Preventive Effects of Eicosapentaenoic Acid on Coronary Artery Disease in Patients With Peripheral Artery Disease – Subanalysis of the JELIS Trial –

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Background: The JELIS trial examined the preventive effects of eicosapentaenoic acid (EPA) on coronary artery disease (CAD) in hypercholesterolemia. Previous investigators have reported that patients with peripheral artery disease (PAD) have a poor prognosis due to the potential risk for CAD. We conducted a subanalysis to examine whether the incidence of CAD was high in patients with PAD and whether EPA prevented the occurrence of CAD.

Methods and Results: Of 18,645 the Japan EPA lipid intervention study (JELIS) patients, 223 had PAD (control group; complicated (n=77), newly diagnosed (n=29), EPA group; complicated (n=96), newly diagnosed (n=21)). We analyzed the incidence of major coronary events (MCE) in the 2 groups. Cox proportional hazard ratio adjusted for baseline risk factor levels was used to test differences between the 2 groups. The incidence of MCE in the control group was significantly higher in patients complicated with PAD and in those newly diagnosed with PAD than in patients without PAD (complicated: hazard ratio 1.97, P=0.039; newly diagnosed: hazard ratio 2.88, P=0.030). As for patients with PAD, the EPA group had a significantly lower MCE hazard ratio than the control group (hazard ratio 0.44, 95% confidence interval 0.19–0.97, P=0.041).

Conclusions: Subanalysis of the JELIS trial demonstrated that in patients with PAD the incidence of CAD was higher than in controls, and that EPA markedly reduced the occurrence of CAD in those patients. (Circ J 2010; 74: 1451–1457)

Key Words: Coronary artery disease; Eicosapentaenoic acid; JELIS; Peripheral artery disease
Lesterol (TC) were reported as important risk factors in the Japanese population. Peripheral artery disease (PAD) is one of the major clinical manifestations of atherosclerosis.\(^4\) PAD deteriorates not only the quality of life of patients but also their prognosis.\(^5\) It is well known that CAD is a major cause of morbidity and mortality in patients with PAD.\(^6\)

We conducted a subanalysis of JELIS to examine whether the incidence of CAD was high in patients with PAD and whether EPA (purity >98%) prevented the occurrence of CAD.

**Methods**

**Study Design and Patients**

The study design of JELIS has been published elsewhere.\(^7\) Briefly, patients with hypercholesterolemia [serum TC levels \(\geq 250\) mg/dl (men: 40–75 years, women: postmenopausal–75 years)] were followed up for 5 years (mean: 4.6 years) using a prospective, randomized, open-label, blinded endpoint evaluation method. In total, 18,645 patients were registered and randomly assigned to either the EPA with statin group (EPA group, n=9,326) or the statin alone group (control group, n=9,319). Those in the control group were treated with simvastatin or pravastatin. Patients in the EPA group received 1,800 mg/day of highly purified EPA-E, in addition to a statin.

The primary endpoint was the occurrence of an MCE, including sudden cardiac death, fatal and nonfatal myocardial infarction (MI), unstable angina, angioplasty/stenting or coronary bypass grafting. Secondary endpoints were all-cause mortality, mortality and morbidity of CAD, stroke, PAD and cancer. PAD was the secondary endpoint in JELIS.

The diagnostic criteria for PAD were established by the JELIS steering committee based on the article written by Kuramochi\(^8\) and it included symptoms (sense of cold skin, pain and intermittent claudication), physical findings (painlessness, vascular bruits and ulcer) and testing including the ankle-brachial index. The JELIS steering committee distributed these criteria to all of the physicians and institutes that participated in the study. At all institutes, the attending physician diagnosed PAD based on these criteria. However, determination of the ankle-brachial index was not mandatory.

**Ethical Considerations**

The study protocol complied with the ethical guidelines of the Helsinki Declaration and all patients gave their informed consent to participate in the study.

**Statistical Analysis**

A 2-sided test with a 5% level of significance was employed. The Wilcoxon 2-sample test was used to compare continuous variables, and a chi-square test was used to compare categorical variables. Survival analysis was conducted using the Kaplan-Meier method and Cox proportional hazards model. The Cox proportional hazards model was adjusted for age, gender, smoking, history of CAD, diabetes mellitus (DM) and hypertension. The time period of patients newly diagnosed with PAD during follow up was defined as the date of diagnosis of PAD to the date of onset of MCE or the end of follow up. All analyses were performed using version 5.0.1a of the JMP statistical software program (SAS Institute, Inc, Cary, NC, USA).

