Eicosapentanoic Acid Reduces Plasma Levels of Remnant Lipoproteins and Prevents in Vivo Peroxidation of LDL in Dialysis Patients

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Abstract. Causative factors of uremia-associated atherosclerosis are complex. However, it is likely that atherogenic lipoproteins accumulated in plasma are involved. Remnant lipoproteins are atherogenic and are frequently observed in uremic plasma. LDL from uremic patients has been shown to be susceptible to in vitro peroxidation, suggesting that oxidized LDL (ox-LDL) could be excessively generated in those patients. No effective treatments to prevent accumulation of both atherogenic lipoproteins in dialysis patients have been published. Eicosapentanoic acid (EPA) may change synthesis and/or catabolism of remnant lipoproteins and increase stability of LDL to peroxidation by altering the fatty acid composition of lipoproteins. A prospective comparative study was conducted to assess the efficacy of EPA on metabolism of remnant lipoproteins and ox-LDL in dialysis patients using two new methods: an immunoaffinity gel separation for quantifying plasma remnant lipoproteins and an enzyme-linked immunosorbent assay for measuring plasma ox-LDL levels, a marker for in vivo LDL peroxidation. Twenty-two hemodialysis and 16 continuous ambulatory peritoneal dialysis patients with relatively high plasma levels of remnant lipoproteins and ox-LDL were randomized to either EPA or placebo. Highly purified EPA, in an ethyl-ester form (ethyl all-cis-5,8,11,14,17-icosapentanoate) with a purity greater than 91%, was administered at a dose of 1800 mg daily. Overall, 3 mo of treatment with EPA significantly reduced the levels of both remnant lipoproteins (52% reduction) and ox-LDL (38% reduction). Additionally, gel filtration chromatography of lipoproteins showed that EPA treatment concomitantly normalized other potential abnormalities in lipoproteins. Treatment compliance was good and no critical adverse effects were observed. In conclusion, EPA administration proved to be effective and safe treatment to decrease plasma remnant lipoproteins and prevent in vivo peroxidation of LDL in dialysis patients.

Atherosclerosis is a major complication that determines the morbidity and mortality in predialysis, dialysis, and kidney transplant patients (1,2). The causative factors of atherosclerosis in dialysis patients are complicated, but atherogenic lipoproteins accumulated in plasma could be related (3). In patients undergoing long-term dialysis, remnant lipoproteins are frequently observed in their plasma (4,5), and LDL may be susceptible to peroxidation in vitro (6,7). We believe that accumulation of remnant lipoproteins as well as of oxidized LDL (ox-LDL) plays a key role in the initiation and acceleration of atherosclerosis in dialysis patients. To date, no pharmacologic approach has been conclusively shown to prevent accumulation of both atherogenic lipoproteins. Our current interest is to design the most appropriate therapeutic strategies to remove excessive atherogenic lipoproteins from uremic patients.

Polyunsaturated fatty acids (PUFA) of fish oil origin, especially eicosapentanoic acid (EPA) (C20:5, ω-3), have played a role in the prevention of atherosclerosis in humans (8,9). Diets enriched with ω-3 PUFA have been shown to increase the removal of remnant lipoproteins from plasma at fasting and/or postprandial state in nonuremic subjects (10,11). Moreover, the fatty acid composition of lipoproteins is thought to be involved in the susceptibility of LDL to in vitro peroxidation (12,13) and, in fact, diets rich in ω-3 PUFA have been shown to increase the stability of LDL to oxidative stress (14). Incorporation of EPA into LDL particles therefore may raise resistance of LDL to peroxidation in vivo. We performed a pilot, controlled, randomized group study to investigate whether EPA reduces plasma levels of remnant lipoproteins and ox-LDL, a new marker of in vivo peroxidation of LDL, in dialysis patients. In addition, the adverse effects of EPA administered in a clinical dose were carefully assessed because drug metabolism often varies among dialysis patients.

We demonstrate here that treatment with EPA reduced both plasma remnant lipoproteins and ox-LDL levels in dialysis patients without inducing adverse reactions.

