Which has the stronger impact on coronary artery disease, eicosapentaenoic acid or docosahexaenoic acid?

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Running head: Impact of EPA and DHA on CAD

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Abstract

It has been suggested that n-3 polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), protect against cardiovascular diseases, and EPA/arachidonic acid (AA) and DHA/AA ratios in the serum has been focused on as risk markers for coronary artery disease (CAD). The purpose of this study was to clarify difference of the clinical significance between the EPA/AA ratio and DHA/AA ratio in patients with CAD. In 369 consecutive patients with confirmed or suspected CAD who underwent diagnostic coronary angiography, we measured serum levels of EPA, DHA and AA, and calculated the EPA/AA and DHA/AA ratios. The EPA/AA ratio was significantly lower in acute coronary syndrome (ACS) patients than in patients with chronic CAD or chest pain syndrome (0.27±0.19 vs 0.44±0.20, respectively, $P<0.01$), whereas the DHA/AA ratio was similar in the two groups (0.78±0.27 vs 0.79±0.37). Multiple logistic regression analysis for discrimination of ACS from other disease entities using various biomarkers related to coronary risk showed that the EPA/AA ratio (odds ratio: 0.0012, 95% confidence interval: 0.00-0.16, $P<0.01$), but not DHA/AA ratio (odds ratio: 1.05, 95% confidence interval: 0.98-1.12), was a significant independent predictive factor. Our findings suggest that the EPA/AA ratio might be more closely associated with pathophysiology of CAD, especially with that of ACS, compared with the DHA/AA ratio.

Keywords: EPA/AA ratio, DHA/AA ratio, coronary artery disease, acute coronary syndrome, risk factor

INTRODUCTION

It has long been noted that consumption of fish or fish oil may prevent cardiovascular events. Since the 1980s various epidemiological studies around the world have suggested that n-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are contained in fish oils, protect against the occurrence of cardiovascular events in comparison with n-6 PUFA as arachidonic acid (AA). Accordingly, supplemental intake of n-3 PUFA is recommended.[1-3] The Japan Eicosapentaenoic acid Lipid Intervention Study (JELIS) showed that administration of high-purity EPA agents in addition to statins reduced the incidence of cardiovascular events.[4] EPA and DHA differ in their biochemical effects and functions, and it is not clear which is more effective for preventing cardiovascular events. Also, the EPA/AA and DHA/AA ratios, which reflect the intake of n-3 PUFA relative to n-6 PUFA, have attracted attention as possible new cardiovascular risk markers. In addition to various conventional risk markers,[5] the EPA/AA and DHA/AA ratios also promise to predict future cardiovascular events. The purpose of the present study was to clarify the clinical significance of the EPA/AA and DHA/AA ratios in patients with coronary artery disease (CAD).

METHODS

Study design

We recruited 369 consecutive patients confirmed or suspected CAD (285 men, 84 women, aged 66±11 yrs) who underwent diagnostic coronary angiography. Those patients who were diagnosed with ST elevation myocardial infarction, non-ST elevation myocardial infarction, and unstable angina were categorized as acute coronary syndrome (ACS). In patients who were without physical coronary artery stenosis an acetylcholine provocation test was performed. Patients in whom coronary artery spasm was provoked were diagnosed as having coronary spastic angina (CSA), and those in whom it was not provoked as having chest pain syndrome. Patients who had taken EPA or DHA as an agent or dietary supplement were excluded from the study. In these 369 patients we measured the serum concentrations of EPA, DHA and AA in venous blood taken before the coronary angiography, and calculated the EPA/AA and DHA/AA ratios. As conventional biomarkers for coronary risk factors, the levels of serum creatinine, hemoglobin A1c, total cholesterol, low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, triglycerides, and uric acid were also measured. Estimated glomerular filtration rate (eGFR) was calculated using the method described in the Japanese Society of Nephrology CKD Practice Guide.[6] The study was approved by the ethics committee of Dokkyo Medical University, and informed consent was obtained from each patient.

