Original Article

Add-on Therapy of EPA Reduces Oxidative Stress and Inhibits the Progression of Aortic Stiffness in Patients with Coronary Artery Disease and Statin Therapy: A Randomized Controlled Study

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Aim: We examined the anti-oxidant mechanisms of combined therapy of eicosapentaenoic acid (EPA) plus statin on the progression of atherosclerosis.

Methods: Patients receiving statin therapy for dyslipidemia and with coronary artery disease (CAD) were assigned randomly in an open-label manner to the EPA (1,800 mg/day) -plus-statin group ($n=25$; combined-therapy group) or to the statin-only group ($n=25$), and followed for 48 weeks. At baseline and 48 weeks after enrollment, oxidative stress, brachial-ankle pulse wave velocity (baPWV) and stiffness parameter β -index of the carotid were measured.

Results: The lipid profile remained unchanged throughout the study. Although the median value of baPWV increased more in the statin-only group than in the combined-therapy group, this difference was not significant ($p=0.29$); however, a decrease in baPWV was associated with combined-therapy treatment by multiple regression analysis adjusted for age and mean blood pressure ($p=0.04$). In addition, the β -index of the carotid was lower in the combined-therapy group than in the statin-only group ($p=0.02$). Furthermore, although the difference in the reduction of the urinary concentration of 8-isoprostane between the two groups did not reach statistical significance, this concentration was significantly lower in the combined-therapy group with higher baseline levels (≥ 183 pg/mL·Cr) of urinary 8-isoprostane ($p=0.004$).

Conclusions: EPA may reduce oxidative stress and inhibit the progression of arterial stiffness more efficiently than statin-only therapy in patients with dyslipidemia and CAD.

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Key words; Eicosapentaenoic acid, Oxidative stress, Arterial stiffness, Coronary artery disease

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Introduction

HMG-CoA reductase inhibitor (statin) therapy reduces mortality and morbidity in patients with coronary artery disease (CAD)^{1, 2}; however, some trials

have also highlighted the significance of residual cardiovascular risk after treatment of low density lipoprotein cholesterol (LDL-C) to target levels, which reflects the complex nature of residual cardiovascular risk³⁾. Evidence for the cardioprotective nature of ω -3 fatty acids, including eicosapentaenoic acid (EPA), a ω -3 polyunsaturated fatty acid (PUFA), indicates that ω -3 fatty acid therapy also shows great promise in the secondary prevention of CAD⁴⁻⁶⁾. According to recent studies, the addition of EPA to statin therapy provides further benefits in preventing cardiovascular events, mainly through cholesterol-independent mechanisms, in patients with hypercholesterolemia, especially for the secondary prevention of CAD^{7, 8)}.

Pulse wave velocity (PWV), a measure of large artery stiffness, is widely used as an indicator of arterial stiffness and as a marker reflecting vascular damage to diagnose arteriosclerosis, and is an important predictor of cardiovascular events^{9, 10)}. In addition, carotid intima-media thickness (IMT) and carotid plaque have been reported to be independent predictors of CAD^{11, 12)}. These noninvasive imaging modalities are useful surrogate markers for future cardiovascular events¹³⁾.

We previously reported that the amount of oxidative stress in the aorta might be an important factor leading to vascular dysfunction, as assessed by arterial stiffness¹⁴⁾, and that the decreased superoxide content of the aorta caused by lipid-lowering drugs through antioxidant mechanisms independent of lipid-lowering effects may inhibit the progression of atherosclerosis and arterial stiffness in persons with hypercholesterolemia¹⁵⁾, suggesting that oxidative stress may be one of the important determinants of arterial stiffness in hypercholesterolemia.

Aim

Although the results of a previous trial demonstrated the efficacy of EPA in preventing CAD in patients with hypercholesterolemia under statin therapy, especially for secondary prevention⁷⁾, the precise mechanisms of this prevention, especially with regard to oxidative stress, in preventing the progression of atherosclerosis following cardiovascular events remain unknown. Thus, in the EPA Lipid Intervention and Atherosclerosis Prevention (ELIA) study, to clarify the anti-oxidant mechanisms of combined therapy of EPA plus statin on the progression of atherosclerosis, we examined the additional effects of EPA on oxidative stress, arterial stiffness and atherosclerosis in patients with CAD who were already receiving statins.