Materials and Methods
Patients and Study Design
The effects of a 3-mo treatment with EPA on plasma levels of remnant lipoprotein and ox-LDL were tested in dialysis patients in a
randomized comparative manner. Highly purified EPA, in an ethyl ester form (ethyl all-cis-5,8,11,14,17-eicosapentanoate) with a purity greater than 91% (Epadel®; Mochida Pharmaceutical, Tokyo, Japan), was administered at a dose of 1800 mg daily (six soft capsules per day). Plasma levels of remnant lipoproteins and ox-LDL were screened in 58 hemodialysis (HD) patients with diabetes mellitus (DM) (57 ± 11 yr), 109 patients without DM (non-DM HD) (50 ± 14 yr), and 44 non-DM patients undergoing continuous ambulatory peritoneal dialysis (CAPD) (46 ± 12 yr). All patients were receiving treatment in the dialysis unit of Tokyo Women’s Medical College or its affiliated hospital. Twenty-two HD (16 DM, 6 non-DM) patients and 16 non-DM CAPD patients with relatively high levels of both remnant lipoproteins (>7.5 mg/dl) and ox-LDL (>150 mg/mg) were enrolled in this study. The patients were randomly allocated to one of two groups: a control group taking placebo (n = 19) or a treatment group with EPA (n = 19), according to the last digit of their medical chart numbers. Patients with even numbers were assigned to the placebo group and those with odd numbers were assigned to the EPA group. The study design consisted of three consecutive periods that lasted a total of 7 mo: the baseline observation (month 0 to month 3), treatment (month 0 to month 3), and washout period (month 3 to month 6) (Figure 1). The clinical profiles and analytic data of the patients at month 0 are summarized in Table 1. There were no significant differences between the groups in any clinical and analytic data shown in the table. HD treatment was performed for 4 h, using conventional bicarbonate-buffered dialysate and regular heparin (9.03 IU/kg for one treatment) in all patients. Dialyzer membranes were made of cellulose triacetate (FB-190U, Nipro, Japan; ultrafiltration rate, 32 ml/mmHg · h; n = 2), polysulfone (PS-1.9UW, Fresenius-Kawasumi, Japan; ultrafiltration rate, 55 ml/mmHg · h; n = 16), and polymethylmethacrylate (BK-1.8P, Toray Medical, Japan; ultrafiltration rate, 36 ml/mmHg · h; n = 4). All CAPD patients were treated with dialysates of 8 to 10 L of peritoneal dialysate solution (Baxter, McGaw Park, IL). Medication of patients included: antihypertensives (64%), calcium carbonate (95%), vitamin D supplementation (68%), and lipid-lowering agents (18%). No medications were discontinued or changed during the period of study or in the previous 4 wk. All ethylenediaminetetra-acetic acid plasma samples were taken at fasting state during the interdialysis period, separated by immediate centrifugation at 1000 × g for 20 min at 4°C, and subsequently kept at 4°C until assayed. The last exchange in CAPD patients was done at least 2 h before the collection of blood samples. The plasma chemistry determinations were performed using an automated multichannel analyzer (Hitachi model 7070, Tokyo, Japan). The medication including recombinant human erythropoietin and insulin that patients were currently taking was not changed throughout the study. The patients were encouraged to maintain their usual diet and physical activities. They were interviewed with regard to any side effects of the medication once a week. Special effort was undertaken to ensure treatment compliance, which was confirmed by measuring the fatty acid concentration in the total lipid extract from plasma by gas chromatography every 3 mo. All participants were informed of the purpose of the study and gave their informed consent in writing. The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board.

