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Clinical pharmacology of n-3 polyunsaturated fatty acids: non-lipidic metabolic and hemodynamic effects in human patients

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Abstract

A high dietary intake of n-3 long chain polyunsaturated fatty acids (PUFA), eicosapentaenoic and docosahexaenoic acids, is associated with a reduced incidence of coronary events. Supplementation with pharmacological doses of the same may improve survival in patients with previous myocardial infarction and established heart failure. Such protective effects may be explained by the action of n-3 PUFA on systemic inflammation, hypertension, endothelial dysfunction, thrombosis, cardiac arrhythmias, heart rate variability and atherosclerotic plaque instability, which are involved in the pathogenesis of these clinical conditions. In this short paper we will review the evidence in support of these pleiotropic effects of n-3 fatty acids.

Keywords: n-3 polyunsaturated fatty acids; Endothelial function; Inflammation; Plaque stability

1. Introduction

Results from epidemiological and clinical studies indicate that a high dietary intake of n-3 long chain polyunsaturated fatty acids (PUFA), eicosapentaenoic and docosahexaenoic acids (EPA and DHA respectively), is associated with a reduced incidence of coronary events, and that the supplementation with pharmacological doses of the same compounds may improve survival in patients with previous myocardial infarction and in patients with established heart failure. Myocardial infarction and heart failure are complex diseases, whose etiology is not completely understood. Systemic inflammation, hypertension, endothelial dysfunction, thrombosis, an increase of chemoattractants, growth factors and adhesion molecules, cardiac arrhythmias, heart rate variability and atherosclerotic plaque instability are involved in the pathogenesis of these clinical conditions; all these possible mechanisms are favorably affected by n-3 PUFA, and these effects have been proposed to explain the protective effects of n-3 fatty acids in patients with heart failure or previous myocardial infarction or in patients at high myocardial infarction risk [1]. These protective effects may derive from variation in cell membranes reactivity, observed when their EPA and DHA content in the phospholipid bilayer is increased by treatments, which can modulate the physical properties of membranes and, more indirectly, can affect the function of membrane receptors. This may occur in a dose response fashion and over days or weeks [2].

2. Platelet aggregation

Platelet aggregation plays a primary role in clot development and wound healing, and is a critical physiological response to vessel injury. It is also involved in thrombus formation, and its inhibition does reduce the incidence of myocardial infarction and ischemic stroke [3]. Platelet aggregation assays are routinely performed with agents that physiologically activate platelets in vivo, such as adenosine diphosphate (ADP), collagen, arachidonic acid, thromboxane A_2, epinephrine, thrombin and serotonin.

The effects of n-3 PUFA on platelet aggregation have been studied in randomized controlled clinical trials, part of which has been included in a recent meta-analysis [4]. The authors of this meta-analysis conclude that supplementation

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significantly reduces ADP-induced platelet aggregation in comparison to placebo, while only a slight, not significant trend toward decreased collagen-induced and arachidonic acid-induced platelet aggregation is observed.

However, in patients with documented stable coronary artery disease (CAD), n-3 PUFA "on top" of a treatment with aspirin and statins, significantly reduced ADP-induced platelet aggregation, glycoprotein (GP) IIb/IIIa plasma antigen levels and activity and P-selectin, with no significant effect on platelet/endothelial cell adhesion molecule, vitronectin receptor, formation of platelet-monocyte microparticles and GP IIb/IIIa receptor blockade, indicating a reduction in platelet activity. These effects have been assessed with two doses of n-3 PUFA (1 and 2 g/day) and are already evident after 1 week of treatment, suggesting that, despite the modest observed reduction of platelet activity biomarkers, n-3 PUFA exert unique effects, different from those of other known antiplatelet agents and potentially "pleiotropic" [5].

n-3 PUFA have also been shown to be effective in improving response to aspirin in aspirin-resistant patients (their effect in these patients is similar to that observed in increasing aspirin dose), and to effectively reduce platelet reactivity in patients with stable CAD [6].