Methods

Ethics Committee Procedure and Consent

The study protocol was approved by the ethics committees of all involved institutions, and the study was undertaken in accordance with the Declaration of Helsinki and Ethics Guidelines for Clinical Studies in Japan. All of the study patients gave their fully informed written consent for study participation.

Study Design and Patient Population

The ELIA study was an investigator-initiated, multi-center study in accordance with the prospective, randomized open blinded endpoint evaluation (PROBE) design of once daily statin versus statin plus EPA (1,800 mg), involving individuals who had experienced cardiovascular disease with dyslipidemia.

Inclusion criteria were outpatient status, age over 20 years and less than 70 years, with dyslipidemia and a previous history of CAD (myocardial infarction, angina pectoris, coronary angioplasty or coronary artery bypass graft surgery) at least 3 months before entering the study, regardless of sex, with total cholesterol ≥ 180 mg/dL or LDL-C ≥ 100 mg/dL and taking any statin. Exclusion criteria for the study were 1) history of myocardial infarction, angina pectoris, coronary angioplasty or coronary artery bypass graft surgery within 3 months prior to enrollment in the study, 2) family history of hypercholesterolemia and secondary hypertension or dyslipidemia, 3) uncontrolled diabetes mellitus with HbA1c $\geq 8.0\%$, 4) severe heart failure (New York Heart Association functional classification IV), 5) severe peripheral arterial disease (Fontaine Class II, III or IV), 6) chronic atrial fibrillation or flutter, 7) bleeding tendency, 8) serious liver dysfunction (aspartate aminotransferase or alanine aminotransferase ≥ 100 IU/L) or serious renal dysfunction (serum creatinine ≥ 2 mg/dL), 9) pregnancy, 10) history of malignancy 5 years prior to study entry, 11) compliance rate $< 80\%$ assessed by a patient interview, 12) other serious illness or significant abnormalities that the investigator judged inappropriate for inclusion in the study.

Eligible patients were randomly assigned to a study arm. Randomization was conducted at the University Hospital Medical Information Network (UMIN) Center, the University of Tokyo Hospital, by the dynamic allocation method (the modified minimization method) after stratification using diabetes and the smoking status as adjusting factors in the minimization calculation.

All of the study patients received a statin once a day; the choice of statin was entrusted to the investi-

gator. The study population was randomly assigned to receive EPA with statin (combined-therapy group) or statin alone (statin-only group), and both groups were followed up for 48 weeks. In the combined-therapy group, we administered a daily dose of 1,800 mg EPA. At each out-patient clinic visit, study doctors provided dietary instructions and monitored medication use, and a questionnaire was given to confirm the adherence to the study medications. Systolic and diastolic blood pressure levels and heart rates were measured twice after the patient had sat quietly for at least 5 minutes at approximately 2-minute intervals, and mean values were used for the study.

Measurement of Arterial Stiffness and Atherosclerosis

We used two different indices to more reliably evaluate arterial stiffness, the brachial-ankle PWV (baPWV) and the stiffness parameter β -index of the carotid, since the baPWV is known to be influenced by blood pressure⁹, whereas the stiffness parameter β -index is a blood pressure-independent patient-specific arterial stiffness measured by ultrasound^{14, 15}. The baPWV was automatically obtained using AT-form PWV/ABI (Omron Colin, Tokyo, Japan), which has been described in detail previously¹⁶⁻¹⁸.

All of the study patients underwent ultrasonography of the carotid artery to assess IMT and the plaque score. Replicate scans were performed by qualified well-trained ultrasonographers at baseline and at 48 weeks to preserve the quality control of image acquisition. At baseline and the 48-week follow-up visit, a B-mode scan was performed with image acquisition at one predefined angle of the far wall of six carotid segments: the right and left common carotid arteries, carotid bifurcations, and internal carotid arteries. All images were transferred to the ultrasonography core laboratory. The stiffness parameter β -index, as measured by carotid ultrasound with a 7.5-MHz transducer, was calculated at a point without plaque, as previously reported^{14, 15, 19}. IMT was evaluated as the distance between the lumen-intimal interface and the medial-adventitial interface, and was measured using two calipers on the frozen frame of a suitable longitudinal image. The upper normal limit for the IMT was defined as 1.0 mm, and the plaque score was calculated by the sum of $\text{IMT} \geq 1.1$ mm. The max IMT of both carotids was used to evaluate atherosclerosis for the study. To assess the severity of atherosclerosis, we also used the plaque score, which was calculated by summing all plaque thickness in both carotid systems²⁰. These indices obtained by carotid ultrasound were assessed by a specialist sonographer in a blind

fashion.

