Original Article

Effects of Eicosapentaenoic Acid on Blood Pressure, Cell Membrane Fatty Acids, and Intracellular Sodium Concentration in Essential Hypertension

Tohru MIYAJIMA^{*, **}, Takeshi TSUJINO^{*}, Komei SAITO^{*, ***}, and Mitsuhiro YOKOYAMA^{*}

This study was designed to clarify the effects of orally administered eicosapentaenoic acid (EPA) on blood pressure, intracellular sodium content, and cell membrane fatty acid composition in patients with essential hypertension. After a 4-week run-in period, a study group of 17 male patients was assigned to an 8-week treatment with EPA (2.7 g/day) or placebo in a randomized, double-blind fashion with a crossover at week 4. Systolic blood pressure (SBP) was lower after treatment with EPA than after treatment with placebo (152.9 ± 17.3 vs. 162.6 ± 20.6 mmHg; $p < 0.01$), while diastolic blood pressure was not statistically different. Compared with the placebo treatment, EPA supplementation resulted in a decrease in intraerythrocyte sodium content (R-Na; 11.17 ± 0.63 vs. 10.44 ± 1.28 nmol/l cells; $p < 0.05$) accompanied by an increase ($p < 0.001$) in erythrocyte membrane EPA content. The increase in membrane EPA content was related to the decrease in SBP ($r = -0.52$, $p < 0.05$) and the decrease in R-Na ($r = -0.57$, $p < 0.02$) during EPA treatment. The decrease in R-Na correlated positively with the decrease in SBP ($r = 0.54$, $p < 0.05$), and correlated negatively with the change in $\text{Na}^+\text{-K}^+$ ATPase activity ($r = -0.59$, $p < 0.02$). However, the change in $\text{Na}^+\text{-K}^+$ ATPase activity did not directly correlate with the change in membrane EPA content. In conclusion, oral EPA supplementation increased membrane EPA content and reduced SBP in patients with essential hypertension. Based on the association between the increase in membrane EPA content and the decrease in intracellular sodium concentration, EPA may lower blood pressure by altering the activities of the membrane sodium transport systems. (*Hypertens Res* 2001; 24: 537–542)

Key Words: eicosapentaenoic acid, essential hypertension, $\text{Na}^+\text{-K}^+$ ATPase, intraerythrocyte sodium concentration, fatty acid

Introduction

Epidemiological studies have suggested an association between increased consumption of a diet rich in fish oils and reduced mortality from coronary heart disease (1, 2). Polyunsaturated fatty acids (PUFA) in fish oil, especially n-3 fatty acids, are known to have beneficial effects against atherosclerotic disease, including reduction of platelet aggregation, improvement of blood lipid profiles, and anti-inflammatory effects (for review, 3). n-3 fatty acids, eicosapentaenoic

acid (EPA) in particular, have been shown to reduce blood pressure in several interventional trials (4–7). Although some studies have failed to show the blood pressure lowering effect of n-3 fatty acids due to an insufficient sample size for detecting relevant blood pressure changes, two meta-analyses concluded that n-3 PUFA supplementation (at least 3 g/day) reduced blood pressure in patients with essential hypertension, but not in normotensive subjects (8, 9). It has been suggested that n-3 fatty acids reduce blood pressure by reducing blood viscosity, altering prostaglandin metabolism, or changing vascular reactivity (10). However, to our knowl-

From ^{*} the First Department of Internal Medicine, Kobe University School of Medicine, Kobe, Japan, ^{**} Department of Internal Medicine, Kenritsu Kakogawa Hospital, Kakogawa, Japan, and ^{***} Saito Clinic, Takarazuka, Japan.

Address for Reprints: Takeshi Tsujino, M.D., First Department of Internal Medicine, Kobe University School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. E-mail: ttsujino@hyo-med.ac.jp

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edge the effects of EPA on how the cell membrane handles sodium *in vivo* have not yet been reported.

It is well established that the properties of cation transport systems like $\text{Na}^+\text{-K}^+$ ATPase are modified by lipid composition and by the bilayer thickness of the cell membrane (11–15). Orally administered EPA is readily incorporated into membrane phospholipid (16); hence, it may affect the cell membrane sodium transport systems. Thus, in the present study, we evaluated how EPA supplementation modifies blood pressure, cell membrane fatty acid composition, and intracellular sodium concentration in patients with essential hypertension. We also examined whether cell membrane $\text{Na}^+\text{-K}^+$ ATPase activity was affected by EPA supplementation, based on the finding that decreased $\text{Na}^+\text{-K}^+$ ATPase activity with increased sodium content may be involved in the pathogenesis of essential hypertension (17–20). This study used a double blind, two-phase crossover design with vegetable oil as the placebo treatment.

