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EFFECTS OF MARINE N-3 FATTY ACIDS ON CIRCULATING LEVELS OF SOLUBLE ADHESION MOLECULES IN PATIENTS WITH CHRONIC HEART FAILURE

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Abstract – Inflammatory markers as circulating soluble cellular adhesion molecules (sCAMs) and high sensitive C-reactive protein (hsCRP) are elevated in patients with chronic heart failure (CHF), and may constitute an increased risk of adverse outcome. Marine n-3 polyunsaturated fatty acids (n-3 PUFA) may have anti-inflammatory effect and reduce levels of sCAMs (soluble intercellular adhesion molecule-1 (sICAM-1), vascular adhesion molecule-1 (sVCAM-1), P-selectin) and hsCRP. In a randomized, controlled trial, 138 patients with NYHA class II-III CHF were allocated to receive a daily supplement of 0.9 g of n-3 PUFA or olive-oil for 24 weeks. After supplementation, no significant changes occurred in sCAMs or hsCRP after adjusting for possible confounders. However, a significant reduction was observed in sP-selectin in patients receiving n-3 PUFA, but this result was only of borderline significance in a between- group analysis. In conclusion, a daily supplement with 0.9 g of n-3 PUFA does not significantly affect plasma levels of sCAMs or hs-CRP in patients with CHF. n-3 PUFA may reduce sP-selectin, indicating a possible effect on platelet (and endothelial) activation. The results also indicate that the low dose of n-3 PUFA used in many intervention trials does not have deleterious effects on sCAMs or hsCRP.

Key words: Heart Failure, n-3 PUFA, P-selectin, ICAM-1, VCAM-1, C-reactive protein.

INTRODUCTION

Despite advances in pharmaceutical therapy of chronic heart failure (CHF), the mortality and morbidity from CHF remain high. CHF is associated with high circulating levels of sCAMs (25,28), hsCRP (24,27) and of several inflammatory cytokines suggesting that chronic low-grade inflammation could play a role in CHF.

Abbreviations: CAM, cellular adhesion molecule; **CHF,** chronic heart failure; **DCMP,** dilated cardiomyopathy; **DHA,** decosahexaenoic acid; **EPA,** eicosapentaenoic acid; **hsCRP,** high sensitive C-reactive protein; **ICAM-1,** intercellular adhesion molecule-1; **IHD,** ischemic heart disease; **LVEF,** left ventricular ejection fraction; **MI,** myocardial infarction;**NYHA,** New York Heart Association; **PUFA,** polyunsaturated fatty acids; **VCAM-1,** vascular adhesion molecule-1.

Dietary intervention with n-3 PUFA may reduce the risk of ischemic heart disease (IHD) (4,11,29), and may possess anti-inflammatory, anti-thrombotic and anti-arrhythmic effects, which may also improve outcome for patients with CHF. A large cohort study of 4738 older persons have suggested that an increased intake of fish is associated with a reduced risk of development of CHF (22), and recently the first clinical endpoint trials with n-3 PUFA to patients with CHF have been published (10).

Marine n-3 PUFA may have an anti-inflammatory effect in healthy subjects but data are limited regarding a possible anti-inflammatory effect of n-3 PUFA in patients with CHF. A randomized controlled trial (RCT) of 14 patients with severe CHF reported a decrease in tumour necrosis factor-alpha in patients receiving a very high daily dose of 8 g of n-3 PUFA as

compared to controls (20). Also, increased intake of n-3 PUFA was reported to be associated with decreased levels of pro-inflammatory cytokine levels in 42 patients with CHF, and furthermore, high levels of these cytokines were associated with an adverse outcome (18). To our knowledge, no trials have reported the effect of n-3 PUFA on CAMs in patients with CHF. However, in healthy subjects or in patients with cardiovascular risk factors or IHD conflicting results of effects of n-3 PUFA on sCAMs have been published. Dose of n-3 PUFA supplements seems to be important with respect to inflammatory parameters as high doses often have increased sCAMs and other proinflammatory cytokines (2,15). However, a beneficial effect of low doses of n-3 PUFA has been reported from epidemiological studies (13) as well as from clinical trials in patients with IHD (4,11)or CHF (10), and a daily supplement of 1 g/day has been advocated by the American Heart Association to patients with IHD (16).

