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ORIGINAL ARTICLE
Imaging

Concomitant Use of Rosuvastatin and Eicosapentaenoic Acid Significantly Prevents Native Coronary Atherosclerotic Progression in Patients With In-Stent Neoatherosclerosis

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Background: In-stent neoatherosclerosis (NA) is a risk for future cardiovascular events through atherosclerotic progression in non-stented lesions. Using optical coherence tomography, this study assessed the efficacy of intensive therapy with 10 mg/day rosuvastatin plus 1,800 mg/day eicosapentaenoic acid (EPA) vs. standard 2.5 mg/day rosuvastatin therapy on native coronary plaques in patients with NA.

Methods and Results: This was a subgroup analysis of the randomized LINK-IT trial, which was designed to compare changes in the lipid index in NA between intensive and standard therapy for 12 months. In all, 42 patients with native coronary plaques and NA were assessed. Compared with standard therapy, intensive therapy resulted in greater decreases in serum low-density lipoprotein cholesterol concentrations and greater increases in serum 18-hydroxyeicosapentaenoic acid concentrations, with significantly greater decreases in the lipid index and macrophage grade in both NA (−24 vs. 217 [P<0.001] and −15 vs. 24 [P<0.001], respectively) and native coronary plaques (−112 vs. 29 [P<0.001] and −17 vs. 1 [P<0.001], respectively) following intensive therapy. Although there was a greater increase in the macrophage grade in NA than in native coronary plaques in the standard therapy group, in the intensive therapy group there were comparable reductions in macrophage grade between NA and native coronary plaques.

Conclusions: Compared with standard therapy, intensive therapy prevented atherosclerotic progression more effectively in native coronary plaques in patients with NA.

Key Words: Atherosclerosis; Eicosapentaenoic acid; Neoatherosclerosis; Optical coherence tomography

It is widely recognized that patients with established coronary artery disease (CAD) who undergo percutaneous coronary intervention (PCI) are at higher risk of secondary cardiovascular events than the primary prevention population. The main etiology of secondary events after stent implantation are late stent failure due to in-stent neoatherosclerosis (NA)[1] and the progression of native coronary plaques in non-stented lesions.

According to previous pathological studies, NA can be characterized by the development of atherosclerotic changes in neointimal tissue within the stented lesion, which has histological similarities with non-stented plaque.[2,3] Thus, it can be hypothesized that progression of NA may be related to native coronary plaques. Indeed, a previous study using serial quantitative coronary angiography revealed that the progression of native coronary plaques was greater in patients with than without NA.[4] In addition, in a recent optical coherence tomography (OCT) study of 175 consecutive patients (314 lesions), we demonstrated that the existence of NA is associated with cardiac death, target lesion revascularization (TLR), and stent thrombosis over a mean (±SD) follow-up period of 50.9±27.7 months after stenting.[1] These results suggest that patients with NA have a higher risk for secondary cardiovascular events, meaning that effective treatment options are needed for such high-risk patients who have NA in previously implanted stents.

In the LesIonal evaluatioN of high-risK patIents with neoatherosclerosis Treated with Rosuvastatin and eicosapentaenoic acid (LINK-IT) trial we demonstrated that the concomitant use of rosuvastatin and eicosapentaenoic acid (EPA) significantly prevented the progression of NA by modifying serum atherogenic lipoproteins and inflammatory

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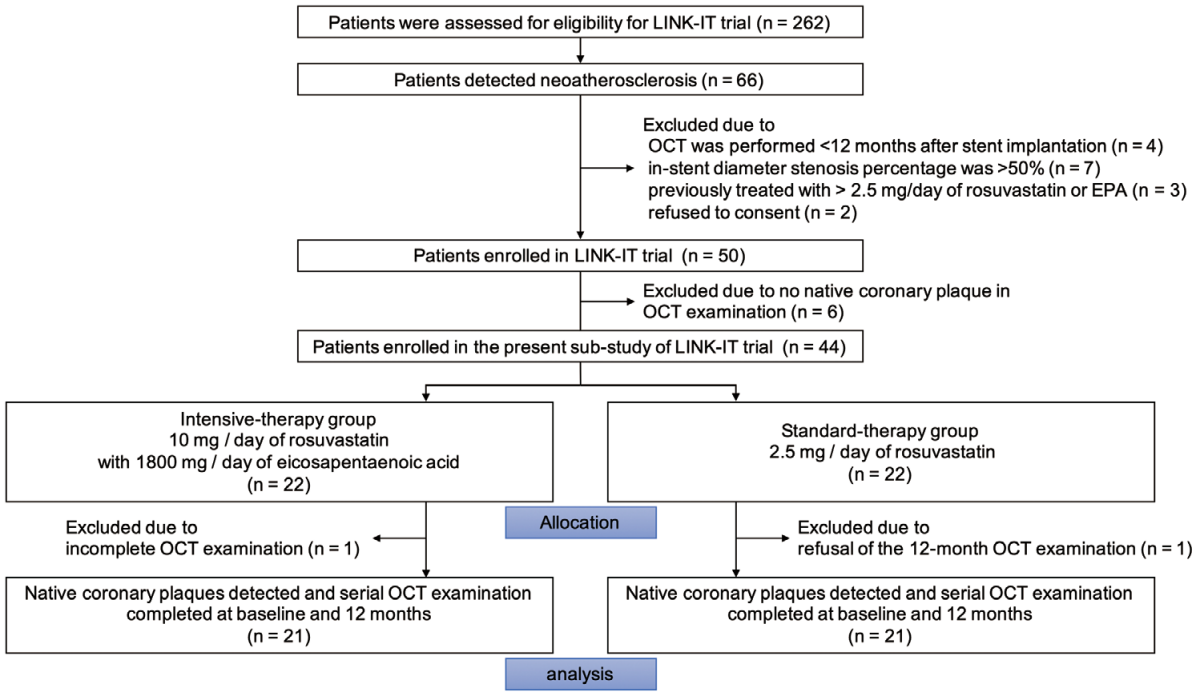

Figure 1. Study flow chart. EPA, eicosapentaenoic acid; LINK-IT, LesIonal evaluatioN of high-risK patIents with neoatherosclerosis Treated with Rosuvastatin and eicosapentaenoic acid trial; OCT, optical coherence tomography.

biomarkers compared with rosuvastatin monotherapy.[5] However, the effects of the concomitant use of rosuvastatin and EPA on native coronary plaques in such high-risk patients with NA remain unclear. Moreover, the relationship between treatment effects on NA and those on native coronary plaques remains unclear. Hence, we conducted the present subgroup analysis of the LINK-IT trial to clarify the local effects of concomitant treatment with rosuvastatin and EPA on native coronary plaques and the relationship between treatment effects on NA and those on native coronary plaques.

