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The efficacy of host response modulation therapy (omega-3 plus low-dose aspirin) as an adjunctive treatment of chronic periodontitis (Clinical and biochemical study)

A randomized, double-blind, placebo-controlled study

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Background and Objective: Regeneration of lost periodontal tissues is considered to be one of the most challenging aspects of periodontal therapy. Our current understanding of the role of the host immuno-inflammatory response in periodontal diseases forms the basis of new therapeutic approaches. The aim of this study was to evaluate the efficacy of systemic administration of omega-3 polyunsaturated fatty acids plus low-dose aspirin as an adjunctive treatment to regenerative therapy of furcation defects.

Material and Methods: Forty patients displaying at least a single grade II furcation defect were enrolled in the study. They were randomly allocated into two groups: an experimental group receiving decalcified freeze-dried bone allograft (DFDBA) + omega-3 polyunsaturated fatty acids combined with low-dose aspirin; and a control group receiving DFDBA + placebo. Clinical parameters were monitored at baseline, and at 3 and 6 mo following therapy, and included plaque index, gingival index, gingival bleeding index, probing pocket depth and clinical attachment level. The biochemical markers assessed in gingival crevicular fluid samples were interleukin-1β and interleukin-10.

Results: The experimental intervention resulted in a greater mean probing pocket depth reduction ($P < 0.001$) and gain in clinical attachment ($P < 0.05$) compared

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with the control at 6 mo. Furthermore, the experimental protocol was able to achieve a significant modulatory effect on the levels of interleukin-1β and interleukin-10 compared with control therapy.

Conclusion: The findings suggest that the combination therapy demonstrated successful reduction of gingival inflammation, reduction of pocket depth and attachment level gain, accompanied by a trend for modulation of the cytokines profile in gingival crevicular fluid.

The ultimate goal of periodontal therapy is to completely restore periodontal tissues lost as a result of periodontal disease. Currently, various techniques have been utilized to achieve this goal in intrabony and furcation defects, including guided tissue regeneration, bone grafting and root surface conditioning, in addition to the use of biologically active agents such as enamel matrix derivative and polypeptide growth factors (1,2).

Several studies have been carried out to evaluate the efficacy of bone allograft materials in the regeneration of periodontal osseous defects. In general, these studies have shown significant radiographic bone fill and significant improvement in probing depth measurements and clinical attachment levels following treatment with demineralized freeze-dried bone allograft (DFDBA) compared with other treatment modalities (3). These studies have attributed the osteoinductive and osteoconductive potentials of DFDBA to bone-inductive components such as bone morphogenetic proteins (4). However, other studies conducted to evaluate the clinical benefits of the material in the treatment of human periodontal furcation defects were unable to demonstrate any statistically significant difference in either pocket depth reduction or clinical attachment gain between grafted and nongrafted sites (5,6). Furthermore, histological studies on the fate of implanted graft materials have provided evidence that DFDBA particles undergo turnover during bone remodeling. They noted that the rate of either DFDBA incorporation into or clearance from the healing site, which could impact the osteogenic potential of the grafted material, was correlated with the level of inflammation (7–10). Moreover, several studies have suggested that the control of local immuno-inflammatory reactions within the healing sites could enhance the regenerative outcomes following various grafting procedures (10,11). Many host-derived mediators and cytokines involved in orchestrating these local immuno-inflammatory processes have been recently utilized by several investigators to monitor therapeutic outcomes following various periodontal treatment modalities (12). Among these cytokines, interleukin (IL)-1β appears to play a central regulatory role in healing events following periodontal therapy (7) through the stimulation of MMP release and fibroblast proliferation, as well as the synthesis of both collagen and collagenase (8). In addition, IL-1β promotes bone resorption through activation of osteoclasts and stimulation of prostaglandin E2 synthesis (9). Studies have demonstrated that IL-1 genotype-positive patients are more prone to periodontal breakdown and are more likely to lose their teeth after conventional periodontal therapy (13). Recent studies have also demonstrated the negative impact of elevated levels of IL-1 on the long-term success and stability of guided tissue regeneration therapy in periodontal intrabony defects (14). Moreover, some studies have reported that elevated levels of this cytokine were correlated with the severity of osteolysis around implants and negatively influenced their longevity (15,16). On the other hand, IL-10 is an anti-inflammatory cytokine with known immunoregulatory functions, including suppression of proinflammatory cytokines and stimulation of IL-1 receptor antagonist (IL-1ra) (17). Some studies have suggested an association between the levels of IL-10 and periodontal status (12), while others did not verify this association (18). Recent research has been focused on new strategies to augment the regenerative outcomes following different periodontal treatment modalities. One strategy is the use of therapeutic agents targeting host response modulation as adjuncts to conventional or surgical treatment modalities for periodontal osseous defects. These agents now include nonsteroidal anti-inflammatory drugs (NSAIDs) (19), tumor necrosis factor-α antagonists (20), tetracyclines (e.g. low-dose doxycycline) (21) and bisphosphonates (22). NSAIDs, including aspirin (acetylsalicylic acid), possess anti-inflammatory properties as a result of their powerful inhibitory effects on cyclooxygenase metabolites such as prostaglandin E2 (23). However, aspirin is unique among current NSAIDs in that when the lipoxin pathway is activated in the presence of aspirin therapy, acetylation of the cyclooxygenase 2 enzyme present at sites of inflammation not only inhibits further production of prostanoids, but also induces the synthesis of 15R-hydroxyeicosatetraenoic acid, which is then transformed into 5(*S*)-epoxytetraene in leukocytes in the presence of 5-lipoxygenase. The 5(*S*)-epoxytetraene intermediate is then transformed to 15-epi-lipoxins, or aspirin-triggered lipoxins, which are more bioactive than native lipoxins and possess more powerful resolving properties (24).

