European **Rev**iew for **Med**ical and **Pharmacol**ogical **Sci**ences 2004; 8: 225-229

Polyunsaturated fatty acids (n-3 PUFAs) and inflammatory bowel disease (IBD): pathogenesis and treatment

A. BELLUZZI

U.O. Gastroenterology, S. Orsola Hospital – Bologna (Italy)

Abstract. – **There is considerable evidence to suggest that polyunsaturated fatty acids (PUFAs) alleviate a number of inflammatory diseases, mainly the fish derivatives, n-3 PUFAs. My aim is to briefly review the literature involving clinical interventions with these lipid compounds in the treatment of Ulcerative Colitis (UC) and Crohn's Disease (CD), Inflammatory Bowel Disease (IBD). Data available are conflicting and the reason for the discrepancies in the findings could reside in the different study designs. Often studies are limited by the choice of placebo and insufficient washout period and direct comparison of trials is hampered by the use of various formulations and dosages of n-3 PUFAs.**

The importance of the n-3 PUFAs formulation in lowering the incidence of side effects along with careful selection of patients and experimental design seems to be associated with benefits. It is possible these fatty acids act by reducing low-grade active inflammation rather than by preventing reinitiation of the inflammatory process from a truly quiescent state. Whether this treatment is applicable to all patients with IBD has not been fully elucidated. Nevertheless, taken together, all these studies suggest the effectiveness of these new therapeutic approaches, not only when the conventional treatment fails or it is not possible to treat chronically, but also, in some instances as first choice.

Key Words:

IBD, Crohn' disease, Ulcerative colitis, Fish oil, n-3 PUFAs.

Backgroun

Over the last few years a growing body of evidence has demonstrated that n-3 PUFAs alleviate a number of inflammatory diseases. This work stems for the early epidemiological observation that high dietary intake of these lipids was associated with a very low incidence of chronic inflammatory conditions in Eskimos[1]. Dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the two major components of fish oil, partially replace arachidonic acid (AA) in a time and dose dependent manner in plasma and cellular phospholipids. These are less readily released upon cell stimulation and reduce the availability of AA for eicosanoid generation. In addition, the n-3 PUFAs reduce the production of the 2-series eicosanoids generated by AA metabolism that are all pro-inflammatory. These include leukotriene B_4 (LTB_4), the most potent chemotactic agent which is responsible of neutrophil recruitment[2], as well as thromboxane A_2 (TXA_2) which is deeply involved in the inflammatory process by increasing vascular permeability, promoting platelet aggregation and causing oedema[3]. In addition, n-3 PUFAs serve as precursors to a class of eicosanoids with limited inflammatory properties, such as leukotriene[4] B_5. It has also been widely demonstrated that n-3 fatty acids, such as EPA and DHA, are able to inhibit inflammatory cytokine production[5]. Cytokines such as interleukin-1 β, interleukin-2, interleukin-6 and tumor necrosis factor (TNF-α), belong to a class of soluble proteins that influence the immune cell system resulting in enhancement of production of chronic inflammatory substances[6,7]. Evenmore Grimble et al. have recently demonstrated that the ability of fish oil to decrease TNF-α production is influenced by inherent TNF-α production and by polymorphisms in the TNF-α and lymphotoxin alfa gene[8]. N-3 PUFAs may also act as free-radical scavengers[9,10].

Moreover, multifocal gastrointestinal infarctions have been suggested as one of the first pathogenic steps in IBD[11], thus suggesting a pivotal role for platelets, as underlined recently by recent observations[12], and possibly for the powerful platelet aggregator thromboxane^3 A_2; treatment with n-3 PUFAs has been shown to decrease platelet responsiveness in patients with IBD[13].