**Results**

**Breakdown of the Study Population (Figure 1)**

In this analysis, we evaluated PAD patients diagnosed at entry and those who were diagnosed during the follow-up period. The total number of PAD patients was 223 (1.2%, 223/18,645). In the control group, the total number of patients
with PAD was 106 (1.1%), the number at baseline was 77 (0.8%) and that of newly diagnosed patients was 29 (0.3%). In the EPA group, these numbers were 117 (1.3%), 96 (1.0%) and 21 (0.2%), respectively.

**Baseline Characteristics of Patients With and Without PAD (Table 1)**

There were no differences in the baseline characteristics of patients in the control group and EPA group, regardless of PAD. In patients complicated with PAD and newly diagnosed with PAD, the mean age, proportion of men, history of CAD and prevalence of DM were significantly higher compared with those without PAD. Conversely, body mass index (BMI) was significantly lower. In patients complicated with PAD, the rate of stroke history, smoking and prevalence of hypertension were significantly higher compared with those without PAD.

In patients newly diagnosed with PAD, the level of low-density lipoprotein (LDL) cholesterol was significantly higher compared with patients without PAD. But there was no significant difference in the level of triglycerides (TG) and baseline plasma concentration of EPA among the 3 groups, and the level of high-density lipoprotein (HDL) cholesterol was significantly lower in both PAD groups.

**Follow-up Characteristics of Patients in the Control Group and EPA Group (Table 2)**

During the follow-up period, the serum levels of LDL cholesterol, HDL cholesterol or TG of patients in the PAD groups did not differ significantly between the controls and those administered EPA. The plasma level of EPA was significantly increased in the EPA group.

In patients without PAD, there were no significant differences in the serum levels of LDL cholesterol or HDL cholesterol between the 2 treatment groups during the follow-up period. However, the serum TG level was significantly decreased and the plasma level of EPA was significantly increased in the EPA group.

**Incidence of MCE (Figure 2)**

In total, 586 MCE occurred during a mean follow up of 4.6 years. Among patients newly diagnosed with PAD, 1 in the control group and 1 in the EPA group had developed an MCE prior to the onset of PAD and did not develop CAD. So we conducted a subanalysis for 584 patients. In the control group, the incidence of MCE was significantly higher in patients complicated with PAD (38.6/1,000 person-years) and newly diagnosed with PAD (55.2/1,000 person-years) than in patients without PAD (7.2/1,000 person-years) (complicated: hazard ratio 1.88, 95%CI 1.13–2.98, P=0.029; newly diagnosed: hazard ratio 2.88, 95%CI 1.13–5.98, P=0.030). While in the EPA group, the incidence of MCE was higher in patients complicated with PAD (18.8/1,000 person-years) and in those newly diagnosed with PAD (10.6/1,000 person-years) than in patients without PAD (6.0/1,000 person-years), the difference was not statistically significant (complicated: hazard ratio 1.00, 95%CI 0.22–17.41, P=0.997; newly diagnosed: hazard ratio 1.17, 95%CI 0.21–21.33, P=0.879).

**Suppressive Effects of EPA on MCE**

As shown in Table 3, the MCE hazard ratio in patients without PAD for the EPA group was 0.82, which was significantly lower compared with those in the control group (95%CI 0.69–0.97, P=0.018). The MCE hazard ratio in patients complicated with PAD for the EPA group was 0.55 compared with PAD for the EPA group was 0.82, which was significant compared with those without PAD. Conversely, body mass index (BMI) was significantly lower. In patients complicated with PAD, the rate of stroke history, smoking and prevalence of hypertension were significantly higher compared with those without PAD.

In patients newly diagnosed with PAD, the level of low-density lipoprotein (LDL) cholesterol was significantly higher compared with patients without PAD. But there was no significant difference in the level of triglycerides (TG) and baseline plasma concentration of EPA among the 3 groups, and the level of high-density lipoprotein (HDL) cholesterol was significantly lower in both PAD groups.

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As shown in Table 3, the MCE hazard ratio in patients without PAD for the EPA group was 0.82, which was significantly lower compared with those in the control group (95%CI 0.69–0.97, P=0.018). The MCE hazard ratio in patients complicated with PAD for the EPA group was 0.55 compared with PAD for the EPA group was 0.82, which was significant compared with those without PAD. Conversely, body mass index (BMI) was significantly lower. In patients complicated with PAD, the rate of stroke history, smoking and prevalence of hypertension were significantly higher compared with those without PAD.

