

Effects of Eicosapentaenoic Acids on Oxidative Stress and Plasma Fatty Acid Composition in Patients with Lupus Nephritis

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Abstract. Eicosapentaenoic acid (EPA) is one of the major components of fish oil, which was reported to have anti-atherogenic, anti-inflammatory and immune suppressive effects. In the present study, highly purified EPA was administered to patients with lupus nephritis and the effects of EPA on urinary 8-isoprostane, a reliable marker of oxidative stress, were investigated in these patients. Six outpatients (1 man and 5 women), with lupus nephritis diagnosed by renal biopsy, were entered in the study. We administered 1800 mg EPA ethyl-ester (purity >95%) daily and examined the urinary 8-isoprostane levels and plasma fatty acid composition before and 3 months after EPA treatment. The urinary 8-isoprostane levels were significantly decreased after the treatment compared with those before the treatment (from 530 ± 113 pg/mg • Cr to 235 ± 49 pg/mg • Cr, $p=0.02$). The EPA levels in the plasma phospholipid (PL) fraction were significantly increased after the treatment (from 3.30 ± 0.64 mol% to 8.01 ± 0.47 mol%, $p<0.001$). Arachidonic acid (AA) levels in the plasma PL fraction were significantly decreased after the treatment (from 9.47 ± 0.28 mol% to 7.33 ± 0.43 mol%, $p<0.001$). The ratios of EPA to AA were significantly increased after the treatment (from 0.35 ± 0.07 to 1.14 ± 0.16 , $p<0.001$). Thus, this preliminary study indicated that EPA might exert beneficial effects on lupus nephritis by decreasing the oxidative stress.

Eicosapentaenoic acid (EPA) is one of the main components of fish oil, which has been reported to have

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anti-atherogenic and anti-inflammatory effects (1). Prickett *et al.* reported that a fish oil-rich diet reduced proteinuria and prolonged the lifespan of NZB/NZW F₁ mice, a model of lupus nephritis (2). Though there have been several reports about the effects of fish oil in patients with lupus nephritis, the results were controversial (3, 4).

On the other hand, it was reported that oxidative stress was increased in patients with systemic lupus erythematosus (SLE), and that the activity of SLE correlated positively with oxidative stress (5). However, there have been no reports about the effects of EPA on oxidative stress in patients with lupus nephritis.

In the present study, highly purified EPA ethyl-ester (purity >95%) was administered to patients with lupus nephritis and the urinary 8-isoprostane, a reliable marker of oxidative stress, and the plasma fatty acid composition were measured.

Patients and Methods

Patients and experimental design. Six outpatients (1 man and 5 women), with lupus nephritis diagnosed by renal biopsy, were entered in this study (Table I). After obtaining their informed consent, 1800 mg EPA ethyl-ester (EPADEL[®] Mochida Pharmaceuticals Co. Ltd., Tokyo, Japan, purity >95%) was administered daily. Blood and urine samples were collected immediately before and 3 months after the treatment with EPA.

Biochemical measurements. Urinary 8-isoprostane levels were measured with an EIA kit (Cayman Chemical Co. Ltd., MI, USA). The fatty acid composition of the plasma phospholipid (PL) fraction was analyzed as follows: total lipids were extracted by the methods of Folch *et al.*, and were separated to PL, triglyceride and free fatty acid fractions by thin-layer-chromatography. Fatty acids in the PL fraction were analyzed by gas-chromatography after transmethylation (6).

Serum immunological markers, such as CH₅₀, serum complements (C₃ and C₄), and anti-double-stranded-DNA antibody (ds-DNA Ab), were analyzed enzymatically, by a latex agglutination method and by ELISA, respectively. Serum total cholesterol (TC) and triglyceride (TG) concentrations were analyzed by enzymatic methods (Determiner TC, TG diagnostic kits from Kyowa Medix,

Table I. Patients' background.

Case	Gender	Age	Medication	Histology
S. A.	Female	25	PSL 10 mg/d MZB 150 mg/d	WHO Va
K. S.	Female	45	mPSL 8 mg/d	WHO Va
K. K.	Female	47	PSL 7 mg/d MZB 200 mg/d	WHO IVa
S. O.	Male	47	mPSL 4 mg/d MZB 150 mg/d	WHO II
Y. O.	Female	28	PSL 17.5 mg/d CYA 150 mg/d	WHO Vb
S. A.	Female	28	mPSL 8 mg/d CYA 150 mg/d	WHO IVa

CYA: Cyclosporin A, mPSL: Methyl-prednisolone, MZB: Mizolibine, PSL: Prednisolone.

