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Effects of Eicosapentaenoic Acid Supplementation in the Treatment of Chronic Hepatitis C Patients

Kayoko TOMIOKA[1], Naoko KAKIBUCHI[2], Yasuko MURAKAMI[3], Takayo KAWAKAMI[3], Kouichi TAKAGUCHI[4], Keiji KITA[4] and Misako OKITA[1,3,*]

[1]*Graduate School of Health and Welfare, and* [3]*Department of Nutritional Science, Faculty of Health and Welfare Science, Okayama Prefectural University, Soja 719–1197, Japan*
[2]*Department of Food and Nutrition, Setouchi Junior College, Takase 767–8511, Japan*
[4]*Department of Internal Medicine, Kagawa Prefectural Central Hospital, Takamatsu 760–8557, Japan*

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Summary Eicosapentaenoic acid (EPA) has been shown to exert anti-inflammatory actions. To evaluate the effects of EPA on chronic hepatitis C, we administered EPA ethyl ester capsules to patients receiving the combination therapy of interferon α-2b and ribavirin. EPA (1,800 mg/d) was supplemented in combination with vitamin E (300 mg/d) and C (600 mg/d) to 5 chronic hepatitis C patients (EPA group). Five patients were administered vitamin E and C but not EPA (control group). Blood samples were obtained before and after 4, 8, 12 and 24 wk of therapy and analyzed for fatty acid compositions of erythrocyte and plasma and serum 8-hydroxy-2′-deoxyguanosine. EPA in erythrocyte membrane rose to 3 fold the basal level in the EPA group, while it decreased significantly in the control group after 24 wk of therapy. Lymphocyte counts in the EPA group increased to 120.8±25.4% after 4 wk of therapy and maintained the basal level throughout therapy, whereas the counts decreased significantly in controls. The serum alanine aminotransferase level was improved significantly in the EPA group. Changes in lymphocyte counts following 24 wk of therapy correlated with the EPA level in erythrocyte. The serum 8-hydroxy-2′-deoxyguanosine level at 24 wk in the EPA group was significantly lower than that in controls. These observations may suggest the beneficial effect of EPA supplementation in the treatment of chronic hepatitis C patients.

Key Words eicosapentaenoic acid, chronic hepatitis C, vitamin E, lymphocyte, 8-hydroxy-2′-deoxyguanosine

Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease and the most common cause of hepatocellular carcinoma (*1*, *2*). Observations of serum antioxidants and oxidative stress markers have shown increased oxidative stress in HCV-infected chronic liver disease (*3–6*). In the previous study (*7*), we observed a decrease in plasma α-tocopherol, increase in plasma thiobarbituric acid reactive substances, and decrease in arachidonic acid and eicosapentaenoic acid (EPA) in the phospholipids of mononuclear cells of cirrhotic patients with HCV and suggested that enhanced oxidation may be a reason for the decrease of these polyunsaturated fatty acids (PUFA).

Immunomodulation by *n*-3 PUFA via lipid raft disruption has been suggested (*8*) and dietary *n*-3 PUFA influences signaling complexes and suppresses T-cell cytokinetics in vivo by altering T-cell raft composition (*9*). EPA has a wide variety of pharmacological actions, preventing neoplastic lesions induced by diethylnitrosoamine (DEN) (*10*), regulating the T-helper 1-type response associated with chronic inflammatory disease, and antagonizing the production of inflammatory eicosanoids from arachidonic acid (*11*). EPA supplementation in HCV chronic hepatitis induced an improvement in ribavirin-related anemia (*12*) and suggested that the increased EPA concentration in the erythrocyte membrane may be associated with increasing membrane fluidity and the viscoelastic properties of erythrocyte membrane as described by Mabile et al. (*13*).