Methods

Study Protocol

Seventeen male outpatients with uncomplicated mild to moderate essential hypertension (mean age $\pm$ SD: 44.8 ± 6.4 years) were included in this study. The subjects' blood pressure in the sitting position was examined every week during the 4-week run-in period, and the subjects who were confirmed to have an office systolic pressure over 140 mmHg or a diastolic pressure over 90 mmHg entered the intervention phase. The patients either had never been treated or had ceased taking their antihypertensive medication at least 2 months before the time of the study. None of the patients had abnormal renal function or electrocardiographic evidence of left ventricular hypertrophy. Secondary causes of hypertension were ruled out by the usual screening tests, including history, physical examination, urinalysis, and blood chemistry. All of the subjects gave their informed consent to participate in the study. The study protocol was approved by the Human Ethics Committee of Kobe University School of Medicine.

After the 4-week run-in period, the subjects were assigned to an 8-week treatment with EPA or placebo. This study phase was randomized and double blind, with a crossover at week 4. A highly purified (>90%) EPA, in soft capsule form (a 300 mg ethyl ester form of EPA/capsule: EPA-E, Mochida Corp., Tokyo, Japan), manufactured from sardine oil, was used in this study. Subjects took 9 capsules of EPA containing 2.7 g of EPA ethyl ester daily in one phase of the study; in the other phase, subjects took 9 capsules of placebo that were identical in appearance to the EPA capsules and contained 2.7 g of salad oil. This placebo was composed of about 20% palmitic acid (16:0), 19% stearic acid (18:0), and 55% linoleic acid (18:2 $n=6$). During the observation period and at the end of each phase of the study, blood pressure recording and venous blood sampling were performed be-

tween 9 and 10 AM after an overnight fast. Blood pressure was measured three times by the same observer using a standard mercury sphygmomanometer after the subject had rested in the supine position for 30 min. The observer had no knowledge of the treatment group to which a patient belonged. Measurements of red cell sodium content, erythrocyte ouabain-sensitive sodium efflux rate constant, and erythrocyte membrane fatty acid composition were performed at the end of the EPA and placebo periods, respectively.

Intraerythrocyte Sodium Content and Sodium Efflux Rate Constant

Red cell sodium content (R-Na) was measured by the method that uses capillary tubes for the microhematocrit, as previously described (17, 18, 21). Two milliliters of venous blood, collected in a heparinized tube, was centrifuged at room temperature. The packed cells were injected into capillary tubes for microhematocrit (75 mm length) and centrifuged at 11,000 rpm for 5 min. The capillary tubes were then cut at the boundary between the erythrocyte and the plasma, and the erythrocyte portions were transferred into 3 ml of lithium solution and hemolyzed. The sodium concentration of the hemolysate was determined by a flame photometer (Hitachi 205, Hitachi Co., Tokyo, Japan). Because 3%(v/v) of erythrocyte portion in a capillary tube was trapped plasma according to the experiment which used ^{131}I -radioactive human serum albumin, R-Na was approximated according to the functions described previously (17) and expressed as mmol/l cells.

The erythrocyte ouabain-sensitive sodium efflux rate constant (K_{os}) was determined by the method of Cumberbatch *et al.* (22). The rate of ouabain-sensitive sodium efflux (mmol/l $\cdot$ cells/h) was measured as one-half of the increase in the R-Na of the whole blood during incubation with ouabain (10^{-4} mol/l) for 120 min at 37°C, which totally inhibits the ouabain-sensitive sodium efflux. The K_{os} (/h) was calculated as the ratio of the efflux to the R-Na before the addition of ouabain.

All of these measurements were performed in duplicate, and the accuracy of the technique was confirmed by a preliminary study, as reported (17). Within 8 pairs of samples obtained on different days, the interassay coefficients of variation were 3.4% and 5.4% for R-Na and K_{os} , respectively.