In patients newly diagnosed with PAD, the level of low-density lipoprotein (LDL) cholesterol was significantly higher compared with patients without PAD. But there was no significant difference in the level of triglycerides (TG) and baseline plasma concentration of EPA among the 3 groups, and the level of high-density lipoprotein (HDL) cholesterol was significantly lower in both PAD groups.
with the control group, but the difference group was not significant (95% CI 0.21–1.35, P=0.189). The MCE hazard ratio in patients newly diagnosed with PAD for the EPA group was 0.32 compared with the control group, but the difference was not significant (95% CI 0.02–2.46, P=0.291).

The suppressive effects of EPA on MCE did not differ among patients without PAD, those complicated with PAD and those newly diagnosed with PAD (interaction P=0.159). Furthermore, among all patients with PAD, whether it was pre-existing or developed during follow up, the incidence of

### Table 2. Serum Lipid Profiles and Plasma Concentration of EPA at Baseline and During the Follow-up Period

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>EPA group</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Average follow-up period</td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td>mg/dl</td>
<td>mg/dl</td>
<td>mg/dl</td>
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<tr>
<td><strong>Complicated with PAD</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LDL-cholesterol</td>
<td>183.2±24.7</td>
<td>127.2±29.3</td>
<td>183.1±26.1</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>53.8±17.7</td>
<td>54.4±14.3</td>
<td>51.8±14.7</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>176.5±88.0</td>
<td>140.7±53.4</td>
<td>186.0±103.1</td>
</tr>
<tr>
<td>EPA (mol %)</td>
<td>2.9±1.6</td>
<td>3.1±1.5</td>
<td>2.8±1.5</td>
</tr>
<tr>
<td><strong>Newly diagnosed with PAD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>187.3±30.8</td>
<td>138.8±29.2</td>
<td>193.5±27.8</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>53.1±16.0</td>
<td>51.5±12.8</td>
<td>55.3±15.8</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>182.3±95.6</td>
<td>231.2±322.8</td>
<td>208.7±181.8</td>
</tr>
<tr>
<td>EPA (mol %)</td>
<td>2.4±1.4</td>
<td>3.0±1.7</td>
<td>2.8±1.8</td>
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<tr>
<td><strong>Without PAD</strong></td>
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<td></td>
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<tr>
<td>LDL-cholesterol</td>
<td>181.7±28.9</td>
<td>136.5±28.6</td>
<td>181.4±29.5</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>58.3±17.0</td>
<td>59.2±15.2</td>
<td>59.9±17.7</td>
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<tr>
<td>Triglyceride</td>
<td>191.1±156.0</td>
<td>163.6±103.0</td>
<td>188.0±143.0</td>
</tr>
<tr>
<td>EPA (mol %)</td>
<td>2.8±1.5</td>
<td>2.9±1.3</td>
<td>2.9±1.6</td>
</tr>
</tbody>
</table>

*P values are given for differences between the control and EPA groups during the follow-up period. Abbreviations see in Table 1.
Preventive Effects of EPA on CAD in Patients With PAD

MCE was 42.9/1,000 person-years in the control group and 17.3/1,000 person-years in the EPA group. The MCE hazard ratio for the EPA group was 0.44 and significantly lower compared with the control group (hazard ratio 0.44; 95%CI 0.19–0.97, P=0.041). The number of patients needed to treat to demonstrate the preventive effect of EPA on MCE was 11 (Figure 3). Four patients in the control group and 1 in the EPA group had developed MCE (unstable angina; n=2, coronary revascularization; n=3) immediately after the allocation in Figure 3. Two patients from the primary prevention subgroup developed unstable angina and 3 patients from the secondary prevention subgroup had coronary revascularization. In addition, Table 4 showed the incidence of CAD in both groups during the follow-up period in patients with PAD.

### Table 3. Suppressive Effects of EPA on MCE

<table>
<thead>
<tr>
<th>No. of events (per 1,000 person-years)</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
<th>Interaction P value</th>
</tr>
</thead>
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<tr>
<td>Control group</td>
<td>0.82</td>
<td>0.69–0.97</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td>EPA group</td>
<td>0.32</td>
<td>0.02–2.46</td>
<td>0.291</td>
<td></td>
</tr>
</tbody>
</table>

HR and P values were adjusted for age, gender, smoking, history of CAD, diabetes and hypertension. MCE, major coronary events; HR, hazard ratio; CI, confidence interval. Other abbreviations see in Table 1.