Measurement of serum fatty acids

Serum fatty acids were assayed by gas chromatography (SRL, Tokyo, Japan). Briefly, total lipids in plasma were extracted according to Folch, followed by hydrolysis to release free fatty acids. Free fatty acids were then esterified with potassium

methoxide/methanol and boron trifluoride/methanol. The methylated fatty acids were analyzed using a GC-17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with an omegawax-250 capillary column (SUPELCO, Sigma-Aldrich Japan, Tokyo, Japan). Reproducibilities (i.e., coefficients of variation) of the determination of serum EPA, DHA, and AA by this method have been reported to be 4.4%, 2.3%, and 3.8%, respectively.[7]

Statistical analysis

Data were expressed as mean±standard deviation. The two groups were compared using Student's unpaired t-test for continuous variables. Multi-group comparisons were performed using one-way analysis of variance (ANOVA) followed by a post-hoc Fisher's Least Significant Difference test. Multiple logistic regression analysis was performed for discrimination of ACS from chronic CAD, such as stable angina pectoris (SAP), old myocardial infarction (OMI), CSA, and chest pain syndrome, using various biomarkers of coronary risk. Statistical significance was defined as $P<0.05$. All statistical analyses were performed using statistical software (Excel To-kei 2012, SSRI Inc., Tokyo, Japan).

RESULTS

The clinical backgrounds of the patients are shown in Table 1. In all subjects lipid metabolism appeared to be well controlled on statin treatment. Serum creatinine, eGFR

and hemoglobin A1c were 0.86±0.30 mg/dl, 71±19 ml/min/1.73m^2, and 6.3±1.2%, respectively.

Both the EPA/AA ratio and DHA/AA ratio were lower in younger patients (<60 yr) than in older patients (≥60 yr) (0.36±0.20 vs 0.47±0.30, respectively, P<0.0001, and 0.72±0.28 vs 0.96±0.33, P<0.001). The EPA/AA ratio was lower in females than in males (0.36±0.25 vs 0.44±0.29, P<0.05), but a gender difference was not seen in the DHA/AA ratio (0.87±0.55 vs 0.91±0.37).

In the disease entities of ACS, SAP, OMI, CSA and chest pain syndrome, the EPA/AA ratios were 0.35±0.13, 0.49±0.20, 0.47±0.20, 0.47±0.23, 0.42±0.19, respectively, and the DHA/AA ratios were 0.78±0.23, 0.95±0.37, 0.86±0.36, 0.95±0.32, 0.95±0.36, respectively. The EPA/AA ratio was significantly lower in the ACS group than in each of the SAP (P<0.05), CSA (P<0.01) and chest pain syndrome (P<0.05) groups. The DHA/AA ratio in the ACS group was significantly lower than in the SAP group (P<0.05) (Fig. 1).

Table 2 shows comparisons of various biomarkers for coronary risk including the EPA/AA and DHA/AA ratios between ACS patients and patients with other disease entities, i.e., chronic CAD such as SAP, OMI and CSA, and chest pain syndrome. In patients with ACS, the EPA/AA ratio was significantly lower, and the DHA/AA ratio tended also to be lower, compared with other patients. Total cholesterol and HDL-cholesterol levels were significantly lower and hemoglobin A1c tended to be higher in the ACS patients compared with others.

Multiple logistic regression analysis for discrimination of ACS from other disease entities showed that only the EPA/AA ratio (odds ratio: 0.0012, 95% confidence

interval: 0.00-0.16, P<0.01), but not DHA/AA ratio (odds ratio: 1.05, 95% confidence interval: 0.98-1.12), was a significant independent predictor (Table 3).

DISCUSSION

In this study we measured the EPA/AA and DHA/AA ratios in patients with definite or suspected CAD. We found that both the EPA/AA ratio and the DHA/AA ratio were lower in young patients than in older patients. The EPA/AA ratio was also lower in females than in males, but a similar difference was not seen in the DHA/AA ratio. Also, the EPA/AA ratio was significantly lower in ACS patients compared with chronic CAD or chest pain syndrome patients, while the difference in the DHA/AA ratio was less significant. In multivariate analysis, the EPA/AA ratio, but not the DHA/AA ratio, was the independent predictor for discriminating ACS from chronic CAD or chest pain syndrome.

It has been shown previously that the EPA/AA ratio is low in young people.[8] In the present study, the DHA/AA ratio was also lower in younger than in older people. This may be a consequence of the westernization of the diet of young Japanese people, providing low n-3 PUFA and high n-6 PUFA intakes. PUFA are essential fatty acids, and their consumption directly affects their plasma concentration. Also, it has been suggested that estrogens affect PUFA metabolism in women, although details are not available.[9] We have also previously reported the gender difference in EPA/AA ratio.[8] Actually it has been well known that the incidence of hypercholesterolemia is higher in

