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Administration of high dose eicosapentaenoic acid enhances anti-inflammatory properties of high-density lipoprotein in Japanese patients with dyslipidemia

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ABSTRACT

Objective: It has been reported that high-density lipoprotein (HDL) loses anti-inflammatory function and promotes atherosclerosis under pathological conditions. However, no pharmacological therapy to improve HDL function is currently available. We aimed to evaluate the effect of oral administration of eicosapentaenoic acid (EPA) on HDL function. **Methods**: Japanese patients with dyslipidemia were treated with EPA (1800 mg/day, 4 weeks), and anti-inflammatory functions of HDL were assessed utilizing in vitro cell-based assays. **Results**: The EPA treatment did not change serum cholesterol and triglyceride levels, but it significantly increased EPA concentrations in the serum and HDL fraction. The EPA/arachidonic acid ratio in the HDL was in proportion to that in the serum, suggesting that the orally administered EPA was efficiently incorporated into the HDL particles. The HDL after EPA treatment showed significantly increased activity of anti-oxidative enzyme, paraoxonase-1. In addition, the EPA-rich HDL significantly improved endothelial cell migration, and markedly inhibited cytokine-induced expression of vascular cell adhesion molecule-1, in human umbilical vein endothelial cells, compared to HDL before the EPA treatment. Moreover, the EPA-rich HDL augmented cholesterol efflux capacity from macrophages. **Conclusion**: Oral administration of EPA regenerated anti-oxidative and anti-inflammatory functions of HDL, and promoted cholesterol efflux from macrophages. Therefore, EPA may transform “dysfunctional HDL” to “functional”, in patients with coronary risk factors.

1. Introduction

Epidemiological studies have revealed that consumption of fish or fish oil is inversely correlated with morbidity and mortality due to coronary artery disease (CAD) [1–5]. The major components of fish oils are long-chain n-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which have a variety of cardioprotective properties. The anti-atherosclerotic effect of PUFA has been explained principally by its action on the plasma lipid profile; n-3 PUFA reduce the plasma concentration of triglycerides (TG) and non-high density lipoprotein cholesterol (HDL-C) by stimulation of peroxisome proliferator-activated receptor-α (PPARα) and by inhibition of sterol regulatory element-binding protein-1 (SREBP-1), but do not substantially decrease low density lipoprotein cholesterol (LDL-C) levels or, sometimes increase LDL-C levels with increased lipoprotein lipase activity in patients with very high TG levels [6]. Supplementation with high doses of purified EPA ethyl ester has been shown to lower major coronary events in Japanese hypercholesterolemic patients [7,8]. The benefits of EPA were greater in high-risk patients with a prior history of CAD (secondary prevention) [7] or multiple coronary risk factors [8]. Interestingly, such benefits were obtained without an effect of lowering LDL-C levels, but were more pronounced in populations consuming low amounts of n-3 PUFA. However, the LDL-C-independent mechanism(s) of this phenomenon remain to be clarified.

Plasma HDL-C concentrations are inversely correlated with the risk for CAD. The cardioprotective effect of HDL is often explained by its reverse cholesterol transport capacity from peripheral cells to the

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liver. Additionally, the HDL particle has a variety of anti-inflammatory and anti-oxidative properties, such as its protection of endothelial function by activating nitric oxide synthase through inhibition of the expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) [9]. Moreover, HDL inhibits the production of inflammatory cytokines and the accumulation of inflammatory cells in the vessel wall [10]. However, recent studies have reported that HDL particles may undergo chemical and oxidative modifications in their protein or lipid constituents under pathological conditions, resulting in the partial or complete loss of atheroprotective effects [11–13]. The transformed HDL is referred to as 'dysfunctional' HDL [9]. Little is known regarding possible pharmacological therapy to improve HDL function. In the present study, we assessed the effects of oral administration of purified EPA on HDL function in patients with coronary risk factor(s).

2. Materials and methods

2.1. Subjects

A total of 28 Japanese patients with dyslipidemia were recruited from Kobe University Hospital (Kobe, Japan) from 2011 to 2012. The background of the participants is described in Table 1. The patients were instructed to maintain a low fat diet before and during the study period and then treated with Epadel-S900® capsules (Mochida Pharmaceutical Co. Ltd., Tokyo, Japan) containing ethyl icosapentate of >98% purity. The EPA capsules were administered 2 times per day orally after meals, for a total daily dose of 1800 mg for 4 weeks. During the study period, participants were directed not to change their regular diet and exercise habits, and doses of regular medications were unchanged. Before and at 1 month after the EPA treatment, fasting blood samples were drawn. Serum was separated by centrifugation, and kept at −80 °C until use. Serum levels of total cholesterol (Tcho), TG, HDL-C, glucose, and hemoglobin A1c were measured by using a standard assay at the Clinical Laboratory of Kobe University Hospital. LDL-C was calculated using the Friedewald formula. Plasma levels of EPA and arachidonic acid (AA) were measured by gas chromatography at SRL, Inc. (Hachioji, Tokyo, Japan). Apolipoprotein (apo) A-1, apoB and apoE were measured using the immunoturbidity method (Sekisui Medical, Tokyo, Japan). RLP-C was measured by immunoadsorption using the RLP-C (Jimro)-II kit (Otsuka Pharmaceutical, Tokyo, Japan).