Methods

Study Design and Population

The LINK-IT trial is a prospective, randomized, open-label, blind-endpoint evaluation, parallel-group, single-center study using OCT to compare changes in the lipid index of NA associated with combined rosuvastatin and EPA treatment vs. standard rosuvastatin monotherapy for 12 months. The LINK-IT trial has been registered with the UMIN Clinical Trials Registry (ID: UMIN000012576). A detailed description of the study design has been published previously.[5] Briefly, the Kobe University Hospital OCT Registry is a single-center registry of consecutive patients undergoing intracoronary OCT imaging. OCT examinations were performed as scheduled follow-up coronary angiography and OCT examination for routine implanted stent follow-up or because of evidence of myocardial ischemia, such as silent myocardial ischemia, stable angina, or acute coronary syndrome. From this registry, we identified patients who underwent OCT examination for evaluation of previously implanted stents from July 2013 to April 2016,[1] and then patients in whom OCT examination detected NA. Patients were excluded if: (1) follow-up OCT imaging was performed <12 months after initial stent implantation; (2) the percentage in-stent diameter stenosis by coronary angiography was >50%; (3) they were already being treated with moderate or high-intensity statin or EPA; and (4) they declined to participate.

Eligible patients with NA were enrolled the LINK-IT trial and randomly assigned in a 1:1 ratio to receive either 10 mg/day rosuvastatin (moderate dose) and 1,800 mg/day EPA (intensive therapy) or 2.5 mg rosuvastatin (standard therapy). OCT was performed baseline and 12 months later to evaluate serial changes in NA.

The present LINK-IT subgroup analysis was conducted to compare OCT findings of native coronary plaques between those receiving intensive therapy (rosuvastatin plus EPA) and those receiving standard therapy (rosuvastatin alone) for 12 months. Patients were eligible for inclusion in the present analysis if: (1) they had NA and had undergone OCT examinations at baseline in the LINK-IT trial; and (2) they had native coronary plaques located >5 mm away from any stent edges causing 25–75% diameter stenosis with the lipid core detected by OCT at baseline. Patients with significant coronary stenosis of the target lesion (>75% by visual estimation on angiogram), those with ischemia revealed by invasive or non-invasive examinations, those who had incomplete OCT examinations or refused the follow-up OCT examinations, those with congestive heart failure or renal insufficiency with baseline creatinine level ≥2.0 mg/dL, except under hemodialysis, and those who did not provide written informed consent were excluded

Table 1. Baseline Characteristics of Patients Receiving Either 10 mg/day Rosuvastatin and 1,800 mg/day EPA (Intensive Therapy) or 2.5 mg Rosuvastatin (Standard Therapy)

	Intensive therapy (n=21)	Standard therapy (n=21)	P value
Age (years)	70.8 [68.4–78.8]	75.3 [68.6–80.5]	0.270
Male sex	17 (81.0)	16 (76.2)	
Medical history			
Hypertension	15 (71.4)	16 (76.2)	1.000
Diabetes	12 (57.1)	8 (38.1)	0.350
Dyslipidemia	19 (90.5)	18 (85.7)	1.000
Familial history of CAD	6 (28.6)	5 (23.8)	1.000
Smoking	11 (52.4)	11 (52.4)	1.000
Angina status at index PCI			0.277
Stable CAD	15 (71.4)	18 (85.7)	
ACS	6 (28.6)	5 (23.8)	
Angina status at baseline OCT			0.818
Routine follow-up	12 (57.1)	10 (47.6)	
Stable CAD	6 (28.6)	7 (33.3)	
ACS	3 (14.3)	4 (19.0)	
Medications at randomization			
Dual anti-platelet therapy	17 (81.0)	16 (76.2)	1.000
Low-intensity statin	13 (61.9)	14 (66.7)	1.000
ACEI/ARB	15 (71.4)	11 (52.4)	1.000
β-blocker	12 (57.1)	9 (42.9)	0.538
Lesion of native coronary plaque			0.357
LAD	8 (47.1)	9 (52.9)	
LCx	3 (33.3)	6 (66.7)	
RCA	10 (62.5)	6 (37.5)	
Lesion of neoatherosclerosis			0.188
LAD	8 (38.1)	10 (47.6)	
LCx	1 (4.8)	4 (19.0)	
RCA	12 (57.1)	7 (33.3)	
Type of stent			0.299
Bare metal stent	5 (23.8)	2 (9.5)	
Sirolimus-eluting stent	6 (28.6)	3 (14.3)	
Paclitaxel-eluting stent	2 (9.5)	3 (14.3)	
Everolimus-eluting stent	8 (38.1)	13 (61.9)	
Stent size (mm)			
Mean stent size	3.0 [3.0–3.5]	3.0 [3.0–3.5]	0.599
Total stent length	24.0 [18.0–33.0]	24.0 [20.0–39.0]	0.222
Duration (months)			
Between index PCI and baseline OCT	67.8 [44.0–100.1]	56.6 [27.2–91.5]	0.333
Between baseline and follow-up OCT	12.7 [11.7–13.2]	13.0 [11.4–14.1]	0.563

Values are presented as the median [interquartile range] or n (%). ACEI, angiotensin-converting enzyme inhibitor; ACS, acute coronary syndrome; ARB, angiotensin receptor blocker; CAD, coronary artery disease; EPA, eicosapentaenoic acid; LAD, left anterior descending artery; LCx, left circumflex artery; OCT, optical coherence tomography; PCI, percutaneous coronary intervention; RCA, right coronary artery.

from the analysis.

The study protocol was approved by the Institutional Review Board of Kobe University Hospital in accordance with the Declaration of Helsinki, and written informed consent was obtained from all patients.

OCT Examination and Analysis

OCT examinations were performed as reported previously.[5] Briefly, OCT images were acquired at baseline and at 12 months using a frequency-domain OCT system (ILUMIEN; Abbott Vascular, Santa Clara, CA, USA). A 0.014-inch standard guide wire was positioned distally in the target vessel, and the OCT catheter (C7 and C8 Dragonfly; Abbott Vascular) was advanced to the distal end of the target lesion. For image acquisition, blood in the lumen was replaced with contrast medium. OCT scans were performed from as far distal as possible to the ostium of each vessel and including the entire length of the lesion of interest using an integrated automated pullback device at a rate of 20 mm/s. If 25–75% diameter stenosis was found angiographically on the vessels without NA, an OCT scan was performed using the same procedure.

