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Original Article

Effect of Eicosapentaenoic Acid Intake on the Relationship between Interleukin-6 and Acute Phase Proteins in Serum in Youths

Naomasa SAKAMOTO, Ichiro WAKABAYASHI, Sachiko YOSHIMOTO, Hidehisa MASUI and Kunihiro SAKAMOTO

Department of Hygiene, Hyogo College of Medicine, Hyogo

Abstract

Twenty male volunteers 20-25 years old were examined to determine their serum concentrations of interleukin-6 (IL-6), acute phase proteins and lipids before, immediately after and one hour after a load of 90 watts for 20 minutes using a Monark ergometer, and the same parameters were reexamined after eicosapentaenoic acid (EPA) intake of 1.125 g/day for 2 weeks. By EPA intake, EPA level of the membranes of red blood cells increased significantly by 75.6% and docosahexanoic acid (DHA) by 53.8%. The IL-6 level increased significantly by 17% and C-reactive protein (CRP) by 11.7%, but fibrinogen (Fbg) decreased by 9.6%. After EPA intake, at one hour after the load, the change rate of IL-6 decreased to that of before EPA. The change rate of α_1-acid glycoprotein (α_1-AGP) increased in the group in which IL-6 was unchanged and did so significantly in the group in which it increased, but tended to increase in the group in which it decreased. Thus the change rate of sialic acids (SA) increased significantly in both the IL-6 unchanged and increased groups. It is suggested that EPA activated IL-6, which was related to the increase of α_1-AGP as an activator of immunity. The change rate of sialic acid (SA) as an index of acute phase protiens was correlated significantly and positively with that of total cholesterol and HDL-cholesterol.

Key words: Eicosapentaenoic acid, Interleukin-6, Acute phase protein, Total-cholesterol, Physical exercise

Introduction

It is known that serum acute phase proteins are increased by physical stress and that, interleukin-6 (IL-6) induces to some extent the production of acute phase proteins in the liver[1-4]. IL-6 is excreted mainly from monocytes and macrophages as well as T and B lymphocytes, fibroblasts, keratinocytes and intima cells of vessels[5-8]. On the other hand, it was recently reported that the IL-6 level is not changed at one hour after physical exercise[9]. There are reports that eicosapentaenoic acid (EPA), which is contained mainly in fish oil, suppresses IL-6 production of macrophages[10, 11]. In our previous study, the EPA content of erythrocytes increased after daily intake of EPA (1.125g per day) for 2 weeks. Changes in serum parameters were evaluated under constant conditions with a physical load by bicycle ergometer of 90 watts for 20 min. We found that fibrinolysis activity was increased by the physical load after the EPA intake, and acute phase proteins seemed to be involved in this increased fibrinolysis (unpublished data). In the present study, we thus focused on IL-6 effects upon acute phase proteins, and investigated whether EPA intake influences the relationship between IL-6 and acute phase proteins.

Reprint requests to:
Naomasa Sakamoto,
Department of Hygiene, Hyogo College of Medicine,
1-1 Mukogawa Nishinomiya, Hyogo 663, Japan
TEL 0798-45-6561
FAX 0798-48-6261

Methods

The subjects were 20 healthy males 20-25 years old, with normotensive, normal blood examination values, no disease history and who gave informed consent. At 1:00 p.m. with fasting, blood and urine samples were collected 3 times, that is, before, immediately after and one hour after physical exercise using a Monark bicycle ergometer with a load of 90 watts, for 20 minutes. After EPA intake of 1.125g/day for 2 weeks using tablets containing 75 mg, which is an established dose with the compliance of subjects in our study, and which resulted in a sufficient increase of the EPA level in red blood cell membranes after two-week intake, the sampling of blood was again done by the method described above. To determine fatty acid content in red

blood cells, dihomo-γ-linolenic acid (DHLA), arachidonic acid (AA), EPA and docosahexanoic acid (DHA) were measured by gas chromatography[12]. To determine acute phase proteins, C-reactive protein (CRP), α_1-antichymotrypsin (α_1-ACT), α_1-acid glycoprotein (α_1-AGP), and haptoglobin (Hp) were measured by nephelometry[13], fibrinogen (Fbg) by thrombin clotting time, sialic acid (SA) by enzymatic assay (Ultrate SIA, Toyobo Co., OSAKA), and IL-6 by enzyme-linked immunosorbent assay (IL-6, Kit, Fuji Rebio, TOKYO)[14]. Blood cell count was measured with a Toa electric cell counter, hemoglobin by the cyanmethemoglobin method, and total cholesterol (T-chol), HDL-chol and triglyceride (TG) by an enzymatic method (Autoanalyser Hitachi 736). The change rate was calculated as the rate of the increased value by the load to the initial value. To clarify the relationship between IL-6 and acute phase proteins, the distribution of the change rate of IL-6 by physical load was divided into three groups, a group in which it decreased (group A), one in which it was unchanged (group B) and one in which increased (group C), by 33.3 and 66.6 percentiles. The change rates of acute phase protein were analyzed by these three groups of IL-6. Statistical analysis was performed with the t-test for differences of the change values of parameters.