Laboratory Measurements

All of the blood and urine samples were collected at the outpatient clinic from patients in a fasting state and were measured by a central laboratory (SRL, Inc., Tokyo, Japan). Serum cholesterol and triglyceride were measured enzymatic ally, high-density lipoprotein cholesterol (HDL-C) was measured by direct measurement, remnant-like particle cholesterol (RLP-C) was measured by immunoseparation assay²¹, antioxidant enzyme superoxide dismutase (SOD) activity was measured by the nitrite method modified by Oyana-gui²², malondialdehyde modified LDL (MDA-LDL), which is one of the surrogate markers for atherogenic oxidized LDL, was measured by enzyme-linked immunosorbent assay²¹, urinary concentration of 8-isoprostane, one of the non-cyclooxygenase-derived prostanoids, was measured by enzyme immunoassay, high-sensitivity C-reactive protein (hs-CRP) was measured by the nephelometry method, and EPA and arachidonic acid (AA), a ω -6 PUFA, were measured by gaschromatography, respectively.

Statistical Analysis

The Wilcoxon 2-sample test was used to compare continuous variables. The χ^2 test was used to compare categorical variables. The change from baseline to 48 weeks after intervention was evaluated using the Wilcoxon signed rank test. Multiple regression analysis was used to evaluate the relations between EPA treatment and baPWV adjusted for age and mean blood pressure. All analyses were performed using version 5.0.1a of the JMP statistical software program (SAS Institute, Inc., Cary, NC, USA). Data are expressed as the mean $\pm$ SD or median (interquartile range). Significance was established at $p < 0.05$.

Results

Patients

From May 2004 until August 2005, 51 patients were enrolled in the study, and follow-up was continued until August 2006. Of these patients, 50 underwent randomization, with 25 assigned to the statin-only group and 25 assigned to the EPA-plus-statin (combined-therapy) group, respectively. We excluded one patient from the analysis because of missing baseline data.

Demographic and clinical characteristics of the patients are listed in **Table 1**. There were no significant differences in the baseline clinical characteristics of the patients, except that the statins chosen by the

Table 1. Baseline characteristics of the study patients

Characteristic	Statin-only (<i>n</i> = 25)	Combined therapy (<i>n</i> = 25)	<i>p</i> value
Age, mean (SD), y	60.9 (7.0)	61.6 (5.6)	0.89
Male, No. (%)	20 (80.0)	21 (84.0)	0.71
BMI, mean (SD), kg/m ²	24.0 (3.6)	25.1 (2.3)	0.12
Coronary artery disease, No. (%)			
OMI	12 (48.0)	16 (64.0)	0.39
AP	10 (40.0)	8 (32.0)	0.56
CABG	4 (16.0)	3 (12.0)	0.68
PCI	12 (48.0)	13 (52.0)	0.78
NYHA class, No.			0.57
I	23	24	
II	1	1	
III	1	0	
Diabetes, No. (%)	8 (32.0)	7 (28.0)	0.76
Hypertension, No. (%)	16 (64.0)	14 (56.0)	0.56
Current smoker, No. (%)	6 (24.0)	5 (20.0)	0.73
Statin, No.	25	25	1.00
Pravastatin	5	14	
Simvastatin	1	2	
Fluvastatin	4	2	(0.02)
Atorvastatin	13	4	
Pitavastatin	1	3	
Antidiabetic agents, No. (%)	4 (16.0)	4 (16.0)	1.00
Sulfonylurea	0	3	(0.04)
Biganides	2	1	(0.55)
α -GI drug	0	2	(0.09)
Thiazolidine	0	1	(0.24)
Insulin	4	0	(0.02)
Antihypertensive agents, No. (%)	20 (80.0)	18 (72.0)	0.51
calcium antagonist	17	7	(0.004)
ACE inhibitor/angiotensin II antagonist	14	16	(0.56)
β -blocker	6	4	(0.48)
Diuretic	2	1	(0.55)
Others	1	0	(0.24)
Antiplatelet/anticoagulants, No. (%)	22 (88.0)	19 (76.0)	0.27
Aspirin	20	19	(0.73)
Ticlopidine	6	2	(0.12)
Cilostazol	1	1	(1.00)
Warfarin	3	0	(0.04)