Vitamin E is one of the natural antioxidants, although the mechanisms of its physiological functions are not completely understood. Vitamin E administration improved the aminotransferase level of patients with HCV-infected chronic liver disease (*14*). We also reported that vitamin E supplementation induced a significant increase in PUFA, both *n*-6 and *n*-3, in the phospholipids of the erythrocyte membrane of HCV-infected patients with chronic hepatitis and this increase was accompanied by an improvement in the serum alanine aminotransferase (ALT) level (*15*). Daily administration of 1,200 IU of vitamin E (800 mg/d of D-α-tocopherol) for 28 d to healthy adult Japanese males reportedly had no unfavorable effects on clinical parameters (*16*). Vitamin C can regenerate α-tocopherol radicals and hence cooperates with α-tocopherol in inhibit-

*To whom correspondence should be addressed.
E-mail: okita@fhw.oka-pu.ac.jp

ing lipid peroxidation (*17*).

In the present study, therefore, we administered EPA ethyl ester combined with vitamins E and C to HCV-infected chronic hepatitis patients receiving combination therapy with IFN α-2b and ribavirin, then observed the effects on clinical observations, the fatty acid composition of erythrocyte membrane phospholipids and serum 8-hydroxy-2′-deoxyguanosine (8-OHdG) level.

SUBJECTS AND METHODS

Subjects. Ten patients with chronic hepatitis C (8 males and 2 females) treated at Kagawa Prefectural Central Hospital were enrolled in the present study. The criteria for enrollment were a persistently elevated serum ALT level for more than 6 mo prior to enrollment, positive for HCV RNA in serum, absence of detectable hepatitis B virus surface antigen and exclusion of all other potential causes of chronic liver disease.

Study design. Patients were randomly assigned to one of 2 treatment groups. Five patients (4 males and 1 female, 52.2±7.0 y) were supplemented with EPA capsules (EPADEL S600, Mochida Pharmaceutical Co. Ltd., Tokyo, Japan), containing 300 mg/capsule EPA ethyl ester. Daily six capsules (1,800 mg/d) were administered orally after three meals (2 capsules after each meal) for 24 wk (EPA group). Five patients (4 males and 1 female, 52.0±7.3 y) were administered vitamin E and C but not EPA (control group). All patients in both groups received 300 mg/d of vitamin E (tocopherol acetate, Juvela, Eisai, Tokyo, Japan) and 600 mg/d of vitamin C (ascorbic acid, HICEE, Takeda Chemical Industries, Osaka, Japan) during the treatment period. IFN α-2b (6 million units of Intron A, Schering-Plough K.K., Osaka, Japan) was injected six times weekly for 2 wk, followed by three times weekly for 22 additional weeks. Daily oral ribavirin (Revetol, Schering-Plough K.K.) (EPA group: 8.83±1.42 mg/kg, control group: 7.36±0.77 mg/kg) was administered for 24 wk. Genotype of the virus (1a/1b/2a/2b) was 0/3/2/0 in the EPA group and 0/2/1/2 in the control group, while the mean viral load was 590±271 KIU/mL in the EPA group and 590±331 KIU/mL in the control. There were no significant differences in body mass index (BMI; 21.0±2.0 in EPA group and 23.0±2.4 in control group).

Blood samples were obtained from each patient immediately before the initiation of therapy (0 wk) and 4, 8, 12 and 24 wk after the start of therapy. These samples were analyzed for α-tocopherol, 8-hydroxy-2′-deoxyguanosine (8-OHdG), and fatty acid composition in phospholipids of erythrocyte membrane and plasma.

Dietary fatty acid intake was calculated from the monthly dietary record for 3 d.

This experiment was designed in accordance with the principles of the Declaration of Helsinki of the World Medical Association and was approved by the Ethics Committee of Kagawa Prefectural Central Hospital. We obtained informed consent from all subjects prior to study participation.

Analytical methods. Blood was drawn into tubes containing disodium ethylenediamine-tetraacetic acid. Plasma was separated after centrifugation of blood samples at 1,600×g for 15 min at 4°C. The erythrocyte layer was drawn off into another tube and washed three times with 5 mM of phosphate buffer (4°C, pH 6.8) for determination of fatty acid composition in erythrocyte membrane phospholipids. All samples were stored at −80°C until assayed.