Results

Total EPA intake of subjects was 15.75g for two weeks.

Table 1 Distribution of fatty acid in red blood cell membranes after EPA intake of 1.125g/day for 2 weeks

	EPA concentration (μg/gHb) (mean±SD)	
	Before EPA intake	After EPA intake
Dihomo-γ-linolenic acid 20:3 n-6	14.50± 9.58	19.13± 14.26*
Arachidonic acid 20:4 n-6	106.63± 105.57	144.91±138.90*
Eicosapentaenoic acid 20:5 n-3	15.50± 13.13	27.22± 26.52**
Docosahexanoic acid 22:6 n-3	37.59± 55.86	57.83± 78.69*

SD: standard deviation
*p<0.05 **p<0.01

Table 1 shows the amounts of fatty acids in membranes of red blood cells after EPA intake. EPA, DHA, DHLA and AA levels increased significantly, by 75.6%, 53.8%, 31.9% and 35.9%, respectively (Table 1). By EPA intake, IL-6 and CRP levels increased significantly, by 17.0% and 11.7%, respectively, but Fbg decreased by 9.6%. Before EPA intake, at one hour after the load, the IL-6 level increased by 6.9% and CRP, α_1-AGP, SA and T-chol levels decreased significantly. After EPA intake, at one hour after the load, the IL-6 level decreased by 7.5%. CRP, α_1-ACT and TG levels decreased singificantly. The level of each parameter at one hour after the load after EPA intake was compared with the level at the same time before EPA intake. The IL-6 level did not change. CRP increased by 17.6% and Fbg decreased by 7.7% (Table 2). The correlation coefficients between IL-6 and α_1-AGP, SA and white blood cell at one hour after the load after EPA intake showed changes to positive from negative values at same time of before EPA intake. SA immediately after the load before EPA intake showed a significant correlations at high levels such as r=0.7-0.5 with α_1-AGP, α_1-ACT, Hp and Fbg, and also with T-chol, HDL-chol, TG and white blood cells. At one hour after the load before EPA intake, SA showed positive and significant correlations with α_1-ACT, T-chol and HDL-chol, and after EPA intake, SA showed positive, significant correlations with α_1-AGP, Hp and T-chol, and a negative one with Fbg (Table 3). To determine the relationships between IL-6 and acute phase proteins, the change rate of each parameter by the load was examined by three subgroups based on the change rate of IL-6 at one hour after the load by EPA intake. Before EPA intake, the change rates of α_1-AGP and Hp of the group C were significantly lower than those of the group B. After EPA intake, the change rates of α_1-AGP, Hp and SA of the group A were significantly lower than those of the group B. The change rate of α_1-AGP of the group B was higher than that before EPA intake ($p<0.08$), the change rate of α_1-AGP of the group C increased significantly compared to that before EPA intake, but the change rate of α_1-AGP of the group A tended to increase from that before EPA intake. The change rates of SA of the groups B and C increased significantly compared to those before EPA intake (Table 4).

Table 2 Distribution of each parameter before, immediately after and one hour after the load by EPA intake (mean±SD)

		Before EPA intake			After EPA intake		
		Before the load	Immediately after the load	One hour after the load	Before the load	Immediately after the load	One hour after the load
Interleukin 6	(pg/ml)	5.43± 1.01	5.53± 1.22	5.71± 1.25*	6.25± 1.82	6.09± 1.88	5.78± 1.39
C-reactive protein	(ng/ml)	128.84± 130.65	117.89± 135.79	110.89± 127.96*	143.89± 210.88	146.44± 230.93	130.44± 214.14**
α_1-acid glycoprotein	(mg/dl)	67.05± 13.98	69.60± 15.68*	65.25± 14.21*	68.10± 18.31	71.00± 21.16*	68.95± 20.3*
α_1-antichymotrypsin	(mg/dl)	29.49± 4.59	30.12± 5.56*	29.34± 4.87**	30.18± 6.06	30.15± 6.13**	29.62± 6.03*
Haptoglobin	(mg/dl)	121.05± 66.08	126.50± 68.75**	117.55± 63.16	124.10± 73.93	130.65± 82.44*	126.05± 80.92
Fibrinogen	(mg/dl)	210.26± 45.51	209.37± 50.37	206.22± 30.97	190.15± 79.60	199.25± 62.79	190.40± 57.59
Sialic acid	(mg/dl)	57.45± 6.04	59.25± 6.12**	56.55± 5.19*	57.50± 7.00	59.05± 7.84**	57.95± 7.84
Total cholesterol	(mg/dl)	164.10± 22.07	167.33± 19.27*	162.62± 20.94*	163.15± 24.34	165.55± 24.46	163.60± 23.06
HDL-cholesterol	(mg/dl)	52.62± 10.33	53.48± 10.56	52.95± 10.50	52.45± 10.86	53.20± 12.29	52.65± 11.69
Triglyceride	(mg/dl)	77.38± 28.60	80.52± 25.43*	70.29± 19.40	80.95± 39.23	87.55± 46.79**	71.80± 32.85**
White blood cell	(/μl)	5985.70±1063.10	6823.80±1482.20**	5981.00±1202.30	5815.00±1527.70	6830.00±1906.80**	5950.00±1344.90

