EDITORIAL

N-3 POLYUNSATURATED FATTY ACID EFFECT IN PERIODONTAL DISEASE: STATE OF ART AND POSSIBLE MECHANISMS INVOLVED

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Anti-inflammatory properties have been widely reported for n-3 polyunsaturated fatty acids (PUFAs) and some studies have been focalized on their possible role in the modulation of gingivitis and alveolar bone resorption in periodontal disease (PD). Increased formation of arachidonic acid-derived inflammatory eicosanoids and augmented oxidative stress are two molecular mechanisms pathogenetically involved in the progression of PD and known to be inhibited by n-3 PUFAs in PD setting. The present review will focus also on other molecular pathways and factors known to be altered in the development of PD and known to be subject to n-3 PUFA modulation in other pathological settings different from PD. Overall, the available findings strongly encourage further experimental studies on animals subject to experimental PD and treated with n-3 PUFAs, long term n-3 PUFA intervention studies on PD patients and molecular studies to identify additional potential molecular routes of n-3 PUFA action in PD.

The hypothesis that diet improvement may be crucial in preventing and slowing progression of inflammatory diseases is now receiving great attention also for periodontal disease (PD) (1). Great interest has been concentrated over the past 25 years on the study of the anti-inflammatory effects of dietary long chain n-3 polyunsaturated fatty acids (PUFAs) present at high levels in fish oils, docosahexaenoic acid (22:6n-3, DHA) and eicosapentaenoic acid (20:5n-3, EPA) in a series of chronic inflammatory pathologies (atherosclerosis, arthritis, asthma, psoriasis, inflammatory bowel diseases) whose incidence is particularly increased in Western society (2). The aim of this review is to analyze published data supporting the hypothesis that n-3 PUFAs may have a role in the prevention and therapy of PD and to examine the possible molecular mechanisms involved in n-3 PUFA action.

n-3 PUFAs and periodontal disease: animal and human studies

A series of animal studies has been carried out to evaluate the possible beneficial role of n-3 PUFAs in PD. Treatment of rats with n-3 PUFAs has been demonstrated to significantly reduce the number of osteoclasts and pre-osteoclasts following pulp exposure (3). Moreover, in models of experimental periodontitis in rats, it has been observed that dietary n-3 PUFAs reduced the gingival tissue levels of Arachidonic acid (AA)-derived inflammatory

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mediators (4). In agreement, it has been reported a reduction in AA and AA-derived pro-inflammatory prostaglandin (PG)E_2 and leukotriene (LT)C_4 in the gingivae of rats fed a diet rich in n-3 PUFAs. Feeding fish oils to rats in a model of experimental tooth movement was also able to reduce the osteoclastic activity and the subsequent bone resorption (5). In a recent study, Kesavalu et al demonstrated the inhibitory effects of dietary n-3 PUFAs on alveolar bone resorption consequent to *Porphyromonas gingivalis* infection (6). Moreover, the newly-described EPA derivative Resolvin E1 has been found to confer dramatic protection against inflammation-induced tissue and bone loss associated with periodontitis in rabbits (7). On the other hand, it has been recently shown that dietary n-3 PUFAs failed to exert beneficial effects in terms of reactive protein C serum levels and inhibition of alveolar bone loss in rats subject to experimental periodontitis (8).

As far as interventional human studies are concerned, a pilot study was conducted (9) in which the anti-inflammatory properties of n-3 PUFAs were evaluated in healthy volunteers subject to experimental gingivitis by assessing the efficacy in terms of improvement of clinical signs. A tendency towards an improvement in inflammatory conditions under n-3 PUFA treatment was demonstrated through biochemical analysis of gingival tissue fragments. More recently, human subjects with periodontitis were supplemented with fish oil as a source of n-3 PUFAs, or with borage oil as a source of the n-6 PUFA γ-linolenic acid, the only n-6 PUFA known to possess anti-inflammatory properties similar to those of EPA and DHA. The authors found an improvement of gingival inflammation with both treatments (10). On the other hand, Eberhard et al (11) failed to observe beneficial effects of a topical application of n-3 PUFAs in patients subject to experimental periodontitis in terms of bleeding on probing frequency, gingivo-crevicular fluid volume and LTB_4 concentration.

Two available epidemiological studies (12-13) demonstrated that a) n-3 PUFA serum levels were lower in patients suffering from periodontal bone loss with respect to control group (12) and b) that the n-3 PUFA fatty acid composition of erythrocyte total phospholipid fraction was inversely related to tooth loss in humans (13).