Total lipid was extracted from plasma and the erythrocyte membrane suspended in saline by the method of Bligh and Dyer (*18*). Phospholipids were separated by one-dimensional thin-layer chromatography using silica gel plates (Silica Gel 60; Merck, Darmstadt, Germany) and a solvent system of petroleum ether/ethyl ether/acetic acid (80 : 20 : 1, v/v). The spots corresponding to phospholipid were scraped from the plates and transmethylated for 2 h at 90°C using 2 mL of acetylchloride/methanol (5 : 50, v/v). A known amount of heptadecanoic acid (17 : 0) was used as an internal standard. Fatty acid methyl esters were analyzed by gas-liquid chromatography (Model GC-14A; Shimadzu, Kyoto, Japan) as described previously (*7*).

α-Tocopherol concentration in plasma was determined according to a modification of the method of Milne and Botnen (*19*). Tocopherols were extracted from plasma with ethanol and *n*-hexane. The extracts were evaporated under nitrogen and re-dissolved in ethanol. The tocopherol level in plasma was quantified by high-performance liquid chromatography on TSK-gel in an ODS-80Ts column (25 cm×4 mm; Tosoh, Tokyo, Japan). The mobile phase was methanol : *n*-butanol (80 : 20, by volume) and included 10 mM of sodium acetate buffer (pH 3.6; 0.1% by volume) at a flow rate of 1.0 mL/min. α-Tocopherol was monitored at an excitation wavelength of 292 nm and an emission wavelength of 320 nm. The tocopherol peaks were identified and quantified against authentic tocopherols used as external standards.

Serum was filtered using an ultrafiltration membrane and 8-OHdG was measured using highly sensitive enzyme-linked immunosorbent assay (ELISA) kits (8-OHdG Check, Japan Institute of the Control of Aging, Hukuroi, Japan). The detectable range of 8-OHdG concentration was 0.125 –10 ng/mL.

Statistical analysis. Values are given as means±SD. A non-parametric method (two-tailed), Wilcoxon's signed rank test, Mann-Whitney *U* test and Freedman's test were performed to analyze the data, as appropriate. $p<0.05$ was considered significant.

RESULTS

Table 1 presents the changes in clinical data during the therapy. There were no significant differences in clinical data between the control and EPA groups at the start of therapy. Erythrocyte counts decreased following 8 wk of therapy in both the groups and 2 of 5 patients in the EPA group and all of the controls showed decreases below the normal level 12 wk after the initiation of therapy. Leukocyte counts in the control group decreased significantly ($p<0.001$) during therapy. Leukocyte counts in the EPA group, however, maintained

Table 1. Changes in clinical data, plasma α-tocopherol and serum 8-OHdG in chronic hepatitis C patients during therapy.