Erythrocyte Membrane Fatty Acid Composition

Erythrocyte ghosts were obtained according to the method described by Hanahan and Ekholm (23). Red cell membrane total lipids were extracted from washed erythrocyte ghosts according to the method of Bligh and Dyer (24). After drying under a nitrogen stream, the samples were hydrolyzed and methylesterified with a 5% hydrogen chloride methanol solution (Wako Corp., Tokyo, Japan) for 1 h at 120°C. Fatty acid analysis was performed by gas chromatography (QP-

Table 1. Supine Blood Pressure, Pulse Rate, Serum Lipids, Serum Electrolytes, Red Cell Sodium Content, and Sodium Pump Activity at the End of the Run-in, Placebo, and EPA Periods

Variables	Run-in	Placebo	EPA
Supine blood pressure (mmHg)			
Systolic	159.4±17.6	162.6±20.6	152.9±17.3 ^{*,††}
Diastolic	100.9±12.1	100.2±11.6	99.1±13.8
Mean	120.4±12.4	121.0±13.9	117.0±13.2
Pulse rate (/min)	72.9±6.2	72.8±6.9	72.5±10.6
Serum lipids (mg/dl)			
Total cholesterol	204.8±32.4	196.2±28.7	185.3±30.3 ^{**,†}
Triglyceride	186.3±110.3	158.2±86.6	123.3±61.8 ^{*,††}
HDL-cholesterol	43.2±15.0	41.8±12.9	42.3±12.7
Serum electrolytes (mEq/l)			
Sodium	142.1±1.5	141.5±2.5	141.9±1.3
Potassium	4.03±0.32	4.05±0.29	3.99±0.30
Chloride	104.9±2.3	104.9±2.6	104.6±2.6

Values are means±SD. EPA, eicosapentaenoic acid; HDL, high density lipoprotein; * $p < 0.05$, ** $p < 0.005$ vs. values in run-in period; † $p < 0.05$, †† $p < 0.02$, ††† $p < 0.01$ vs. values in placebo period.

Table 2. Red Cell Sodium Content and Na⁺-K⁺ ATPase Activity (K_{os}) at the End of the Placebo and EPA Periods

	Placebo period	EPA period
Red cell sodium (mEq/l·cells)	11.17±0.63	10.44±1.28 [†]
K _{os} (/h)	0.098±0.025	0.110±0.032

Values are means ± SD. EPA, eicosapentaenoic acid. † $p < 0.05$ vs. values in placebo period.

1000EX, Shimadzu, Kyoto, Japan). The column was a 2 m long×0.53 mm internal diameter (film thickness, 1.5 μm) fused silica megabore column. Peaks were identified in comparison with the known standards (Wako Corp., Tokyo, Japan), and quantitative determinations were carried out by measuring the mass chromatogram peak areas.

Other Measurements

Serum concentrations of sodium, potassium, chloride, triglyceride, total cholesterol, and high density lipoprotein (HDL) cholesterol were measured by the autoanalyzer.

Statistical Analysis

Values were expressed as mean±SD. The differences between the mean values were tested by Student's *t*-test or Wilcoxon signed rank test when appropriate. Correlations of the data were determined with a least-squares fit linear regression analysis. Multiple regression analyses were performed using SAS statistical software. A *p*-value less than 0.05 was considered to be significant.

Table 3. The Fatty Acid Composition of the Red Cell Membrane by Percent of Total Fatty Acids during the Placebo and EPA Periods

	Placebo period	EPA period
12:0 (%)	0.24±0.08	0.25±0.10
14:0 (%)	0.73±0.53	0.68±0.49
16:0 (%)	27.09±2.91	26.13±1.84
18:0 (%)	18.12±2.46	17.84±0.89
18:1 (%)	13.11±1.62	11.66±1.64 [#]
18:2 (%)	8.13±1.11	6.98±0.82 [#]
20:0 (%)	0.30±0.11	0.41±0.38
20:4 (%)	9.44±2.06	10.19±1.09
20:5 (%)	1.51±0.90	3.20±0.84 [#]
22:0 (%)	1.57±0.28	1.54±0.26
22:3 (%)	0.89±0.45	1.09±0.39 [†]
22:6 (%)	7.82±2.59	9.02±1.51 [†]
24:0 (%)	5.53±0.58	5.60±0.71
24:1 (%)	5.35±0.63	5.40±0.77

Values are means±SD. EPA, eicosapentaenoic acid. † $p < 0.05$, # $p < 0.005$ vs. values in placebo period.

Results

Systolic blood pressure values measured with the subject in the supine position were significantly lower after treatment with EPA than before randomization ($p < 0.05$) or after treatment with placebo ($p < 0.01$) (Table 1). Diastolic blood pressure, mean blood pressure, and pulse rate were not affected by either treatment. Serum triglyceride and total cholesterol levels after EPA administration were significantly reduced compared with those at the baseline and after the placebo treatment phase. There was no difference between the two treatment phases in the plasma concentrations of HDL cho-

Fig. 1. A scatterplot shows the correlation of the membrane EPA content of the red cell membranes in the placebo period with the change in systolic blood pressure after EPA supplementation.

lesterol or serum electrolytes. As compared with values after treatment with placebo, R-Na after the EPA treatment was decreased ($p < 0.05$). K_{os} was slightly higher after the EPA treatment than after the treatment with the placebo, but the difference was not statistically significant (Table 2).