Table 1. Baseline characteristics of the patients

<table>
<thead>
<tr>
<th>Category</th>
<th>EPA Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of patients</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>age (yr)</td>
<td>54 ± 11</td>
<td>51 ± 13</td>
</tr>
<tr>
<td>gender (F/M)</td>
<td>2/17</td>
<td>3/16</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.1 ± 0.5</td>
<td>20.8 ± 0.9</td>
</tr>
<tr>
<td>EPO (yes/no)</td>
<td>15/4</td>
<td>16/3</td>
</tr>
<tr>
<td>DM (yes/no)</td>
<td>8/11</td>
<td>8/11</td>
</tr>
<tr>
<td>modality of dialysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-DM/DM HD</td>
<td>3/8</td>
<td>3/8</td>
</tr>
<tr>
<td>non-DM/DM CAPD</td>
<td>8/0</td>
<td>8/0</td>
</tr>
<tr>
<td>years on dialysis</td>
<td>8.3 ± 5.1</td>
<td>9.1 ± 6.9</td>
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Analytical data in plasma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EPA Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>hematocrit</td>
<td>0.32 ± 0.012</td>
<td>0.31 ± 0.022</td>
</tr>
<tr>
<td>albumin (g/L)</td>
<td>34.3 ± 6.1</td>
<td>36.0 ± 4.4</td>
</tr>
<tr>
<td>cholesterol (mmol/L)</td>
<td>5.09 ± 1.61</td>
<td>4.89 ± 1.79</td>
</tr>
<tr>
<td>triglycerides (mmol/L)</td>
<td>2.92 ± 1.64</td>
<td>3.07 ± 1.87</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>0.82 ± 0.18</td>
<td>0.79 ± 0.29</td>
</tr>
<tr>
<td>fasting glucose (mmol/L)</td>
<td>5.30 ± 0.211</td>
<td>5.66 ± 0.305</td>
</tr>
<tr>
<td>hemoglobin A₁c</td>
<td>0.059 ± 0.002</td>
<td>0.061 ± 0.007</td>
</tr>
</tbody>
</table>

a Data are expressed as mean ± SD. EPA, eicosapentanoic acid; BMI, body mass index; EPO, erythropoietin therapy; DM, diabetes mellitus; HD, hemodialysis; CAPD, continuous ambulatory peritoneal dialysis; HDL-C, high density lipoprotein cholesterol.

b Data of patients with DM.
Quantification of Plasma Remnant Lipoproteins Using an Immunoaffinity Gel Mixture Method

Remnant lipoproteins in fasting plasma were separated using an immunoaffinity gel mixture containing specific anti-apolipoprotein (apo) B100 (JI-H) and anti apo A-I antibodies coupled to CNBr-Sepharose 4B (RLP detection kit; JIMRO II, Japan ImmunoResearch Laboratories, Takasaki, Japan). Briefly, 10 μl of plasma was added to 600 μl of a gel suspension solution that contained 50 μl of apo B100 gel and 50 μl of apo A-I gel. This reaction mixture was gently shaken for 60 min at room temperature to ensure complete mixing. Then the tubes were allowed to stand for 10 min, and 30 μl of the supernatant (unbound lipoproteins termed RLP) was withdrawn to determine cholesterol concentration. The cholesterol level in RLP, which is designated RLP-C, was determined using an enzymatic assay in an automatic analyzer. Using HPLC, Nakajima et al. (15) confirmed that RLP-C can serve as a quantitative marker of plasma levels of chylomicron remnant (extrinsic remnant lipoprotein) plus β-very low density lipoprotein (VLDL) or intermediate density lipoprotein (IDL) (intrinsirc remnant lipoprotein). The intra- and interassay coefficients of variation of this assay kit were 6.1% (at 4.9 mg/dl) to 3.5% (at 19.8 mg/dl) and 5.6% (at 19.7 mg/dl) to 10.2% (at 5.9 mg/dl), respectively (16).

Measurement of Plasma Ox-LDL Levels Using Enzyme-Linked Immunosorbent Assay

Plasma ox-LDL levels were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) with the specific monoclonal antibody (FOH1a/DLH3) against peroxidized phosphatidylcholine that forms a complex with apo B protein of the LDL particles, described previously by Itabe et al. (17) (Vessel Research Laboratory, Kanagawa, Japan). In brief, 100 μl of 1600-fold diluted plasma was applied to each well of a microtiter plate coated with the monoclonal antibody and incubated for 18 h at 4°C. After washing the wells, 100 μl of sheep anti-human apo B antibody was added to each well and the plate was incubated for 2 h at 37°C. After washing the wells again, 100 μl of alkaliphosphatase (ALP)-conjugated goat anti-sheep IgG antibody was added to each well and the plate was incubated for another 2 h at 37°C. The reactivity of ALP was measured after adding p-nitrophenylphosphate to each well and incubating the microtiter plate at 37°C for 30 min. Absorbance was measured on an ELISA plate reader with the filter at 405 nm. The measured plasma ox-LDL concentration was corrected for the apo B100 concentration of the plasma. This corrected value was used as the plasma ox-LDL level in this study. Apo B100 concentration of plasma was measured by immunoturbidimetry.

