Nutrition 29 (2013) 127–131

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Comparison of eicosapentaenoic acid concentrations in plasma between patients with ischemic stroke and control subjects

Yoshimori Ikeya M.D. [a], Naoto Fukuyama M.D., Ph.D. [a], Waichi Kitajima M.D., Ph.D. [c], Yoichi Ogushi Ph.D. [b], Hidezo Mori M.D., Ph.D. [a,*]

[a] Department of Physiology, Tokai University School of Medicine, Shimokasuya, Isehara, Kanagawa, Japan
[b] Medical Informatics, Tokai University School of Medicine, Shimokasuya, Isehara, Kanagawa, Japan
[c] Fureai Machida Hospital, Oyamagaoka, Machida, Tokyo, Japan

ARTICLE INFO

Article history:
Received 12 November 2011
Accepted 2 May 2012

Keywords:
ω-3 Fatty acids
Eicosapentaenoic acid
Ischemic stroke
Cardioembolism
Large-artery atherosclerosis
Prevention of ischemic stroke

ABSTRACT

Objective: ω-3 fatty acids, including eicosapentaenoic acid (EPA), prevent ischemic stroke. However, the clinical importance of EPA for ischemic stroke and its subtype has not been fully elucidated.
Methods: In a cross-sectional study, we determined whether ω-3 fatty acids were predictive factors for ischemic stroke. We compared common clinical parameters among 65 patients with ischemic stroke and 65 control subjects. The parameters included blood chemistry data; concentrations of EPA, docosahexaenoic acid, and arachidonic acid (AA); EPA/AA ratio; smoking; alcohol intake; fish consumption more than four times per week; and the incidence of underlying diseases. The comparisons were performed using the Mann-Whitney *U* test, and multiple logistic regression analysis was applied to the significant factors in the non-parametric test. We also applied the same approach to the ischemic stroke subtypes, cardioembolism and large-artery atherosclerosis.
Results: In the multiple logistic regression analysis after the Mann-Whitney *U* test, a lower EPA concentration was one of the significant risk factors for ischemic stroke, as were a lower body mass index, lower high-density lipoprotein cholesterol, and smoking (sensitivity 0.846, specificity 0.831, positive predictive value 0.833). In the analysis of subtypes, a lower EPA/AA ratio and a lower body mass index were the significant risk factors for cardioembolism (sensitivity 0.800, specificity 0.733, positive predictive value 0.750). However, large-artery atherosclerosis was not related to the EPA concentration or the EPA/AA ratio.
Conclusions: In this study, the plasma EPA concentration and the EPA/AA ratio were potential predictive risk factors for ischemic stroke, especially for cardioembolism. Further prospective studies are necessary.

Introduction

The ω-3 fatty acids found in fish oil have preventive effects on coronary artery disease and stroke [1–16]. Yokoyama et al. [2] found that eicosapentaenoic acid (EPA) supplementation in hypercholesterolemic patients prevents major coronary events in Japan. A meta-analysis of randomized trials has indicated that supplementation with the marine ω-3 fatty acids EPA and docosahexaenoic acid (DHA) decreases the rate of death from coronary heart disease [15].

Some reports have suggested that ω-3 fatty acids decrease the risk of cardiac arrhythmias [16–19]. Mozaffarian et al. [20] indicated that fish intake decreases the incidence of atrial fibrillation. Calò et al. [21] suggested that the supplementation of ω-3 fatty acids decreases the incidence of atrial fibrillation after coronary artery bypass graft surgery.

With regard to stroke, many reports have suggested that the consumption of ω-3 fatty acids from fish and rapeseed oil has a preventive effect [8–13,22]. Furthermore, recent biochemical research has shown that ω-3 fatty acids have a neuroprotective effect after brain ischemia [23,24]. A recent randomized controlled trial by Tanaka et al. [13] also showed the beneficial effects of EPA on the recurrence risk of ischemic events in Japanese hypercholesterolemic patients with a history of stroke. In addition, Park et al. [14] reported that a low level of ω-3 fatty acids in erythrocytes is

* Corresponding author. Tel.: +81-463-93-1121, ext. 2532; fax: +81-463-93-6684.
E-mail address: coronary@is.icc.u-tokai.ac.jp (H. Mori).