A sex specificity of n-3 PUFA supplementation on platelet aggregation has been hypothesized based on the results obtained in a double-blind, randomized, placebo controlled trial conducted in 94 healthy men and women treated, for 4 weeks, with EPA-rich (1000 mg EPA plus 200 mg DHA) or DHA-rich (200 mg EPA plus 1000 mg DHA) capsules per day. In a subgroup analysis, EPA rich treatment (but not DHA rich treatment) was able to reduce platelet aggregation in men, as compared with placebo, while in women an opposite pattern of activity was observed (DHA rich, but not EPA rich treatment, was effective). Gender differences in treatment interactions were also observed with plasma levels of hemostatic markers (thromboxane B_2, P-selectin, von Willebrand Factor activity, plasminogen activator inhibitor-1 or PAI-1) and with the uptake of n-3 (plasma EPA concentrations significantly increasing to the same extent in men and women supplemented with EPA, and plasma DHA concentrations increasing significantly more in women than in men in the DHA supplementation group). These results suggest that a reduction of thrombotic disease risk is more likely to be observed in men after EPA supplementation, and in women after DHA supplementation [7].

3. Blood pressure

A blood pressure (BP) lowering effect of n-3 PUFA was first hypothesized following the observations of Bang and Dyerberg on a negative association between plasma n-3 levels and diastolic BP in Greenland Inuits [8]. Systematic reviews of published clinical trials have allowed to conclude that the effect of n-3 PUFA on BP is dose dependent (with a minimal effective dose of 3 g/day) and can be estimated to be of −0.66/−0.35 mm Hg per n-3 PUFA gram for systolic and diastolic pressure, respectively [9].

Metaregression analysis of double blind randomized trials has confirmed a small but significant antihypertensive effect of high median doses (3.7 g/day), administered for more than 2 weeks, on both systolic blood pressure (SBP) and diastolic blood pressure (DBP). These effects can be larger in subjects more than 45 years old and in hypertensive patients (BP greater than 140/90 mm Hg) [10]. Age and the presence of hypertension seem to independently affect BP sensitivity to fish oil.

The antihypertensive effect of n-3 fatty acids, with SBP and DBP reduction and improvement of pulse pressure and of basal heart rate, has been positively associated with baseline BP values, but inversely associated with patients age, in a retrospective evaluation of long-term effect of a supplementation with 2 g of n-3 PUFA in hypertriglyceridemic patients with untreated normal-high BP [11].

Mori initially individuated DHA, rather than EPA, as the long chain PUFA responsible for BP lowering effects [12], providing evidence to support its influence on both endothelial dependent and endothelium independent relaxation of forearm blood vessels. On the other hand, Nestel [13] reported improvements in arterial compliance following alternate days supplementation with 3 g of EPA or DHA for 7 weeks. The major findings of this study were an improvement in systolic and pulse pressures and of total vascular resistance of about 36% with EPA and of 27% with DHA.

Also in type 2 diabetes patients DBP and factor VII plasma levels are affected by n-3 PUFA supplementation for an average of 8.5 weeks, as shown in a meta-analysis of 12 trials involving 847 patients [14]; the absence of BP pressure effects with n-3 fatty acids in other studies on diabetic subjects may be attributed to inadequate experimental designs and/or to the use of insufficient doses of n-3.

Heart rate is also affected by n-3 PUFA supplementation, as observed in postmenopausal women treated with 2.8 g/day of DHA for 4 weeks [15]. In this study no changes in BP were observed, while heart rate was reduced on average by 7%. In another study, performed in healthy non-smoking men aged 36–56 years, DHA, but not EPA, at an intake level of 4 g/day, decreased heart rate but not BP [16]. Echocardiography in a subsample of enrolled men showed an improved left ventricular diastolic filling in both EPA and DHA groups, compared with placebo.

A meta-analysis of clinical trials published from 1966 to 2005 [17] has concluded that heart rate reduction by fish oil supplementation is more efficient in controlled trials with higher baseline heart rate or longer term duration, providing firm evidence that n-3 PUFA affect cardiac electrophysiology in humans.

4. Endothelial function

Disturbance in endothelial cell functions is a key event in the development of atherosclerosis. Endothelial function (or dysfunction) can be monitored by measuring the so called flow-mediated dilation (FMD), but also vessel diameter, compliance and distensibility of specific arteries and/or the plasma concentrations of markers like soluble intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, P selectins and NO [18].