Abbreviations: OMI, old myocardial infarction; AP, angina pectoris; CABG, coronary artery bypass grafting; PCI, percutaneous coronary intervention; NYHA, New York Heart Association; α -GI, α -glucosidase inhibitor; ACE, angiotensin-converting enzyme. No. indicates number of patients. Parentheses indicate the results of the χ^2 test. Statin was prescribed as a single drug in each patient. Concomitant drugs such as antidiabetic or antihypertensive agents and antiplatelet/anticoagulants were prescribed as combination therapy in some patients in both groups.

investigators were not the same for all patients; pravastatin was prescribed in the statin-only group, whereas atorvastatin was more preferably prescribed in the combined-therapy group, although all patients in both

groups were prescribed a statin as standard therapy. Good adherence of the study patients to the study drug protocol (i.e., receipt of at least 80% of a study medication) was maintained throughout the study in

Table 2. Levels of blood pressure, lipids, remnant like protein cholesterol, malondialdehyde modified low-density lipoprotein, urinary 8-isoprostane, and C-reactive protein in patients with coronary artery disease at baseline and after 48 weeks of treatment with study medications

Characteristic	Group	Baseline	48 weeks	Amount of change from baseline	<i>p</i> value [†]
SBP, mmHg	Statin-only	131 ± 15	128 ± 17	2.7	0.72
	Combined therapy	128 ± 14	125 ± 17	5.3	
DBP, mmHg	Statin-only	77 ± 9	74 ± 9	0.8	0.62
	Combined therapy	76 ± 7	75 ± 9	1.3	
Total CHO, mg/dL	Statin-only	193 ± 28	201 ± 41	9.4	0.10
	Combined therapy	186 ± 28	180 ± 27	-3.1	
LDL-C, mg/dL	Statin-only	106 ± 19	109 ± 32	6.8	0.06
	Combined therapy	110 ± 23	107 ± 21	-5.1	
HDL-C, mg/dL	Statin-only	57 ± 9	61 ± 14	4.4	0.13
	Combined therapy	52 ± 11	52 ± 12	0.1	
non HDL-C, mg/dL	Statin-only	137 ± 28	140 ± 38	5.0	0.34
	Combined therapy	137 ± 27	128 ± 25	-3.2	
Triglyceride, median (IQR), mg/dL	Statin-only	121 (92-206)	113 (95-141)	9.1	0.06
	Combined therapy	87 (71-134)	98 (69-124)	-9.3	
RLP-C, mg/dL	Statin-only	6.3 ± 4.4	6.8 ± 9.2	0.7	0.11
	Combined therapy	4.3 ± 5.4	3.6 ± 2.1	0.6	
SOD activity, U/mL	Statin-only	3.0 ± 1.9	3.0 ± 2.1	0.2	0.66
	Combined therapy	3.9 ± 2.5	4.2 ± 3.4	0.0	
MDA-LDL, U/L	Statin-only	117.0 ± 63.5	121.9 ± 38.2	5.0	0.71
	Combined therapy	113.8 ± 32.2	118.0 ± 28.1	9.3	
Urinary 8-isoprostane, pg/mg · Cr	Statin-only	239 ± 117	219 ± 120	-21.6	0.16
	Combined therapy	246 ± 198	154 ± 72	-100.1	
hs-CRP, ng/mL	Statin-only	574 ± 829	956 ± 1553	508.6	0.03
	Combined therapy	2102 ± 2148	3932 ± 9183	1674.2	

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; CHO, cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; RLP-C, remnant like protein cholesterol; SOD, superoxide dismutase; MDA-LDL, malondialdehyde modified low-density lipoprotein; hs-CRP, high sensitive C-reactive protein; Cr, creatinine. Data are the mean ± SD unless specified. IQR, interquartile range.

[†] *P* values are for differences between the two treatment groups in the amount of change from baseline using the Wilcoxon signed rank test.

both groups.

