Relationship between Arteriosclerosis Obliterans and the Ratio of Serum Eicosapentaenoic Acid to Arachidonic Acid

Shoji Fukuda, MD, PhD, Shunichiro Fujioka, MD, Shigeru Hosaka, MD, PhD, Sam Akita, MD, Sosuke Kimura, MD, PhD, Kayo Kurotani, PhD, and Tetsuya Mizoue, MD, PhD

Purpose: A low ratio of serum eicosapentaenoic acid to arachidonic acid (EPA/AA) has been associated with coronary artery disease. We retrospectively examined serum concentrations of polyunsaturated fatty acids in patients with arteriosclerosis obliterans (ASO) and in non-atherosclerotic patients.

Methods: From April 2011 to March 2012, serum EPA/AA was retrospectively examined in 33 consecutive outpatient patients with ASO complicated by intermittent claudication and 21 outpatients with hypercholesterolemia without ASO as controls. The Student’s t-test was used for continuous variables and Chi-square test for categorical variables, with analysis of covariance adjusting for age, sex, body mass index, smoking, alcohol, and diabetes.

Results: The ASO group were significantly different with regard to mean age (71.5 vs. 63.9 year-old, p = 0.03), body mass index (21.3 vs. 24.1 kg/m², p = 0.002) and morbidity of diabetes mellitus (51.5 vs. 4.8%, p = 0.0004). Serum EPA/AA was significantly decreased in ASO (0.36 vs. 0.61, p = 0.03), when adjusted for age, sex, body mass index, smoking status and alcohol drinking, but was not statistically significant when adjusted for diabetes.

Conclusion: Patients with ASO were more likely to have a low EPA/AA ratio and non-diabetic patients with ASO had a significantly reduced EPA/AA.

Keywords: Arteriosclerosis obliterans, eicosapentaenoic acid, arachidonic acid, eicosapentaenoic acid/arachidonic acid ratio

Introduction

Development of coronary artery disease (CAD) is influenced by dietary fatty acid composition as well as hypertension, smoking, hypercholesterolemia and type-2 diabetes. Epidemiological and clinical evidence suggest that long-term consumption of long-chain n-3 polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA), can improve CAD-associated mortality. The Japan EPA Lipid Intervention Study (JELIS) was a prospective, randomized, open-label, blinded endpoint evaluation trial in which the preventive effects of EPA on CAD in patients with dislipidemia were investigated. In a subgroup analysis of patients with peripheral artery disease (PAD), Ishikawa, et al. found a significant reduction in the rate of major coronary event (MCE) following EPA treatment and suggested that EPA could influence the grade of atherosclerosis. In this retrospective study, we compared serum concentrations of polyunsaturated fatty acids...
in patients with ASO to those in patients with non-atherosclerotic disease.

Materials and Methods

This retrospective study was approved by the Ethical Committee of the National Center for Global Health and Medicine. This study included 33 consecutive outpatients with ASO complicated by intermittent claudication and 21 outpatients with hypercholesterolemia without ASO, as controls, who were evaluated at the Department of Cardiovascular Surgery from April 2011 to March 2012. The clinical and laboratory results of these patients were obtained from our hospital database. None of the patients in the present study were taking the purified EPA drug, ethyl eicosapentate.

Serum measurements were performed by gas chromatography at the central laboratory (BML, Tokyo, Japan). In particular, plasma fatty acid composition was determined by capillary gas chromatography. Plasma lipids were first extracted by Folch’s procedure. Then, fatty acids (with tricosanoic acid, C23:0, used as the internal standard) were methylated with boron trifluoride and methanol. Finally, the methylated fatty acids were analyzed using the SHIMAZU GC-2010 gas chromatograph (Shimadzu Corporation, Kyoto, Japan) and a BPX70 capillary column (0.22 mm ID × 30 m; 0.25 μm thickness; SGE Analytical Science Pty Ltd., Australia).

The Student’s t-test for continuous variables and the Chi-square test for categorical variables were performed. The analysis of covariance (ANCOVA) was used to analyze the relationship between ASO and EPA/AA ratio, adjusted for age, sex, body mass index (BMI), smoking status (non-smoker or 20 cigarettes smoking/day), alcohol drinking (non-drinker or habitual drinker) and type 2 diabetes mellitus (DM). A p value of <0.05 was regarded as statistically significant. All analyses were completed with STATA statistical software version 10.0 (Stata Corp, College Station, TX, USA).