		Group	0 wk	4 wk	8 wk	12 wk	24 wk	Freidman's test p
Erythrocyte	($\times10^4/\mu$L)	Control	442±23	404±60	397±56*	390±44*	379±51*	0.011
		EPA	458±69	445±80	414±75*	418±72*	399±68*	0.023
Leukocyte	(/μL)	Control	4,540±873	3,360±844	3,340±802	3,140±929	2,960±607*	0.001
		EPA	5,460±737	5,100±394##	4,840±695#	4,560±1,024#	4,520±1,108#	ns
Neutrophil	(/μL)	Control	1,780±503	1,420±245	1,387±354*	1,303±378*	1,331±307*	ns
		EPA	2,055±710	1,456±637*	1,602±792	1,467±903*	1,540±612*	ns
Lymphocyte	(/μL)	Control	2,095±671	1,477±595*	1,485±673*	1,334±675*	1,219±640**	0.001
		EPA	2,579±1,097	2,928±862#	2,473±750#	2,366±679#	2,305±570#	ns
Platelet	($\times10^4/\mu$L)	Control	13.5±4.2	13.8±3.4	12.9±2.9	12.4±2.3	12.8±1.8	ns
		EPA	18.3±8.2	16.5±7.1	16.4±6.7	17.5±7.6	17.7±6.1	ns
Hemoglobin	(g/100 mL)	Control	14.2±1.0	12.9±1.9*	12.8±1.9	12.7±1.5**	12.2±1.5*	ns
		EPA	14.6±1.5	13.9±1.7*	12.9±1.7**	12.8±1.5**	12.3±1.4**	ns
ALT	(IU/L)	Control	73±47	33±18	27±12	24±12*	24±14	ns
		EPA	90±46	63±60	45±43*	42±33*	33±29*	0.005
Albumin	(g/100 mL)	Control	4.4±0.5	4.4±0.4	4.3±0.3	4.1±0.3	4.1±0.4	ns
		EPA	4.0±0.4	4.0±0.4	3.9±0.4	4.0±0.5	4.0±0.5	ns
Total cholesterol	(mg/100 mL)	Control	143±19	139±16	141±22	139±12	143±15	ns
		EPA	156±19	156±34	139±32	150±30	136±33	ns
α-Tocopherol	(mg/mL)	Control	8.3±2.0	14.6±1.6*	16.7±4.4*	14.3±1.8*	11.4±0.6*	0.036
		EPA	7.8±2.0	16.2±3.2*	17.9±1.9**	17.7±4.8**	13.8±4.9*	0.001
8-OHdG	(ng/mL)	Control	0.36±0.07	0.34±0.09	0.61±0.15	1.18±0.29*	0.29±0.05	ns
		EPA	0.35±0.07	0.29±0.03	0.52±0.15*	0.86±0.50	0.20±0.09*#	ns

Mean±SD. ##$p<0.01$, #$p<0.05$; compared with control group, Mann-Whitney U test.
**$p<0.01$, *$p<0.05$; compared with 0 wk, Wilcoxon's test.
ALT, alanine aminotransferase; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

Table 2. Dietary fatty acid intake (g/d) during therapy.

	Group	0 wk	4 wk	8 wk	12 wk	24 wk
18:1	Control	15.4±3.7	15.6±2.2	14.6±2.7	15.7±3.1	13.7±3.3
	EPA	11.5±1.4	12.6±1.5	12.3±1.7	16.7±3.4	14.7±3.9
18:2*n*-6	Control	9.0±2.7	9.1±1.3	7.6±1.4	8.4±1.6	5.8±1.2
	EPA	6.0±1.0	7.2±1.2	6.4±1.2	5.9±2.1	8.3±1.7
18:3*n*-3	Control	1.7±0.6	1.6±0.4	1.3±0.3	1.5±0.2	0.9±0.2
	EPA	0.9±0.2	1.1±0.4	1.0±0.2	1.3±0.2	1.4±0.4
20:5*n*-3	Control	0.4±0.2	0.4±0.2	0.4±0.1	0.4±0.1	0.5±0.2
	EPA	0.5±0.2	0.5±0.2	0.5±0.2	0.7±0.5	0.3±0.1
22:6*n*-3	Control	0.9±0.3	0.8±0.3	0.7±0.2	0.8±0.2	0.8±0.4
	EPA	0.9±0.3	0.8±0.2	0.7±0.2	0.9±0.6	0.4±0.1
Total SFA	Control	12.3±1.9	11.6±1.5	11.6±1.2	12.7±1.7	12.3±2.4
	EPA	10.5±1.4	10.4±1.5	10.5±2.1	15.0±3.2	11.3±3.0

Mean±SD.
SFA, saturated fatty acid.

the basal level and were significantly higher than those in the control group during therapy. Lymphocyte counts increased to 120.8±25.4% after 4 wk of the therapy in the EPA group and maintained the basal level throughout therapy. In the control group, lymphocyte counts decreased significantly (55.6±15.5%) after 24 wk of therapy. Hemoglobin concentrations were decreased in both groups and there were no significant differences between the groups. There was significant improvement in the serum ALT level during the therapy ($p<0.005$) in the EPA group, but not in the control group. Neutrophils decreased insignificantly in both groups, and platelet counts did not change in either group during therapy. Plasma α-tocopherol peaked 8 wk after the initiation of therapy in both the control and EPA group. Serum albumin and total cholesterol concentrations did not change during therapy in either group. The serum 8-OHdG level increased significantly following 8 wk of therapy in the EPA group and 12 wk in the control group. After 24 wk of therapy, the 8-

Table 3. Changes in fatty acid composition (mol%) of phospholipids in erythrocyte membrane during therapy.