It has also been demonstrated that fish oil supplementation improves the nutritional state of rats in which a short bowel syndrome, a clinical condition that may also affect CD patients after multiple surgical bowel resections, was induced. These results were obtained by inducing enterocyte hyperplasia, which markedly increases the mucosal surface area with a corresponding increase in enteral absorption[14]. In this respect, it has recently been shown that increasing dietary polyunsaturated fat intake in IBD patients enhances by 65% the absorption and the utilisation of saturated fat, such as palmitic acid, improving the overall nutritional condition[15].

n-3 PUFAs Trials

Investigation of n-3 PUFAs in inflammatory bowel disease (IBD), ulcerative colitis (UC) and Crohn's disease (CD), began at the end of the eighties. Shoda et al.[16] provided epidemiological evidence relating to the incidence of IBD in Japan. They showed that increasing incidence of CD was strongly correlated with, among others, the ratio of n-6 to n-3 fatty acid intake (r = 0.792) where increased dietary intake of n-6 with less n-3 PUFAs, may contribute to the development of CD.

From the clinical point of view, the first evidence of clinical benefit came from McCall[17] who, in an open study, treated 6 patients with active UC by giving 3-4 g of EPA daily (16-24 capsules of fish oil as triacylglycerol) for 12 weeks and obtained a significant improvement in symptoms and in histological appearance, along with a significant fall in neutrophil leukotriene B_4 production.

The first prospective, controlled and double blind study was published by Lorenz et al.[18] who treated 39 patients with IBD, of which 29 had CD, in different stages of clinical activity in a 7-month controlled, crossover trial. Patients were randomised to receive either 3.2 g daily of n-3 PUFA or olive oil as placebo. Conventional treatment was discontinued whenever possible. Otherwise it was minimized and kept constant for at least 3 weeks before the study and until completion. Between the two treatments there was a one-month wash out-period. At the end of the study, the clinical activity expressed by Crohn's Disease Activity Index (CDAI)[19] was unchanged in-patients with CD after n-3 PUFAs supplementation .

Hawthorne et al.[20] published the first large placebo controlled study in 1992. In this study 96 UC patients in different activity stages were enrolled and were given 4.5 g daily of EPA as triacylglycerol for 1 year. The patients in the placebo group received olive oil. In-patients with active disease at entry, it was possible to demonstrate a significant steroid-sparing effect, but fish oil failed to prevent clinical relapse in the group of patients enrolled in remission. Remarkably, the leukotriene B_4 production in stimulated neutrophils was reduced by more than 50%.

Stenson et al.[21] carried out a randomised double-blind placebo-controlled crossover study with 5.4 g of n-3 PUFA as triacylglycerol (18 capsules daily), or olive oil as placebo, in 24 active UC patients.

The patients received treatment for 4 months followed by 1 month of washout. The study demonstrated that fish oil was able to induce a significant gain in body weight, significantly improve the histology score and reduce the leukotriene B_4 production in rectal dialysates by 60%. No significant steroid-sparing effect was found compared with placebo and the improvement in the endoscopy score did not reach a significant level (p = 0.06). Aslan and Tridafilopoulos[22] carried out a similar placebo-controlled crossover trial by giving 4.2 g of n-3 PUFA daily or corn oil as placebo. Seventeen active UC patients received treatment for 3 months followed by 2 months of wash-out. In 72% of patients, a steroid-sparing effect was seen and in 56% the activity score of the disease improved significantly. Improvement of the histology score did not reach statistical significance.

More recently Loeschke et al.[23] presented data on a placebo-controlled trial in the prevention of UC relapse. 64 patients in remission were randomised to receive 5.1 g of n-3 PUFAs as ethyl-esters or maize oil as placebo

for 2 years. The ongoing treatment with 5-aminosalicylic acid was allowed for 3 months. Interestingly after 3 months of study, the fish oil group had fewer relapses than the placebo group ($p < 0.02$) but this beneficial effect was lost by the end of the study (2 years). This leads to speculation that fish oil and 5-aminosalicylic acid may have synergetic effects and also that patient compliance in the fish oil group decreased during the study and could have affected the clinical outcome.

Lorenz-Meyer et al.[24] published data from a large, placebo-controlled trial in 204 CD patients. Patients were included after an acute relapse of their disease in which remission (CDAI < 150) was obtained under steroid therapy. Patients were randomised to receive either n-3 PUFA (n = 70) (5.1 g daily of fish oil as ethyl-esters), a carbohydrate-reduced diet, (72 g/daily) (n = 69) or placebo (corn oil)(n = 65) for 1 year. Low dose prednisolone was given to all patients for the first 8 weeks of the trial and then discontinued. On an intent-to-treat analysis, none of the treatments were able to prevent clinical flare-up, but the diet poor in carbohydrates seemed to be effective ($p < 0.05$), although it had the highest number of drop-outs, 20 of 69 (35%).