Higher FMD values are predictive of a decreased risk of cardiovascular events in the follow-up. According to Goodfellow [19], treatment with marine n-3 fatty acids results in a significant improvement in FMD, in addition to a significant reduction in triglycerides, in subjects with hypercholesterolemia. Endothelium-independent dilation was not affected by marine n-3 fatty acids treatment.

An improvement in FMD has also been observed in patients with peripheral artery disease supplemented with 1 g/day n-3 for 3 months [20]. However, regular n-3 PUFA supplementation in apparently healthy young and middle-aged individuals did not consistently improve endothelial function. Improvements may, however, be observed in older individuals and in patients with cardiovascular risk factors including overweight, dyslipidemia and type 2 diabetes [21].

Two recent meta-analyses [22,23] have combined and analyzed the results of clinical trials evaluating the efficacy of n-3 supplementation on endothelial function. In the metanalysis by Xin and coworkers, the effect of fish oils on endothelial function was affected by the quality of the studies considered (good/poor), by the characteristics of patient enrolled (diabetic or not, as an example), by trial length (more or less than 12 weeks) and by type of protocol (randomized, double-blinded, placebo controlled or not). In normoglycemic participants and in subjects with lower DBP (less than 75 mm Hg) n-3 treatment seems to be associated with significant improvements of FMD, while FMD of diabetic patients or of patients with higher DBP had no apparent benefit from n-3 supplementation.

The meta-analysis by Wang, performed on 16 eligible studies involving 901 participants, concludes that supplementation with n-3 fatty acids significantly improves the endothelial function, by increasing FMD by 2.3% at a dose ranging from 0.45 to 4.5 g/day, without affecting endothelium-independent dilation. Sensitivity analyses indicated that the protective effect of n-3 on endothelial function was robust. No significant change in endothelium-independent vasodilation was observed after n-3 PUFA.

Two recent clinical trials have provided new information on the relationship between n-3 and endothelial function in high risk patients. The supplementation with 2 g/day of n-3 ethylesters (84% EPA + DHA) in healthy male cigarette smokers has been shown to acutely increase endothelial t-PA release and to improve endothelial vasomotor function in response to intrabrachial infusions of acetylcholine, substance P and sodium nitroprusside, vs placebo (olive oil) [24]. In another high risk population (obese adults) 4 g of the same n-3 preparation for 12 week, administered with a 25% dietary caloric restriction, induced an improvement in SBP, heart rate and plasma triglycerides, and an increase in elasticity of both large and small arteries, measured using pulse contour analysis. These changes were demonstrated against the background of the favorable effects of weight loss alone on DBP, insulin sensitivity, and plasma concentrations of HDL cholesterol and adiponectin [25].

Longer term n-3 supplementation has been shown by Dangardt and coworkers [26] to further improve vascular function in obese adolescents, characterized by a marked inflammatory status. After a 3 months supplementation with 1.2 g/day of n-3 fatty acids, the number of lymphocytes and monocytes and the levels of TNF-α, IL-1 and IL-6 decreased, possibly contributing to the observed improvement of endothelial function. These effects paralleled the increased incorporation of n-3 in serum phospholipids, which may beneficially influence NO release, leukocyte adhesion, free radical formation, and inflammatory status. However, authors underline that the dosage they used may be relatively low for obese children with large amount of adipose tissue.

Only a limited number of studies has evaluated the individual effects of EPA and DHA on FMD, or compared the effects of these two fatty acids.