Tokyo, Japan). HDL-C was measured directly in the serum (Determiner HDL-C diagnostic kits from Kyowa Medix) using an automated procedure with a Hitachi 7450 (Hitachi Medical, Tokyo, Japan). Serum lipoprotein (a) [Lp(a)] concentrations were measured using a commercially available ELISA kit (TintElize Lp(a) kit, Biopool). The assay, which uses polyclonal antibodies raised against purified Lp(a), has been shown to be specific, sensitive and reproducible. Serum remnant-like particles-cholesterol (RLP-C) concentrations were analyzed using the methods developed by Nakajima *et al.* (7). Briefly, serum was added to an immunoaffinity mixed gel suspension containing anti-apo B-100 and anti-apo A-I. Cholesterol levels in the supernatant were measured using a Mercko test diagnostic kit (Kanto Kagaku, Tokyo, Japan).

Statistical analysis. Results were expressed as means±SE. Statistical analysis was performed using the paired *t*-test. *P*<0.05 was considered significant.

Results

Table I shows the characteristics of the patients included in the study. Five of the six patients were treated with steroid and immunosuppressive therapy.

Changes in urinary 8-isoprostane levels are shown in Figure 1. The urinary 8-isoprostane levels were significantly decreased after treatment with EPA (from 530±113 pg/mg•Cr to 235±49 pg/mg•Cr, *p*=0.02).

Changes in urinary protein excretion levels are shown in Figure 2. The urinary protein excretion levels non-significantly decreased after the treatment (from 0.44±0.11 g/g•Cr to 0.27±0.06 g/g•Cr, *p*=0.1).

The fatty acid composition of the plasma PL fraction is shown in Table II. The EPA levels were significantly

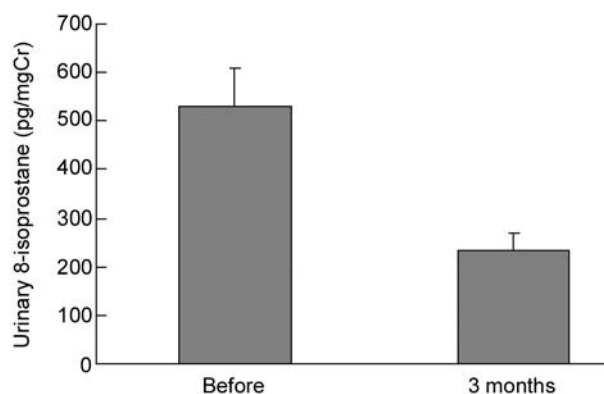


Figure 1. Changes in urinary 8-isoprostane levels after EPA administration. Six patients with lupus nephritis, diagnosed by renal biopsy, were treated with 1800 mg EPA ethyl-ester daily. Urinary samples were obtained before and 3 months after the treatment. Urinary 8-isoprostane levels were analyzed by using a EIA kit. Data were expressed as mean±SE. Urinary 8-isoprostane levels were significantly decreased after the treatment with EPA (from 530±113 pg/mg • Cr to 235±49 pg/mg • Cr, *p*=0.02)

increased after the treatment with EPA, while the arachidonic acid (AA) levels were significantly decreased after the treatment. Thus, the ratios of EPA to AA were significantly increased after the treatment. However, there was no significant correlation between urinary 8-isoprostane levels and EPA, AA, or the ratios of EPA to AA.

Changes in the immunological parameters and the serum lipid profile are shown in Table III and Table IV, respectively. These results did not show any significant changes after the treatment.

No serious adverse reactions were observed in any patients during EPA treatment.

Discussion

The administration of pure EPA suppressed the urinary 8-isoprostane levels in patients with lupus nephritis. The mechanism(s) of this effect are not clear, but the following may explain the results: i) EPA up-regulates the gene expression of antioxidant enzymes and down-regulates genes associated with the production of reactive oxygen species (8); ii) EPA suppresses the production of oxidative stress by decreasing the production of pro-inflammatory cytokines (9).

The recent discovery of 8-isoprostane, which is one of the non-enzymatic prostaglandin-like products of free radical peroxidation of AA, has allowed for the direct assessment of *in vivo* lipid peroxidation (10). There is now good evidence that quantitation of 8-isoprostane provides a reliable measure of *in vivo* oxidative stress (11, 12).

SLE is a chronic progressive autoimmune disorder with a wide spectrum of clinical and immunological abnormalities.

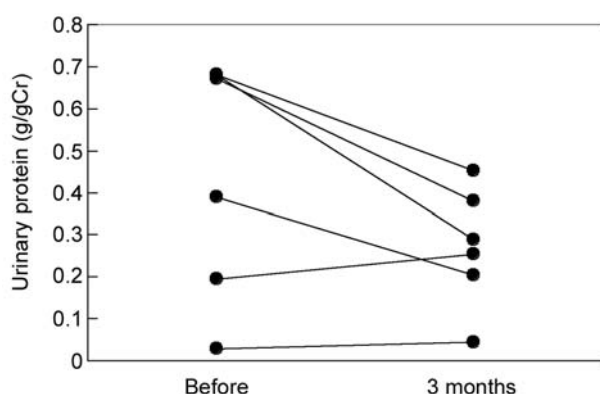


Figure 2. Changes in urinary protein excretion levels after EPA administration. Six patients with lupus nephritis, diagnosed by renal biopsy, were treated with 1800 mg EPA ethyl-ester daily. Urinary samples were obtained before and 3 months after the treatment. Urinary protein excretion levels were analyzed enzymatically. Urinary protein excretion levels after the treatment with EPA tended to be lower than those before the treatment (from 0.44 ± 0.11 g/g • Cr to 0.27 ± 0.06 g/g • Cr, $p=0.1$).

