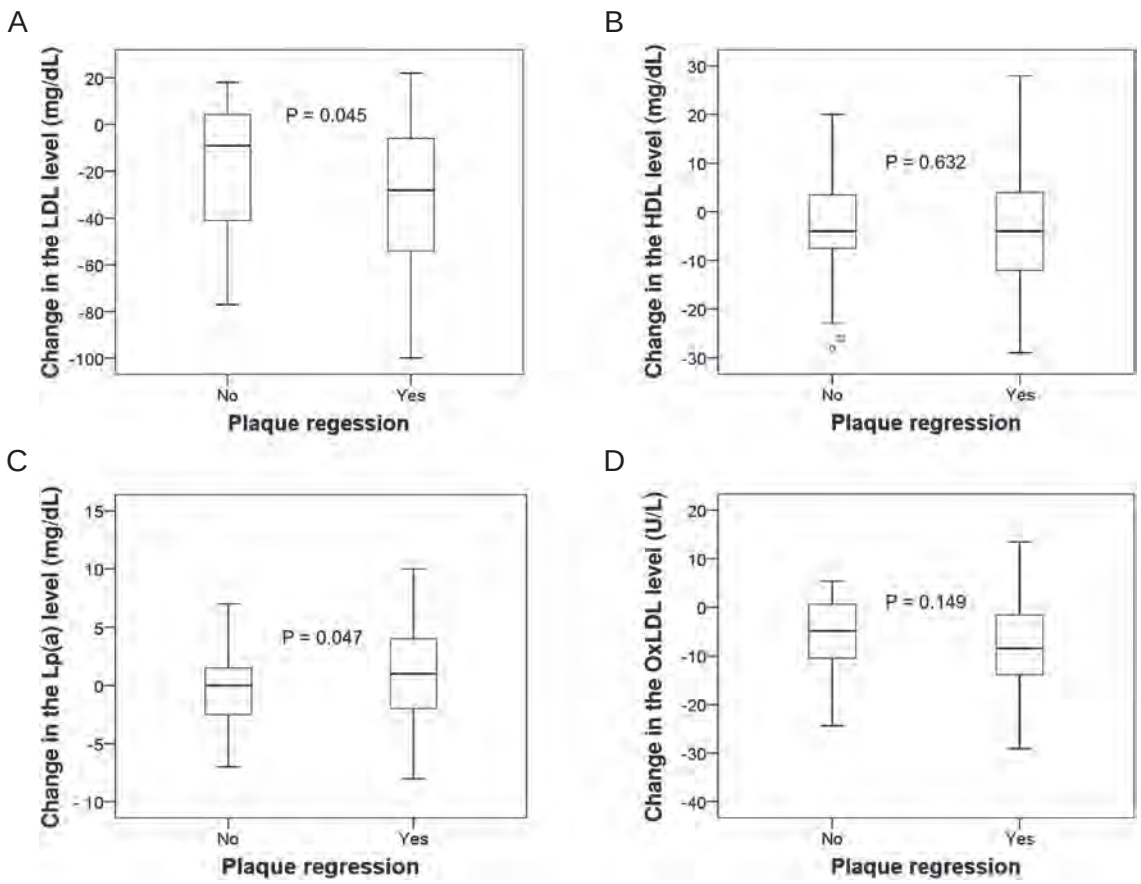

Fig. 2. Changes in lipid and biomarker levels according to plaque regression based on TAV.

Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein; Lp(a), lipoprotein (a); oxLDL, oxidized LDL cholesterol.

in HDL and oxidized LDL levels were not significantly different between patient groups with and without plaque regression.