	Group	0 wk	4 wk	8 wk	12 wk	24 wk	Freidman's test p
16:0	Control	26.71±3.67	27.70±2.36	29.41±1.81	30.00±1.90	30.76±2.13	ns
	EPA	27.42±3.02	26.33±4.32	29.21±3.46	29.25±2.82	29.06±1.54	ns
18:0	Control	15.97±1.07	15.64±1.38	18.92±6.85	17.52±4.59	18.91±3.76	ns
	EPA	15.48±0.35	15.70±1.43	15.81±0.83	15.54±0.79	17.92±2.96	ns
18:1	Control	13.26±0.37	13.31±0.88	12.06±2.22	12.11±2.78	12.44±1.32	ns
	EPA	13.03±1.84	14.05±0.91	13.92±1.80	13.85±1.39	11.79±2.00	ns
18:2*n*-6	Control	9.35±1.44	8.00±1.98	8.88±1.53	9.14±1.90	8.20±1.70	ns
	EPA	8.31±2.22	6.90±0.71	6.36±0.87	8.09±1.23	9.02±2.49	ns
20:3*n*-6	Control	1.24±0.21	1.08±0.36	1.02±0.27	0.95±0.34	0.98±0.20	ns
	EPA	1.19±0.18	1.13±0.53	0.87±0.22	0.89±0.17	0.77±0.19*	ns
20:4*n*-6	Control	9.76±1.64	8.97±2.35	8.19±3.00	7.40±1.46	7.42±0.94	ns
	EPA	8.84±1.33	8.34±2.12	7.98±1.59	7.96±0.97	5.84±1.18*	0.010
20:5*n*-3	Control	1.30±0.22	1.21±0.42	1.03±0.28	0.99±0.16	0.82±0.14*	0.050
	EPA	1.04±0.46	2.56±0.76**#	2.89±0.84**##	3.39±0.98**##	2.25±0.80*##	0.011
22:5*n*-3	Control	1.38±0.44	1.31±0.48	1.31±0.55	1.10±0.19	1.11±0.19	ns
	EPA	1.53±0.39	2.03±0.46*#	2.57±0.43**##	2.79±0.36**##	2.36±0.55*##	0.027
22:6*n*-3	Control	5.39±1.20	5.33±1.70	4.86±2.14	4.77±1.78	4.24±0.89	ns
	EPA	5.72±1.08	5.24±1.30	4.84±0.71	4.84±0.71	3.38±1.06*	ns
AA/EPA	Control	7.80±2.29	8.27±3.30	8.07±2.50	7.63±1.89	9.18±0.87	ns
	EPA	11.15±7.76	3.64±1.93**#	2.96±1.03**#	2.57±1.09**##	2.78±0.73*##	0.006

Mean±SD. **p<0.01, *p<0.05; compared with 0 wk, Wilcoxon's test.
##p<0.01, #p<0.05; compared with control group, Mann-Whitney U test.
AA, alachidonic acid; EPA, eicosapentaenoic acid; ns, not significant.

Table 4. Changes in fatty acid composition (mol%) of plasma phospholipids during therapy.