*p<0.05 **p<0.01 Significant difference from before the load by paired t-test

Table 3 Correlation coefficients between change rates of IL-6, CRP and SA and the change rate of each parameter immediately after and one hour after the load by EPA intake

	Before EPA intake						After EPA intake					
	Immediately after the load			One hour after the load			Immediately after the load			One hour after the load		
	IL-6	CRP	SA	IL-6	CRP	SA	IL-6	CRP	SA	IL-6	CRP	SA
Interleukin-6	1.00	-0.03	-0.06	1.00	0.04	-0.06	1.00	0.22	0.42*	1.00	-0.02	0.29
C-reactive protein	-0.03	1.00	0.34	0.04	1.00	-0.12	0.22	1.00	0.33	-0.02	1.00	0.01
α_1-acid glycoprotein	0.03	0.00	0.68***	-0.19	0.00	0.38	0.04	-0.19	0.28	0.15	-0.25	0.41*
α_1-antichymotrypsin	-0.14	-0.06	0.71***	-0.25	-0.28	0.54*	-0.02	0.03	0.43*	0.06	-0.37	0.17
Haptoglobin	-0.19	0.05	0.67**	-0.37	0.43*	-0.19	-0.33	0.21	0.32	0.05	0.10	0.46*
Fibrinogen	0.06	0.11	0.52*	-0.22	0.14	-0.19	-0.59**	-0.08	0.24	-0.17	-0.05	-0.40*
Sialic acid	-0.06	0.34	1.00	-0.06	-0.12	1.00	0.42*	0.33	1.00	0.29	0.01	1.00
Total cholesterol	0.13	0.11	0.83***	0.24	0.25	0.69***	-0.21	0.12	0.52*	0.17	-0.02	0.53*
HDL-cholesterol	0.31	-0.19	0.44*	-0.16	0.13	0.47*	-0.13	0.37	0.23	-0.22	0.16	0.31
Triglyceride	-0.09	0.46*	0.63**	-0.14	0.24	0.13	0.13	0.33	0.55*	0.06	0.01	-0.07
White blood cells	0.31	-0.26	0.50*	-0.42*	0.30	-0.06	-0.06	0.13	0.50*	0.18	-0.20	0.18

*p<0.05 **p<0.01 ***p<0.001

Table 4 Distribution of change rates of parameters by three groups divided by the change rate of IL-6 at one hour after the load before or after EPA intake. Mean±SD

	Decreased group (-0.360~-0.020) a		Unchanged group (-0.019~-0.090)		Increased group (0.091~0.950)	
IL-6	Before	After	Before	After	Before	After
	-0.1871±0.1177 (7)[b]	-0.2205±0.1376 (8)	0.0305±0.0294 (6)	-0.0141±0.0234 (7)	0.4682±0.2905 (7)	0.2124±0.0661 (5)
C-reactive protein	-0.0861±0.3063	-0.2016±0.2329	-0.2897±0.3063	-0.2137±0.2771	-0.0590±0.1057	-0.2064±0.1373
α_1-acid glycoprotein	-0.0360±0.0499	-0.0225±0.0353	0.0075±0.0355	0.0480±0.0374	-0.0475±0.0332	0.0045±0.0481
α_1-antichymotrypsin	-0.0081±0.0337	-0.0294±0.0214	0.0129±0.0358	-0.0036±0.0391	-0.0201±0.0178	-0.0189±0.0394
Haptoglobin	-0.0035±0.0723	-0.0336±0.0562	-0.0039±0.0430	0.0892±0.1426	-0.0611±0.0329	-0.0099±0.0708
Fibrinogen	0.1065±0.2789	0.2363±0.5387	0.0043±0.0863	0.0864±0.4944	0.0197±0.0856	-0.0032±0.5063
Sialic acid	-0.0161±0.0342	-0.0156±0.0198	-0.0061±0.0231	0.0288±0.0221	-0.0191±0.0194	0.0172±0.0355