0899-9007/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nut.2012.05.003

a risk factor for hemorrhagic stroke and ischemic stroke. In the present study, we determined whether the EPA concentration is a predictive factor for ischemic stroke by comparing clinical data, including the EPA concentration and the EPA/arachidonic acid (AA) ratio, between patients with ischemic stroke and control subjects. We used a non-parametric test and analyzed the factors with significant differences through a multiple logistic regression analysis.

Materials and methods

Subjects

From June 28, 2009 through December 20, 2011, we recruited 65 consecutive patients who were admitted to Fureai Machida Hospital (Tokyo, Japan) for their first ischemic stroke. All patients were in the subacute phase (defined as ≤60 d from the onset of ischemic stroke) and were 70 ± 10 y of age. All patients with ischemic stroke were diagnosed based on their medical history, brain magnetic resonance imaging results, brain magnetic resonance angiographic results, cardiac ultrasonographic results, Doppler ultrasonographic results of the carotid artery, and/or electrocardiographic results. Fifteen patients were diagnosed with cardioembolism and 50 patients were diagnosed with large-artery atherosclerosis using the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification system [25]. Patients with previous stroke were excluded because of their use of secondary preventive therapy for stroke, and patients with small-artery occlusion of unknown etiology were excluded because the number of cases was insufficient to analyze the relation of the occlusion to the plasma EPA concentration.

We also recruited 65 age-matched control subjects (>50 y old) from the outpatient clinic of the hospital; these subjects had no history of ischemic stroke and were not taking medications containing EPA, DHA, or AA. Of these 65 subjects, 38 consecutive patients with chronic cardiovascular or metabolic disease (as listed in Table 1) agreed to join the protocol from July 23, 2010 through January 8, 2011. In addition, 27 consecutive patients with neurologic symptoms (e.g., headache, dizziness, numbness, and forgetfulness) but with no brain disease (as determined by brain imaging and neurologic findings) agreed to a blood examination for EPA, DHA, and AA. Of the 65 control subjects, 15 subjects presented with atrial fibrillation (AF), which was identified by a 12-lead electrocardiogram or Holter electrocardiogram, and were considered to be specific controls for cardioembolism. The remaining 50 patients without AF were considered controls for large-artery atherosclerosis.

Table 1
Profiles of 65 patients with ischemic stroke and 65 control subjects

Patient profile	Ischemic stroke ($n = 65$)	Control ($n = 65$)	*P*
Men/women	44/21	32/33	<0.05
Age (y)	70 ± 11	70 ± 10	NS
BMI (kg/m^2)	21 ± 3	23 ± 4	<0.001
SBP (mmHg)	132 ± 13	129 ± 12	NS
DBP (mmHg)	80 ± 10	76 ± 10	NS
LDL-C (mg/dL)	114 ± 32	116 ± 23	NS
HDL-C (mg/dL)	43 ± 11	60 ± 14	<0.001
TG (mg/dL)	124 ± 58	118 ± 69	NS
HbA1c (%)	6.4 ± 1.3	6.0 ± 0.6	NS
EPA (μg/mL)	61.5 ± 25.7	98.6 ± 50.2	<0.001
DHA (μg/mL)	142.0 ± 35.2	154.8 ± 47.0	NS
AA (μg/mL)	166.2 ± 39.8	167.2 ± 35.4	NS
EPA/AA	0.39 ± 0.18	0.61 ± 0.31	<0.001
Smoking	19 (29%)	4 (6%)	<0.001
Alcohol intake	37 (57%)	38 (58%)	NS
>400 mL/d	22 (34%)	13 (20%)	NS
Fish consumption >4 times/wk	14 (22%)	32 (49%)	<0.001
Underlying diseases			
HBP	29 (45%)	25 (38%)	NS
Dys LP	16 (25%)	25 (38%)	NS
DM	23 (35%)	8 (12%)	<0.01
AF	15 (23%)	15 (23%)	NS

AA, arachidonic acid; AF, atrial fibrillation; BMI, body mass index; DBP, diastolic blood pressure; DHA, docosahexaenoic acid; DM, diabetes; Dys LP, dyslipidemia; EPA, eicosapentaenoic acid; HBP, high blood pressure; HDL-C, high-density lipoprotein cholesterol; hemoglobin A1c, HbA1c; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triacylglycerols
Values are presented as number (percentage) or mean ± SD.

The study protocol described below was approved by the institutional review board of the Tokai University School of Medicine.