The fatty acid compositions of red cell membrane phospholipid at the ends of the EPA period and of the placebo period are shown in Table 3. After EPA supplementation, cell membrane EPA (C20:5) content was increased in all patients ($p < 0.001$) in association with an increase ($p < 0.05$) in docosahexaenoic acid (C22:6) content, as compared with the values measured after placebo supplementation. On the other hand, the oleic acid (C18:1) and linoleic acid (C18:2) content was increased ($p < 0.005$) at the end of the placebo period as compared to the end of the EPA period.

The change in systolic blood pressure after EPA supplementation correlated positively with erythrocyte membrane EPA content during the placebo period ($r = 0.56$, $p < 0.05$, Fig. 1).

Table 4 summarizes the correlations among the changes in systolic blood pressure, erythrocyte membrane EPA, R-Na, and K_{os} after EPA supplementation. The increase in erythrocyte membrane EPA content after EPA supplementation correlated inversely with the change in systolic blood pressure during the EPA period ($r = -0.52$, $p = 0.03$), and with the

change in R-Na ($r = -0.57$, $p = 0.01$). The change in R-Na correlated neither with the change in oleic acid nor with that in linoleic acid (data not shown). The decrease in R-Na was inversely related to the change in Na^+/K^+ ATPase activity measured as K_{os} ($r = -0.59$, $p = 0.01$) and was positively related to the decrease in systolic blood pressure ($r = 0.54$, $p = 0.03$). However, the change in erythrocyte membrane EPA content did not significantly correlate to the change in K_{os} .

The change in erythrocyte membrane EPA content, the change in R-Na, and the change in K_{os} were introduced into a multivariate model to establish whether they had an independent effect on the change in systolic blood pressure. However, none of these changes reached statistical significance (Table 5a). The change in K_{os} and the change in erythrocyte membrane EPA content were independently related with the change in R-Na (Table 5b).

Discussion

The present results indicated that orally administered EPA supplied as ethyl ester for 4 weeks significantly reduced systolic blood pressure. This finding was consistent with the conclusions of two previous meta-analyses, which found that n-3 PUFA supplementation (at least 3 g/day) had a weak but significant blood-pressure-lowering effect in patients with essential hypertension (8, 9). With regard to the effect on diastolic blood pressure, however, no significant change was observed in either phase. The relatively low dose of EPA (2.7 g/day) administered and the small number of subjects in our study may account for this lack of effect on diastolic blood pressure.

With regard to the efficacy of EPA supplements for reducing systolic blood pressure, we observed a significant and a greater decrease in blood pressure in patients with low erythrocyte membrane EPA content at the placebo period, and in patients who showed a greater increase in erythrocyte membrane EPA content after EPA supplementation. These findings are consistent with the findings of Bønaa *et al.* (5), in which fish oil had no effect on blood pressure in subjects who had a high baseline plasma concentration of n-3 fatty acids; the decrease in blood pressure after fish oil supplementation was related to the increase in plasma phospholipid

Table 4. Correlations among the Changes in Systolic Blood Pressure, EPA Content of Red Cell Membrane, Red Cell Sodium Content, and Na^+/K^+ ATPase Activity (K_{os}) after EPA Supplementation

	Change in SBP		Change in membrane EPA		Change in R-Na	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Change in membrane EPA	-0.52	0.03	—	—	—	—
Change in R-Na	0.54	0.03	-0.57	0.01	—	—
Change in K_{os}	-0.45	NS	0.07	NS	-0.59	0.01

SBP, systolic blood pressure; EPA, eicosapentaenoic acid; R-Na, red cell sodium content; K_{os} , erythrocyte ouabain-sensitive sodium efflux rate constant.