Offline OCT analysis was performed using a dedicated imaging review system (Abbott Vascular). Serial OCT

Table 2. Laboratory Results for Patients Receiving Either 10 mg/day Rosuvastatin and 1,800 mg/day EPA (Intensive Therapy) or 2.5 mg Rosuvastatin (Standard Therapy)

	Change between baseline and follow-up						Absolute change		
	Intensive therapy (n=21)			Standard therapy (n=21)			Intensive therapy (n=21)	Standard therapy (n=21)	P value
	Baseline	Follow-up	P value	Baseline	Follow-up	P value			
TC (mg/dL)	155 (138, 184)	133 (125, 157)	0.007	145 (136, 168)	138 (130, 162)	0.218	−20 (−38, −7)	−2 (−24, 23)	0.237
LDL-C (mg/dL)	90 (79, 110)	68 (59, 79)*	<0.001	89 (71, 102)	82 (70, 104)	0.817	−21 (−33, −12)	1 (−9, 21)	<0.001
HDL-C (mg/dL)	47 (42, 54)	45 (37, 51)	0.118	43 (40, 51)	43 (39, 53)	0.275	−2 (−4, 1)	−1 (−5, 2)	0.791
TG (mg/dL)	132 (99, 159)	128 (91, 163)	0.199	124 (93, 145)	117 (96, 150)	0.745	−17 (−32, 16)	0 (−26, 20)	0.466
EPA (μg/mL)	125 (79, 157)	260 (174, 310)*	0.001	94 (58, 155)	129 (83, 156)	0.467	149 (62, 189)	12 (−4, 66)	0.005
EPA/AA ratio	0.41 (0.28, 0.64)	0.90 (0.50, 0.96)*	0.014	0.41 (0.29, 0.52)	0.42 (0.21, 0.57)	0.722	0.26 (−0.03, 0.48)	−0.07 (−0.28, 0.16)	0.026
18-HEPE (pg/mL)	21 (18, 31)	122 (64, 156)*	<0.001	30 (12, 45)	32 (21, 39)	0.362	99 (56, 129)	1 (−5, 20)	<0.001
TNF-α (pg/mL)	0.70 (0.62, 1.00)	0.70 (0.53, 0.99)*	0.143	1.25 (0.95, 1.35)	1.36 (0.95, 1.79)	0.086	−0.09 (−0.14, 0.03)	0.09 (−0.06, 0.53)	0.021
hs-CRP (mg/dL)	0.05 (0.04, 0.15)	0.04 (0.03, 0.07)*	0.007	0.07 (0.04, 0.09)	0.12 (0.07, 0.20)	0.168	−0.02 (−0.08, −0.01)	0.04 (0.01, 0.10)	0.001
HbA1c (%)	6.5 (5.9, 7.4)	6.6 (6.0, 7.9)	0.581	6.2 (5.8, 6.9)	6.3 (5.9, 6.9)	0.311	0.1 (−0.2, 0.4)	0 (−0.1, 0.2)	0.686

Values are given as the median (interquartile range). *P<0.05 compared with standard therapy. AA, arachidonic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; HEPE, hydroxyeicosapentaenoic acid; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor-α.

images at baseline and the 12-month follow-up were reviewed in a blinded manner with regard to treatment allocation, and target lesions were matched based on the distance from landmarks, such as branches, ostium, calcifications, and stent edges. All in-stent frames and native coronary plaque frames were analyzed by 2 experienced observers who were blinded to the clinical information.

Plaque characterization was assessed by visual estimation of the target lesion at 0.2-mm intervals. Lesions were diagnosed as NA+ if they contained lipid-laden neointima, neointima with calcification, a thin cap fibroatheroma-like neointima, or neointimal rupture in at least 3 consecutive cross-sections.[1,5] Native coronary plaques were defined as coronary plaques located >5 mm away from any stent edges causing angiographic 25–75% diameter stenosis with the lipid core ≥90° detected by baseline OCT. If there were multiple plaques that met the definition of native coronary plaques, the most proximal one was included as a target native coronary plaque.

Plaque tissue was characterized using previously validated criteria.[6] Cholesterol crystals were defined as thin, linear regions of high intensity within a plaque. Fibrous cap thickness was defined as the minimum thickness of the signal-rich layer overlying the native coronary plaque and NA. Thin cap fibroatheroma was defined as a plaque with a fibrous cap thickness <65 μm and a lipid arc ≥90°.[6] Serial changes in fibrous cap thickness were evaluated by measuring the minimum fibrous cap thickness at baseline and the fibrous cap thickness of the same site at follow-up. Lipid length and lipid arc were measured on the longitudinal reconstructed view and the cross-sectional image, respectively. The lipid core detected by OCT was defined as a diffusely bordered signal-poor region with high attenuation. The lipid core arc was measured at 0.2-mm intervals throughout the native coronary atherosclerotic plaque and NA segments to estimate quantitative changes. The mean lipid core arc was calculated for each lesion. Then, the lipid index was calculated by multiplying the mean lipid core arc by the lipid core longitudinal length.[7]

Macrophage accumulation was defined as confluent or punctate highly backscattering focal regions in the artery wall.[8] We also performed macrophage grading at 0.2-mm intervals to assess the quantitative changes in macrophage accumulation based on axial and circumferential distribution as follows: Grade 0, no macrophages; Grade 1, localized macrophage accumulation (<30°); Grade 2, clustered accumulation ≥30° and <90°; Grade 3, clustered accumulation ≥90° and <270°; and Grade 4, clustered accumulation ≥270° and <360°.[8] Macrophage grade was evaluated as the summation of Grades 0–4 across all NA and native coronary atherosclerotic cross-sections.

Blood Samples

Blood samples were collected in the fasting state before OCT examination at baseline and at the 12-month follow-up. Serum total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol, triglycerides, EPA, EPA/arachidonic acid (AA) ratio, and HbA1c levels were measured at baseline and at the 12-month follow-up. Serum concentrations of high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor (TNF)-α, and the n-3 polyunsaturated fatty acid metabolite 18-hydroxyeicosapentaenoic acid (HEPE) were measured to investigate the potential relationships between plaque stabilization and inflammation. 18-HEPE, an active mono-oxygenated metabolite of EPA, was measured by liquid chromatography/tandem mass spectrometry-based lipid mediator metabololipidomics.

Table 3. OCT Measurements in Patients Receiving Either 10 mg/day Rosuvastatin and 1,800 mg/day EPA (Intensive Therapy) or 2.5 mg Rosuvastatin (Standard Therapy)