a: distribution range
b: sample number
*p<0.05

Discussion

EPA is one of the essential fatty acids of n-3 series for physical activity. But it was pointed out that, as a side-action, EPA increases the superoxidant level of fatty acid in serum[2, 10]. EPA is absorbed from the small intestine, and stored mainly in red blood cells. The EPA level in red blood cells may thus reflect the amount of EPA intake. But the physiological activity of EPA in red blood cells is unclear[2, 11]. EPA intake may increase the absorption of the n-6 series fatty acids. Tamura et al.[10] reported that EPA had a suppressive action on the production of cytokines and the immunoreaction in inflammation by suppressing IL-6 production of macrophages. Meydani et al.[11] reported that EPA suppressed not only the production of IL-1 and TNF, but also that of IL-6 and IL-2 by monocytes. In this study, by EPA intake, IL-6 level showed an increasing tendency by 17.0% and CRP by 11.7%, but Fbg decreased by 9.6%. No significant correlation between the change rate of EPA concentration in membranes of red blood cells and that of IL-6 for 2 weeks interval could be found, but a negative correlation to Fbg was observed. Thus, EPA may possibly suppress the production of Fbg. The correlation coefficients between the change rate of IL-6 and those of other parameters were analyzed at one hour after the load before and after EPA intake. The correlation coefficient after EPA intake changed to positive values from negative values in parameters such as α_1-AGP, α_1-ACT, Hp, SA, and white blood cells. Those results suggested that EPA was related to IL-6 activity and thus promoted the synthesis of acute phase proteins in the liver[3]. IL-6 is used as a marker of inflammation similar to CRP, α_1-ACT, and HP[1, 6, 15-17]. In this study there was a tendency for the change rate of IL-6 to be negatively correlated with those of CRP and positively correlated with those of α_1-ACT and HP. These correlations might be related to certain properties of the parameters; CRP is a mediator of inflammation, α_1-ACT is a repair factor and HP is a scavenger during inflammation[4, 15, 16]. The activity of IL-6 is promoted by the glucocorticoid level[3, 5]. Previously, at the same physical load, the cortisol level was increased by 28.2% immediately after the load and decreased by 15% one hour after the load. In this study, the IL-6 level increased by 3.6% immediately after the load and by 6.9% one hour after the load. It seemed that the IL-6 level was not correlated with the cortisol level. Before EPA intake, the change rates of α_1-AGP and Hp of the group C were significantly lower than

those of the group B, which seemed to be related to physical load. After EPA intake, the change rate of IL-6 tended to decrease, the change rates of α_1-AGP and Hp of the group B increased and were significantly higher than those of the group A. The change rate of α_1-AGP in the group B increased to that before EPA intake ($p<0.08$), and it significantly increased in the group C compared to that before EPA intake and showed a positive change rate. But the change rate of α_1-AGP in the group A tended to increase. And the change rate of Hp in the group C increased as compared to that before EPA intake, but still showed the negative change rate. As result, the change rate of SA as the indicator of acute phase proteins increased significantly from that before EPA in both groups B and C. Thus, it semmed that EPA activated IL-6 and that this then activated the synthesis of acute phase proteins especially of α_1-AGP, as an activator of immunity, in co-operation with EPA. Hag et al.[18] reported α_1-ACT, α_1-AGP and Hp to be correlated significantly with SA with higher levels, but not with CRP. Kosaka et al.[19] reported α_1-AGP and α_1-ACT to contain many sialic compounds and the amount of sialic acid induced by acute phase protein to be 40-50% in the serum. In this study, immediately after the load, the change rate of SA showed a significant and positive correlation of r=0.7 with acute phase proteins except CRP. Orekhov et al.[20] reported that the desialyated LDL level in serum was high in hypertensive patients. Lindberg et al.[21] reported that the relative risk of death from cardiovascular disease increased with increasing serum SA concentrations, and that the SA level might reflect the existence or the activity of an arteriosclerotic process. The change rate of SA after the load was correlated significantly and positively with that of T-chol, irrespective of EPA intake, and also positively correlated with HDL-chol, but no relation between SA and TG could be found[18, 22].

In conclusion, after EPA intake, the IL-6 level increased significantly. The change rate of α_1-AGP increased in the group B and increased significantly in the group C, but tended to increase in the group A. The change rate of SA increased significantly in both the groups with unchanged and changed IL-6 level. The SA level, as an index of acute phase proteins, was positively, significantly correlated with T-chol and HDL-chol.

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