This investigation conforms to the principles outlined in the Declaration of Helsinki. The study protocols complied with the Guidelines of the Ethical Committee of the Kobe University Hospital of Kobe, and was approved by the Institutional Review Board of Kobe University Hospital. Written informed consent for participation was obtained from all subjects before the study.

Table 1
Patient characteristics at baseline.

Variable	Value
Male, *n* (%)	21 (75)
Age (years)	71.5 ± 11.8
Body mass index (kg/m^2)	23.8 ± 2.1
Hypertension, *n* (%)	13 (46.4)
Diabetes mellitus, *n* (%)	9 (32.1)
Hyperuremia, *n* (%)	1 (3.6)
Coronary artery disease, *n* (%)	5 (17.9)
Chronic kidney disease, *n* (%)	4 (14.3)
Peripheral artery disease, *n* (%)	2 (7.1)
Alcohol consumption, *n* (%)	18 (64.3)
Statin internal use, *n* (%)	12 (42.9)
Current smoking, *n* (%)	6 (21.4)

Patients with dyslipidemia were enrolled ($n = 28$) for evaluation of EPA effect on HDL quality. Data represent mean ± SD.

2.2. HDL purification

The HDL fraction was isolated as described previously [14]. Briefly, we used D_2O-sucrose as basic solution. The serum samples were separated to isolate HDL (1.063–1.210) by ultracentrifugation. Manufactured HDL were stored individually in sealed tubes at 4 °C in the dark. Acetyl LDL was generated by incubating LDL with acetic anhydride, and then used in the cholesterol efflux assay.

2.3. Measurement of paraoxonase (PON) activity

The PON1 (arylesterase) activity was analyzed spectrophotometrically with serum and HDL-associated PON1 as previously reported [15]. Briefly, the arylesterase activity was measured with phenyl acetate (Sigma–Aldrich, St. Louis, MO, USA) as a substrate. Samples were mixed with eserine (10 μmol/L) to inhibit cholinesterase activity at room temperature for 10 min. After that, the samples were stirred with phenyl acetate (10 mmol/L) in 890-μL assay buffer containing 10 mmol/L Tris–HCl, pH 8.0 and 1 mmol/L $CaCl_2$. The increase in absorbance was continuously recorded at 270 nm for a total time of 2 min and an interval time of 60 s.

2.4. Measurement of HDL-mediated cholesterol efflux

Assays of cellular cholesterol efflux were performed as previously described [15,16] with minor modifications. In brief, human monocyte cell line THP-1 (RIKEN, Tsukuba, Japan) cells were cultured on 24-well tissue culture plates and grown in RPMI medium (Wako, Osaka, Japan) containing 10% fetal bovine serum (FBS). Cells were plated at a density of 8×10^5 cells per mL in 24-well culture dishes and converted to macrophages by phorbor myristate acetate (200 nmol/L, Sigma–Aldrich) for 72 h. The THP-1 macrophages were washed once with PBS and the medium was replaced with the RPMI-0.2% bovine serum albumin (BSA) with ^{3}H-cholesterol (1 μCi/mL) and acetylated LDL (25 μg/mL) for a final volume of 500 μL/well. Twenty-four hours later, the cells were washed with PBS and the culture medium was replaced with RPMI-0.2% BSA containing Liver X receptor agonist (T0901317, 10 μmol/L, Sigma–Aldrich) to induce the expression of ATP-binding cassette transporter A1 (ABCA1) and G1 (ABCG1) [17]. After incubation for 16 h at 37 °C, the medium was replaced with RPMI-0.2% BSA and 25 μg protein/mL HDL to stimulate cholesterol efflux reaction. The cells were incubated for 4 h at 37 °C. At the end of the assay, the radioactivity in the supernatants and whole cell extracts was measured and expressed as the percentage of total radioactive counts removed from the cells during the efflux period.

2.5. Wound-healing cell migration assay

Human umbilical vein endothelial cells (HUVECs) were plated on 6-well plates (25×10^4 cells/well) in RPMI containing 10% FBS and endothelial growth factors to be confluent. A wound was made on the confluent monolayer of HUVECs by manually scratching with a yellow pipette tip. After washing with PBS, the cells were incubated with RPMI containing 1% FBS and 100 μg protein/mL-HDL for an additional 20 h. The cells were then fixed with 10% formalin, stained with 0.5% crystal violet stain, and viewed under an inverted microscope (Nikon, Tokyo, Japan). The cells that had migrated past the wound edge were photographed and quantified in 5 random high power (40 ×) fields.

2.6. Evaluation of VCAM-1 expression

The confluent monolayer of HUVECs on 6-well plates was serum-deprived in RPMI-1% FBS, and then incubated with RPMI-1%