	Comparisons of OCT measurements between baseline and follow-up						Comparisons of absolute changes from baseline to follow-up across treatment groups		
	Intensive therapy (n=21)			Standard therapy (n=21)			Intensive therapy (n=21)	Standard therapy (n=21)	P value
	Baseline	Follow-up	P value	Baseline	Follow-up	P value			
Native coronary plaque									
Lesion length analyzed (mm)	4.7 (3.8, 5.0)	4.5 (3.4, 5.0)	0.160	4.1 (3.2, 5.9)	4.1 (3.3, 6.0)	0.485	0.0 (−0.2, 0.0)	0.0 (−0.1, 0.1)	0.226
Minimum lumen area (mm^2)	4.14 (3.26, 5.90)	4.90 (3.24, 5.62)	0.094	4.48 (3.10, 7.40)	4.91 (2.76, 7.61)	0.816	0.13 (−0.28, 0.33)	0.06 (−0.30, 0.45)	0.715
Fibrous cap thickness (μm)	150 (90, 190)	190 (150, 240)*	<0.001	140 (60, 190)	150 (80, 210)	0.088	50 (40, 60)	20 (10, 30)	<0.001
Lipid length (mm)	4.2 (2.9, 5.4)	3.3 (2.8, 4.8)	<0.001	3.4 (1.7, 4.6)	3.4 (1.6, 4.9)	0.182	−0.4 (−0.7, −0.2)	0.0 (−0.2, 0.2)	<0.001
Lipid arc (°)									
Maximum	167 (135, 255)	156 (133, 189)	0.005	203 (125, 281)	199 (126, 323)	0.150	−15 (−76, 3)	0 (−3, 37)	0.005
Mean	131 (115, 157)	114 (106, 129)	0.001	143 (103, 202)	144 (104, 202)	0.094	−19 (−38, −4)	7 (−4, 18)	<0.001
Lipid index	593 (403, 730)	380 (312, 619)	<0.001	660 (219, 825)	601 (242, 898)	0.078	−112 (−213, −73)	29 (−22, 97)	<0.001
Macrophage grade	39 (29, 62)	24 (9, 37)	<0.001	44 (30, 58)	51 (21, 66)	0.364	−17 (−24, −11)	1 (−4, 4)	<0.001
Neoatherosclerosis									
Lesion length analyzed (mm)	24 (17, 33)	24 (18, 32)	0.589	24 (20, 39)	24 (20, 40)	0.511	0.0 (−1.5, 0)	0.0 (−1.0, 0.3)	0.661
Minimum stent area (mm^2)	5.90 (4.18, 7.69)	5.77 (4.63, 7.07)	0.565	5.44 (4.16, 6.53)	5.46 (4.29, 6.53)	0.794	−0.05 (−0.38, 0.30)	0.15 (−0.50, 0.71)	0.597
Minimum lumen area (mm^2)	3.06 (2.77, 4.23)	3.18 (2.43, 4.06)	0.254	3.26 (2.69, 4.46)	3.18 (1.26, 4.90)	0.114	0.12 (−0.38, 0.31)	−0.28 (−1.23, 0.36)	0.302
Fibrous cap thickness (μm)	120 (90, 140)	160 (110, 180)	0.004	120 (90, 140)	130 (100, 140)	0.644	30 (10, 40)	10 (−10, 20)	<0.001
Lipid length (mm)	4.6 (1.8, 12.0)	4.4 (1.8, 10.2)	0.012	3.4 (1.6, 6.7)	4.2 (2.5, 7.6)	0.001	−0.2 (−1.6, 0.0)	0.6 (0.1, 1.3)	<0.001
Lipid arc (°)									
Maximum	177 (88, 252)	202 (68, 282)*	0.568	204 (122, 292)	290 (141, 360)	0.002	−1 (−26, 8)	16 (2, 102)	0.001
Mean	102 (70, 137)	104 (76, 129)*	0.668	134 (100, 184)	157 (105, 224)	0.001	−4 (−16, 22)	24 (4, 44)	0.007
Lipid index	581 (126, 1,448)	541 (114, 1,448)	0.290	430 (141, 1,125)	749 (277, 1,522)	<0.001	−24 (−118, 1)	217 (80, 397)	<0.001
Macrophage grade	48 (16, 119)	42 (3, 91)	0.004	42 (18, 71)	69 (33, 97)	<0.001	−15 (−23, −3)	24 (5, 27)	<0.001

Values are given as the median (interquartile range). *P<0.05 compared with standard therapy. Abbreviations as in Table 1.

Outcome Measures

The primary purpose of this analysis was to clarify changes in the lipid index or macrophage grade of native coronary plaques from baseline to the 12-month follow-up. Several OCT findings of native coronary plaques and NA, including minimum lumen area, mean and maximum lipid arc, and lipid length, were also assessed, and correlations between the lipid index or macrophage grade in native coronary plaques and those in NA were evaluated.

Clinical Events

A detailed definition of clinical events is available elsewhere.[5] Myocardial infarction (defined on the basis of increases in creatine kinase and creatine kinase-MB according to the third universal definition of myocardial infarction),[9] ischemic-driven TLR, ischemic-driven target vessel revascularization, and major adverse cardiac events (defined as the composite of death, myocardial infarction [MI], and TLR) were assessed 12 months after baseline OCT. TLR and target vessel revascularization were considered in the presence of symptomatic vascular stenosis ≥75%. Fractional flow reserve was measured under maximum hyperemia induced by intravenous adenosine infusion ($180\,\mu g \cdot kg^{-1} \cdot min^{-1}$) to objectively evaluate the level of ischemia in these cases. If the fractional flow reserve was less than or equal to the ischemic threshold (≤0.80), revascularization was performed.

Statistical Analysis

Quantitative data are shown as the median and interquartile range (IQR). Categorical variables are presented as frequencies and were compared between groups using the

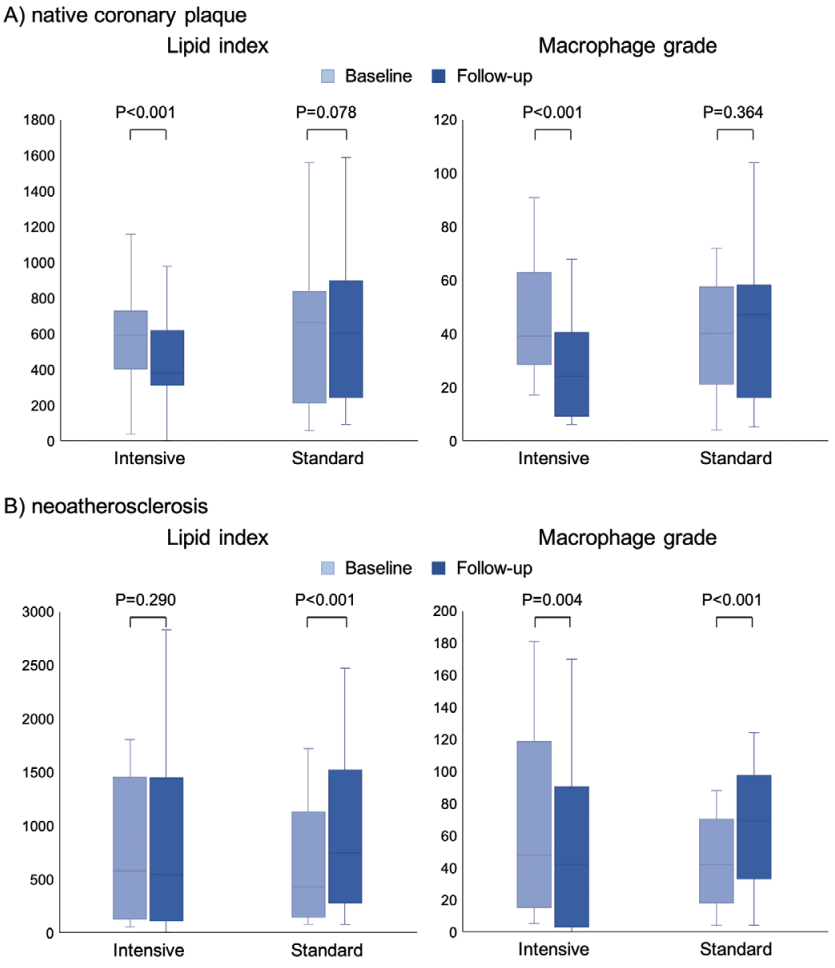

Figure 2. Changes in plaque characteristics of (**A**) native coronary plaque and (**B**) neoatherosclerosis on optical coherence tomography between baseline and the 12-month follow-up in patient groups receiving either 10 mg/day rosuvastatin (moderate dose) and 1,800 mg/day eicosapentaenoic acid (intensive therapy) or 2.5 mg rosuvastatin (standard therapy). (**A**) The lipid index and macrophage grade of native coronary plaque were significantly decreased in the intensive therapy group, but not standard therapy group, during treatment. (**B**) There was no significant change in the lipid index in the intensive therapy group during the treatment period, but the lipid index increased significantly in the standard therapy group. Macrophage grade decreased significantly in the intensive therapy group, but increased significantly in the standard therapy group. The boxes show the interquartile range, with the median value indicated by the horizontal line; whiskers show the range.