Neointimal Hyperplasia of PCI Target Lesions

Angiographic and procedural data for index PCI target lesions are presented in **Table 5**. There were no significant differences in baseline or procedural data between the two treatment groups. Follow-up angiography at 9 months showed similar stenosis diameter, late loss, and in-stent restenosis rates between the patient groups. The IVUS parameters of the stented

Table 5. Angiographic and procedural data for index PCI target lesions

	Group I Probucol & cilostazol (n=45)	Group II Cilostazol only (n=46)	p-value
PCI target lesion			0.996
Left anterior descending	25 (55.6%)	26 (56.5%)	
Left circumflex	12 (26.7%)	12 (26.1%)	
Right coronary	8 (17.8%)	8 (17.4%)	
Lesion type B2/C	27 (60.0%)	26 (56.5%)	0.737
Lesion length (mm)	17.0 ± 6.1	18.8 ± 7.1	0.219
Pre-PCI data			
Reference diameter (mm)	3.1 ± 0.4	3.2 ± 0.4	0.529
Minimum lumen diameter (mm)	1.0 ± 0.4	1.1 ± 0.4	0.728
Diameter stenosis (%)	67.3 ± 11.6	66.5 ± 12.4	0.781
Post-PCI data			
Minimum lumen diameter (mm)	2.8 ± 0.4	2.9 ± 0.4	0.511
Diameter stenosis (%)	9.9 ± 8.2	9.6 ± 6.3	0.864
Stent diameter (mm)	3.2 ± 0.4	3.2 ± 0.4	0.908
Stent length (mm)	22.2 ± 5.9	22.3 ± 5.4	0.916
Follow-up data			
Reference diameter (mm)	3.1 ± 0.4	3.2 ± 0.4	0.358
Minimum lumen diameter (mm)	2.4 ± 0.7	2.4 ± 0.5	0.935
Diameter stenosis (%)	22.1 ± 15.8	23.0 ± 12.9	0.779
Late loss (mm)	0.4 ± 0.4	0.5 ± 0.4	0.564
In-stent restenosis	3 (6.7%)	1 (2.2%)	0.361*
In-segment restenosis	3 (6.7%)	1 (2.2%)	0.361*

PCI, percutaneous coronary intervention. *p-value determined by Mann-Whitney U test.

segments are shown in **Table 6**. The vessel, lumen, and persistent plaque volumes post-procedure and at 9-month follow-up did not differ between the two groups. Neointimal volume, neointimal volume index, and percent neointimal obstruction were not significantly different between group I and group II.

Clinical Outcomes and Adverse Events

Clinical outcomes are summarized in **Table 7**. There were no significant differences in the incidence of major adverse clinical events (1.2% vs. 4.3%, p=1.000), a composite of all-cause death, MI and target vessel revascularization (TVR); incidence of death (0.0% vs. 0.0%, p=1.000); TVR (2.2% vs. 4.3%, p=1.000); or target lesion revascularization (2.2% vs. 2.2%, p=1.000) between group I and group II. There were no cases of MI or stent thrombosis in either group. The cause of death in one mortality case in group II was related to hepatic cell carcinoma diagnosed one month after enrollment and was considered unrelated to the study drugs. Various adverse events potentially related to the study drugs among initially enrolled patients are presented in **Table 8**. There was a trend toward a lower frequency of drug-related adverse events in group I than group II (20.3% vs. 35.0%, p=0.114). However, only a small number of patients (one patient in group I and three patients in group II) discontinued the treatment due to adverse events. There was no incidence of significant bleeding event in both patient groups. Each group had only one case of skin bruise that was well tolerated by the patients.

Discussion

In the present study, plaque volume regression was observed in more than 70% of individuals in both treatment groups. There was approximately a 7-8% decrease in TAV for both groups. However, VH-IVUS revealed no significant differences in plaque volume changes or changes in plaque composition between the two groups.

The reduction of TAV might be due to anti-atherosclerotic effects of statin and/or cilostazol.