Results

Demographic data and metabolic parameters are presented in Table 1. The control group consisted of 13 patients with venous thrombosis, 7 patients with lower limb varices, and one patient with lymphedema. Compared to non-atherosclerotic patients, those with ASO were more likely to be older (71.5 vs. 63.9, p = 0.03) and diabetic (51.5% vs. 4.8%, p = 0.0004). However, patients with ASO were more likely to have a smaller BMI (21.3 vs. 24.1, p = 0.002).

Concentrations of EPA (62.6 vs. 105.7, p = 0.03) and docosahexaenoic acid (DHA) (117.7 vs. 163.2, p = 0.02) and EPA/AA ratio (0.36 vs. 0.61, p = 0.03) were significantly reduced in patients with ASO. When adjusted for age, sex, BMI, smoking status, alcohol drinking, and DM, however, there was no significant difference in serum EPA/AA (0.39 vs. 0.57, p = 0.12).

Furthermore, there were no significant differences in dihomo-gamma-LA and arachidonic acid, when adjusted for age, sex, BMI, smoking status and alcohol drinking (Table 2).

Discussion

Derived from triglycerides as natural fat or phospholipids, fatty acids are carboxylic acids with a long aliphatic chain, and are either saturated or unsaturated. Fatty acids are important sources of energy as they yield large quantities of ATP. Recent reports have determined that unsaturated fatty acids can improve CAD.3–5 Therefore, we hypothesized that fatty acids are associated with ASO.

We retrospectively analyzed serum concentrations of polyunsaturated fatty acids in patients with ASO and

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>ASO</th>
<th>p1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects, n (%)</td>
<td>21 (38.9)</td>
<td>33 (61.1)</td>
<td></td>
</tr>
<tr>
<td>Age (year), mean (SD)</td>
<td>63.9 (14.2)</td>
<td>71.5 (9.8)</td>
<td>0.03</td>
</tr>
<tr>
<td>Male (%)</td>
<td>20.4</td>
<td>79.6</td>
<td>0.05</td>
</tr>
<tr>
<td>BMI (kg/m²), mean (SD)</td>
<td>24.1 (2.7)</td>
<td>21.3 (3.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>23.8</td>
<td>57.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Current alcohol drinking (%)</td>
<td>42.9</td>
<td>36.4</td>
<td>0.98</td>
</tr>
<tr>
<td>Morbidity of diabetes (%)</td>
<td>4.8</td>
<td>51.5</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

1Student’s t-test for continuous variables and chi-square test for categorical variables
patients with non-atherosclerotic disease. Patients with ASO were more likely to have a lower BMI, although normal BMI was noted in both ASO and non-atherosclerotic patients. This finding is likely due to the obesity paradox,8, 9) which suggests that low BMI is partially mediated by malnutrition and systemic inflammation due to PAD.10) The serum EPA/AA ratio was significantly reduced in ASO patients without DM compared to patients with non-atherosclerotic disease, suggesting an association between EPA/AA ratio and ASO. We hypothesized that EPA treatment may improve ASO. To further evaluate effect of EPA in ASO patients, we have started a randomized control trial.

In the subgroup analysis of JELIS,6) Ishikawa, et al. found a higher incidence of MCE in PAD-complicated patients and in those newly diagnosed with PAD (complicated group: hazard ratio 1.97, p = 0.039; newly diagnosed group: hazard ratio 2.88, p = 0.030).7) Both JELIS and the subgroup analysis suggest that there is likely an association between PAD, ASO, and EPA. However, the direct relationship between ASO and EPA remains unclear.

This study has some limitations. First, this was a retrospective study so the information available is limited to the hospital database. Second, there were relatively few patients in this study, and these patients resided in different areas in or around Tokyo. Third, there was a disproportionate number of men in the ASO group, which is reflective of the disease itself. Therefore, no definitive conclusions can be drawn. Nevertheless, this is the first study examining the relationship between EPA/AA ratio and ASO in consecutive Japanese patients. The findings of this study may suggest treatment options for ASO.

Conclusion

Patients with ASO were more likely to have a low EPA/AA ratio and non-diabetic patients with ASO had a significantly reduced EPA/AA.

Disclosure Statement

This study was financially supported by a grant from the National Center for Global Health and Medicine (23–113).

References