Further Examination of Lipoprotein Characteristics Before and After EPA Treatment Using Fast Performance Liquid Chromatography

Total lipoproteins were isolated from fasting plasma by sequential preparative ultracentrifugation at month 0 and month 3 in the nine EPA-treated patients who showed a greater than 50% reduction in RLP-C levels (they were termed good responders). Two hundred microliters of total lipoproteins was size-fractionated using a Superose 6 gel filtration column on a fast performance liquid chromatography (FPLC) system (Pharmacia, Uppsala, Sweden) (18). Cholesterol and triglyceride concentrations of each fraction were assayed using the diagnostic kits from Kyowa Medix (Determiner TC 555 or TG 555; Kyowa Medix, Tokyo, Japan).

Statistical Analyses

All data are presented as the mean ± SD unless otherwise indicated. Statistical analyses were performed using t test. Differences with a P value <0.05 were considered statistically significant.

Results

Fasting Levels of Plasma RLP-C and Ox-LDL in Dialysis Patients

Plasma RLP-C Levels.

The mean values of RLP-C were 6.6 ± 2.9 mg/dl (n = 58), 5.9 ± 3.3 mg/dl (n = 44), and 4.5 ± 2.2 mg/dl (n = 109) in DM HD, non-DM CAPD, and non-DM HD patients, respectively (the upper value of the reference range, 7.5 mg/ml; data are from Japan ImmunoResearch Laboratories). The RLP-C levels were significantly greater in DM HD and CAPD patients than in non-DM HD patients (P < 0.001). No significant difference was observed between DM HD and CAPD patients. RLP-C levels showed a significant correlation with plasma triglyceride (TG) levels (P < 0.01), but not with plasma total cholesterol (TC) levels.

Plasma Ox-LDL Levels.

Plasma ox-LDL levels of non-DM HD patients (161.9 ± 103.9 ng/mg, n = 109) and DM HD patients (209.3 ± 126.2 ng/mg, n = 58) were significantly higher than those of CAPD patients (128.0 ± 85.3 ng/mg, n = 44) (reference range, 150.9 ± 56.4 ng/mg; data are from Vessel Research Laboratory). No significant difference was observed between non-DM and DM HD patients. There was no specific correlation between plasma ox-LDL levels and RLP-C, TC, or TG ones. Both fasting levels of RLP-C and ox-LDL were elevated in DM HD patients.

Reduction of Fasting Levels of Plasma Lipids after EPA Treatment

Plasma lipid levels at fasting state throughout the study in the overall group are summarized in Table 2. The changes of plasma levels of RLP-C and ox-LDL during the study in HD and CAPD subgroups are depicted separately in Figure 2, A and B, respectively. The ages were matched between the HD placebo and treated subgroups; however, those in the EPA groups were significantly younger than those of HD placebo patients and CAPD patients (48.9 ± 11.7 vs. 20.3 ± 11.7 yr). There were no significant differences in baseline plasma lipid levels between the EPA and placebo groups (subgroups). All plasma lipid levels remained constant throughout the study in the placebo groups (subgroups); however, those in the EPA groups (subgroups) changed as follows.

Plasma RLP-C Levels.

Treatment with EPA for 3 mo decreased plasma RLP-C levels in all patients. The mean reductions (reduction rates) were 6.7 ± 5.0 mg/dl (52%) in the overall group, 7.6 ± 5.9 mg/dl (56%) in the HD subgroup, and 5.5 ± 3.1 mg/dl (46%) in the CAPD subgroup. The reduction was abolished after the 3-mo washout period in each group (subgroup).

Plasma Ox-LDL Levels.