In our study, all patients received simvastatin, a rather moderate potency statin, and both treatment groups achieved mean LDL levels of approximately 70

Table 6. Post-procedural and 9-month follow-up IVUS data for stented PCI target lesions

	Group I Probucol & cilostazol ($n=45$)	Group II Cilostazol only ($n=46$)	p-value
Vessel volume (mm^3)			
Baseline	326.3 ± 113.6	342.4 ± 115.7	0.599
Follow-up	324.1 ± 106.6	340.0 ± 109.5	0.488
Δ	− 2.2 ± 28.6	1.6 ± 37.5	0.668
Lumen volume (mm^3)			
Baseline	151.4 ± 56.2	159.6 ± 52.8	0.578
Follow-up	130.7 ± 49.0	142.1 ± 50.5	0.392
Δ	− 20.7 ± 17.7*	− 17.5 ± 17.3*	0.489
Peristent plaque volume (mm^3)			
Baseline	174.7 ± 65.2	182.6 ± 70.0	0.571
Follow-up	177.9 ± 61.1	186.6 ± 63.3	0.548
Δ	3.2 ± 18.0	4.1 ± 25.9	0.887
Neointimal volume (mm^3)	16.2 ± 13.2	14.8 ± 10.6	0.605
Neointimal volume index (mm^3/mm)	0.7 ± 0.5	0.7 ± 0.4	0.707
Percent neointimal volume (%)	11.3 ± 7.2	9.6 ± 6.6	0.289

*p-value determined by Mann-Whitney U test.

Table 7. Clinical outcomes

	Group I Probucol & cilostazol ($n=45$)	Group II Cilostazol only ($n=46$)	p-value*
Clinical outcomes			
MACE[1]	1 (2.2%)	2 (4.3%)	1.000
Death	0 (0.0%)	0 (0.0%)	1.000
MI	0 (0.0%)	0 (0.0%)	1.000
TVR	1 (2.2%)	2 (4.3%)	1.000
TLR	1 (2.2%)	1 (2.2%)	1.000
Stent thrombosis	0 (0.0%)	0 (0.0%)	1.000

[1]MACE is defined as a composite of death, MI, and target vessel revascularization. Abbreviations: MACE, major adverse cardiac event; MI, myocardial infarction; TVR, target vessel revascularization; TLR, target lesion revascularization; All p-values were determined by Fisher's exact test. P-value of 1.0 by Fisher's exact test indicates a p-value close to 1.0.

mg/dL at 9-month follow-up, although a greater reduction in LDL cholesterol level was achieved with the combination therapy than with cilostazol monotherapy. Patients with plaque regression showed a greater reduction in LDL levels than those without plaque regression. However, there was no significant linear relationship between the extent of LDL reduction and percent change in TAV. Multivariate regression analysis demonstrated that the presence of DM was the only independent determinant of change in TAV. Our observation that the presence of DM contributed to the increase in TAV is plausible since DM is known to be a major risk factor for atherosclerosis. ACS showed a trend toward significant association with reduction of TAV in the multivariate analysis ($p=0.055$). Previous studies have also shown that stabilization of ACS results in negative remodeling and a reduction in plaque volume in coronary artery lesions[19, 20]. Furthermore, we found a trend toward a negative linear relationship between percent change in Lp(a) level and percent change in TAV: patients with plaque regression showed a greater increase in Lp(a) levels than

Table 8. Adverse events related to study drugs and reasons for discontinuation of treatment

	Group I Probucol & cilostazol (n=59)	Group II Cilostazol only (n=60)	p-value
Adverse events related to study drugs			
Number of patients	12 (20.3%)	21 (35.0%)	0.114
Number of events	15	31	
QTc interval (>470 ms)	2 (3.4%)	1 (1.7%)	0.619*
AST/ALT >3× ULN	0 (0.0%)	1 (1.7%)	1.000*
Palpitation	1 (1.7%)	1 (1.7%)	1.000*
Diarrhea/nausea	4 (6.8%)	2 (3.3%)	0.439*
Headache/dizziness	6 (10.2%)	18 (30.0%)	0.014
Dizziness	0	4 (6.7%)	0.119*
Weakness	2 (3.4%)	2 (3.3%)	1.000*
Muscle pain	0 (0.0%)	1 (1.7%)	1.000*
Blurred vision	0 (0.0%)	1 (1.7%)	1.000*
Discontinuation of treatment	9 (11.9%)	11 (20.0%)	0.597
Reasons			
Adverse events	1 (1.7%)	5 (5.0%)	0.207*
AST/ALT elevation	0 (0.0%)	1 (1.7%)	1.000*
Palpitation	1 (1.7%)	0 (0.0%)	0.496*
Headache	0 (0.0%)	1 (1.7%)	1.000*
Fever	0 (0.0%)	1 (1.7%)	1.000*
Malignancy	0 (0.0%)	2 (3.3%)	0.496*
Consent withdrawal	2 (3.4%)	2 (3.3%)	1.000*
Loss of follow-up	1 (1.7%)	0 (0.0%)	0.496*
Protocol violations	5 (8.5%)	4 (6.7%)	0.743*
Inadequate enrollment	3 (5.1%)	2 (3.3%)	1.000*
Non-compliance	2 (3.4%)	2 (3.3%)	1.000*