	Group	0 wk	4 wk	8 wk	12 wk	24 wk	Freidman's test p
18:2*n*-6	Control	18.85±4.76	20.44±1.31	20.45±2.29	20.60±1.46	20.29±1.20	ns
	EPA	16.84±2.49	16.00±3.85#	14.48±3.20##	14.80±3.58##	16.12±1.70##	ns
20:3*n*-6	Control	2.18±0.38	1.54±0.28	1.78±0.30	1.90±0.50	1.84±0.30	ns
	EPA	2.27±0.78	1.55±0.70	1.40±0.86	1.35±0.63	1.37±0.56*	0.017
20:4*n*-6	Control	7.12±1.86	5.91±1.11	6.37±0.82	6.08±1.08	5.72±1.29*	ns
	EPA	7.57±0.96	6.49±0.76*	5.96±1.26*	6.39±1.04*	5.40±1.39*	0.008
20:5*n*-3	Control	1.40±0.65	1.79±1.23	1.50±0.71	1.57±0.64	1.32±0.59	ns
	EPA	1.91±1.11	6.15±2.71**#	5.47±1.44**##	6.33±1.90**##	4.63±1.84**#	0.007
22:5*n*-3	Control	0.96±0.45	0.76±0.17	0.75±0.08	0.69±0.10	0.63±0.06	ns
	EPA	0.87±0.31	1.93±0.80**#	1.65±0.60**##	1.60±0.45*##	1.19±0.34*#	0.029
22:6*n*-3	Control	6.24±2.82	4.80±0.36	5.74±0.84	5.03±1.05	4.42±0.47	ns
	EPA	6.57±1.80	6.75±1.59#	5.88±1.58	6.23±1.79	4.34±0.55*	ns

Mean±SD. **p<0.01, *p<0.05; compared with 0 wk, Wilcoxon's test.
##p<0.01, #p<0.05; compared with control group, Mann-Whitney U test.
ns, not significant.

OHdG level was reduced to the basal level in the control group and decreased significantly in the EPA group, and the level in the EPA group was significantly lower than that in the control group.

No significant differences were observed in dietary fatty acid intake between the control and EPA groups during therapy (Table 2).

Changes in fatty acid composition in the phospholipids of erythrocyte membranes during the therapy are presented in Table 3. In the control group, EPA decreased gradually and a significant decrease was observed after 24 wk of the therapy. In the EPA group, EPA increased significantly after 4 wk of EPA supplementation and the level was elevated to 3 fold the basal level after 12 wk. Docosapentaenoic acid (DPA) also increased significantly following EPA supplementation. Inversely, arachidonic acid decreased after EPA supplementation and the arachidonic acid/EPA ratio, therefore, decreased markedly.

Table 4 presents changes in the fatty acid composition of plasma phospholipids. EPA supplementation induced significant increases in EPA and DPA levels similarly to those in the erythrocyte membrane; on the other hand, dihomo-γ-linoleic acid (20:3*n*-6) and

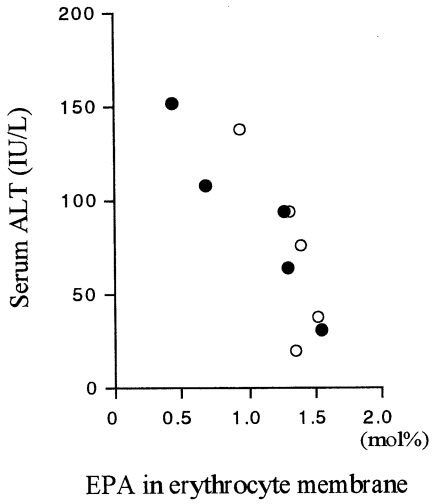

Fig. 1. Correlation between the serum alanine aminotransferase (ALT) and eicosapentaenoic acid (EPA) in phospholipids of erythrocyte membranes in the patients with chronic hepatitis C, EPA group (●) and control group (○), before therapy. (n=10, r=−0.859, p<0.01).

