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Effect of n-3 polyunsaturated fatty acid supplementation in patients with rheumatoid arthritis: a 16-week randomized, double-blind, placebo-controlled, parallel-design multicenter study in Korea☆,☆☆

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Abstract

N-3 polyunsaturated fatty acids (PUFA) have anti-inflammatory effects and may be useful for the treatment of inflammatory diseases such as rheumatoid arthritis (RA).We examined the efficacy of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) supplementation on RA on top of standard anti-inflammatory treatment. Patients with RA were randomized into two groups in a double-blind, placebo-controlled, parallel-design multicenter study. One hundred nine patients received five capsules of either n-3 PUFA (2.090 g of EPA and 1.165 g of DHA) or high-oleic-acid sunflower oil for 16 weeks. Eighty-one patients completed the study, and no adverse effects were reported. Dietary intake did not change significantly during the study. There were significant increases in n-3 PUFA and EPA levels in erythrocytes in the n-3 PUFA group versus the placebo group, but decreases in n-6 PUFA, 18:2n6, 20:4n6 and 18:1n9 levels in the n-3 PUFA group versus the placebo group. N-3 PUFA supplementation had no significant effects on nonsteroidal anti-inflammatory drug (NSAID) requirements, clinical symptoms of RA or the concentration of cytokines, eicosanoids and bone turnover markers. However, n-3 PUFA supplementation significantly decreased NSAID requirements and leukotriene B_4 levels in patients who weighed more than 55 kg. Our results suggest that n-3 PUFA supplementation has no significant effect on RA but may decrease the requirement for NSAIDs in Korean patients with RA who weigh more than 55 kg.

Keywords: Bone turnover; Inflammation; n-3 polyunsaturated fatty acids; Multicenter clinical trial; Rheumatoid arthritis

1. Introduction

Rheumatoid arthritis is one of the most common autoimmune joint diseases and is characterized by chronic inflammation of the small and large joints, mediated by the excessive production of eicosanoids and cytokines [1]. Treatment of RA with nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids and disease-modifying antirheumatic drugs (DMARDs) improves symptoms, but may lead to side effects such as osteoporosis, diabetes mellitus, weight gain and increased adiposity, and even death [2,3].

An epidemiological study in Greenland Eskimos suggested that n-3 PUFA intakes from seafood may have a potential anti-inflammatory effect; in this population, the n-3 PUFA intake was higher and the prevalence of autoimmune and inflammatory conditions was lower than those of Western populations [4]. Clinical trials of n-3 PUFA supplementation have also reported an improvement in the number of tender joints on physical examination [5–12], the Ritchie Articular Index [11,13,14], morning stiffness [6,8,9,11,12] and NSAID requirements [10,15–17]. A meta-analysis of randomized controlled trials confirmed that n-3 PUFA supplementation improved clinical symptoms of RA [18]. However, most previous studies were performed in small patient groups in a single-center setting, and the subjects have generally been Caucasian subjects with a European background. Koreans consume >50 g/day of fish, which is higher than the daily fish

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consumption of Western populations [19]; thus, the Korean population may be an interesting population in which to investigate the effect of n-3 PUFA on RA.

In addition, n-3 PUFA consumption has been suggested to influence bone health through anti-inflammatory mechanisms [20], but only a few studies have determined bone turnover in patients with RA [21,22]. Thus, our objective in this study was to determine whether n-3 PUFA supplementation reduced clinical symptoms, inflammatory markers, NSAIDS requirements and levels of bone turnover markers in Korean patients with RA.

2. Subjects and methods

2.1. Study design

The study was designed as a double-blind, randomized, multicenter, placebo-controlled, parallel-group trial. At the trials' coordinating center, a computer-generated block sequence balanced by the participating center randomly assigned subjects in blocks of 2. All the investigators, patients and research staff were blinded to the treatment codes. Patients were assigned to take five capsules a day of either n-3 PUFA(Ropufa 75 n-3 ethyl ester; DSM Nutritional Products, Switzerland) containing 2.09 g eicosapentaenoic acid (EPA) and 1.165 g docosahexaenoic acid (DHA) or placebo containing sunflower oil with oleic acid (DSM Nutritional Products, Switzerland) for 16 weeks. Patients were asked to maintain their usual diet during the study, and 3-day dietary records were collected to monitor dietary changes from baseline through 8 and 16 weeks after taking the supplement. Dietary intake was calculated using Can-pro 4.0 (the Korean Nutrition Society, Seoul, Korea).

2.2. Subjects

Patients with RA diagnosed based on the American College of Rheumatology classification criteria were recruited consecutively from Hanyang University Hospital in Seoul, Eulji University Hospital in Daejun, the Catholic University Hospital in Daegu and the Maryknoll Medical Center in Busan between December 2010 and December 2011 [23]. Patients receiving NSAIDs, glucocorticoids or DMARDs were eligible if the dosage had been stable for at least 3 months prior to entering the study. Patients were excluded if they were pregnant, lactating, under the age of 18 years or over the age of 80 years, taking supplements containing n-3 PUFA, had a white blood cell count $\leq 3.5\times10^9$/L, hemoglobin (Hb) level ≤8.5g/dl, platelet count $\leq 100\times10^9$/L, creatinine level ≥2.0 mg/dl, and aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels ≥2.5 times the upper limit of normal. This study was conducted according to guidelines laid out in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Institutional Review Board of Hanyang University Hospital, Eulji University Hospital, Catholic University Hospital and Maryknoll Medical Center. Written informed consent was obtained from all patients prior to enrollment in the study.