Abbreviations: QTc interval, corrected QT interval; AST, aspartate aminotransferase; ALT, alanine aminotransferase. *p-value determined by Fisher's exact test. P-value of 1.0 by Fisher's exact test indicates a p-value close to 1.0.

those without plaque regression. This finding appears to be contradictory since Lp(a) is generally known as a biomarker of increased cardiovascular risk. However, increases in Lp(a) levels under statin therapy were also observed in the REVERSAL study[21]. It was suggested that Lp(a) may bind and remove oxidized fatty acids from oxidized phospholipids, thus acting as a scavenger[22]. However, the mechanism and clinical impact of increased Lp(a) levels during treatment remain uncertain. Although Lp(a) levels were increased in the cilostazol monotherapy group, both probucol and cilostazol have been reported to exert no effect on Lp(a) level[13, 23]. Oxidized LDL levels were significantly decreased in both treatment groups. However, a greater reduction in oxidized LDL levels occurred under the combination therapy. This observation may reflect reduced oxidation of LDL cholesterol by probucol as the previous study reported[24]. However, this cannot be validated in the present study since additional markers of oxidative stress were not evaluated.

In the present study, the use of probucol decreased not only LDL cholesterol and apoB levels, but also HDL cholesterol and apoA1 levels, in agreement with previous findings. Probucol is thought to reduce HDL cholesterol by enhancing RCT through increased expression of the hepatic HDL-receptor SR-B1 and activation of CETP[6, 25, 26]. Increased expression of SR-B1 in the liver increases hepatic uptake of HDL cholesterol, resulting in increased biliary excretion of cholesterol. Activation of CETP increases transfer of cholesterol esters from HDL to triglyceride-rich lipoprotein particles in exchange for triglyceride, thereby reducing the circulating HDL cholesterol concentration. Probucol-activated CETP increases preβ1-HDL (lipid-poor apoA1), which participates in the cholesterol efflux, and decreases HDL particle size[27]. A pre-

vious study showed that probucol treatment resulted in a greater reduction in HDL_{2b} than in HDL_{2a} and HDL_3, and that CETP mass increased by 20%[28]. We also observed a trend toward a decrease in HDL_{2b} and a significant increase in the HDL_{2a} subtype in the combination treatment group. In the cilostazol monotherapy group, HDL cholesterol and apoA1 levels were increased in association with a significant shift in HDL subtypes from HDL_{2a} and HDL_3 to the larger HDL subtype, HDL_{2b}. Generally, the larger triglyceride-rich HDL_2 subfraction is thought to confer better protection against coronary artery disease than the smaller HDL_3 fraction[29]. However, we found no relationship between plaque regression and a given HDL subtype in our study. In a recent study, Nakaya *et al.*[30] reported that cilostazol exerts its anti-atherosclerotic effects by promoting RCT through increased ABCA1/G1 expression in macrophages. Specifically, cilostazol was shown to induce macrophage ABCA1 and ABCG1 expression in a cAMP/PKA-independent manner and stimulate apoA1- and HDL-mediated cholesterol efflux, resulting in promotion of RCT *in vivo*. In our study, we confirmed the effects of probucol and cilostazol on various lipid and biochemical parameters. However, the mechanisms by which probucol or cilostazol treatment lead to plaque volume regression could not be fully clarified in the present study.