Supplementation with EPA alone (1.8 g/day for 3 months) increased endothelium-dependent forearm blood flow response in untreated hypertriglyceridemic males [27], whereas DHA alone (1.2 g/day for 6 weeks) improved endothelium-dependent FMD in hyperlipidemic children [28]. Comparison of the vasodilatory effects of high doses of EPA and DHA (4 g/day for 6 weeks) in overweight mildly hyperlipidemic males showed that DHA, but not EPA, increased forearm blood flow in response to acetylcholine, which activates NO release; however, it also increased forearm blood flow when acetylcholine was injected together with an inhibitor of endothelial NO synthase, or with nitroprusside as donor of exogenous NO, suggesting that the increase in forearm blood flow caused by DHA may be at least in part independent of endothelial function [29]. Similarly, DHA (but not EPA) decreased vasoconstriction in response to norepinephrine, which is again endothelial cell independent. Overall, results from these limited number of studies may suggest that EPA effects on FMD are largely endothelial cell dependent, and those of DHA are at least in part endothelial cell independent [30]. The results of the cited meta-analyses [22,23], on the other hand, suggest that the effect of the combination of these fatty acids is limited to endothelial dependent vasodilation, and no significant effect on endothelium independent vasodilation is observed.

5. Inflammation

Inflammation process is now recognized as a crucial player in the natural history of atherosclerosis, which facili-

tates plaques formation, evolution and rupture. Fatty streaks (the earliest lesions) only consist of monocyte derived macrophages and T lymphocytes, whose adhesiveness is increased by endothelial injury, which infiltrate the arterial wall endothelium. The injured endothelium also produces vasoactive molecules, cytokines and growth factors, resulting in further promotion of the inflammatory response and of migration and proliferation of smooth muscle cells into the area of inflammation [2]. Plasma markers of inflammation include an increase in the number of circulating leukocytes, acute phase proteins (C-reactive protein or CRP, serum amyloid A, fibrinogen), cytokines and their soluble receptors (TNF-α, IL-1, IL-6, IL-7, IL-8 and IL-18, interferon gamma), adhesion molecule (ICAM-1, VCAM-1, E and P selectin) and PAI-1. An increase in the concentration of insulin and a decrease in the concentrations of leptin and adiponectin are also associated with inflammation. Plasma levels of CRP, one of the most commonly used markers of inflammation, are strictly associated with relative risk for cardiovascular diseases [31].

In addition to modulating inflammatory gene expression in immune cells, anti-inflammatory effects of n-3 long chain PUFA are mediated to a large extent through eicosanoids. The earliest anti-inflammatory action of marine n-3 described in humans was the reduction in synthesis and tissue levels of arachidonic acid-derived eicosanoids like PGE_2 and LTB_4, reflecting, in part, the decrease in arachidonic acid content and the EPA action as an inhibitor of arachidonic acid metabolism via the cyclooxygenase and lipoxygenase enzymes, and in part the lower biological activity of the EPA-derived mediators as compared to those produced starting from arachidonic acid [32].

Other anti-inflammatory pathways of PUFA n-3 have recently been investigated and described. In recent studies EPA and DHA have been identified as precursors of new classes of potent anti-inflammatory and inflammation-resolving mediators, known as resolvins, from EPA and DHA (e.g. resolvin E_1 and resolvin D_1) and protectins from DHA (e.g. protectin D_1) [33].

While a number of epidemiological and observational studies has demonstrated that lower n-3 PUFA levels are associated with higher serum IL-6, TNF-α, and CRP [34,35], comparisons of supplemented and placebo treated groups in n-3 PUFA randomized controlled trials have not always produced reliable serum cytokine differences. In fact, the strongest support for n-3 PUFA anti-inflammatory properties mainly comes from studies with older, hypertriglyceridemic or diabetic individuals, with elevated inflammatory markers, while that cytokine production in healthy people seems to be relatively insensitive to long-chain n-3 PUFA [36].

An anti-inflammatory effect, as an example, has been demonstrated in the adipose tissue in a population of severely obese non-diabetic patients treated with 3.36 g of n-3 PUFA for 8 weeks [37]. Authors observed a statistically significant reduction in the expression of most of the analyzed inflammatory genes in subcutaneous adipose tissue at the end of the study, together with an increased production of anti-inflammatory eicosanoids in visceral adipose tissue and in subcutaneous adipose tissue, and with a decrease in circulating IL-6 and triglyceride concentrations. These results are particularly interesting due to the important role of adipose tissue inflammation in determining obesity-related systemic inflammation, which predisposes patients to the development of metabolic and cardiovascular disease.