Chi-squared or Fisher exact tests (for an expected cell value <5). Continuous variables are presented as the median and IQR and were compared using the Mann-Whitney U-test (between-group comparison) or Wilcoxon signed-rank test (within-group comparisons between baseline and the 12-month follow-up). Relationships between changes in biomarkers, the lipid index, and macrophage grade during follow-up, and relationships between lipid index or macrophage grade in native coronary plaques and those in NA were investigated using simple regression analysis. Univariate and multivariate linear regression analyses were performed to identify independent factors associated with changes in the lipid index or macrophage grade. Variables with P<0.2 on simple regression analysis, age, and sex were included in multivariate linear regression analysis. Two-sided P<0.05 was considered significant. Statistical analyses were performed using SPSS version 25.0 (IBM, Armonk, NY, USA).

Results

Patients and Lesion Characteristics

Figure 1 shows the disposition for the patient population. Among 262 patients screened in the Kobe University Hospital OCT Registry, NA was detected in 66 by OCT examination. The prevalence of NA and median stent duration for bare metal stents and sirolimus-, paclitaxel-, and everolimus-eluting stents were 27% (11/41) at 8.4 (IQR 6.5–10.8) years, 34% (16/47) at 5.9 (IQR 3.6–8.4) years, 24% (8/33) at 5.4 (IQR 4.8–6.4) years, and 22% (31/141) at 2.4 (IQR 1.4–4.3) years, respectively. Sixteen patients were excluded because follow-up OCT imaging was performed <12 months after initial stent implantation (n=4), the percentage in-stent diameter stenosis by coronary angiography was >50% (n=7), the patients were already receiving moderate or high-intensity statin or EPA (n=3), or because they declined to participate (n=2). Thus, 50 patients with NA were enrolled the LINK-IT trial. Of these patients, 6 were not eligible for the present analysis because of the absence of native coronary plaques. In addition, 2 patients were excluded from the present analysis because of incomplete OCT examination and refusal to undergo the OCT procedure at 12 months (n=1 each). This left 42 patients with NA and de novo native coronary plaques (21 patients each in the intensive and standard therapy groups) for analysis. Baseline patient and lesion characteristics did not differ between the 2 groups (**Table 1**).

Laboratory Results

Laboratory results at baseline and the 12-month follow-up are given in **Table 2**. There were no significant differences in laboratory results between the 2 groups at baseline,

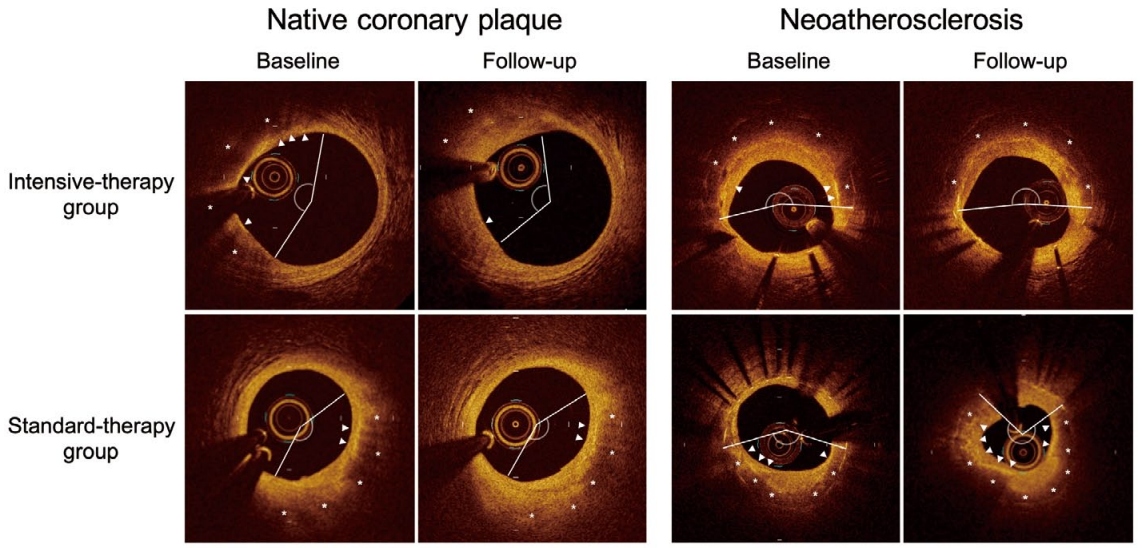

Figure 3. Representative optical coherence tomography images of native coronary plaque and neoatherosclerosis at baseline and the 12-month follow-up in patient groups receiving either 10 mg/day rosuvastatin (moderate dose) and 1,800 mg/day eicosapentaenoic acid (intensive therapy) or 2.5 mg rosuvastatin (standard therapy). In native coronary plaques, the lipid arc and macrophage accumulation decreased during the treatment period in the intensive therapy group, but did not change in the standard therapy group. In neoatherosclerosis, there was no change in the lipid arc and macrophage accumulation decreased in the intensive therapy group, whereas in the standard therapy group both the lipid arc and macrophage accumulation increased. Asterisks indicate the lipid-laden neointima or intima, white arrowheads indicate macrophages, and the white lines indicate the lipid arc.

whereas at the 12-month follow-up serum LDL-C, TNF-α, and hs-CRP concentrations were significantly lower and serum EPA and 18-HEPE concentrations were significantly higher in the intensive than standard therapy group. In the intensive therapy group, serum LDL-C and hs-CRP concentrations were significantly decreased and serum EPA and 18-HEPE concentrations were significantly increased from baseline to the 12-month follow-up. In contrast, there were no significant changes in these parameters in the standard therapy group from baseline to the 12-month follow-up. As indicated in **Table 2**, the changes in serum LDL-C, EPA, 18-HEPE, TNF-α, and hs-CRP concentrations, as well as the EPA/AA ratio, were significantly greater in the intensive than standard therapy group.

OCT Findings of Native Coronary Pplaques

In all, 42 native coronary plaques were analyzed in 42 patients (21 plaques each in the intensive and standard therapy groups). There were no significant differences in OCT findings between the 2 groups at baseline (**Table 3**). The number of thin cap fibroatheromas decreased numerically from baseline to follow-up in both the intensive therapy (from 5 [24%] to 1 [5%]) and standard therapy (from 6 [29%] to 3 [14%]) groups. Moreover, there were no patients with newly emerged cholesterol crystals in the intensive therapy group, compared with 2 patients in the standard therapy group.