Previous studies have demonstrated that both probucol and cilostazol are effective in preventing restenosis after PCI, with or without stent implantation[7, 31]. Sekiya *et al.*[32] reported that cilostazol and probucol combination therapy reduces restenosis after stent implantation more effectively than either cilostazol or probucol alone. However, our study showed no significant difference in neointimal growth at PCI target lesions after stenting between combination therapy and cilostazol monotherapy. Therefore, probucol and cilostazol combination therapy seems to have less impact on neointimal hyperplasia and restenosis in patients treated with drug-eluting stents than in patients treated with bare-metal stents.

There are several limitations to be considered in the present study. First, there was no control group treated with statin alone or without statin. Therefore, we cannot rule out that regression of coronary plaques was mainly caused by simvastatin effect on LDL reduction. Pleotropic effect of statin may have also lead to plaque regression in both treatment groups. Second, the size of the study population was relatively small and the follow-up duration may have been short to detect significant differences in plaque regression between the two treatment groups. Third, we analyzed a relatively short segment of coronary artery that may not be representative of the entire coronary artery system. Fourth, the combination treatment group in our study received a smaller dose (500 mg daily) of probucol compared to previous studies[1, 31]. Thus, we cannot exclude the possibility that a higher dose of probucol might have lead to a greater reduction in plaque volume. However, the present study was able to show effects of probucol on various biomarkers, suggesting that the dose of probucol was not insufficient.

Conclusions

The present study did not demonstrate the superiority of cilostazol and probucol combination therapy in reducing coronary plaque volume compared with cilostazol monotherapy. However, plaque regression was achieved in the majority of individuals in both treatment groups. A larger study population and longer-term follow-up are required to fully assess the efficacy of cilostazol and probucol combination therapy.

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Conflicts of Interest

None.

References

1) Sawayama Y, Shimizu C, Maeda N, Tatsukawa M, Kinukawa N, Koyanagi S, Kashiwagi S, Hayashi J: Effects of probucol and pravastatin on common carotid atherosclerosis in patients with asymptomatic hypercholesterolemia. Fukuoka Atherosclerosis Trial (FAST). J Am Coll Cardiol, 2002; 39: 610-616

2) Yamashita S, Matsuzawa Y: Where are we with probucol:

a new life for an old drug? Atherosclerosis, 2009; 207: 16-23
3) Yamashita S, Hbujo H, Arai H, Harada-Shiba M, Matsui S, Fukushima M, Saito Y, Kita T, Matsuzawa Y: Long-term probucol treatment prevents secondary cardiovascular events: a cohort study of patients with heterozygous familial hypercholesterolemia in Japan. Journal of atherosclerosis and thrombosis, 2008; 15: 292-303
4) Franceschini G, Sirtori M, Vaccarino V, Gianfranceschi G, Rezzonico L, Chiesa G, Sirtori CR: Mechanisms of HDL reduction after probucol. Changes in HDL subfractions and increased reverse cholesteryl ester transfer. Arteriosclerosis, 1989; 9: 462-469
5) Ishigami M, Yamashita S, Sakai N, Hirano K, Arai T, Maruyama T, Takami S, Koyama M, Kameda-Takemura K, Matsuzawa Y: High-density lipoproteins from probucol-treated patients have increased capacity to promote cholesterol efflux from mouse peritoneal macrophages loaded with acetylated low-density lipoproteins. Eur J Clin Invest, 1997; 27: 285-292
6) Hirano K, Ikegami C, Tsujii K, Zhang Z, Matsuura F, Nakagawa-Toyama Y, Koseki M, Masuda D, Maruyama T, Shimomura I, Ueda Y, Yamashita S: Probucol enhances the expression of human hepatic scavenger receptor class B type I, possibly through a species-specific mechanism. Arterioscler Thromb Vasc Biol, 2005; 25: 2422-2427
7) Weintraub WS: The vascular effects of cilostazol. Can J Cardiol, 2006; 22 Suppl B: 56B-60B
8) Nakamura T, Houchi H, Minami A, Sakamoto S, Tsuchiya K, Niwa Y, Minakuchi K, Nakaya Y: Endothelium-dependent relaxation by cilostazol, a phosphodiesteras Ⅲ inhibitor, on rat thoracic aorta. Life Sci, 2001; 69: 1709-1715
9) Kim KY, Shin HK, Choi JM, Hong KW: Inhibition of lipopolysaccharide-induced apoptosis by cilostazol in human umbilical vein endothelial cells. J Pharmacol Exp Ther, 2002; 300: 709-715
10) Okutsu R, Yoshikawa T, Nagasawa M, Hirose Y, Takase H, Mitani K, Okada K, Miyakoda G, Yabuuchi Y: Cilostazol inhibits modified low-density lipoprotein uptake and foam cell formation in mouse peritoneal macrophages. Atherosclerosis, 2009; 204: 405-411
11) Ishizaka N, Taguchi J, Kimura Y, Ikari Y, Aizawa T, Togo M, Miki K, Kurokawa K, Ohno M: Effects of a single local administration of cilostazol on neointimal formation in balloon-injured rat carotid artery. Atherosclerosis, 1999; 142: 41-46
12) Tani T, Uehara K, Sudo T, Marukawa K, Yasuda Y, Kimura Y: Cilostazol, a selective type Ⅲ phosphodiesterase inhibitor, decreases triglyceride and increases HDL cholesterol levels by increasing lipoprotein lipase activity in rats. Atherosclerosis, 2000; 152: 299-305
13) Elam MB, Heckman J, Crouse JR, Hunninghake DB, Herd JA, Davidson M, Gordon IL, Bortey EB, Forbes WP: Effect of the novel antiplatelet agent cilostazol on plasma lipoproteins in patients with intermittent claudication. Arterioscler Thromb Vasc Biol, 1998; 18: 1942-1947
14) Yoshikawa T, Mitani K, Kotosai K, Nozako M, Miyakoda G, Yabuuchi Y: Antiatherogenic effects of cilostazol and probucol alone, and in combination in low density lipoprotein receptor-deficient mice fed with a high fat diet. Horm Metab Res, 2008; 40: 473-478
15) Park SY, Lee JH, Shin HK, Kim CD, Lee WS, Rhim BY, Shin YW, Hong KW: Synergistic efficacy of concurrent treatment with cilostazol and probucol on the suppression of reactive oxygen species and inflammatory markers in cultured human coronary artery endothelial cells. Korean J Physiol Pharmacol, 2008; 12: 165-170
16) Ko YG, Kim BK, Lee BK, Kang WC, Choi SH, Kim SW, Lee JH, Lee M, Honda Y, Fitzerald PJ, Shim WH: Study design and rationale of "Synergistic effect of combination therapy with cilostazol and ProbUcol on plaque stabilization and lesion REgression (SECURE)" study: a double-blind randomised controlled multicenter clinical trial. Trials, 2011; 12: 10
17) Anderson JL, Adams CD, Antman EM, Bridges CR, Califf RM, Casey DE Jr, Chavey WE, 2nd, Fesmire FM, Hochman JS, Levin TN, Lincoff AM, Peterson ED, Theroux P, Wenger NK, Wright RS, Smith SC Jr, Writing Group M, Accf/Aha Task Force M: 2011 ACCF/AHA Focused Update Incorporated Into the ACC/AHA 2007 Guidelines for the Management of Patients With Unstable Angina/Non-ST-Elevation Myocardial Infarction: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation, 2011; 123: e426-579
18) Nicholls SJ, Ballantyne CM, Barter PJ, Chapman MJ, Erbel RM, Libby P, Raichlen JS, Uno K, Borgman M, Wolski K, Nissen SE: Effect of two intensive statin regimens on progression of coronary disease. N Engl J Med, 2011; 365: 2078-2087
19) Okazaki S, Yokoyama T, Miyauchi K, Shimada K, Kurata T, Sato H, Daida H: Early statin treatment in patients with acute coronary syndrome: demonstration of the beneficial effect on atherosclerotic lesions by serial volumetric intravascular ultrasound analysis during half a year after coronary event: the ESTABLISH Study. Circulation, 2004; 110: 1061-1068
20) Hiro T, Kimura T, Morimoto T, Miyauchi K, Nakagawa Y, Yamagishi M, Ozaki Y, Kimura K, Saito S, Yamaguchi T, Daida H, Matsuzaki M: Effect of intensive statin therapy on regression of coronary atherosclerosis in patients with acute coronary syndrome: a multicenter randomized trial evaluated by volumetric intravascular ultrasound using pitavastatin versus atorvastatin (JAPAN-ACS [Japan assessment of pitavastatin and atorvastatin in acute coronary syndrome] study). J Am Coll Cardiol, 2009; 54: 293-302
21) Choi SH, Chae A, Miller E, Messig M, Ntanios F, DeMaria AN, Nissen SE, Witztum JL, Tsimikas S: Relationship between biomarkers of oxidized low-density lipoprotein, statin therapy, quantitative coronary angiography, and atheroma: volume observations from the REVERSAL (Reversal of Atherosclerosis with Aggressive Lipid Lowering) study. J Am Coll Cardiol, 2008; 52: 24-32
22) Tsimikas S, Tsironis LD, Tselepis AD: New insights into the role of lipoprotein(a)-associated lipoprotein-associated phospholipase A2 in atherosclerosis and cardiovascular disease. Arterioscler Thromb Vasc Biol, 2007; 27: 2094-