Laboratory Results

The difference in EPA/AA ratio between the two groups was low at baseline, whereas the EPA/AA ratio in the combined-therapy group increased substantially from the baseline to 24 weeks after the administration of EPA compared with the statin-only group (statin-only group versus combined-therapy group: 0.69 ± 0.38 versus 0.85 ± 0.46 at baseline, not significant, 0.69 ± 0.39 versus 1.57 ± 0.72 at 24 weeks, $p < 0.0001$, respectively).

Table 2 summarizes the laboratory results in the ELIA study. There were few differences in the amount of change of blood pressure, total cholesterol, LDL-C, HDL-C, non HDL-C, triglyceride, RLP-C, SOD

activity and MDA-LDL between the two groups, whereas the amount of change of hs-CRP was significantly less in the statin-only group than in the combined-therapy group. In addition, although the reduction of urinary concentration of 8-isoprostane was greater in the combined-therapy group than in the statin-only group, this difference did not reach statistical significance.

Effects of Combined Therapy of EPA Plus Statin on Arterial Stiffness and Max IMT and Plaque Score of the Carotid Artery

Table 3 summarizes the effects of EPA on the baPWV, β -index of the carotid, max IMT and plaque score of the carotid artery in CAD and statin-therapy patients. There was no difference in the amount of

Table 3. Effects of adding EPA treatment to the statin therapy on baPWV, β -index of the carotid artery, max IMT and plaque score of the carotid artery in patients with coronary artery disease at baseline and after 48 weeks of the treatment of study medications

Characteristic	Group	Baseline	48 weeks	Amount of change from baseline	<i>p</i> value [†]
baPWV, cm/sec	Statin-only	1615 (1458-1816)	1670 (1585-1793)	90.9	0.29
	Combined therapy	1514 (1332-1656)	1552 (1385-1684)	44.1	
Carotid β -index	Statin-only	7.1 (5.2-11.4)	7.6 (6.0-12.9)	-0.0	0.02
	Combined therapy	9.4 (5.6-12.4)	5.5 (4.5-8.1)	-2.7	
Max IMT, mm	Statin-only	1.0 (0.9-1.3)	1.1 (0.9-1.5)	0.06	0.50
	Combined therapy	1.3 (0.9-1.4)	1.2 (0.8-1.5)	0.09	
Plaque score	Statin-only	1.3 (0.8-6.3)	1.0 (0.0-4.2)	-1.1	0.60
	Combined therapy	1.9 (0.0-6.2)	1.9 (0.0-3.0)	-0.5	

Abbreviations: baPWV, brachial-ankle pulse wave velocity; IMT, intima-media thickness. The baPWV was automatically obtained using AT-form PWV/ABI (Omron Colin, Tokyo, Japan). The stiffness parameter β -index as measured by carotid ultrasound was calculated at a point without plaque. Max IMT of the carotid artery was obtained by carotid ultrasound. Data are expressed as the median (interquartile range). [†]*p* values are for differences between the two treatment groups in the amount of change from baseline using the Wilcoxon signed rank test.

Table 4. Multiple regression analysis of the relationship between baPWV and other associated variables

	β	SE	<i>t</i> value	<i>p</i> value
Combined therapy, yes/no	-75.4	34.7	-2.17	0.04
Age, years	12.8	5.7	2.25	0.03
Mean blood pressure, mmHg	5.3	3.6	1.48	0.15

Abbreviations: baPWV, brachial-ankle pulse wave velocity; β , standardized regression coefficients; SE, standard error. Combined-therapy; EPA (1,800 mg/day) plus statin. Mean blood pressure was calculated from diastolic blood pressure + [(systolic blood pressure-diastolic blood pressure)/3].

change in baPWV between the two groups, although the increase in the median value of the baPWV from baseline in the statin-only group was greater than that in the combined-therapy group. The decrease in the median value of the β -index of the carotid from baseline to 48 weeks was significantly lower in the combined-therapy group than in the statin-only group. There were no differences in the amount of change of the max IMT and plaque score between the two groups.

Furthermore, the results of multiple regression analysis adjusted for age and mean blood pressure showed a significant association between the decrease in baPWV and combined-therapy treatment (Table 4).