The lipid index decreased significantly from baseline to follow-up in the intensive therapy group (from 593 [IQR 403–730] to 380 [IQR 312–619]; P<0.001), but not in the standard therapy group (from 660 [IQR 219–825] to 601 [IQR 242–898]; P=0.078; **Figure 2A**). Macrophage grade decreased significantly from baseline to follow-up in the intensive therapy group (from 39 [IQR 29–62] to 24 [IQR 9–37]; P<0.001), but did not change in the standard therapy group (from 44 [IQR 30–58] to 51 [IQR 21–66]; P=0.364; **Figure 2A**). The decreases in the lipid index and macrophage grade were significantly greater in the intensive than standard therapy group (**Table 3**).

OCT Findings of NA

In all, 42 NA were analyzed in 42 patients (21 each in the intensive and standard therapy groups). There were no patients with neointimal calcification or neointimal rupture. All 42 patients had a lipid-laden neointima. There were no significant differences in OCT findings between the 2 groups at baseline (**Table 3**).

The lipid index did not decrease significantly in the intensive therapy group from baseline to the 12-month follow-up (from 581 [IQR 126–1,448] to 541 [IQR 114–1,448]; P=0.290), but increased significantly in the standard therapy group (from 430 [IQR 141–1,125] to 749 [IQR 277–1,522]; P<0.001; **Figure 2B**). The macrophage grade decreased significantly in the intensive therapy group (from 48 [IQR 16–119] to 42 [IQR 3–91]; P=0.004), but increased significantly in the standard therapy group (from 42 [IQR 18–71] to 69 [IQR 33–97]; P<0.001; **Figure 2B**). The changes in the lipid index and macrophage grade were significantly lower in the intensive than standard therapy group (**Table 3**).

Representative OCT images of native coronary plaque and NA are shown in **Figure 3**.

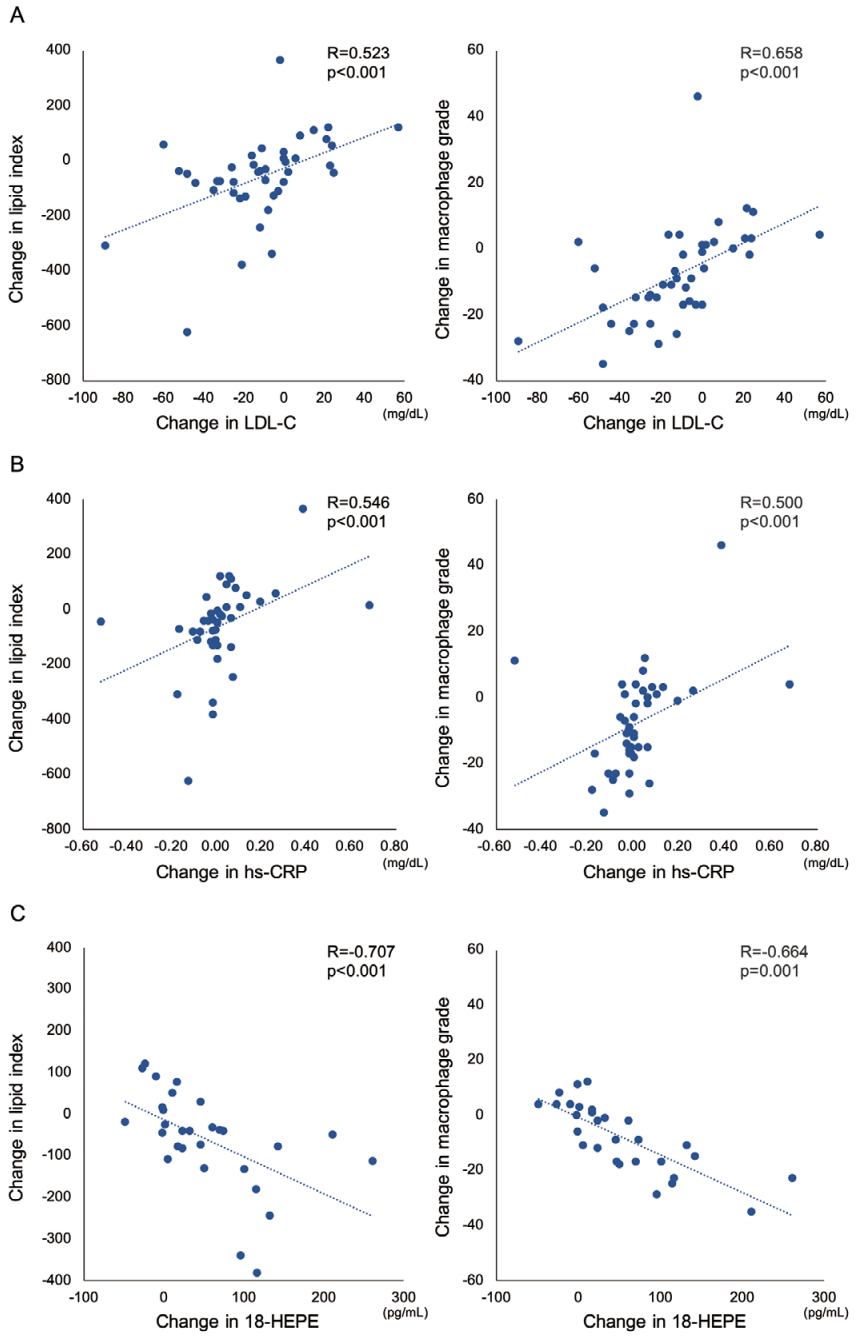

Figure 4. Correlation between changes in native coronary plaque characteristics on optical coherence tomography and the relevant biomarkers at the 12-month follow-up. Serum (**A**) low-density lipoprotein cholesterol (LDL-C), (**B**) high-sensitive C-reactive protein (hs-CRP), and (**C**) 18-hydroxyeicosapentaenoic acid (18-HEPE) concentrations were significantly correlated with changes in the lipid index and macrophage grade.

Relationships Between OCT Findings of Native Coronary Plaque or NA and Laboratory Results

Regarding native coronary plaques, multivariate analysis demonstrated that changes in serum LDL-C and 18-HEPE concentrations were independently associated with changes in the lipid index and macrophage grade (**Supplementary Table 1**). Moreover, changes in the lipid index were positively correlated with changes in serum LDL-C (R=0.523; P<0.001; **Figure 4A**) and hs-CRP (R=0.546; P<0.001; **Figure 4B**), and negatively correlated with changes in 18-HEPE concentrations (R=−0.707; P<0.001; **Figure 4C**). Changes in the macrophage grade were positively correlated with changes in serum LDL-C (R=0.658; P<0.001; **Figure 4A**) and hs-CRP (R=0.500; P=0.001; **Figure 4B**), and negatively correlated with changes in 18-HEPE concentration (R=−0.664; P=0.001; **Figure 4C**).