Overall, the available studies demonstrate how knowledge is still incomplete in the field of n-3 PUFAs and PD. However, on the basis of many encouraging results obtained so far, it seems highly advisable to expend additional efforts to clarify the exact role of these fatty acids in the prevention and therapy of PD.

n-3 PUFAs and PD: possible molecular mechanisms involved

The molecular mechanisms known for being involved in PD and, at the same time, regulated by n-3 PUFAs are examined first (see paragraphs a and b). Afterwards, some molecular mechanisms (paragraphs c-g) recognized crucial to the development and progression of PD and known to be modulated by n-3 PUFAs in pathological settings different from PD are discussed.

a) Alteration in the levels and quality of PUFA derivatives: eicosanoids and docosanoids

It is believed that n-3 PUFAs attenuate PD progression by altering the pattern of AA (20:4n-6)-derived pro-inflammatory mediators, known to play a key role in the development of PD. In particular, LTB_4, PGE_2 and $PGF_2\alpha$ are considered important factors in the development of gingivitis and PD. It is known that EPA and DHA may alter AA oxidative metabolism competing with AA for acylation in membrane phospholipids. Moreover, EPA competes with AA for the cyclooxygenase (COX) and lipooxygenase enzymes, and produces eicosanoids with lower biological activity than those derived from AA. Finally, both DHA and EPA down-regulate the expression of the inducible form of COX, COX-2, which becomes overexpressed during periodontal inflammation (14). In a model of endotoxin-induced periodontitis of the rat, n-3 PUFAs have been shown to attenuate the progression of periodontitis by modulating the gingival levels of PGE_2, $PGF_2\alpha$ and LTB_4 (4). Similar results were observed also in human experimental gingivitis (9). Moreover, novel DHA- and EPA-derived eicosanoids and docosanoids, comprised in the classes of resolvins, docosatriens and protectines (7), and possessing powerful anti-inflammatory and protective properties particularly evident in the

resolution phase of inflammation, have been recently discovered. The EPA derivative Resolvin E1 confers dramatic protection against inflammation-induced tissue and bone loss associated with periodontitis in rabbit (7).

b) Modulation of oxidative stress

The oxidative stress caused by the excessive generation of reactive oxygen species (ROS) by leukocytes, fibroblasts, gingival cells and osteoclasts within periodontal tissues, is considered a crucial component in the abnormal response to plaque in PD. Recently (15), it was demonstrated that neutrophils from patients with chronic periodontitis exhibit not only hyper-reactivity following stimulation, but also an increased baseline ROS release. Moreover, it has been proven that ROS generated by neutrophil myeloperoxidase system *in vitro* is able to lyse gingival epithelial cells, and that ROS can activate osteoclast functions and differentiation. Furthermore, lower total antioxidant capacity was found in serum and gingival crevicular fluid of periodontitis subjects as compared with healthy controls (16) and analysis on dogs revealed significant negative correlation between total antioxidant capacity of serum and gingival crevicular fluid and gingival inflammation. It has been found that *Treponema denticola*, a PD pathogen, lowers the antioxidant reduced glutathione (GSH) levels within periodontal cells and that oxidative stress further depletes GSH. Finally, the level of GSH is significantly reduced in gingival crevicular fluid from periodontitis patients (17). A decreased pro-inflammatory cytokine gene expression and up-regulation of the antioxidant enzymes catalase and superoxide dysmutase have been recently shown in experimental PD of rats fed a n-3 PUFA diet (18). These results seem particularly interesting since, even though n-3 PUFAs are highly peroxidable, they may act as antioxidants, either up-regulating enzymatic and non-enzymatic antioxidant factors, or acting as precursors to novel metabolites such as neuroprotectins and resolvins, shown to be bioactive in inhibiting oxidative stress (19).

c) Down-regulation of cellular adhesion molecules (CAMs) in inflammation

Inflammatory mediators induce the expression of adhesion molecules in endothelial cells. Periodontopathogens are able to induce over-expression of CAMs in human endothelial cells *in vitro,* and increased endothelial CAM expression and augmented endothelial activation has been described in PD (20).

Modulatory effects of n-3 PUFAs on CAMs as observed in other settings

DHA is able to decrease the expression of VCAM-1, ICAM and selectins in saphenous vein endothelial cells activated by pro-inflammatory cytokines (21). Furthermore, in a population study it was shown that high dietary n-3 PUFA intake was associated with lower levels of soluble ICAM, VCAM and E-selectin (22).

d) Modification of metalloproteinase (MMP) expression

Overexpression of MMPs occurs when pathological destruction of tissues takes place. A key role in the loss of tooth-supporting structures in the course of PD is thought to be played by MMP-1, -2, -8, -9, -13 and -14. They are produced as a host response to periodontopathogens by circulating inflammatory or residential cells. Since they are present in different oral fluids (23), their wide use as biological non-invasive markers for periodontitis has been hypothesized.