Recently, several studies have evaluated the beneficial effects of fish oil as rich dietary sources of omega-3 polyunsaturated fatty acids (ω-3 PUFAs) on diverse physiological processes in the body and on a variety of chronic inflammatory diseases, including periodontal diseases (25). However, studies

investigating their effects on wound healing/regeneration following therapy are still lacking. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the major ω-3 PUFAs in fish oil, were found to alter cellular functions of polymorphonuclear leukocytes, modulate lymphocyte proliferation and enhance endogenous host antioxidant capacity. Moreover, they were shown to competitively inhibit the production of arachidonic acid metabolites via the cyclooxygenase and lipoxygenase pathways, thus reducing the synthesis of proinflammatory arachidonic acid metabolites accounting for the potent pro-resolving properties of ω-3 PUFAs (26). In addition, metabolism of ω-3 PUFAs results in the production of the pro-resolving lipid mediators, resolvins and protectins with anti-inflammatory and immunoregulatory actions, through regulating the trafficking of inflammatory cells to the sites of inflammation and blocking proinflammatory cytokine production, thus enhancing clearance of inflammation within the lesion to promote tissue regeneration (27,28). Interestingly, studies have demonstrated that metabolism of ω-3 PUFAs, when taken concurrently with aspirin, yields products (resolvins and protectins) that are more potent and stable than those endogenously produced (29). El-Sharkawy *et al.* (30), have recently evaluated the therapeutic benefits of ω-3 PUFAs plus low-dose aspirin as a new strategy targeting the host modulatory response. This regimen was utilized to augment the clinical outcomes of standard periodontal therapy (scaling and root planing) in a 6 mo clinical study on patients with chronic periodontitis. They concluded that this adjunctive treatment provided a sustainable, low-cost intervention for treatment of inflammatory periodontal diseases.

The aim of this study was to test the hypothesis that the systemic administration of ω-3 PUFAs plus low-dose aspirin would augment the outcomes following regenerative therapy of furcation defects in periodontitis patients, as assessed by both clinical parameters and biologic markers involved in the healing events.

Material and methods

Patient selection

This parallel-design, double-blind study was conducted on 40 patients (25 men and 15 women), 35–60 years of age (mean ± standard deviation: 42.6 ± 9.7 years). All patients were selected from the Periodontics Clinic, Faculty of Dentistry, October 6 University (Cairo, Egypt).

Eligible patients fulfilled the following criteria: suffering moderate to severe chronic periodontitis with at least a single grade II furcation defect according to Glickman's classification (31), determined by both clinical and radiographic examinations; free from systemic diseases known to influence the periodontal condition; and not receiving any medication known to affect the periodontal status. Moreover, the enrolled subjects had not received any periodontal therapy for a minimum of 6 mo prior to the study (32). Exclusion criteria for this study included: known hypersensitivity or allergy to one of the used medications; pregnancy or lactation; heavy smoking (≥ 10 cigarettes/d); a history of alcohol abuse; and participation in other clinical trials.