The hypothesis of the present study is that supplementation with marine n-3 PUFA will reduce markers associated with inflammation in patients with CHF. We here report the effect of 24 weeks of dietary supplementation with 0.9 g marine n-3 PUFA on plasma levels of sCAMs and hsCRP in patients with CHF.

MATERIAL AND METHODS

Materials

The present work is a sub-study of a prospective planned randomized, double-blind, placebo controlled, multicenter trial to determine the effect of ethyl esters of eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA) on the balance of the autonomic nervous system in patients with CHF. From May, 2001 to November 2002, 138 patients with CHF were enrolled in Italy within the Salvatore Maugeri Foundation-Institute of Care and Scientific Research, at the Departments of Cardiology in Montescano, Gussago, Pavia,Telese, Tradate and Veruno. Eligible patients had CHF caused by IHD or dilated cardiomyopathy (DCMP) together with: 1) a left ventricular ejection fraction (LVEF) < 0.40, 2) CHF in New York Heart Association (NYHA)-class II-III, 3) stable oral medication for at least three month prior to randomization, 4) age 19-80 years. Exclusion criteria were: 1) atrial flutter or fibrillation, 2) frequent ectopic activity, 3) pacemaker rhythm, 4) the use of anti-arrhythmic drugs (beta-blockers excluded), 5) coronary angioplasty or coronary bypass grafting within 3 months or planned within the study period, 6) myocardial infarction (MI) or unstable angina pectoris within 3 months, 7) type I diabetes mellitus, 8) serious concomitant illness, 9) laboratory findings suggesting the presence of other serious illness, 10) history of an allergic response to n-3 PUFA, 11) history of alcohol or drug abuse, and 12) treatment with any investigational compound within 30 days.

Patients were randomized to one soft gelatine capsule a day containing 0.9 g of EPA and DHA as ethyl ester or 1 g of olive oil contained in similar capsules both for 24 weeks. Capsules were provided by Società Prodotti Antibiotici S.p.A., via Biella 8, 20143 Milano, Italy.

The trial was conducted according to GCP guidelines and the study protocol was approved by Ethics Committee of each participating centre and signed informed consent was obtained from all the participants.

Blood Samples

Venous blood samples were collected from antecubital vein after an overnight fast. Blood were centrifuged within 5 minutes at 400 x g for 10minutes and two quantities of 1 ml of the plasma fraction were stored at -80°C until analysis for sCAMs and n-3 PUFA levels. Samples were transferred on dry ice to the Department of Cardiology in Aalborg, Denmark for analyses of sCAMs and hsCRP.

Determination of plasma levels of VCAM-1, ICAM-1, E-selectin and P-selectin were performed using commercially available test-kits (From R&D Systems Europe, Ltd.: Catalogue Numbers: DVC00 (sVCAM-1), BBE 1B (sICAM-1), BBE 6(sP-selectin)). The inter-assay precision (CV%) of the ELISA assays for sP-selectin, sE-selectin, sICAM-1 and sVCAM-1 are 4.9-5.6%, 5.7-8.8%, 3.3-4.8% and 5.5-7.8%, respectively. Intra-assay precision (CV%) of the ELISA assays for sP-selectin, sE-selectin, sICAM-1 and sVCAM-1 are 7.9-9.9%, 4.7-5.0%, 6.0-10.0% and 2.3-3.6% (given by the manufacturer). Samples from each patient were analyzed in the same analytical run in duplicates with the mean of the measurements reported.