men than women under 40 years old but that this trend reverses as women enter into menopause when the level of estrogen production decreases, since estrogen has a known mechanism to lower LDL-cholesterol level and raise HDL-cholesterol level. The gender difference in the mortality rate from CAD lessens at menopause and disappears when they are in the 80's. It suggests that women's essential fatty acid metabolism may also be affected around the time their sex hormone balances undergo change. The oxidative metabolism of estrogen is catalyzed by hepatic cytochrome P450 (CYP450) predominantly, and dietary fish oil increases the concentration of CYP450 and some subtypes of CYP450 in rat,[10] moreover the n-3 PUFA supplementation may decrease the metabolism of estradiol.[11] Whereas alpha-linolenic acid is converted to subtype of long chain n-3 PUFA such as EPA and DHA by desaturase and elongase enzymes in the liver. The concentrations of long chain n-3 PUFA may positively associate with circulating concentration of estrogen and progesterone, but negatively with testosterone. This may suggest sex hormones may act to modify plasma and tissue n-3 PUFA content, possibly by altering the expression of desaturase and elongase enzymes. Therefore, the findings in the present study that the EPA/AA ratio was lower in female patients than in male patients, but that such a gender difference was not seen in the DHA/AA ratio, might be a consequence of an effect of sex hormones on PUFA metabolism.

Although a number of epidemiological studies have found that consumption of fish or fish oils prevented early onset of cardiovascular events,[12] the mechanism by which fish oil might suppress atherogenesis is not understood. Arita et al. [13] demonstrated that the strong anti-inflammatory and anti-oxidative actions of both EPA and DHA metabolites, such as rezolvins and protectins, have the potential to suppress

atherogenesis. However, it is not known which metabolites of EPA or DHA are more effective for atherosclerosis prevention. On the other hand, the PUFA with 20 carbon chain, EPA and AA, which are present in cell membranes, are released by phospholipase A and transported into the metabolic cascade. Then they are transformed into eicosanoids such as prostaglandins or thromboxanes by cyclooxygenase, or into leukotrienes by lipoxygenase, which mediate their biological activities.[14-16] The AA-derived eicosanoids act as promoters of platelet aggregation and inflammatory cell migration, whereas EPA-derived eicosanoids do not do so and are thought to be anti-atherosclerotic. However, DHA, which is a PUFA with 22 carbon chain, can never be involved in such a metabolic cascade. Therefore, EPA and DHA are potentially different in terms of their biological properties. The Japanese JELIS trial demonstrated that administration of a high-purity EPA agent reduced the onset of CAD. In particular, an inhibitory effect of the EPA agent on the onset of ACS, demonstrated by sub-analysis of the JELIS data, merits attention.[17] Since ACS is pathologically assumed to be characterized by plaque destabilization, plaque rupture and plaque erosion, in which inflammation and oxidative stress play a key role,[18] anti-inflammatory and anti-oxidative properties of the EPA might contribute not a little to its effect on inhibiting onset of ACS. Regarding DHA, however, there are no evidence of inhibitory effects on ACS onset. Domei et al [19] reported that the risk of developing cardiovascular events after percutaneous coronary intervention was increased in association with a low EPA/AA ratio, but was independent of the DHA/AA ratio. Lee et al [20] reported that a low EPA/AA ratio, but not a low DHA/AA ratio, was predictive of total cardiovascular death in patients with ACS. It has also been reported that the concentration of EPA in

erythrocytes, but not that of DHA, predicted the incidence of in-hospital death after onset of ACS.[21] In our present study, the EPA/AA ratio but not the DHA/AA ratio was strongly associated with the presence of ACS in patients with CAD, supporting previous data. From our results, we can envision that interventions with EPA agents or supplemental EPA are likely to confer greater benefit for plaque stabilization to prevent ACS in CAD patients, compared with DHA agents or DHA supplements .

Conclusion

The EPA/AA ratio might be more closely associated with pathophysiology of CAD, especially with that of ACS, compared with the DHA/AA ratio.

CONFLICT OF INTEREST

The authors declare no conflict of interest

ACKNOWLEDGEMENT

This study is a joint effort of many investigators and staff members whose contribution is greatly acknowledged.

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Figure legends

Figure 1 Comparisons of the EPA/AA and DHA/AA ratios between each disease entity of acute coronary syndrome (ACS), stable angina pectoris (SAP), old myocardial infarction (OMI), coronary spastic angina (CSA), and chest pain syndrome (CPS). The EPA/AA ratio was significantly lower in the ACS group compared with each of the SAP ($P<0.05$), CSA ($P<0.01$) and CPS ($P<0.05$) groups. The DHA/AA ratio in the ACS group was significantly lower than in the SAP group.