Study design

Initial visit— Subjects who met the inclusion/exclusion criteria were assigned numbers in ascending order by the study coordinator and completed a written medical history, which was confirmed verbally. Vital signs were taken, together with a comprehensive periodontal examination, by a single periodontist (S.A.R.) who also carried out motivation sessions and performed full-mouth supragingival and subgingival scaling and root planing for all enrolled patients. This initial phase of periodontal therapy was performed using periodontal scalars and curettes in two sessions completed 4–6 wk before commencement of the study and the subjects were given detailed instructions on self-performed plaque control. Clinical measurements, gingival crevicular fluid sampling, as well as reinforcement of oral hygiene at baseline and during follow-up evaluations were carried out by the same periodontist who remained masked to treatment allocation.

All subjects were randomly assigned by the study coordinator (H.T.), using a coin toss, to receive one of the two treatments. They were treated either with regenerative therapy of the furcation defect using DFDBA (AlloSource, Denver, CO, USA) adjuncted by omega-3 (Omega 3 plus; SEDICO Pharmaceutical Co., 6 October City, Egypt) (1 g, three times daily) plus low-dose aspirin [Aspocid; Chemical Industries Development (CID), Giza, Egypt] (75 mg of acetylsalicylic acid once daily) for 6 mo (experimental group), or with the same regenerative therapy and placebo pills (control group). Each omega-3 capsule provided 300 mg of DHA and 150 mg of EPA essential fatty acids. To achieve lot consistency over the 6 mo of the study, sufficient medication of a single lot was ordered. Coded medication or placebo packages containing two bottles each (omega-3 + aspirin or placebos) were prepared by the university pharmacy and given to the study coordinator who was the only person who had access to them.

The bottles were not decoded until after all the follow-up evaluations and final statistical analyses were completed, to ensure a proper double-blind study protocol. No adverse effects of the medications were reported by the study coordinator. The investigators in the current study were working independently of each other, and without discussing the clinical aspects of the study.

Checking subject's compliance

1 All subjects took part in motivation sessions during which oral hygiene instructions were given. The purpose of these sessions was to ensure that the subjects could maintain an appropriate level of oral hygiene before starting active treatment. These sessions were repeated until subjects showed the ability to maintain good plaque control.
2 Reinforcement of oral hygiene habits was carried out during reassessment appointments and at 4-wk intervals through telephone calls.

3 The necessary amounts of the medications for the first half of the study period were dispensed by the study coordinator. To check the adherence of individuals to the drug regimen, an extra number of pills were put in each bottle. During the 3- and 6-mo reassessments the unused medications were collected and counted.

All patients were informed of the objectives and nature of the study, including benefits and risks, and then their full signed consents were obtained prior to entry into the study. This study complied with the Helsinki Declaration of 1964, as revised in 2004 and approved by the Committee on Ethics Involving Human Subjects, of Cairo University, Egypt. There were no conflicts of interest in this study.

Clinical parameters

The clinical parameters plaque index (33), gingival index (34), gingival bleeding index (35), probing pocket depth and clinical attachment level were measured at the treated sites immediately before the surgical procedure (baseline) and after surgery, at 3- and 6-mo visits. These measurements were recorded using a Williams graduated periodontal probe and a curved, color-coded, blunt # 2, Nabers furcation probe (Thompson, Dental Mfg. Co. Missoula, MT, USA) and were rounded to the nearest millimeter. Pocket depths were measured from the gingival margin to the depth of the pocket, and clinical attachment levels were measured from the cemento–enamel junction to the depth of the pocket. Only one furcation entrance per tooth per subject was included in this study.

Gingival crevicular fluid sampling

The filter papers (Whatman chromatography paper; Whatman Lab Sales Ltd., Maidstone, Kent, UK) used for sample collection were pre-cut into strips of 2 mm × 8 mm (36). The strips were sterilized by autoclaving for 20 min. Each strip was weighed in an empty sterilized Eppendorf tube using a four-digit electronic scale (Eppendorf North America Inc., Hauppauge, NY, USA). The participants were instructed not to eat for a minimum of 2 h before the collection of gingival crevicular fluid. The selected tooth was isolated with cotton rolls, supragingival plaque was removed carefully without disturbance of the soft tissues, and the tooth was dried with a gentle stream of air for 5 s (37). Two filter paper strips (one for IL-10 determination and one for IL-1β determination) were used when obtaining each gingival crevicular fluid sample (38). The filter paper strips were introduced simultaneously into the furcation defect until mild resistance was felt and then left for 60 s to ensure collection of adequate quantities of gingival crevicular fluid. Filter paper strips contaminated with blood or saliva were discarded (39,40). The strips were reweighed following sample collection and the difference between the weights before and after collection represented the mass (mg) of the collected fluid on each filter paper.