Regarding NA, similar correlations were seen between the OCT findings and laboratory results (**Supplementary Figure**). Changes in the lipid index of NA were positively correlated with changes in serum LDL-C (R=0.478; P=0.001) and hs-CRP (R=0.312; P=0.044), and negatively correlated with changes in 18-HEPE concentrations (R=−0.619; P<0.001). Changes in the macrophage grade of NA were positively correlated with changes in serum

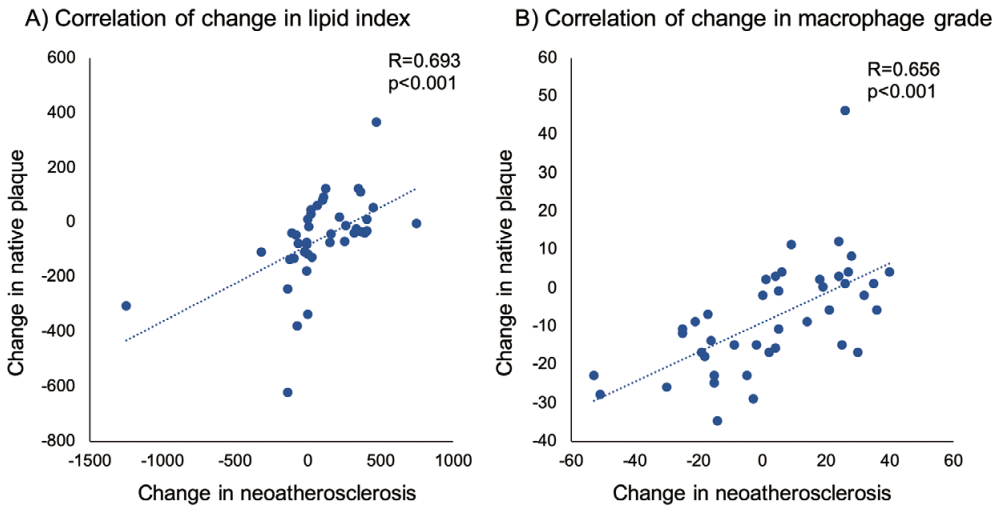

Figure 5. Correlations of changes in plaque characteristics on optical coherence tomography between native coronary plaques and neoatherosclerosis. There were significant correlations in changes in the lipid index and macrophage grade between native coronary plaques and neoatherosclerosis.

LDL-C (R=0.438; P=0.004) and hs-CRP (R=0.351; P=0.023), and negatively correlated with changes in 18-HEPE concentrations (R=−0.632; P<0.001).

Differences in Changes in OCT Findings Between Native Coronary Plaque and NA

Figure 5 shows the relationship between changes in the lipid index and macrophage grade of NA and those in native coronary plaque. Changes in both the lipid index (R=0.693; P<0.001) and macrophage grade (R=0.656; P<0.001) of NA were positively correlated with those of native coronary plaque. Comparisons of absolute changes in OCT findings between NA and native coronary plaque in each treatment group are summarized in **Supplementary Table 2**. There was no significant difference in minimum lumen area changes between NA and native coronary plaque in both the intensive and standard therapy groups. In the intensive therapy group, the change in the lipid index was significantly smaller for NA than native coronary plaque (median −24 [IQR −118, 1] vs. −112 [IQR −213, −73]; P=0.009). There was no significant difference in the decrease in macrophage grade between NA and native coronary plaque (−15 [IQR −23, −3] vs. −17 [IQR −24, −11]; P=0.290). In the standard therapy group, the increases in the lipid index and macrophage grade were significantly greater for NA than native coronary plaque (lipid index: 217 [IQR 80, 397] vs. 29 [IQR −22, 97], respectively [P<0.001]; macrophage grade: 24 [IQR 5, 27] vs. 1 [IQR −4, 4], respectively [P<0.001]).

Clinical Events

Overall, no patient experienced cardiac death or MI in either group. Seven patients required TLR of an in-stent lesion (6 in the standard therapy group), which corresponded to the NA segment. All these patients with symptomatic angina at follow-up OCT examination underwent ischemic-driven TLR. Of these patients, 2 had 99% in-stent restenosis at the NA segment, and 5 had 75–90% in-stent restenosis at the NA segment with ischemia proven by invasive functional testing. There were no cases of plaque rupture or thrombi at the NA segment. In contrast, no patients required TLR of native coronary plaque.

Discussion

The main findings of the present study can be summarized as follows. Compared with standard therapy, intensive therapy with rosuvastatin and EPA resulted in greater decreases in serum LDL-C levels and greater increases in serum 18-HEPE concentrations, with significantly greater decreases in the lipid index and macrophage grade not only in NA, but also in native coronary plaques. These changes were positively correlated with changes in serum LDL-C concentrations, and negatively correlated with serum 18-HEPE concentrations. Moreover, the therapeutic effects of intensive and standard therapy on NA were poorer than those on native coronary plaques.

Effect of Statins and EPA Therapy on Native Coronary Plaques in Patients With NA

Taniwaki et al demonstrated that, angiographically, atherogenic progression in minimum lumen area of untreated native coronary artery segments between baseline and the 5-year follow-up was more pronounced in patients with than without NA.[4] Thus, patients with NA were considered to be at high risk for atherosclerotic progression of native coronary plaques. In the present study, we demonstrated that intensive therapy with rosuvastatin and EPA suppressed atherosclerotic progression of de novo native plaque in patients with NA significantly more than standard rosuvastatin monotherapy. Moreover, the changes in serum LDL-C and 18-HEPE concentrations were independently associated with decreases in the lipid index and macrophage grade. Therefore, we currently consider that both the decrease in LDL-C and the anti-inflammatory

effect of 18-HEPE played key roles in suppressing the progression of NA and native coronary plaque in this study.

Previous intravascular ultrasound studies demonstrated that plaque regression could be obtained when LDL-C was reduced to <70–80 mg/dL.[10] In the present study, there was a significant reduction in the lipid index and macrophage grade in the intensive therapy group in which median LDL-C concentrations decreased to 68 mg/dL; in contrast, in the standard therapy group in which median LDL-C concentrations were 82 mg/dL, there was no significant reduction in the lipid index or macrophage grade.

Another key factor in the reductions in lipid index and macrophage grade may be the anti-inflammatory effect of 18-HEPE, an active mono-oxygenated metabolite of EPA. A recent experimental study suggested that 18-HEPE decreases atherosclerotic plaque size and necrotic core formation by suppressing proatherogenic signaling in macrophages.[11] In the present study, the decreases in the lipid index and macrophage grade in native coronary plaque were significantly correlated with decreases in serum LDL-C concentrations and increases in 18-HEPE concentrations, respectively (**Figure 4**). In addition, the decreases in the lipid index and macrophage grade in NA were significantly correlated with decreases in serum LDL-C concentrations and increases in 18-HEPE concentrations (**Supplementary Figure**).

Taking these observations together, we currently consider that, compared with standard-dose statin monotherapy, intensive lipid-lowering therapy with EPA plus moderate-dose statin resulted in native plaque stabilization in patients with NA through further lipid-lowering and anti-inflammatory effects, possibly improving clinical outcomes in a secondary prevention population.