In a group of patients with CD, we investigated the ability of a new fish oil derivative to modify the phospholipids fatty acid profile in plasma and in red blood cells by means of its fatty acid absorption and incorporation[25]. This new fish oil formulation had two main characteristics: it was a free fatty acid mixture of 45% EPA and 20% of DHA and was coated in three different manners to minimize the fish oil side effects. The pills were coated with a special gastroresistant coating, to avoid breaking the capsules in the stomach and to obtain the delivery of the n-3 PUFAs to the first part of the small intestine. Our data showed that the free fatty acid mixture seems to be much better absorbed compared with the traditional triacylglycerol mixture and the double mechanism of release enables better absorption and incorporation. We then investigated the possible beneficial effects of this new n-3 PUFA preparation in the maintenance of remission in patients with CD[26].

Our patients were in clinical remission for less than 24 months before the study, and all had some laboratory evidence of inflammation. Patients of this type have about a 75% greater risk of relapse compared with patients who are in remission longer and have normal laboratory tests[27]. 78 patients were enrolled in the one-year study. The patients were randomly assigned to receive either three enteric-coated capsules of fish oil three times daily or three enteric-coated capsules of identical appearance containing 500 mg of placebo three times daily. The placebo contained a mixed acid triglyceride of fractionated fatty acids: 60 percent capryl acid and 40 percent capric acid. The fish oil capsules used contained 500 mg of a new marine lipid concentrate in free fatty acid form (40% EPA and 20% DHA) resulting in daily doses of 1.8 g of EPA and 0.9 g of DHA. The capsules were specially coated to resist gastric acid for at least 30 minutes and to disintegrate within 60 minutes. After 1 year, in the fish oil group (39 patients) 1 patient withdrew (moved away), 4 dropped out because of diarrhoea and 11 relapsed. In the placebo group (39 patients) 1 patient withdrew (moved away), 1 dropped out because of diarrhoea and 27 relapsed (intent-to-treat analysis: relapse rate 41 percent in fish oil group versus 74 percent in placebo group, difference 33 percent, 95 percent confidence interval, 13 to 54, $p = 0.003$). In the five patients with diarrhea, this began within the first month of treatment and did not improve when the daily intake of capsules was reduced. There were no other side effects. After one year of treatment 59 percent of the patients in the fish oil group were still in remission, as compared with only 26 percent in the placebo group ($p = 0.006$). Multivariate logistic regression analysis indicated that only fish oil treatment reduced the likelihood of relapse (odds ratio 4.2; 95% confidence interval, 1.6 to 10.7).

As you might probably know, two multicentre, well designed studies – EPIC-1 and EPIC-2 – are ongoing in North America and Europe to test whether a 70 % enteric coated EPA-DHA free fatty acid preparation (Epanova) is efficacious in two subgroup of patients affected by Crohn's disease. I think these studies will definitely establish if omega-3 fatty acids have a significant effect and can therefore be used for treating inflammatory bowel diseases.

Conclusions

My overall opinion about the majority of trials published so far on n-3 PUFAs for treating IBD patients is that they are not really comparable; n-3 PUFAs were in most cases added to other ongoing treatments such as 5-asa, steroids, immunosuppressors.

The obvious consequence of this overlap of treatments is the impossibility to comment on the real efficacy of n-3 fatty acids *per se*.

1. In the different studies, diverse kinds of fish oil derivatives preparations (concentrations, chemical form-ethyl-esters, tryglicerides,free fatty-coating) which are characterized by different intestinal absorption and incorporations into tissues (due to many annoying side effects influencing patients' compliance) were used[28-30]. As a consequence, the n-3 PUFAs dose assumed daily is not comparable.
2. Although samples are apparently homogeneous two different populations of patients were enrolled, as Crohn's patients in remission differ depending on the previous remission time they have had. Indeed, a stable remission, which means more than 2 years needs to be distinct from a remission occuring after an acute relapse[31]. These two subpopulations of Crohn's disease patients are both in remission but they cannot be campared since their risk of a new flare-up in the 12 following months is significantly different.
3. The placebos used in most of these studies were not real placebo, it is now very well known that olive oil[32-35], corn oil etc cannot be considered as such[36].
4. Some of these studies were not well designed: the "cross-over design" cannot be used for testing n-3 fatty acids because the "carry over effect" of the omega-3 PUFAs lasts more than 10 weeks (inhibition of cytokine production such as IL-1 beta and TNF alfa)[5] and therefore the wash out period (1 month) of these previous studies was not long enough.