The individual response of plasma ox-LDL levels to the treatment showed considerable variability, but the mean reduction reached statistical significance in each group (subgroup). The magnitudes of the reduction (rate) were 114 ± 132 ng/mg (38%) in the overall group,
Table 2. Plasma lipid levels throughout the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>At Baseline</th>
<th>After EPA Treatment</th>
<th>After Washout</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Month −1</td>
<td>Month 0</td>
<td>Month 3</td>
</tr>
<tr>
<td>RLP-C (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA (n = 19)</td>
<td>12.2 ± 1.35</td>
<td>12.9 ± 1.52</td>
<td>6.16 ± 0.90&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>placebo (n = 19)</td>
<td>11.7 ± 1.58</td>
<td>12.2 ± 0.96</td>
<td>11.9 ± 0.95</td>
</tr>
<tr>
<td>Ox-LDL (ng/mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA (n = 19)</td>
<td>298 ± 25.5</td>
<td>300 ± 23.4</td>
<td>186 ± 24.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>placebo (n = 19)</td>
<td>299 ± 31.7</td>
<td>291 ± 24.7</td>
<td>293 ± 21.6</td>
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<tr>
<td>Total cholesterol</td>
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<tr>
<td>mmol/L</td>
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<td></td>
<td></td>
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<tr>
<td>EPA (n = 19)</td>
<td>5.11 ± 0.41</td>
<td>5.09 ± 0.37</td>
<td>4.26 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>placebo (n = 19)</td>
<td>5.07 ± 0.51</td>
<td>4.89 ± 0.41</td>
<td>5.05 ± 0.43</td>
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<tr>
<td>Triglycerides</td>
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<td></td>
</tr>
<tr>
<td>mmol/L</td>
<td></td>
<td></td>
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<tr>
<td>EPA (n = 19)</td>
<td>3.14 ± 0.47</td>
<td>2.92 ± 0.38</td>
<td>1.69 ± 0.27&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>placebo (n = 19)</td>
<td>2.94 ± 0.35</td>
<td>3.07 ± 0.43</td>
<td>3.02 ± 0.33</td>
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<tr>
<td>HDL-C (mmol/L)</td>
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</tr>
<tr>
<td>EPA (n = 19)</td>
<td>0.84 ± 0.03</td>
<td>0.82 ± 0.04</td>
<td>0.81 ± 0.05</td>
</tr>
<tr>
<td>placebo (n = 19)</td>
<td>0.76 ± 0.05</td>
<td>0.79 ± 0.07</td>
<td>0.78 ± 0.07</td>
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</table>

* Values are expressed as mean ± SEM. RLP-C, a quantitative marker of remnant lipoproteins described in Materials and Methods; ox-LDL, oxidized low density lipoprotein level corrected for the apolipoprotein B100 concentration. Other abbreviations as in Table 1.

<sup>a</sup> p < 0.01 versus month 0.

<sup>b</sup> p < 0.01 versus month 3.

<sup>c</sup> p < 0.05 versus month 3.

143 ± 157.7 ng/mg (40%) in the HD subgroup, and 74.8 ± 79.7 ng/mg (36%) in the CAPD subgroup. The plasma level of ox-LDL returned to the pretreatment level after the washout period in each group.

Other Plasma Lipids. The EPA treatment significantly decreased both plasma levels of TC and TG, but did not change plasma HDL cholesterol (HDL-C) levels (Table 2).

Qualitative Changes of Lipoproteins after EPA Treatment, Evaluated Using FPLC Analysis

FPLC analyses revealed that EPA treatment induced a qualitative alteration of lipoproteins, as well as a reduction of remnant lipoprotein levels in good responders to EPA treatment (n = 9). Summary data of nine patients are presented in Figure 3, A and B. Before treatment, (1) the lipoproteins enriched with TG were widely distributed from the IDL to LDL fractions (3A); (2) the lipoproteins corresponding to the IDL fractions were prominent and enriched with both TG and cholesterol (Figure 3, A and B); and (3) the LDL fractions included small particles rich in cholesterol (Figure 3B). Treatment with EPA normalized most of these qualitative abnormalities of lipoproteins and thus reversed the lipoprotein distribution pattern to an almost normal one in which three peaks of VLDL, LDL, and HDL were clearly identified.

Treatment Compliance and Side Effects

Nineteen patients were monitored for adverse effects of EPA for 3 mo. One patient complained of a mild headache and diarrhea but these symptoms soon disappeared without any treatment (the patient completed the study). The patients with placebos did not complain of any symptoms. No bleeding was observed even in the HD patients despite the fact that EPA is known to inhibit platelet aggregation at a clinical dose (19). The total doses of heparin were not changed throughout the study in any HD patient. Blood cell counts and chemistry were examined regularly twice a month. Hematopoietic status of the EPA group remained stable. A significant decrease in platelet count, which was reported previously when fish oil was used in hyperlipidemic HD patients, was not observed in this study (20). There were no changes in erythropoietin doses during the study. No additional treatment was required to control anemia, glycemia, and BP throughout the study. Treatment compliance was good and it was confirmed by the statistically significant changes of EPA concentration in the fatty acids (15.3 ± 9.98 μg/ml at month 0; 162.9 ± 28.2 μg/ml at month 3; and 50.0 ± 16.6 μg/ml at month 6). None of the patients dropped out of the study.