CRP was measured using a highly sensitive assay using a BMII nephelometer (Dade Behring Marburg GmbH, Marburg Germany). The assay employs time fixed kinetic measurements at 840 nm as previously described (19). Plasma lipids and lipoproteins were measured by routine laboratory methods with calculation of LDL cholesterol. Plasma fatty acid composition was measured at the Department of Pharmacological Sciences, University of Milan by gas-liquid chromatography (DANI 8610, Monza, Italy) and expressed as a percentage of total fatty acid contents.

Statistical analysis

A power calculation based on experiences from populations with IHD (9) or CHF (25) was performed. Plasma samples were available from 138 patients, randomized in the ratio 1:1. Assuming a SD of 0.25 of population mean, and a reduction in sCAMs of 0.5 of SD to be clinically important, our study would have a power of approximately 83 % to detect such a difference.

Differences between the two intervention groups were tested by unpaired t-test and proportions were tested using test for equality of proportions. hsCRP are significantly positively skewed and all calculations with hsCRP were performed using a logarithmic scale. Thus, results on hsCRP are reported as medians with 95% confidence intervals and differences as ratio of medians with 95% confidence intervals. Baseline correlations between continuous numerical variables were tested in a multiple regression model with baseline sP-selectin, sICAM-1, sVCAM-1 or ln(hs-CRP) as dependent variables and LVEF or n-3 PUFA, and age, body mass index, systolic blood pressure, total cholesterol as independent variable with gender, diabetes mellitus, smoking status and prior MI as indicator variables.

Within group differences in sCAMs and ln(hs-CRP) were tested using paired t-test. Baseline distributions were tested using Shapiro-Wilk's test of normality. In case of non-normality within group differences were also tested

using Wilcoxons signed rank test. However, we present the results of the paired t-test in the analysis because this method gives an estimate of the size of the difference, but the results of the signed rank tests are also given in parentheses.

Between groups differences were tested using t-test. Due to non-normality of sCAMs and hsCRP at baseline, we also performed signed rank tests. Finally, a multiple regression analysis was conducted in order to adjust for differences in baseline concentrations of sCAMs between intervention groups

A p-value below 0.05 (two-tailed) was considered significant. Statistical analyses were performed using STATA statistical software, version 9.2.

RESULTS

No differences in the characteristics of the patients, prior cardiac history, laboratory tests and medications were observed between the two groups at baseline (Table 1). There was a significant negative association between LVEF and sICAM-1 (standardized beta coefficient: -2.2 [-4.3;-0.2]) and sP-selectin (-2.1 [-4.1 ;-0.2]) and, but no association between other sCAMs and LVEF.

The content of EPA and DHA in plasma increased significantly in the n-3 PUFA group, indicating a good compliance to the n-3 PUFA supplement.

Plasma levels of sCAMs and hsCRP, and EPA and DHA at baseline and after 24 weeks of supplementation are given in Table 2. No significant differences between groups in sCAMs or hsCRP were found after supplementation, but the 13 % decrease in sP-selectin in the n-3 PUFA group was borderline significant (p=.06). Adjustments for differences in the baseline levels further decreased the strength of the association (p=.17). However, sP-selectin decreased significantly in the n-3 PUFA group after the supplement (p=0.02, paired t-test). sVCAM-1 and hs-CRP decreased in both groups after the supplements, and the decrease was significant within the control group but not in the between group analysis. No change in plasma sICAM-1 was observed.

DISCUSSION

The present study is the first larger randomized, placebo-controlled trial of the effect of dietary supplementation with a low dose of 0.9 g marine n-3 PUFA on sCAMs and hsCRP in patients with CHF. No significant effect was observed on plasma levels of sCAMs or hs-CRP in those randomized to the n-3 PUFA after adjusting for possible confounders. However a borderline significant reduction in sP-selectin was observed in the group receiving n-3 PUFA in the between group analysis, and the difference was significant within the n-3 PUFA group.