In hypercholesterolemic patients treated with a combination of statins and n-3 PUFA for 23 weeks, a significant reduction of plasma IL-6, in addition to inhibition of platelet function [38] and improvement of the daytime BP, was observed.

In hypertriglyceridemic men, aged 39–66 years, higher doses of DHA (3 g/day) for more than 3 months proved effective in reducing CRP (−15/−25%) and IL-6 concentrations; a reduction in the number of circulating neutrophils was also observed [39]. Notably, their effect on CRP was comparable to that obtained with statins in randomized clinical trials.

The anti-inflammatory effect of n-3 PUFA in chronic heart failure is dose dependent, as demonstrated in Austrian patients with non-ischaemic chronic heart failure undergoing optimal therapy: both the administered doses (1 and 4 g/day) significantly reduced monocyte-platelet aggregates in a dose-dependent manner and negatively affected tissue factor, but only the higher dose significantly decreased P-selectin and exhibited modest anti-inflammatory effects with a significant reduction of IL-6 and a trend-wise reduction of TNF-α and prothrombin fragment F1.2 [40].

6. Adhesion molecules

Measurement of the circulating concentrations of soluble VCAM-1, ICAM-1, E and P selectins can provide information on endothelial activation. However, only few studies have examined the effects of purified EPA and/or DHA on these markers. Overall, results are inconclusive, even if they suggest that EPA may be more effective than DHA in affecting the circulating concentrations of adhesion molecules.

In fact, a high dose of DHA alone (3 g/day) for a long time period (90 days) did not alter the circulating concentrations of soluble ICAM-1, soluble VCAM-1 and E-selectin in hypertriglyceridemic subjects [39]. On the other hand 4 g/day of EPA and DHA supplement (mixture of 465 mg and 375 mg per gram, respectively) for 7 months significantly decreased the plasma concentrations of soluble ICAM-1 and soluble E-selectin, with no changes in soluble VCAM-1 concentration, in hypertriglyceridemic subjects [41]; a 4 week treatment with 1.8 g/day of EPA significantly decreased plasma concentrations of E selectin in diabetic patients, and not significantly P selectin plasma levels; finally, in healthy subjects, 6.6 g/day of a 1:1 mixture of

EPA and DHA for 12 weeks, decreased E-selectin without affecting adhesion molecules [42].

7. Plaque stability

A study by Calder's group, published a decade ago [43], led to hypothesize a specific effect of n-3 PUFA on plaque stability; this effect might explain the reduction in non-fatal and fatal cardiovascular events in some relatively short-term studies performed using these fatty acids [44].

The administration of fish oil providing 1.4 g/day of EPA and DHA or sunflower oil capsules for 7–189 days (median 42), to patients scheduled to undergo carotid endarterectomy, was associated in the Thies study, with higher proportions of EPA and DHA in the lesion, thicker fibrous caps, reduced abundance of macrophages and plaque vulnerability, the primary determinant of thrombosis-mediated acute cardiovascular events [43].

More recently Cawood and coworkers, in another randomized clinical trial, treated with two capsules per day of n-3 PUFA (0.81 g EPA and 0.675 g DHA) or placebo patients on a waiting list for carotid endarterectomy, for a shorter time period (21 days on the average) [45]. Plaque from n-3 treated group showed higher proportion of EPA in phospholipids, inversely associated with plaque instability and inflammation and with the number of T cells in the plaque. Moreover plaques from supplemented patients had significantly lower levels of mRNA for matrix metalloproteinases (MMP-7 and MMP-12) and for IL-6 and ICAM-1. However, plaque stability was not different between the two groups, probably in association with the lack of incorporation of DHA in plaque phospholipids, as well as the short duration of the study.

In a series of patients referred to an emergency unit for an acute heart attack, Amano observed an increasing amount of fibrous tissue in arterial lesions (and conversely a decreasing presence of "soft" plaque tissue) with increasing proportion of n-3 PUFA in patients serum, suggesting the contribution of low n-3 to the incidence of acute coronary syndrome [46].