Protocol

First, we measured common clinical parameters (body mass index [BMI] and blood pressure); lipid levels (low-density lipoprotein cholesterol, high-density lipoprotein [HDL] cholesterol, and triacylglycerols); glycosylated hemoglobin; and plasma EPA, DHA, and AA concentrations. Second, we investigated lifestyle habits (smoking, alcohol intake, and frequency of fish consumption) and underlying diseases (hypertension, dyslipidemia, and diabetes) using a questionnaire. A non-parametric test was then used to compare these values between the 65 patients with ischemic stroke and the 65 control subjects. To determine which factors were related to the risk for ischemic stroke, we applied a multiple logistic regression analysis to the significant factors for ischemic stroke defined by prior non-parametric tests. We also applied the same approach to the ischemic stroke subtypes (cardioembolism and large-artery atherosclerosis) using a similar procedure. We used a receiver operating characteristic curve analysis to evaluate the cutoff values of the EPA concentrations and the EPA/AA ratios for ischemic stroke, cardioembolism, and large-artery atherosclerosis.

Measurement of variables

The clinical parameters were measured as follows. Height and weight were measured the same day that blood samples were collected from the patients with ischemic stroke and the control subjects. The blood pressure in the patients with ischemic stroke was measured (with the patients seated) at the brachial artery at the time of hospital admission using an automated sphygmomanometer (Panasonic, Osaka, Japan). The control subjects' blood pressure was measured using the same method at the time of their consultation.

The blood chemistry data were measured as follows. All blood samples were obtained from the patients in the morning and in a fasting state. The plasma fatty acid composition (EPA, DHA, AA, and EPA/AA) was determined by capillary gas chromatography. Briefly, plasma lipids were extracted using the Folch procedure, after which fatty acids with tricosanoic acid (C23:0) used as the internal standard were methylated with boron trifluoride and methanol. The methylated fatty acids were analyzed using a Shimadzu GC-2010 gas chromatograph (Shimadzu Corporation, Kyoto, Japan) and a BPX70 capillary column (0.22 mm inner diameter × 30 m, 0.25 μm thickness; SGE Analytical Science Pty Ltd., Melbourne, Australia). Serum low-density lipoprotein cholesterol, HDL cholesterol, and triacylglycerols were measured using enzymatic methods with a commercial kit (Sekisui Medical Co., Ltd., Tokyo, Japan) on an AU400 chemistry immuno analyzer (Olympus Co., Tokyo, Japan). Glycosylated hemoglobin was measured using ion-exchange high-performance liquid chromatography on a HLC-723G glycohemoglobin analyzer (Tosoh, Tokyo, Japan).

Information derived from a questionnaire concerning lifestyle and underlying diseases was analyzed as follows. We investigated smoking, alcohol intake, fish consumption more than four times per week, and history of hypertension, dyslipidemia, and diabetes in the entire study group of 130 subjects. If the patients had stopped smoking or stopped drinking alcohol more than 5 y before the blood examination, they were classified as non-smokers or non-drinkers in this research. Twenty-three patients (ischemic stroke group, 19; control group, 4) were smokers (Table 1). Seventy-five patients (ischemic stroke group, 37; control group, 38) were drinkers (Table 1). Of the drinkers, 35 patients (ischemic stroke group, 22; control group, 13) had an alcohol intake more than 400 mL/d (Table 1). Forty-six patients (ischemic stroke group, 14; control group, 32) said they consumed fish more than four times per week (Table 1). Regarding underlying diseases, 54 patients (ischemic stroke group, 29; control group, 25) had a history of hypertension, 41 patients (ischemic stroke group, 16; control group, 25) had a history of dyslipidemia, and 31 patients (ischemic stroke group, 23; control group, 8) had a history of diabetes (Table 1).

Statistical analyses

We used computer software (SPSS Statistics 19, IBM, New York, USA) for all statistical analyses. Mann–Whitney *U* tests were used to compare clinical parameters between the 65 patients with ischemic stroke and the 65 control subjects, between the 15 patients with cardioembolism and the 15 control subjects with AF, and between the 50 patients with large-artery atherosclerosis and the 50 control subjects without AF. The clinical parameters included sex; age; BMI; blood pressure; concentrations of low-density lipoprotein cholesterol, HDL cholesterol, triacylglycerols, glycosylated hemoglobin, EPA, DHA, and AA; the EPA/AA ratio; smoking; alcohol intake; fish consumption more than four times per week; and the incidences of underlying diseases (hypertension, dyslipidemia, and diabetes).

A multiple logistic regression analysis was applied for all factors with significant differences in the Mann–Whitney *U* test analysis to confirm the