Table 5. Multiple Regression Analyses for Determinants of the Change in Systolic Blood Pressure and Red Cell Sodium Content

(a) For determinants of the change in systolic blood pressure

Dependent variable: Change in SBP		
Independent variables	β	p
Change in membrane EPA	-12.97	NS (0.11)
Change in R-Na	0.3	NS (0.93)
Change in K_{os}	109.9	NS (0.17)

 $r^2=0.44$, $p=0.049$

(b) For determinants of the change in red cell sodium content

Dependent variable: Change in R-Na		
Independent variables	β	p
Change in membrane EPA	-1.44	0.0058
Change in K_{os}	-21.2	0.0048

$r^2=0.63$, $p=0.0010$. Data are expressed as partial regression coefficient (β) and p value. SBP, systolic blood pressure; EPA, eicosapentaenoic acid; R-Na, red cell sodium content; K_{os} , erythrocyte ouabain-sensitive sodium efflux rate constant.

n-3 fatty acids concentration. It is well known that membrane lipids are in dynamic equilibrium with plasma lipids (25), and that membrane phospholipids, one of the most important cell membrane components, are readily exchanged with plasma phospholipids (26). Thus, it is possible that a subset of hypertensive patients with low plasma and erythrocyte membrane concentrations of phospholipid n-3 fatty acids is particularly likely to benefit from EPA supplementation. The increase in erythrocyte membrane EPA content due to orally administered EPA might be causally related to the decrease in systolic blood pressure in our patients.

In the present study, EPA supplementation resulted in a significant reduction in R-Na, accompanied by an increase in cell membrane n-3 polyunsaturated fatty acid content (EPA and docosahexaenoic acid). Moreover, the change in erythrocyte membrane EPA content was negatively correlated with the change in R-Na. On the other hand, an increase in cell membrane n-6 linoleic acid and in monounsaturated oleic acid contents was observed at the end of placebo period (placebo capsules contained palmitic acid, stearic acid, and linoleic acid). However, no significant correlations were found between the change in R-Na and the changes in the membrane content of oleic acid or linoleic acid after EPA supplementation. These findings suggested that membrane EPA content could affect intracellular sodium concentration.

Many previous studies have shown abnormal membrane sodium transport systems, such as Na^+-K^+ ATPase, Na^+-Li^+ countertransport, or Na^+-K^+ cotransport, to be present in blood cells in essential hypertension (17–20, 27, 28). Among these, some investigators, including the present authors, showed an increased sodium content in blood cells of

hypertensive subjects (17–20), but the other investigators did not (27, 28). It might be difficult to detect increased intracellular sodium content because increased intracellular sodium concentration might be compensated for by other transporter systems such as an Na^+-Ca^{2+} exchange. In the present study, univariate analysis suggested a correlation between R-Na and systolic blood pressure, but multiple regression analysis failed to show that R-Na was an independent determinant of systolic blood pressure. To establish whether R-Na could be an independent determinant of blood pressure, larger scale studies are necessary.

If cationic changes in circulating blood cells do reflect changes in the vascular smooth muscle cells, the increased intracellular sodium may contribute to the increase in intracellular calcium content through the Na^+-Ca^{2+} exchange system (29) or the sodium-dependent calcium release from the mitochondria (30), which, in turn, may induce an enhancement of vascular reactivity to various extracellular stimuli. Although we did not evaluate intracellular calcium levels in the present study, it is possible that, if the decrease in intracellular sodium is related to the decrease in cytosolic free calcium, the decrease in intracellular sodium could be involved in the hypotensive mechanism of oral EPA supplementation.

Former interventional studies (13–15) have suggested that intracellular cationic contents can be affected by changes in membrane phospholipid composition through modification of the transmembrane cationic transport systems. Among the many sodium transport systems, we investigated the alteration of Na^+-K^+ ATPase by EPA supplementation based on the finding that an increased unsaturation of the phospholipid acyl chain, particularly the n-3 fatty acids, was known to increase cell membrane fluidity (31, 32), which in turn can modulate the membrane-bound Na^+-K^+ ATPase activity (33). In a previous *in vitro* study, EPA worked to potentiate the Na^+-K^+ ATPase activity of human aortic smooth muscle cells (34). We showed that both the increase in membrane EPA content and the change in Na^+-K^+ ATPase (K_{os}) were related to the decrease in R-Na, but these relations were found to be independent by a multiple regression analysis. We also could not find a direct correlation between the change in erythrocyte membrane EPA content and the change in K_{os} . These data suggest that cell membrane EPA might affect intracellular sodium concentration via the transport systems independent of Na^+-K^+ ATPase. The mechanisms by which the alteration of the membrane fatty acid composition modulates membrane sodium handling remain to be elucidated.

In conclusion, the present study demonstrated that highly purified EPA administration (2.7g/day) over a duration of 4 weeks reduced systolic blood pressure in patients with essential hypertension in association with an increase in the EPA content in the erythrocyte membrane. The increase in membrane phospholipid n-3 polyunsaturated fatty acids might cause a decrease in intracellular sodium concentration

through a modification of the sodium transport systems other than $\text{Na}^+\text{-K}^+$ ATPase, which may account for the blood pressure lowering effect of EPA supplementation.

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