I think the bottom line is that the number of well designed available trials is at the moment insufficient to allow any conclusions on the efficacy of omega-3 fatty acids, waiting for the EPIC-1 and EPIC-2 studies.

References

1) *Kromann N, Green A.* Epidemiological studie in the Upernavik district, Greenland: incidence of some chronic diseases 1950-1974. Acta Med Scand 1980; 208: 401-406.
2) *Ford-Hutchinson AW.* Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leucocytes. Nature 1980; 286: 264.
3) *Rampton DS, Collins CE.* Review article: thromboxanes in inflammatory bowel d isease-pathogenic and therapeutic implications. Aliment Pharm & Ther 1993; 7: 357-367.
4) *Lee TH, Hoover RL, Williams D, Sperling RI, et al.* Effect of a dietary enrichment with eicosapentaenoic acid and docosahexaenoic acids on in vitro polymorphonuclear and monocyte leukotriene generation and polymorphonuclear leukocyte function. N Engl J Med 1985; 312: 1217-1224.
5) *Endres S, Ghorbani R, Kelly VE, et al.* The effect of dietary supplementation with n-3 fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Engl J Med 1989; 320: 265-270.
6) *Calder PC.* N-3 polyunsaturated fatty acids and cytokine production in health and disease. Ann Nutr Metab 1997; 41: 203-234.
7) Endres S, von Schacky C. N-3 polyunsaturated fatty acids and human cytokine synthesis. Current Opinion in Lipidology 1996; 7: 48-52.
8) *Grimble RF, Howell WM, O'Reilly GO, et al.* The ability of fish oil to suppress tunor necrosis alfa production by peripheral mononuclear cells in healthy men is associated with polymorphisms in genes that influence tumor necrosis factor alfa production. Am J Clin Nutr 2002; 76: 454-459.
9) *Payan DG, Wong MYS, Chernov-Rogan T, et al.* Alterations in Human Leukocyte function induced by ingestion of eicosapenatenoic acid. J Clin Immunol 1986; 6: 402-410.
10) *Wang HH, Hung TM, Wei J, Chiang AN.* Fish oil increases antioxidant enzyme activities in macrophages and reduces atherosclerotic lesions in apoE-knockout mice. Cardiovasc Res 2004; 61: 169-176.
11) *Wakefield AJ, Sawyerr AM, Dhillon AP, et al.* Pathogenesis of Crohn's disease: multifocal gastrointestinal infarction. Lancet 1989; 1: 1057-1062.

12) *Danese S, Motte Cd Cde L, Fiocchi C.* Platelets in inflammatory bowel disease: clinical, pathogenic, and therapeutic implications. Am J Gastroenterol 2004; 99: 938-945.

13) *Jaschoenek K, Clemens MR, Scheurlen M.* Decreased responsiveness of platelets to a stable prostacyclin analogue in patients with Crohn's disease. Reversal by n-3 polyunsaturated fatty acids. Thromb Res 1991; 63: 667-672.

14) *Vanderhoof JA, Park JHY, Herrington MK, Adrian TE.* Effects of dietary menhaden oil mucosal adaptation after small bowel resection in rats. Gastroenterology 1994; 106: 94-99.

15) *French MA, Parrot AM, Kielo ES, et al.* Polyunsaturated fat in the diet may improve intestinal function in patients with CD. Biochim Biophys Acta 1997; 1360: 262-270.

16) *Shoda R, Matsueda K, Yamato S, Umeda N.* Epidemiologic analysis of Crohn disease in Japan: increased dietary intake of n-6 polyunsaturated fatty acids and animal protein relates to the increased incidence of Crohn disease in Japan. Am J Clin Nutr 1996; 63: 741-745.