### Figure 3.
Kaplan-Meier curves for the incidence of major coronary events (MCE) in patients with peripheral arterial disease (PAD). CI, confidence interval; EPA, eicosapentaenoic acid; NNT, number needed to treat. The exact time-period (year) was defined as the date of diagnosis of PAD to the onset of MCE or the end of follow up in patients newly diagnosed with PAD during follow up. The exact time-period (year) was defined as the date of the start of the present study to the onset of MCE or the end of follow up in patients complicated with PAD. Hazard ratios and P-values were adjusted for age, gender, smoking, history of coronary artery disease, diabetes and hypertension.

### Table 4. Incidence of CAD During Follow-up Period in Patients With PAD

<table>
<thead>
<tr>
<th>No. of events (per 1,000 person-years)</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n=106)</td>
<td>0.44</td>
<td>0.19–0.97</td>
<td>0.041</td>
</tr>
<tr>
<td>EPA group (n=117)</td>
<td></td>
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<td></td>
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</tbody>
</table>

Items of account:
- Sudden cardiac death: 4 (10.4) vs. 1 (2.0), P=0.19, 0.01–1.33, 0.099
- Fatal MI: 1 (2.6) vs. 2 (4.0), P=1.81, 0.17–39.43, 0.623
- Non-fatal MI: 3 (7.9) vs. 1 (2.0), P=0.27, 0.01–2.15, 0.225
- Unstable angina: 8 (21.2) vs. 5 (10.4), P=0.56, 0.17–1.71, 0.310
- CABG or PTCA: 12 (32.5) vs. 6 (12.4), P=0.40, 0.14–1.05, 0.064

Combined endpoint:
- Coronary death or MI: 7 (18.5) vs. 3 (6.0), P=0.35, 0.08–1.28, 0.114
- Coronary death: 5 (13.0) vs. 3 (6.0), P=0.48, 0.10–1.98, 0.308
- Non fatal coronary events: 14 (38.5) vs. 7 (14.6), P=0.41, 0.15–0.98, 0.045

HR and P values were adjusted for age, gender, smoking, history of CAD, diabetes and hypertension. MI, myocardial infarction; CABG, coronary artery bypass grafting; PTCA, percutaneous transluminal coronary angioplasty. Other abbreviations see in Tables 1, 3.

†We redundantly counted the incidence.
The non-fatal coronary events hazard ratio for the EPA group was 0.41, which was significantly lower than the control group (hazard ratio 0.41; 95%CI 0.15–0.98, P=0.045).

Discussion

Epidemiology

The results of this study showed that the prevalence of PAD during the 4.6 years in the JELIS trial was 1.2% (223/18,645). Fujiwara et al reported that the prevalence of arteriosclerosis obliterans was 2.7% in inhabitants of rural communities in Japan.8 Ojiri et al reported that the prevalence of PAD in the elderly was 20.6% and the death rate as well as the rate of acute MI (AMI) as the cause of death were higher for people with PAD.9 The prevalence of PAD varies depending on the population studied, and the diagnostic method and criteria used. Criqui et al reported that the incidence of PAD was 11.7%.6 Kannel reported that the biennial incidence of intermittent claudication in the Framingham Heart Study was 7.1/1,000 for men and 3.6/1,000 for women, and increased with age in both sexes up to the age of 75.10 To know the true incidence of PAD in Japan, we should implement more sophisticated methods to diagnose PAD in a large-scale prospective clinical trial.

Risk Factors

Norgren et al reported in TASC II that gender, age, smoking, DM, hypertension and dyslipidemia were risk factors for PAD.9 These findings were comparable with the results of the JELIS trial. However, the level of LDL cholesterol and TG did not differ between patients with and without PAD in the JELIS trial. We recruited patients with hypercholesterolemia, so the level of LDL cholesterol was not expected to be different. However, the level of HDL cholesterol was significantly lower in the patients with PAD compared with those without PAD. Yamazaki et al found in the REACH registry that the prevalence of DM in Japanese patients with PAD was 41.1%.11 In the present study, the prevalence of DM in patients complicated with PAD at baseline and those newly diagnosed with PAD was 29.5% and 56.0%, respectively. These rates were comparable to those found by Kannel.10 The prevalence of DM in patients complicated with PAD at baseline was more than twice that of patients without PAD. Qadan et al reported that the prevalence of metabolic syndrome was more than 95% in patients with advanced PAD, and that DM was the most prevalent component followed by hypertension and low HDL cholesterol.12 In the FRENA registry, it was found that the patients with PAD showed an inverse correlation between BMI and cardiovascular mortality.13 In the present study, the BMI of patients with PAD was lower than that of patients without PAD. But as the difference was so small, its clinical significance was obscure.