8. Intima media thickness

Intima media thickness (IMT) has long been used as a marker of atherosclerotic involvement of arterial walls, and as a surrogate endpoint to evaluate regression and/or progression of atherosclerotic lesions [47]. A number of observational human studies have indicated the existence of an inverse association between n-3 PUFA intake and atherosclerosis, measured as IMT, while results from human interventional studies with marine PUFA have been quite variable [48].

Changes in the lumen of atherosclerotic coronary arteries, determined by angiography, did not differ between treated and control patients in the Harvard Atherosclerosis Reversibility Study on patients with CAD taking fish oil supplements (12 g/day) or n-3 PUFA (6 g/day) or a placebo for 2 years [49]. In the Study on Prevention of Coronary Atherosclerosis by Intervention with Marine Omega-3 fatty acids (SCIMO), patients with angiographically proven CAD taking a fish oil supplement (1.65 g EPA + DHA for 3 months and then 3 g/day for 21 months) or a placebo, suffered less cardiovascular events during the trial, but the loss in luminal diameter was similar in the two groups (n-3 and placebo) [50]. Differences in arterial lumen reduction between men and women without known CAD, on the opposite, have been observed in the Multi-Ethnic Study of Atherosclerosis: men in the highest quartile of plasma n-3 PUFA had the lowest diameter of brachial artery (that was not correlated with n-3 PUFA for women), while women in the highest quartile of plasma n-3 PUFA had the lowest change in percent FMD [51]. In conclusion, the association between n-3 PUFA intake and the risk of atherosclerosis seems to vary according to sex and the artery examined.

The effect of 1.8 g of highly purified EPA has been assessed on the progression of diabetic macroangiopathy, in an open-label randomized prospective trial on 81 Japanese subjects with type 2 diabetes [52]. During the 2.1 years study period, mean IMT and max IMT showed a significant annual decrease in EPA treated group, as compared with the control group. Also brachial-ankle pulse wave velocity improved throughout the follow up. Multiple regression analysis showed that the administration of EPA was a significant and independent factor inversely associated with mean IMT in diabetic patients.

The clinical literature regarding the effect of n-3 fatty acids on measures of vascular structure and function, including coronary artery restenosis after angioplasty, carotid IMT and exercise capacity has been evaluated in a systematic review [53]. The risk ratio of coronary artery restenosis was reduced with fish oil by a non-significant 13% (RR: 0.87; 95% CI 0.73–1.05) across 12 randomized controlled trials. Three randomized trials and 3 uncontrolled studies also reported small non-significant improvements in exercise capacity associated with fish oil treatment. The difficulty to draw firm conclusions regarding the effects of n-3 fatty acids on arterial wall structure has been attributed by the authors to the short length of most studies, and specifically to the absence of intervention studies lasting more than 2 years.

9. Conclusions

n-3 PUFA have shown, in experimental studies performed in humans, to possess a wide array of favorable effects on risk factors for atherosclerosis and on endothelial function, considered a sort of "obliged" intermediate step of the atherogenetic process. The available evidence suggests that such effects should translate into an improvement of the natural history of the atherosclerotic disease, and thus into a reduction of the incidence of the clini-

cal consequences of the atherosclerotic disease (essentially myocardial infarction and ischemic stroke).

The effects of n-3 fatty acids on blood pressure, inflammatory parameters and platelet aggregation, specifically, can be expected, based on the published literature, to reduce the cardiovascular risk of patients. Similarly, the improvement of endothelial function, observed also in patients treated with drugs of recognized efficacy (as statins, ACE inhibitors, antihypertensive drugs), is likely to improve the cardiovascular prognosis of treated patients.

Accumulating evidence suggests that the effects of EPA and DHA, in this context, may be not perfectly overlapping: but to maximize the protective effects described in this paper a balanced mix of these long chain PUFA can represent, today, the preferable option.

The data on the metabolic and hemodynamic effects of n-3 PUFA, combined with the effects on the lipid profile described elsewhere in this volume, can help to put in the proper context the information obtained from randomized clinical trials performed using n-3 PUFA in human patients with different pathological conditions.

Conflict of interest

AP declares the following conflict of interest: speaker for Sigma Tau industries. FM has no conflict of interest to report.

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