2099
23) Naruszewicz M, Selinger E, Dufour R, Davignon J: Probucol protects lipoprotein (a) against oxidative modification. Metabolism, 1992; 41: 1225-1228
24) Bird DA, Tangirala RK, Fruebis J, Steinberg D, Witztum JL, Palinski W: Effect of probucol on LDL oxidation and atherosclerosis in LDL receptor-deficient mice. J Lipid Res, 1998; 39: 1079-1090
25) Fruebis J, Gonzalez V, Silvestre M, Palinski W: Effect of probucol treatment on gene expression of VCAM-1, MCP-1, and M-CSF in the aortic wall of LDL receptor-deficient rabbits during early atherogenesis. Arterioscler Thromb Vasc Biol, 1997; 17: 1289-1302
26) Lau AK, Leichtweis SB, Hume P, Mashima R, Hou JY, Chaufour X, Wilkinson B, Hunt NH, Celermajer DS, Stocker R: Probucol promotes functional reendothelialization in balloon-injured rabbit aortas. Circulation, 2003; 107: 2031-2036
27) Miida T, Seino U, Miyazaki O, Hanyu O, Hirayama S, Saito T, Ishikawa Y, Akamatsu S, Nakano T, Nakajima K, Okazaki M, Okada M: Probucol markedly reduces HDL phospholipids and elevated prebeta1-HDL without delayed conversion into alpha-migrating HDL: putative role of angiopoietin-like protein 3 in probucol-induced HDL remodeling. Atherosclerosis, 2008; 200: 329-335
28) Miida T, Yamaguchi T, Tsuda T, Okada M: High prebeta1-HDL levels in hypercholesterolemia are maintained by probucol but reduced by a low-cholesterol diet. Atherosclerosis, 1998; 138: 129-134
29) Colvin PL, Parks JS: Metabolism of high density lipoprotein subfractions. Curr Opin Lipidol, 1999; 10: 309-314
30) Nakaya K, Ayaori M, Uto-Kondo H, Hisada T, Ogura M, Yakushiji E, Takiguchi S, Terao Y, Ozasa H, Sasaki M, Komatsu T, Ohsuzu F, Ikewaki K: Cilostazol enhances macrophage reverse cholesterol transport in vitro and in vivo. Atherosclerosis, 2010; 213: 135-141
31) Tardif JC, Cote G, Lesperance J, Bourassa M, Lambert J, Doucet S, Bilodeau L, Nattel S, de Guise P: Probucol and multivitamins in the prevention of restenosis after coronary angioplasty. Multivitamins and Probucol Study Group. N Engl J Med, 1997; 337: 365-372
32) Sekiya M, Funada J, Watanabe K, Miyagawa M, Akutsu H: Effects of probucol and cilostazol alone and in combination on frequency of poststenting restenosis. Am J Cardiol, 1998; 82: 144-147