Discussion

EPA treatment significantly reduced plasma levels of remnant lipoproteins and ox-LDL without inducing adverse reactions in the dialysis patients. In addition, this reduction was accompanied by qualitative changes of lipoproteins that could contribute to the prevention of atherosclerosis.

There have been no simple methods for quantifying plasma remnant lipoproteins. The immunoaffinity column method that Nakajima et al. (15) have recently developed is a rapid and accurate quantitative one for routine clinical use. Lipoprotein particles separated by this method, which are termed RLP, include both extrinsic and intrinsic remnant lipoproteins (15,21). The exact reason why the JI-H (anti-apo B 100)
antibody recognizes VLDL and LDL but not partially degraded VLDL remnants has not yet been established. Nakajima et al. (22) and Marcoux et al. (23) have recently characterized RLP and indicated that those particles include chylomicron remnants and a fraction of larger VLDL remnants, both enriched in apo E, a characteristic of remnant lipoproteins, suggesting that apo E can compete for binding of the antibody to its epitope on apo B 100. They also showed that an increase in the amount of apo C-III relative to apo B, in addition to an increase in apo E relative to apo B, is a characteristic feature of particles that are not recognized by the anti-apo B 100 (JI-H) monoclonal antibody. There is a large amount of evidence that RLP-C (cholesterol level of RLP) can serve as a good quantitative marker for plasma remnant lipoproteins (15,16). Moreover, recent studies have demonstrated that RLP per se are likely to be atherogenic because of the following: (1) RLP are assimilated by macrophages, which can turn into foam cells (24); (2) RLP stimulate platelet aggregation in vitro (25); and (3) RLP-C levels are statistically relevant to the incidence of coronary artery disease or sudden cardiac death (26,27).

Clinical trials of several pharmacologic agents have been conducted in an attempt to reduce plasma remnant lipoprotein levels. On a theoretical basis, fibric acids could be most suitable for reducing plasma levels of TG-rich lipoproteins, including remnant lipoproteins, because this type of drug stimulates the catabolism of VLDL mainly through activation of lipoprotein lipase or hepatic lipase (28,29). However, their use has been discouraged because of worries about side effects such as myolysis and elevation of creatine kinase (30,31). Nishizawa et al. (32) showed that pravastatin, a 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitor, reduced IDL and LDL in CAPD patients. However, their study was not performed in a randomized comparative manner, and the efficacy of the drug was limited only to hyperlipidemic CAPD patients. Based on the principal action of HMG-CoA reductase inhibitors, patients with predominant elevation of cholesterol rather than triglycerides could be candidates for drugs of this category (33,34). For dialysis patients, there seem to be currently no hypotriglyceridemic drugs that have a better risk/benefit ratio than EPA.

Data from our study provided evidence that EPA induces a...
solid and safe reduction of plasma remnant lipoprotein levels in dialysis patients. Disorders of lipid metabolism between HD and CAPD patients are quite different and could be implicated in their diabetic status (1,2,35). Our findings suggested that modalities of dialysis and diabetic conditions of HD patients do not influence efficacy of EPA on remnant lipoprotein levels. Moreover, the treatment with EPA concurrently normalized other potential abnormalities in lipoprotein size and content. EPA has various beneficial effects that contribute to the prevention of atherosclerosis (36–38). Among them, the main characteristic of EPA is its ability to decrease plasma TG-rich lipoprotein levels through the following mechanisms: (1) changes in the rates of synthesis and/or catabolism of lipoproteins; (2) changes in the fatty acid composition of lipoproteins; and (3) increasing fecal excretion of steroids (8,39). Indeed, Hamazaki et al. (20) demonstrated that fish oil rich in EPA safely reduced serum total cholesterol, triglycerides, and phospholipid levels in hyperlipidemic HD patients. Those findings were almost compatible with ours; however, they did not clarify its effect on serum remnant lipoprotein levels. Our findings added importantly to the validity of the affect of EPA on the remnant lipoprotein metabolism in dialysis patients.