Modulatory effects of n-3 PUFAs on MMPs, as observed in other settings

n-3 PUFAs are able to down-regulate the expression of MMP-13 and -3 in cultured explants of human osteoarthritic cartilage, while in rat placenta DHA suppresses the production of MMP-2 and -9. The down-regulation of several MMPs by n-3 PUFAs has also been reported to explain the anti-invasive and anti-metastatic effects of these fatty acids in tumors (24).

e) Modulation of transcription factor (NF-kB) expression and activity

The transcription factor NF-kB is activated in neutrophils stimulated by LPS derived from PD pathogen bacteria, and the same heat-killed bacteria induce apoptosis in human gingival epithelial cells, through NF-kB activation. Moreover, NF-kB has been found overexpressed in inflammatory sites of

human PD (25).

Modulatory effects of n-3 PUFAs on NF-kB as observed in other settings

n-3 PUFAs decrease the activity of NF-kB in LPS-stimulated inflammatory leukocytes. Moreover, transgenic mice able to endogenously biosynthesize n-3 PUFAs from n-6 PUFAs, possess a decreased NF-kB activity, a reduced expression of pro-inflammatory cytokines and higher resistance towards colitis (26).

f) Modulation of Osteoprotegerin/ Receptor activator of NF-kB ligand (RANKL)/RANK axis

RANKL and its receptor, RANK, have been recognized recently as key factors in the cytokine-activated pathways allowing osteoclastogenesis. The interaction of RANK and RANKL initiates a signaling cascade that results in differentiation and maturation of osteoclast precursor cells to active osteoclasts. Osteoprotegerin (OPG), a soluble TNF-receptor-like molecule, is the natural inhibitor of osteoclast differentiation. OPG binds to RANKL with high affinity and blocks RANKL from interacting with RANK. Overexpression of RANKL has been observed in human periodontal tissues which are sites of inflammation (27). The increase of RANKL/OPG ratio, was recently observed in the crevicular fluid of patients with periodontitis (28).

Effects of n-3 PUFAs on OPG/RANKL/RANK in other settings

Long term feeding with a diet rich in n-3 PUFAs decreases RANKL mRNA and enhances OPG mRNA expression in lymph nodes of mice affected by arthritis, inhibiting inflammation and bone loss in joints (29). Moreover, bone loss in ovariectomized mice is significantly attenuated by feeding diets enriched with fish oil, possibly through the decreased activation of RANKL in T cells.

g) Modification of Mitogen Activated Protein Kinase (MAPK) pathways

LPS from a PD pathogen, *Actinobacillus actinomycetemcomitans,* has shown to activate all the families of MAPKs inducing the *in vitro* synthesis of pro-inflammatory cytokines and other compounds involved in inflammation, such as chemokines, MMPs and PGs. In particular, the chemokine Monocyte Chemoattractant Protein-1, expressed in vascular endothelial cells of inflamed gingival tissues, and playing an important role in PD pathogenesis, was up-regulated in endothelial cells infected by PD pathogens through a mechanism involving the activities of the MAPKs p38 and JNK (30). Furthermore, the increased "tube formation" *in vitro,* expression of increased angiogenesis, was observed in endothelial cells exposed to *Porphyromonas gingivalis* LPS and took place through the activation of ERK1/2 (31), a MAPK related to the aberrant angiogenesis associated with chronic periodontitis.

Effects of n-3 PUFAs on MAPKs in other settings

n-3 PUFAs act as potent and efficacious broad-spectrum protein-kinase inhibitors (32). In particular, inhibition of JNK, by n-3 PUFAs was observed in normal cells activated by inflammatory stimuli. However, controversial results have been so far obtained on the effect of n-3 PUFAs on p38 activity and no effects, either activating or inhibiting, have been obtained so far. Moreover, EPA and DHA are able to inhibit angiogenesis decreasing ERK1/2 activation in colon cancer cells (32).

CONCLUSIONS

On the basis of the studies available, somehow controversial, additional experimental and human investigations seem essential to better clarify the real beneficial effects of n-3 PUFAs in the prevention and therapy of PD. Some possible molecular mechanisms by which n-3 PUFAs may prevent or slow progression of PD have already been clearly characterized. However, n-3 PUFA modulation of other molecular mechanisms known to have a crucial role in the progression and development of PD need to be more directly studied in the setting of PD. Often, these mechanisms are already known to be negatively regulated by n-3 PUFAs, but in pathological settings different from PD.

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