17) *McCall TB, O'Leary D, Bloomfield J, et al.* Therapeutic potential of fish oil in the treatment of ulcerative colitis. Aliment Pharmacol Therap 1989; 3: 415-424.

18) *Lorenz R, Weber PC, Szimnau P, et al.* Supplementation with n-3 fatty acids from fish oil in chronic inflammatory bowel disease–a randomized, placebo-controlled, double-blind cross-over trial. J Int Med 1989; 225 (Suppl): 225-232.

19) *Best WR, Becktel JM, Singleton JW, Kern F.* Development of a Crohn's disease activity index. Gastroenterolology 1976; 70: 439-444.

20) *Hawthorne AB, Daneshmend, Hawkey CJ, et al.* Treatment of ulcerative colitis with fish oil supplementation: a prospective 12 months randomized controlled trial. Gut 1992; 33: 922-928.

21) *Stenson WF, Cort D, Rodgers J, Burakoff R, et al.* Dietary supplementation with fish oil in ulcerative colitis. Ann Intern Med 1992; 116: 609-614.

22) *Aslan A, Triadafilopoulos G.* Fish oil fatty acid supplementation in active ulcerative colitis: a double-blind, placebo-controlled, crossover study. Am J Gastroenterol 1992; 87: 432-437.

23) *Loeschke K, Ueberschaer B, Pietsch A, et al.* N-3 fatty acids retard early relapse in ulcerative colitis. Dig Dis Sci 1996; 41: 2087-2094.

24) *Lorenz-Meyer H, Bauer P, Nicolay C, et al.* Omega-3 fatty acids and carbohydrate diet for maintenance of remission in Crohn's disease. Scan J Gastroenterol 1996; 31: 778-785.

25) *Belluzzi A, Brignola C, Campieri M, et al.* Effects of a new fish oil derivative on fatty acid phospholipid-membrane pattern in a group of Crohn's disease patients. Dig Dis Sci 1994; 39: 2589-2594.

26) *Belluzzi A, Brignola C, Campieri M, et al.* Effect of an enteric-coated fish oil preparation on relapses in Crohn's disease. New Engl J Med 1996; 334: 1557-1616.

27) *Brignola C, Iannone P, Belloli C, et al.* Prediction of relapse in patients with Crohn's disease in remission: a simplified index using laboratory tests, enhanced by clinical characteristics. Eur J Gastroenterol Hepatol 1994; 6: 955-961.

28) *Appel LJ, Miller ER, Seidler AJ, Whelton PK.* Does supplementation of diet with "fish oil" reduce blood pressure? A meta-analysis of controlled following coronary angioplasty. Am J Prev Med 1992; 8: 186-192.

29) *O'Connors GT, Malenka DJ, Olmstead EM, et al.* A meta-analysis of randomized trials of fish oil in prevention of restenosis following coronary angioplasty. Am J Prev Med 1992; 8: 186-192.

30) *Kunzel U, Bertsch S.* Klinsche erfahrungen mit einem standardiserten fischolkonzenntrat. Feldstudie mit 3.958 hyperlipamischen patienten in der praxis des niedergelassenen arztes. Fortschr. Med 1990; 108: 437-442.

31) *Brignola C, De Simone G, Belloli C, et al.* Steroid treatment in active Crohn's disease: a comparison between two regimens of different duration. Aliment Pharmacol Ther 1994; 8: 465-468.

32) *Budiarso IT.* Fish oil versus olive oil. Lancet 1990; 336: 1313-1314. Letter.

33) *Grimble RF.* Interaction between nutrient, proinflammatory cytokines and inflammation. Clin Sci 1996; 91: 121-129.

34) *Petroni A, Blasevich M, Salami M, et al.* Inhibition of platelet aggregation and eicosanoids production by phenolic components of olive oil. Thromb Res 1995, 78: 151-160.

35) *Petroni A, Blasevich M, Papini N, et al.* Inhibition of leukocyte leukotriene-B4 production by an olive oil-derived phenol identified by mass-spectrometry. Thromb Res 1997; 87: 315-322.

36) *Horrobin D.F.* The regulation of prostaglandin biosynthesis by the manipulation of essential fatty acid metabolism. Rev Pure Appl Pharmacol Sci 1983; 4: 339-432.