P-selectin is mainly believed to be a marker of platelet activation. Thus, the observed reduction in sP-selectin indicates that n-3 PUFA supplementation may reduce platelet activation. Platelet activity may play an important role for the occurrence of clinical events in patients with CHF (12), but to date no trials of anti-platelet regimens to patients with CHF in sinus rhythm have shown conclusive benefit on tromboembolic events. However, several abnormalities in platelet function have been reported in patients with CHF (6). Elevated sP-selectin have been reported among 74 patients with stable CHF , and interestingly, only sP-selectin was independently associated with major cardiac events after a mean follow-up of 240 days (28). In contrast, another trial of 120 patients with CHF followed for 2 years reported no prognostic association between sP-selectin and a composite endpoint of death and cardiovascular hospitalisations (5). Also elevated hsCRP (23,27) and sICAM-1 (25) have been associated to an adverse outcome in patients with CHF.

No previous studies have investigated the effect of n-3 PUFA on plasma levels of sCAMs or hsCRP in patients with CHF. In vitro studies have shown that DHA (26) and EPA (7) decrease the expression of cell-associated CAMs after cytokine activation. We have previously reported the effect of different doses of marine n-3 PUFA on sCAMs (8) and hsCRP (19) in healthy subjects. In those studies, sP-selectin was significantly reduced only in subjects given 6.6 g n-3 PUFA daily, but not in subjects given 2.0 g n-3 PUFA (8). No effect was observed on sICAM-1 or sVCAM-1 (8) or in hsCRP (19). Dietary intervention studies with fish oils have shown diverging results with respect to reducing sCAMs in patients with atherosclerotic disease(15,21) . In patients with IHD, supplementation of 1.0 g n-3 PUFA for three months had no effect on sP-selectin or other markers of inflammation (17). In contrast, a high dose of 5.1 g/day n-3 PUFA supplemented to patients with IHD increased sVCAM-1 and sE-selectin, but not sICAM-1 (15). It was also reported that a high intake of n-3 PUFA may have proinflammatory effects and increase sVCAM-1 whereas moderate intake may have

Table 1. Baseline characteristic of the patients with CHF, divided according to randomization to either n-3 PUFA or olive oil. Results are given as mean ± SD or exact numbers (n (proportion)).

	n-3 PUFA N=69	Olive oil N=69
Men, n	57 (.83)	61 (.88)
Age (years)	58 ± 10	61 ± 8
Body Mass Index	27.1 ± 0.5	27.1 ± 0.4
NYHA class II/III (n)	58/11	63/6
LVEF	32 ± 6	32 ± 6
DCMP	21 (.30)	23 (.33)
History of IHD, n	48 (.70)	46 (.67)
Previous MI	43 (.62)	43 (.62)
Previous CABG	29 (.42)	36 (.52)
Diabetes mellitus (n)	9 (.13)	4 (.6)
Hypertension (n)	32 (.46)	27 (.39)
Hypercholesterolemia (n)	51 (.73)	45 (.65)
Current smoking, n	9 (.13)	12 (.17)
Plasma lipids (mmol/l)		
Total cholesterol	4.73 ± 1.05	4.87 ± 1.00
LDL cholesterol	2.81 ± 0.91	2.95 ± 0.91
HDL cholesterol	1.16 ± 0.33	1.20 ± 0.34
Triglycerides	1.67 ± 0.74	1.81 ± 1.14
Medications		
Beta blockers, n	64 (.92)	58 (.84)
RAS Inhibitors [1], n	64 (.92)	67 (.97)
Aspirin, n	38 (.55)	37 (.53)
Statins, n	39 (.57)	36 (.52)

[1]RAS: Angiotensin Converting Enzyme inhibitor or Angiotensin II Receptor blocker.
All comparisons between groups were not statistically significant.