The mass was converted to a volume (in mL) by assuming that the density of gingival crevicular fluid is approximately 1 (41).

Elution of gingival crevicular fluid from strips

One-hundred and fifty milliliters of phosphate-buffered saline, pH 7.4,was added to each plastic Eppendorf tube, using a sterilized pipette. The plastic Eppendorf tubes were then centrifuged at 3000 *g* for 5 minutes. After removal of the strips the supernatants were transferred into other sterilized plastic Eppendorf tubes and stored at −38°C until required for the assay procedures.

IL-1β and IL-10 assay

The amounts of IL-1β and IL-10 in the gingival crevicular fluid samples were assayed by solid-phase sandwich ELISAs using commercial kits from Diaclone, Tepnel Research (Besançon Cedex, France). The sensitivity of both ELISA kits was < 5 pg/mL, and all assay procedures were carried out according to the manufacturer's instructions. The absorbance of each well was assessed using a spectrophotometer set to a wavelength of 450 nm. The amount of each cytokine in the samples was determined from IL-1β and IL-10 calibration curves (15.6–500 pg/mL and 15–300 pg/mL, respectively) prepared by plotting the absorbance vs. the concentration of the cytokine (expressed as pg/mL) in the original samples (per 60-s sample time). The total amounts of each cytokine (pg/site) were obtained by multiplying the concentrations with the corresponding gingival crevicular fluid volumes (42).

Surgical phase

All surgical procedures were performed by the same periodontist (E.M.A.) who remained masked to treatment assignments. Following local anesthesia and intrasulcular incision, the mucoperiosteal flap was elevated. The incision and reflected flap were extended to include at least one tooth mesial and distal to the treated site. Soft-tissue debridement was performed at surgical sites along with root planing using hand and ultrasonic instruments. DFDBA (particle size 125–710 μm, human cortical powder) was prepared by pouring the graft material into a sterile dappen dish and reconstituted with sterile saline to obtain wet putty dough. The graft material was then placed in the furcation defect using a sterile carrier and condensed using a titanium-plated condenser to minimize the dead spaces between the DFDBA; this process was repeated until a slight overfilling of the defect was achieved. The mucoperiosteal flap was then replaced at its original position and sutured using the interrupted or horizontal mattress technique, without tension, to achieve primary wound closure over the graft material and furcation entrance, then a periodontal dressing was placed over the surgical area.

Coded medication or placebo packages were supplied to all participants following the surgical procedure. All subjects were instructed to refrain from brushing and flossing at the surgical sites for 2 wk postoperatively. During this time, they rinsed twice daily with 0.12% chlorhexidine gluconate

Table 1. Results of repeated-measures analysis of variance for the effect of treatment protocol and time on clinical parameters

	Group					
	Experimental group			Control group		
Variable	Baseline	3 mo	6 mo	Baseline	3 mo	6 mo
Plaque index	1.48 ± 0.6	0.79 ± 0.38†	1.10 ± 0.51†	1.37 ± 0.6	0.92 ± 0.41†	1.12 ± 0.35†
Gingival index	1.83 ± 0.52	0.94 ± 0.50†	0.78 ± 0.48†	1.62 ± 0.65	1.08 ± 0.41†	1.25 ± 0.46*†
Gingival bleeding index	1.61 ± 0.48	0.59 ± 0.22†	0.48 ± 0.24†	1.72 ± 0.31	0.68 ± 0.24†	0.79 ± 0.32*†
Probing pocket depth	5.6 ± 0.8	3.7 ± 0.8†	3.4 ± 0.6†	5.8 ± 0.7	4.4 ± 0.7*†	4.3 ± 0.6*†
Clinical attachment level	4.4 ± 0.8	3.2 ± 0.6†	2.9 ± 0.6†	4.6 ± 0.7	3.7 ± 0.7*†	3.5 ± 0.5*†

Values are mean ± standard deviation.
*Significant difference between the two groups at $p \leq 0.05$.
†Significant change over time compared with the baseline measurement at $p \leq 0.05$.