2.3. Clinical assessment

At baseline, height; duration of disease; medical history; family history; and exercise, smoking and alcohol habits were assessed. At baseline and at 8 and 16 weeks after supplementation, weight, waist circumference, breathing rate, body temperature, blood pressure, pulse rate, duration of morning stiffness, physician's global assessment (PhyGA), concomitant medications, patient's global assessment (PatGA), Korean Health Assessment Questionnaire (KHAQ) score and pain scale were assessed. Adverse events and compliance with supplementation were monitored by questioning the patients and counting the number of remaining capsules.

2.4. Laboratory measurements

Blood samples were collected in EDTA and SST tubes at baseline and at the end of the study by venipuncture. Erythrocytes, plasma and serum were stored at −80°C until analysis. Counts of white blood cells, erythrocytes and platelets, and levels of Hb, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), AST, ALT, high-sensitivity C-reactive protein (hs-CRP), total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, creatinine, blood urea nitrogen, calcium, inorganic phosphorus, osteocalcin, bone specific alkaline phosphatase (BSAP) and C-terminal telopeptide of type 1 collagen (CTX) were measured by Green Cross Reference Lab.(Yongin-si, Gyeonggi-do, Korea).

Prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4), tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) levels in serum were measured using high-sensitivity enzyme-linked immunosorbent assay kits (Quantikine; R&D systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Coefficients of variation for PGE_2, LTB_4, TNF-α and IL-6 were 4.1%, 3.4%, 2.7% and 2.2%, respectively.

Erythrocyte fatty acid analysis was performed as described previously [24]. Briefly, lipids were extracted, methylated to form fatty acid methyl esters (FAMEs) and analyzed by gas chromatography on a GC2010 (Shimadzu Scientific Instrument, Tokyo, Japan) equipped with a 100-m SP-2560 column (Supelco, Bellefonte, PA, USA). FAME composition is reported as the percentage by weight of total identified FAMEs. The Omega-3 Index is the sum of EPA and DHA in erythrocytes, and the coefficient of variation for the Omega-3 Index was 5.8%.

2.5. Statistical analysis

Power calculations suggested that with a sample size of 40 patients per group, it would be possible to detect a mean difference in NSAID requirements of >20% with a power of 80% at $P<.05$, assuming a standard deviation (S.D.) of 35%. Continuous variables are expressed as mean values and S.D., and categorical variables are expressed as frequencies. To confirm that no selection bias was present, we used the independent t test to compare continuous variables and the χ^2 test to compare categorical variables between the n-3 PUFA group and the placebo group at baseline. Mean changes from baseline to 16 weeks between the n-3 PUFA group and the placebo were compared using the independent t test. If the data were not normally distributed, the Mann–Whitney U test was used. To identify the effects of n-3 PUFA on RA, we used analysis of covariance (ANCOVA) with adjustment for baseline measurements. P values <.05 were considered statistically significant. Statistical analyses were performed using SPSS, version 18.0 (SPSS Inc., Chicago, IL, USA).

3. Results

One hundred nine patients were enrolled and randomized, and 81 completed the study. Fourteen subjects in each group dropped out: 27 for patient's request and 1 patient due to participation in another clinical trial. Compliance was 96.7%±4.8% (77.6%–100%) in the n-3 PUFA group and 96.0%±4.5% (83.5%–100%) in the placebo group (P=.489). No study-related adverse effects were reported during the study.

The baseline characteristics of the n-3 PUFA and placebo groups are shown in Table 1. There were no significant differences in age, sex, body mass index (BMI), duration of disease, family history of RA, exercise, smoking, or drinking between the groups. However, patients in the n-3 PUFA supplementation group had a more extensive medical history than those in the placebo group, which was due mainly to nine patients with hypertension in the n-3 PUFA group vs. only one patient with hypertension in the placebo group. There was no significant difference in dietary intake at baseline between the groups, and there were no significant changes in diet during the study between the two groups (data not shown). The average fish intake was 60±42 g/day in the n-3 PUFA group and 64±46 g/day in the placebo group (P=.426).

Clinical indicators of disease activity, physical examinations and vital signs are shown in Table 2. N-3 PUFA supplementation had no significant effects on NSAID requirements, physician and patient assessments, pain, morning stiffness, KHAQ, waist circumference, BMI or vital signs (Table 2). However, body weight was significantly different between groups after adjusting for baseline body weight. Laboratory indicators are shown in Table 3. N-3 PUFA had no significant effects on blood chemistry, liver function, lipid profile,

Table 1
Baseline characteristics of subjects

	N-3 PUFA (n=41)	Placebo (n=40)	P value[a]
Age (years)	49.24±10.46	47.63±8.78	.453
Female, n (%)	38 (92.68)	37 (92.50)	.975
BMI (kg/m^2)	22.59±2.99	22.21±2.65	.553
Disease duration (years)	9.50±7.86	7.30±5.99	.161
Medical history of disease, n (%)	22 (53.66)	10 (25.00)	.008
Family history of disease, n (%)	7 (17.07)	6 (15.00)	.799
NSAIDs, n (%)	31 (75.61)	35 (87.50)	.168
Glucocorticoids, n (%)	34 (82.93)	33 (82.50)	.959
DMARDs, n (%)	37 (90.24)	37 (92.50)	.718
Exercise habits, n (%)	17 (41.46)	15 (37.50)	.715
Smoking habits, n (%)	6 (14.63)	9 (22.50)	.362
Alcohol drinking habits, n (%)	22 (53.66)	13 (32.50)	.055

Values are mean±S.D. or number of subjects (percentage), as appropriate.
[a] P values were evaluated by independent t tests or χ^2 tests, as appropriate.