In the present analysis, the patients with PAD, regardless of whether it was pre-existing or newly diagnosed during follow up, were at a significantly higher risk of developing CAD compared with those without PAD. Especially in patients newly diagnosed with PAD, we found the risk of CAD increased by 188%. The reasons for an increased risk for CAD were considered to be male gender, higher mean age, prevalence of CAD, diabetes and lower HDL-C levels.

Saito et al reported the preventive effects of EPA on CAD in patients with hypercholesterolemia and multiple risk factors.3 In the present study, patients with PAD had greater risk factors such as the proportion of men, and the prevalence of CAD, diabetes and dyslipidemia. EPA might have significantly reduced the incidence of CAD. Matsuzaki et al also indicated that EPA prevented secondary CAD, especially in patients at high risk, such as those with a previous history of MI or interventions (percutaneous transluminal coronary angioplasty or coronary artery bypass grafting).14

Mechanisms

The fatty acid composition of membrane lipids greatly influences membrane function and the dietary fatty acid profile may change the membrane composition.15 The membrane composition was found to be more responsive to n-3 and n-6 polyunsaturated fatty acids.

EPA, a polyunsaturated n-3 fatty acid, is taken up by the membrane of systemic cells, leading to an alteration of cell properties and the manifestation of its diverse pharmacological actions. These include anti-hyperlipidemic actions,16,17 anti-inflammatory actions,18,19 reduction of platelet aggregation,20,21 vasodilation,22 enhancement of red cell deformability,23 stabilization of plaque,24,25 and so on.

Recently, Mita et al reported that EPA improved the carotid intima-media thickness in patients with type 2 diabetes or metabolic syndrome after 2.1 years of treatment.26 Ueeda et al demonstrated a significant correlation between n-3 polyunsaturated fatty acid levels and the extent of soft plaque in AMI patients.27 Taken together, it seems likely that, through these pharmacological actions, EPA would suppress the systemic progression of atherosclerosis, especially soft plaque in patients with PAD, resulting in marked suppression of the occurrence of CAD.

Furthermore, Mii et al reported that the graft patency rate following autogenous vein grafting for the treatment of lower limb PAD was significantly higher in the EPA-treated group than in the untreated control group.28 EPA could thus be a valid means for treating PAD itself.

Study Limitations

In patients with pre-existing or newly diagnosed PAD, the incidence of MCE in the EPA group was lower than that in the control group, although the difference was not significant, which was underpowered statistically to show an effect because of the small number of patients with complicated and newly diagnosed PAD.

The attending physician diagnosed PAD based on the diagnostic criteria. However, clinical manifestations, severity and ABI were not reported. In the JELIS trial, all patients were administered statins. It was reported that statin use reduced the occurrence of CAD and improved the walking performance and symptoms such as intermittent claudication.29 Therefore, statin use may have affected the results of the present study.

Although all patients were prescribed a statin, the level of LDL cholesterol during the follow-up period was 130 mg/dl and did not meet the current treatment target value.30 It is possible that EPA prevents CAD in patients with PAD independent of its control of LDL cholesterol levels because EPA has multiple effects in addition to lipid lowering effects as previously mentioned.

Conclusions

This subanalysis of the JELIS trial showed that the incidence of MCE was distinctly higher in patients with PAD, and that the administration of EPA reduced the risk for MCE in those patients.
Acknowledgments

We are indebted to all those who participated in this trial and to the large number of physicians, nurses and hospital staff for their long-term commitment to the study.

Disclosures

Conflict of interest: This study was supported by grants from Mochida Pharmaceutical Co Ltd, Tokyo, Japan. The marketed capsules containing 300 mg EPA ethyl ester were supplied by Mochida Pharmaceutical Co Ltd. The results of this study were presented at the 73rd Annual Scientific Meeting of the Japanese Circulation Society, Osaka, Japan, 20–22 March 2009. Y. Ishikawa did not receive any financial support from Mochida Pharmaceutical Co Ltd, regarding his participation in the annual scientific meeting. None of the committee members or investigators received remuneration for performing the study. Clinical trial registration information: NCT00231738 (http://www.clinicaltrials.gov).

References