Table 2. Effects of daily Supplements with 0.9 g of n-3 PUFA for 24 weeks to patients with Chronic Heart Failure. (mean ± SD)

	Baseline	6 months	Difference	P within group *	P between groups †
sICAM-1 ‡					
n-3 PUFA	276 ± 56	282 ± 69	6 ± 48	0.33 (0.44)	
Control	279 ± 73	272 ± 76	-6 ± 38	0.19 (0.10)	**0.12** (0.12)
sVCAM-1 ‡					
n-3 PUFA	702 ± 229	686 ± 215	-16 ± 100	0.20 (0.18)	
Control	700 ± 219	667 ± 206	-33 ± 127	0.03 (0.09)	**0.37** (0.32)
sP-selectin ‡					
n-3 PUFA	116 ± 80	99 ± 55	-18 ± 63	0.02 (0.06)	
Control	101 ± 54	101 ± 59	0 ± 46	0.99 (0.83)	**0.06** (0.17)
hsCRP §			Ratio of medians		
n-3 PUFA	2.61[1.92;3.52]	2.14[1.61;2.82]	1.22[.98;1.51]	0.07 (0.17)	
Control	1.61[1.23;2.11]	1.21[.96;1.54]	1.32[1.06;1.64]	0.01 (0.05)	**0.59** (0.05)
EPA ‖					
n-3 PUFA	.71 ± .04	1.71 ± .08	1.00 ± .08	<0.001	
Control	.75 ± .06	.78 ± .04	.03 ± .05	0.79 (0.96)	**<0.001**
DHA ‖					
n-3 PUFA	2.11 ± .08	2.94 ± .10	.82 ± .11	<0.001	
Control	2.16 ± .10	2.13 ± .11	.03 ± .10	0.77 (0.96)	**<0.001**

* Paired t-test (in parenthesis: signed rank test). † unpaired t-test (in parenthesis: regression analysis with each sCAM or hsCRP as independent variable with intervention group as indicator variable and baseline value as dependent variable). ‡ ng/mL. § mg/L. ‖ Percent of total fatty acid in plasma.

anti-inflammatory effect and decrease sVCAM-1 among elderly subjects at high risk for coronary artery disease (2). A possible explanation for the observed pro-inflammatory effect could be that n-3 PUFA are highly susceptible to oxidation, which may lead to an increase of oxidized LDL-cholesterol (1), a known stimulus for CAMs expression (3). In a study by Mehra et al (20), 14 patients with severe CHF (NYHA III-IV) were randomized to 18 weeks of 8 g/day n-3 PUFA or placebo. In the n-3 PUFA group, there was a significant reduction in TNF-alpha, indicating reduced inflammation. In contrast, supplementation with 3.4 g/day of n-3 PUFA to patients after a heart transplant increased plasma levels of TNF-alpha and decreased IL-10, thus indicating a pro-inflammatory effect (14). These divergences regarding a possible anti-inflammatory effect of n-3 PUFA could indicate a dose-response relation that might differ between different patient populations. In the present study, the dose of 0.9 g of EPA and DHA might have been too low to induce a significant change in sCAMs. However , our study used similar dose as used in the GISSI Prevenzione trial (1 g) (11) and the GISSI Heart Failure trial (1 g) (10), a large-scale trial designed to investigate the effects of 1 g of n-3 PUFA on mortality and morbidity in patients with symptomatic CHF. In the GISSI Heart Failure trial, the beneficial effect of n-3 PUFA on the clinical endpoints required supplementation for more than 1 year before the effect showed in the separation of the survival curves. Thus, the 24 weeks of supplements of n-3 PUFA could be to short time for a significant effect on anti-inflammatory markers.

Limitations: This study is the largest to date examining the effect of n-3 PUFA on inflammatory markers in patients with CHF. However the number of patients is still modest and this may explain that observed decrease in sP-selectin was only of borderline significance (type 1 error).

In conclusion, a daily supplement with 0.9 g of n-3 PUFA does not significantly affect levels of sCAMs or hs-CRP in patients with CHF. There might be a lowering of sP-selectin by n-3 PUFA, indicating a possible effect on platelet (and endothelial) activation, but further studies are warranted to clarify this and its possible clinical relevance. The results also indicate that a dose of 1 g/day of n-3 PUFA used in larger intervention trials does not have deleterious effects on sCAMs or hsCRP.

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Other articles in this theme issue include references (30-41).

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