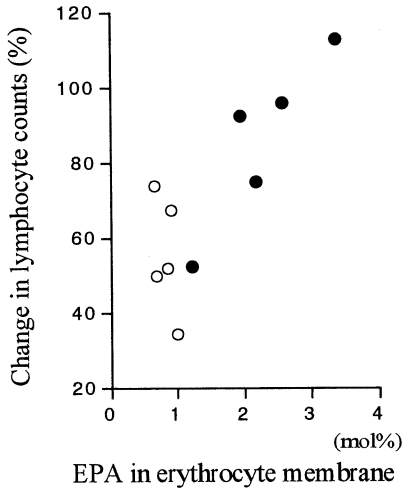

Fig. 2. Correlation between the change in lymphocyte counts and eicosapentaenoic acid (EPA) in phospholipids of the erythrocyte membrane in the EPA group (●) and control group (○) after 24 wk of therapy. Correlation was observed in all patients (n=10, r=0.709, p<0.05) and in the EPA group (n=5, r=0.829, p<0.05).

arachidonic acid decreased significantly following EPA supplementation. Linoleic acid remained lower than that in the control group. Palmitic (16:0), stearic (18:0), lignoceric, oleic (18:1n-9) and tetracosenoic acid did not change during therapy in either group and there were no significant differences in these fatty acid compositions between the control and EPA groups.

Correlation among clinical data and fatty acid composition in the phospholipids of the erythrocyte membrane and plasma are shown in Figs. 1 and 2. Before the start of therapy, serum ALT activity correlated negatively with EPA levels both in the erythrocyte membrane (r=−0.859, p<0.01; Fig. 1) and plasma (r=−0.626, p<0.05). After 24 wk of therapy, considering all individuals as a group, lymphocyte counts correlated negatively with plasma arachidonic acid/EPA ratio (r=−0.624, p<0.05) and arachidonic acid levels in the erythrocyte membrane (r=−0.648, p<0.05). In each subgroup, however, there were no such correlations. Changes in lymphocyte counts following 24 wk of therapy correlated significantly with EPA levels both in the erythrocyte membrane (r=0.709, p<0.05; Fig. 2) and in plasma (r=0.672, p<0.05) in all patients as a group, and also in the EPA group (p<0.05).

DISCUSSION

Effects of EPA supplementation combined with vitamins E and C in chronic hepatitis C patients receiving combination therapy with IFN α-2b and ribavirin were evaluated in the present study. As shown in Tables 3 and 4, we found significant increases of EPA in plasma and erythrocyte membrane phospholipids of the patients who received daily oral EPA ethyl ester capsules. Plasma α-tocopherol concentrations also increased in all patients at 4 wk after initiation of therapy and thereafter. These results indicated good compliance with orally administered EPA and vitamin E.

Ribavirin is a purin nucleoside analogue and combination therapy with IFN α-2b and ribavirin is reported to show a higher sustained viral response than IFN α-2b monotherapy (*20*). In the course of therapy with IFN α-2b and ribavirin, however, hematologic abnormality such as hemolytic anemia has been observed as a common side effect. Although the mechanism of hemolysis remains uncertain, it may be related to ribavirin accumulation in erythrocyte (*21*). A role of membrane oxidative damage was also suggested (*22*).

We observed that EPA supplementation prevented a decrease of leukocyte and lymphocyte counts known as a side effect of the combination therapy. Poynard et al. (*20*) observed a decrease in leukocyte counts during IFN therapy either in combination with rivabirin or as monotherapy. Our present results agree with their observation in which a decrease in leukocyte counts observed in the patients treated with combination therapy was largely as the result of a decrease in lymphocyte counts. A vigorous T-cell response is seen in the majority of patients with clearance of HCV infection in response to IFN-α treatment (*23*), and such an immune response is much less frequent in patients who failed to respond to treatment. Maintenance in the lymphocyte counts observed in the EPA group should significantly influence the improvement of ALT level.

A high fish oil diet (15% oil/diet, w/w) induced oxidative senescence of erythrocytes linked to the degeneration of spleen cells and decrease in mitogen-induced blastogenesis in spleen cells in mice (*24*). In their study, the mice received an extremely high level of EPA, about 10 times that in our human study. Several studies report no effect of various dose of long-chain n-3 PUFA on lymphocyte proliferation (*25–27*). However, it was reported that long-chain n-3 PUFA caused a dose-dependent increase in proliferation of T cells when the fish oil was used in combination with an antioxidant

mix (*28*). It is suspected that activation of lymphocyte generation may be induced by moderate supplementation of EPA and that vitamins E and C supplementation together with EPA may suppress the formation of lipid peroxides in patients receiving combination therapy. It may be important to determine the appropriate dose of EPA in combination with vitamins E and C.