Effects of Combined Therapy of EPA Plus Statin on Urinary Concentration of 8-Isoprostane

To further explore the effect of EPA on oxidative stress, we examined the effect of EPA on urinary concentration of 8-isoprostane based upon the baseline urinary concentration of 8-isoprostane divided by the median value at 183 pg/mL·Cr (Fig. 1). There was little difference in both higher and lower urinary con-

centrations of 8-isoprostane between the statin-only group and the combined-therapy group at baseline. Interestingly, the subgroup with a higher urinary concentration of 8-isoprostane in the combined-therapy group at 48 weeks was significantly lower than at baseline, and the concentration became significantly lower than at 48 weeks in the statin-only group. On the other hand, in the subgroups with a lower urinary concentration of 8-isoprostane, the concentration remained unchanged at 48 weeks compared with those values at baseline.

Adverse Events

After randomization, 1 patient in the combined-therapy group and 1 patient in the statin-only group experienced nonfatal myocardial infarction and unstable angina pectoris, respectively. None of the patients involved in the study showed side effects of the study medications.

Discussion

Several lipid-independent mechanisms in addi-

Fig. 1. Effects of adding EPA treatment to statin therapy on urinary concentration of 8-isoprostane in patients with coronary artery disease.

Each group was divided into two subgroups by the median value (183 pg/mL·Cr) based upon the baseline urinary concentration of 8-isoprostane. In subgroups with a higher urinary concentration of 8-isoprostane, add-on therapy of EPA induced a significant lowering effect on the concentration compared with the statin-only group. In contrast, there was little effect of EPA observed in the subgroups with lower urinary concentrations of 8-isoprostane. Data express the box and schematic plot. The line drawn across the box indicates the median. The lower line of the box is at the first quartile (Q1), and the upper line is at the third quartile (Q3). The whiskers are lines that extend from the top or bottom of the box to adjacent values within $\pm 1.5 \times (Q3 - Q1)$. Closed square outlier. * $p = 0.002$ versus baseline in the combined-therapy group. S, statin-only group; C, combined-therapy group.

tion to decreased triglycerides, including anti-inflammatory effects, modulation of autonomic function, vasodilation, decreased blood pressure and plaque stabilization, have been suggested to result from the association between ω -3 PUFAs and the risk of cardiovascular disease⁽⁴⁾. In the present study, we clearly demonstrated that combined EPA-plus-statin therapy was superior to statin-only therapy for inhibition of the progression of arterial stiffness independent of lipid lowering.

The study population in the present study showed good adherence to the study protocol. The EPA/AA ratio was significantly higher in the combined-therapy group than in the statin-only group. In previous studies, the EPA/AA ratio was significantly lower in CAD patients than in healthy volunteers, and

platelet aggregation, serotonin release, the amount of thromboxane B₂, a cyclooxygenase product synthesized from exogenous AA, and insulin resistance were increased by decreasing the ratio of EPA to AA^(23, 24). In addition, the results of a previous study demonstrated that in good adherers with previous CAD, EPA substantially reduced the risk compared with statin-only therapy⁽²⁵⁾. These results suggest that the increase in the EPA/AA ratio in the present study may explain the favorable effects of EPA in the combined-therapy group.

It has been reported that baPWV is an index of arterial stiffness with similar characteristics to aortic PWV⁽²⁶⁾, and is a marker of severity of atherosclerotic vascular damage as well as a predictor of prognosis in patients with cardiovascular disease⁽²⁷⁾; however, PWV

is strongly influenced by blood pressure and age⁹⁾. Blood pressures were unchanged throughout the study, and there was no difference in the amount of change in baPWV between the two groups, although the increase in the median baPWV value from baseline was greater in the statin-only group than in the combined-therapy group. However, multiple regression analysis adjusted for age and blood pressure clearly demonstrated an association between a decrease in baPWV and combined-therapy treatment, as shown in **Table 4**. The β -index of the carotid at 48 weeks was also significantly lower in the combined-therapy group than in the statin-only therapy group. Previous studies demonstrated that EPA supplements attenuated age-related increases in arterial stiffness in patients with dyslipidemia²⁸⁾ and improved arterial stiffness and were less influenced by blood pressure in patients with metabolic syndrome²⁹⁾. These results may support our findings regarding arterial stiffness, suggesting that EPA supplements attenuate age-related increases in arterial stiffness in patients with CAD and statin therapy.