Differences in OCT Findings Between Native Coronary Plaques and NA

In the present study we demonstrated that changes in the lipid index and macrophage grade of native coronary plaques were positively correlated with those of NA (**Figure 5**). These data suggest that, at least to some extent, there is a relationship between the treatment effect of lipid-lowering therapy on atherosclerotic progression in native coronary plaque and these effects in NA, which is probably due to the common mechanisms of atherosclerotic development, such as lipoprotein retention and macrophage accumulation. In addition, we found that responses of NA to lipid-lowering therapy tended to be poorer than those of native coronary plaque. In the standard therapy group, despite lipid-lowering therapy with 2.5 mg/day rosuvastatin, increases in the lipid index (217 vs. 29; $P<0.001$) and macrophage grade (24 vs. 1; $P<0.001$) were significantly greater for NA than native coronary plaque (**Figure 2**; **Supplementary Table 2**). Although the detailed mechanism remains unknown, we hypothesize that, compared with native coronary plaque, the atherogenicity of NA enhanced by the following mechanism would weaken the anti-atherogenic effects of lipid-lowering therapy. It is well known that stent implantation, especially with drug-eluting stents, causes incomplete maturation of the regenerated endothelium. These immature endothelia are covered with poorly formed cell-to-cell junctions that allow great amounts of lipoproteins to enter the subendothelial area to form a large lipid core. Furthermore, in stented lesions, the high expression of cell adhesion molecules enables monocytes to migrate into the subendothelial area, where monocytes are converted into foamy macrophage.[12] Interestingly, the intensive therapy induced a similar reduction in macrophage grade in NA and native plaque (**Supplementary Table 2**), whereas standard therapy was less effective in NA. These findings may suggest that, by taking 2 different approaches with statin and EPA, the intensive lipid-lowering therapy used in the present study may suppress atherosclerotic progression not only in native coronary plaque, but also in NA. Further studies with a larger sample size and longer-term follow-up are needed to confirm our proposal.

Study Limitations

The present subgroup analysis of a randomized trial had several limitations. First, there is a potential selection bias because the analysis was based on a small number of patients who underwent OCT examination in single center. However, there was no significant difference in the baseline characteristics between the 2 groups. Second, because the intensive therapy group received a higher statin dose (10 vs. 2.5 mg daily) and EPA, the study design does not enable us to determine which medication played a central role in the suppression of NA and native coronary plaque progression we observed. Because the LINK-IT trial was the first interventional study of NA and it was uncertain whether modification of the lipid profile could suppress NA progression, we selected an increased statin dose and EPA as the maximum intensive therapy available in Japan. Currently, we hypothesize that both the increased statin dose and additional EPA therapy played important roles because the changes in serum LDL-C, hs-CRP, and 18-HEPE concentrations were independently associated with changes in the lipid index and macrophage grade (**Supplementary Table 1**).

Conclusions

In patients with NA, intensive therapy with 10 mg rosuvastatin and EPA significantly suppressed atherosclerotic progression in both native coronary plaques and NA by modifying the lipid profile and anti-inflammatory metabolites. The findings of this study may provide a mechanistic insight into the effects of moderate-dose statin plus EPA therapy in reducing cardiovascular events in patients with NA.

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Disclosures

K.H. is a member of *Circulation Journal*' Editorial Team. The other authors have no conflicts of interest to declare.

IRB Information

This study was approved by the Institutional Review Board of Kobe University Hospital (Reference no. 250052).

Data Availability

The deidentified participant data will not be shared.

References

1. Kuroda M, Otake H, Shinke T, Takaya T, Nakagawa M, Osue T, et al. The impact of in-stent neoatherosclerosis on long-term clinical outcomes: An observational study from the Kobe University Hospital optical coherence tomography registry. *EuroIntervention* 2016; **12:** e1366–e1374.
2. Nakazawa G, Otsuka F, Nakano M, Vorpahl M, Yazdani SK, Ladich E, et al. The pathology of neoatherosclerosis in human coronary implants bare-metal and drug-eluting stents. *J Am Coll Cardiol* 2011; **57:** 1314–1322.
3. Romero ME, Yahagi K, Kolodgie FD, Virmani R. Neoatherosclerosis from a pathologist's point of view. *Arterioscler Thromb Vasc Biol* 2015; **35:** e43–e49.
4. Taniwaki M, Windecker S, Zaugg S, Stefanini GG, Baumgartner S, Zanchin T, et al. The association between in-stent neoatherosclerosis and native coronary artery disease progression: A long-term angiographic and optical coherence tomography cohort study. *Eur Heart J* 2015; **36:** 2167–2176.
5. Kuroda K, Otake H, Shinohara M, Kuroda M, Tsuda S, Toba T, et al. Effect of rosuvastatin and eicosapentaenoic acid on neoatherosclerosis: The LINK-IT trial. *EuroIntervention* 2019; **15:** e1099–e1106.
6. Tearney GJ, Regar E, Akasaka T, Adriaenssens T, Barlis P, Bezerra HG, et al. Consensus standards for acquisition, measurement, and reporting of intravascular optical coherence tomography studies: A report from the International Working Group for Intravascular Optical Coherence Tomography Standardization and Validation. *J Am Coll Cardiol* 2012; **59:** 1058–1072.
7. Xie Z, Tian J, Ma L, Du H, Dong N, Hou J, et al. Comparison of optical coherence tomography and intravascular ultrasound for evaluation of coronary lipid-rich atherosclerotic plaque progression and regression. *Eur Heart J Cardiovasc Imaging* 2015; **16:** 1374–1380.
8. Tahara S, Morooka T, Wang Z, Bezerra HG, Rollins AM, Simon DI, et al. Intravascular optical coherence tomography detection of atherosclerosis and inflammation in murine aorta. *Arterioscler Thromb Vasc Biol* 2012; **32:** 1150–1157.
9. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. *J Am Coll Cardiol* 2012; **60:** 1581–1598.
10. Tsujita K, Sugiyama S, Sumida H, Shimomura H, Yamashita T, Yamanaga K, et al. Impact of dual lipid-lowering strategy with ezetimibe and atorvastatin on coronary plaque regression in patients with percutaneous coronary intervention: The multicenter randomized controlled PRECISE-IVUS trial. *J Am Coll Cardiol* 2015; **66:** 495–507.
11. Laguna-Fernandez A, Checa A, Carracedo M, Artiach G, Petri MH, Baumgartner R, et al. ERV1/ChemR23 signaling protects against atherosclerosis by modifying oxidized low-density lipoprotein uptake and phagocytosis in macrophages. *Circulation* 2018; **138:** 1693–1705.
12. Otsuka F, Finn AV, Yazdani SK, Nakano M, Kolodgie FD, Virmani R. The importance of the endothelium in atherothrombosis and coronary stenting. *Nat Rev Cardiol* 2012; **9:** 439–453.

Supplementary Files

Please find supplementary file(s);
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