EPA level in erythrocyte membrane decreased gradually during the therapy in the control group. Dietary EPA intake did not decrease during therapy. Considering the report of De Franceschi et al. (*22*) that ribavirin treatment in vitro induced increases in erythrocyte malondialdehyde, the decrease of EPA in erythrocyte membrane was probably due to enhanced peroxidation induced by ribavirin.

The EPA level in phospholipids of the erythrocyte membrane increased to 2.5 fold the basal level in the EPA group. It was reported that oral administration of EPA reduced whole blood viscosity and increased erythrocyte deformability (*29*). EPA-enriched erythrocyte membrane may prevent the hemolytic anemia as suggested by Ide et al. (*12*). Excess intake of PUFA in fish oil has been shown to lead to acceleration of membrane lipid-peroxidation of erythrocyte (*30*). However, a moderate increase in membrane *n*-3 PUFA such as EPA induced a higher incorporation of α-tocopherol into the erythrocyte membrane and protected erythrocyte against hemolysis in normotriglyceridemic subjects (*13*).

Before the start of therapy, serum ALT activity in the present patients correlated negatively with EPA levels both in the erythrocyte membrane and plasma. The phospholipids in mononuclear cells are characterized by the predominance of PUFA such as arachidonic acid and EPA, esterified in position 2 of glycerol. Upon activation of phospholipases by physiologic or pathophysiologic stimuli, arachidonic acid and EPA are released and oxidized by cyclooxygenase and lipoxygenase. Accelerated synthesis of prostaglandin E_2 from arachidonic acid causes inhibition of the proliferation and cytokine production of Th1-cells, mediators of cellular immunity (*31*). Immunomodulation by EPA via competitive inhibition of arachidonic acid metabolism may explain the negative correlation between EPA and ALT levels, and arachidonic acid and lymphocyte counts, supporting the possibility of EPA supplementation as an adjunctive treatment for chronic hepatitis C.

We determined 8-OHdG as an indicator of oxidative DNA damage and showed an earlier increase during therapy and a significant decrease with improvement of clinical parameters in the EPA group. Farinati et al. (*3*) reported higher 8-OHdG levels in leukocytes from chronic active HCV-related hepatitis patients than in those from normal control subjects and HCV-related compensated cirrhosis and suggested that oxidative DNA damage appears strongly in an early stage of HCV-infection. Ribavirin enhances host antiviral immunity by enhancing antiviral type 1 cytokine expression in human T cells, which is important in the early phase of viral infection (*32*). Type 1 cytokines, interleukin (IL)-2, IFN-γ and tumor necrosis factor (TNF)-α induce activation of cytotoxic lymphocytes and recruitment of natural killer cells and macrophages, which induce lysis of HCV-infected cells. Although the exact mechanism remains unclear, the different pattern of 8-OHdG levels in the EPA group during treatment suggests an effect of EPA on immunomodulation of HCV-infected patients treated with IFN-α and ribavirin. EPA may also work as an antioxidant as suggested by Mabile et al. (*13*). We did not observe the α-tocopherol concentration in lymphocytes. However, we demonstrated a 2.37-fold increase in α-tocopherol in the erythrocyte membrane following 2 wk of D-α-tocopherol (500 mg/d) administration in a previous report (*15*). Therefore, α-tocopherol may be highly incorporated in lymphocyte and other cell membranes and may suppress oxidative damage to DNA.

In conclusion, EPA supplementation with vitamins E and C prevented a decrease in the lymphocyte counts, improved serum ALT, and induced an earlier response and a decrease in serum 8-OHdG in patients with chronic hepatitis C during combination therapy of IFN α-2b and ribavirin. Based on these observations, it was suggested that EPA supplementation with antioxidative-vitamins may have some useful effects on combination therapy for HCV-related chronic hepatitis, but more detailed research should be performed to confirm this suggestion.

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