There are no established methods to estimate the status of in vivo peroxidation of LDL. Serum levels of end products of lipid peroxidation have been available for this purpose (40,41). However, we question whether those measurements directly reflect the current formation of ox-LDL in vivo and whether the reliability and specificity of them have been fully assessed on a biochemical basis. Thus, more specific methodology is required on this issue. Itabe et al. (42) recently established and characterized a specific and sensitive monoclonal antibody against human ox-LDL, which has enabled us to measure even low concentrations of circulating ox-LDL using ELISA (17). The metabolism of ox-LDL in vivo, however, still remains unclear. Conventionally, ox-LDL has been assumed to be generated and metabolized in the subendothelial space mainly through scavenger receptors on cells of the monocyte-macrophage lineage (43). However, our findings and other recent reports showed that ox-LDL or malondialdehyde-modified LDL actually exist in the circulation (17,44). Those ox-LDL could be the ones released back into the circulation from the subendothelium and/or those generated in the circulation. We believe that plasma ox-LDL levels may be a good indicator of the actual in vivo balance between generation and removal of ox-LDL. Additional studies are now under way to consolidate the significance of this method in our laboratory.

The data on plasma ox-LDL levels indicated that in vivo peroxidation of LDL was enhanced in HD patients compared with CAPD patients. Oxidation of LDL is a lipid peroxidation process in which PUFA contained in the phospholipids of lipoproteins are attacked by free radicals (45). Susceptibility of LDL to in vitro peroxidation therefore may in part depend on its compositional characteristics of the antioxidant and fatty acid content. Additionally, in vivo, the intrinsic generation of oxidative stress should be involved. HD per se generates excessive amounts of oxidative stress possibly through leukocyte activation due to repeated contact of blood with dialysis membranes (46). The fact that HD patients had higher plasma levels of ox-LDL than the CAPD patients may suggest that they are under enhanced oxidative stress.

EPA treatment mildly but significantly decreased the plasma levels of ox-LDL. We propose three reasons why EPA reduced the plasma ox-LDL levels. (1) EPA incorporated into cellular

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**Figure 3.** Qualitative changes of lipoproteins before and after EPA treatment evaluated using fast performance liquid chromatography (FPLC) analysis. Summary data from nine patients who showed good responses to the EPA treatment in RLP-C levels are presented. Two hundred microliters of total lipoproteins was size-fractionated using a gel filtration column on the FPLC system. Triglycerides and cholesterol concentration of each fraction is presented on the y-axis in A and B, respectively. ○, triglyceride concentration before treatment; ●, triglyceride concentration after the treatment; □, cholesterol concentration before treatment; and ■, cholesterol concentration after treatment.
phospholipids stabilized LDL against peroxidation process. The fatty acid composition in LDL is, at least in part, involved in its stability (12,13). The susceptibility of EPA to in vitro peroxidation in aqueous solution is lower than that of other PUFA such as linoleic, linolenic, and arachidonic acids (47), and EPA in human fat tissue is remarkably stable to peroxidation during storage (48). Moreover, incorporated EPA can decrease the compositional rate of arachidonic acid as a potential substrate for peroxidation (36). In fact, the EPA:arachidonic acid ratio increased 2.8-fold after the 3-mo treatment with EPA (0.57 ± 0.11 to 1.61 ± 0.43; P < 0.01) in our study. (2) EPA raised the ability to scavenge environment oxidative stress. Previous reports suggested that EPA can help to remove a variety of reactive oxygen species induced by Fenton reactions from plasma possibly by increasing the amount or activity of hepatic peroxisomal catalase, which is a scavenger of oxygen species in vivo (49,50). (3) It is likely that the action of the α-tocopherol fraction in the EPA is involved in part in the prevention of LDL peroxidation (51). However, EPADEL® (highly purified EPA in an ethyl-ester form) that we tested in this study only contains 0.2% of α-tocopherol (0.6 mg in a capsule) as an antioxidant. This means that the patients took 3.6 mg of α-tocopherol with EPA in a day during the study. The sole or synergetic effect of daily use of a tiny amount of α-tocopherol on prevention of oxidative stress is still equivocal, but it may be an issue to be considered.

In conclusion, EPA treatment remarkably reduced elevated plasma levels of remnant lipoproteins in dialysis patients. This reduction was accompanied by the normalization of other qualitative abnormalities of lipoproteins. Furthermore, our findings suggested that EPA serves as a buffer against peroxidation of LDL in those patients. We strongly recommend EPA treatment for dialysis patients showing dyslipidemia in view as it may contribute to the prevention of atherosclerosis.

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