Recent studies have suggested that excess oxidative stress induced by hyperlipidemia may play a critical role in the progression of atherosclerosis^{30, 31)}. We previously reported that arterial stiffness in patients with hypercholesterolemia may be preceded by arterial sclerosis³²⁾, and the smooth muscle phenotype of the aortic tunica media may be one of the important determinant factors of aortic stiffness^{14, 33)}. Furthermore, statin inhibits the increase in oxidative stress and halts the progression of arterial stiffness independent of lipid lowering *in vivo*^{14, 15)}. In the present study, although the reduction of the urinary concentration of 8-isoprostane was greater in the combined-therapy group than in the statin-only group, the difference between groups did not reach statistical significance. However, we clearly demonstrated a significant lowering effect of the urinary concentration of 8-isoprostane in combined-therapy group patients with a higher baseline concentration of urinary 8-isoprostane. The formation of 8-isoprostane in lipid bilayers may contribute to alterations in the fluidity and integrity of cellular membranes, inducing oxidant injury, and the level of 8-isoprostane reflects *in vivo* oxidative stress³⁴⁾, suggesting that the inhibitory effects of EPA on the observed changes in baPWV and the β -index of the carotid in the combined-therapy group might have been due to the modification of oxidative stress, mainly in patients with CAD and statin therapy with a higher oxidative stress condition, which may have caused functional changes in the vasculature by the addition of EPA to the statin therapy beyond lipid

modification.

The levels of MDA-LDL and RLP-C in patients with CAD were higher than those in the control group, suggesting that the association between high levels of remnant lipoprotein cholesterol and high oxidized LDL levels might be linked to atherogenesis in patients with CAD²¹⁾. Nakamura *et al.* previously reported that the administration of EPA to patients with hyperlipidemia and statin therapy for about 30 months induced a significant decrease in serum triglyceride and RLP-C³⁵⁾. In another study, treatment with a ω -3 fatty acid for 4 weeks had no effect on hs-CRP levels or triglyceride levels in patients with CAD³⁶⁾. In addition, after statin (pitavastatin) treatment for 12 months, significant decreases in 8-hydroxy-2'-deoxyguanosine, which is a marker of oxidative DNA damage, MDA-LDL and aortic stiffness, were observed in type 2 diabetic patients, and the change of aortic stiffness significantly correlated with the change of MDA-LDL³⁷⁾. In the present study, RLP-C, SOD activity and MDA-LDL remained unchanged by combined therapy compared with statin-only therapy. It is still unknown why the level of hs-CRP in the combined-therapy group was higher at both baseline and 48 weeks, and why there was a significant difference in the level hs-CRP between the two groups. Nonetheless, the high level of hs-CRP did not affect aortic stiffness based upon the results obtained in the study.

Study Limitations

First, the follow-up period of the present study was only 48 weeks. It is likely that more time is needed to see the effects of EPA in addition to statin therapy, since the prevention of cardiovascular events by the addition of EPA required more than 2 years⁷⁾. Our study demonstrated that the effect of EPA on aortic stiffness may precede the inhibition of cardiovascular events. Second, in this study, the choice and applied dosage of statin was entrusted to each investigator, and the study period was relatively short. These differences may have caused different results for these indices between the present study and others³⁵⁻³⁷⁾. Furthermore, the number and class of anti-diabetic and antihypertensive drugs, and antiplatelet/anticoagulants were different between the two groups, although these drugs were not changed throughout the study; we cannot exclude the possibility that this difference in drugs may have affected the results. In addition, the importance of central pressure and derived central hemodynamic indices compared with peripheral pressure for cardiovascular outcomes and all-cause mortal-

ity was reported recently³⁸⁾. Since in our study we used peripheral blood pressure to evaluate arterial stiffness, it is possible that we would have obtained different results had we used central blood pressure. Finally, this study had an exploratory and PROBE design, and the sample size was small, so the results of the study may have been influenced by these factors. Further study will be necessary to confirm our results.

Conclusion

Combined EPA-plus-statin therapy may reduce oxidative stress and inhibit the progression of arterial stiffness more efficiently than statin-only therapy, independent of cholesterol lowering in patients with dyslipidemia and a previous history of CAD.

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