Table II. Changes in fatty acid composition of plasma phospholipid fraction (mol%) after the EPA administration.

	Before	3 months
Linoleic acids (18:2n-6)	19.54 ± 0.78	$16.98 \pm 1.06^*$
Dihomo- γ -linolenic acids (20:3n-6)	1.69 ± 0.14	1.42 ± 0.15
Arachidonic acids (AA; 20:4n-6)	9.47 ± 0.28	$7.33 \pm 0.43^{***}$
α -Linolenic acids (18:3n-3)	0.22 ± 0.02	0.25 ± 0.03
Eicosapentaenoic acids (EPA; 20:5n-3)	3.30 ± 0.64	$8.01 \pm 0.47^{***}$
Docosapentaenoic acids (22:5n-3)	0.88 ± 0.06	$1.98 \pm 0.15^{***}$
Docosahexaenoic acids (22:6n-3)	7.03 ± 0.48	$5.43 \pm 0.23^{**}$
EPA/AA	0.35 ± 0.07	$1.14 \pm 0.16^{***}$

Six patients with lupus nephritis, diagnosed by renal biopsy, were treated with 1800 mg EPA ethyl-ester daily. Blood samples were obtained immediately before and 3 months after the treatment. The fatty acid composition of the plasma phospholipid fraction was analyzed by gas-chromatography. Data were expressed as means \pm SE. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Although the pathogenesis of SLE is probably multifactorial, the inflammatory nature of SLE implies that a state of oxidative stress may exist in this disease, which may contribute to immune cell dysfunction, auto-antigen production and auto-antibody reactivity (5). In fact, it was reported that oxidative stress was increased in patients with SLE and lupus nephritis (5, 13), and that the activity of SLE correlated positively with oxidative stress (5). In the present study, the administration of pure EPA decreased the urinary 8-isoprostane levels in patients with lupus nephritis. Consequently, these results suggested that EPA might depress oxidative stress in lupus nephritis patients.

Table III. Changes in the immunological parameters after the EPA administration.

	Before	3 months
Anti-ds-DNA Ab (IU/mL)	18.0 ± 9.3	27.0 ± 13.5
CH ₅₀ (mg/dL)	29.8 ± 2.6	29.1 ± 2.3
C ₃ (mg/dL)	72.4 ± 4.2	72.4 ± 5.2
C ₄ (mg/dL)	14.6 ± 1.7	13.7 ± 1.9

Six patients with lupus nephritis, diagnosed by renal biopsy, were treated with 1800 mg EPA ethyl-ester daily. Blood samples were obtained immediately before and 3 months after the treatment. Immunological parameters that indicated the activity of lupus nephritis were measured. Data were expressed as means \pm SE. There were no significant differences during the experiment. Anti-ds-DNA Ab: Anti-double-stranded-DNA antibody.

Table IV. Changes in serum lipid profile (mg/dL) after EPA administration.

	Before	3 months
Total cholesterol	190.6 ± 6.9	186.4 ± 12.9
Triglyceride	132.1 ± 25.4	128.1 ± 19.3
HDL-cholesterol	65.2 ± 5.9	65.3 ± 6.5
Lp(a)	22.0 ± 8.3	20.4 ± 8.0
RPL-cholesterol	5.2 ± 0.7	5.8 ± 0.8

Six patients with lupus nephritis, diagnosed by renal biopsy, were treated with 1800 mg EPA ethyl-ester daily. Blood samples were obtained before and 3 months after the treatment. The serum lipid profile was analyzed enzymatically. Data were expressed as means \pm SE. There were no significant differences during the experiment.

In the present study, immunological parameters, such as ds-DNA Ab and serum complements, did not show any significant changes. These results are compatible with the reports of Clark *et al.* (3, 4).

The serum lipid profile did not show any significant changes after EPA administration. Recently, we reported that RPL-cholesterol levels were decreased after EPA administration in type 2 diabetic patients (14). In the present study, the pre-treatment levels of serum lipids were very low in all patients. If serum lipid levels before treatment had been higher, the lipid lowering effects of EPA might have been more obvious.

In conclusion, we administered highly purified EPA to six patients with lupus nephritis for 3 months, and investigated the effects of EPA on urinary 8-isoprostane. The urinary 8-isoprostane levels after the EPA treatment were significantly decreased compared to those before the treatment. Consequently, EPA might have beneficial effects for patients with lupus nephritis by decreasing the oxidative stress.

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