(Antiseptol; Kahira Pharm. and Chem. Ind. Co., Cairo, Egypt). Thereafter, gentle brushing with a soft toothbrush was initiated.

Postsurgical evaluation

Gingival crevicular fluid sampling and clinical soft tissue measurements, including plaque index, gingival index, gingival bleeding index, probing pocket depth and clinical attachment level were carried out at 3 and 6 mo postsurgery.

Statistical analysis

Data were presented as mean and standard deviation. Repeated-measures analysis of variance was used to study the effect of therapeutic modality and time on different variables. The significance level was set at $p \leq 0.05$. Data were explored using the Kolmogorov–Smirnov test of normality and were found to have a normal (parametric) distribution. Statistical analysis was performed using SPSS 16.0 (Statistical Package for Scientific Studies) for Windows (SPSS Inc., Chicago, IL, USA).

Results

Beneficial clinical impact of a regimen of ω-3 PUFAs + aspirin on clinical parameters

In the present study, both treatment modalities achieved a statistically significant reduction in the mean scores of plaque index, gingival index and gingival bleeding index at the treated sites in both groups during follow-up evaluations compared with baseline. However, by the end of the study the reduction of the mean gingival index and gingival bleeding index scores with the resultant dampening of inflammation were statistically different and more obvious in the experimental group compared with the control group ($p < 0.05$; Table 1).

At baseline, the mean probing pocket depth measurements were not significantly different between both groups ($p > 0.05$; Table 1). Meanwhile, the experimental intervention resulted in a mean probing pocket depth reduction of 1.9 mm vs. 1.5 mm in the control group by 3 mo ($p < 0.05$) and 2.3 mm vs. 1.6 mm, respectively, by 6 mo ($p < 0.001$; Table 1).

As regards changes in clinical attachment level, the results revealed a statistically significant reduction at 3 and 6 mo in both groups. Moreover, as observed with changes in probing pocket depth, the experimental sites showed a 1.2-mm gain in clinical attachment vs. 0.9 mm for the control group by 3 mo ($p < 0.05$) and 1.5 mm vs. 1.1 mm, respectively, by 6 mo ($p < 0.05$; Table 1).

Regulatory effect of ω-3 PUFAs + aspirin on gingival crevicular fluid levels of IL-1β and IL-10

The gingival crevicular fluid samples of both experimental and control sites showed a statistically significant reduction in the concentrations, as well as the total amounts, of IL-1β at 3 mo compared with baseline levels, with no significant difference between the two groups (Table 2). At 6 mo, although both treatments resulted in a statistically significant reduction of IL-1β, a greater and significant reduction of the cytokine concentrations ($p < 0.05$), as well as of the total amounts ($p < 0.001$), were achieved following the experimental protocol of DFDBA + ω-3 PUFAs + aspirin compared with DFDBA + placebo (Table 2).

On the other hand, the mean concentrations of IL-10 in the control group showed a nonsignificant change at 3 and 6 mo from baseline levels. Moreover, despite the increase in mean concentrations of IL-10 detected following the experimental protocol at 6 mo ($p < 0.05$), no significant differences were observed between the two groups (Table 2).

All patients enrolled in the present study returned for scheduled maintenance visits. There were neither dropouts nor major deviation in the use of medications.

Discussion

Regeneration of the lost attachment apparatus in molars with periodontal furcation defects still presents a great therapeutic challenge to many clinicians. In the past few years, various treatment modalities aimed at regeneration within periodontal furcation defects have been evaluated, including coronally positioned flaps, guided tissue regeneration, bone grafting and various combinations of these techniques (2,43). Despite the clinical success of DFDBA, variability in the reported clinical outcomes and limited efficacy in controlled studies have

Table 2. Results of repeated-measures analysis of variance for the effect of treatment protocol and time on the volume of gingival crevicular fluid and the cytokine levels

	Group					
	Experimental group			Control group		
Variable	Baseline	3 mo	6 mo	Baseline	3 mo	6 mo
Gingival crevicular fluid volume IL-1β	0.577 ± 0.13	0.335 ± 0.07†	0.195 ± 0.06†	0.591 ± 0.14	0.393 ± 0.06*†	0.408 ± 0.07*†
IL-1β concentration	211.7 ± 76.8	83.6 ± 52.1†	52.4 ± 36.4†	198.8 ± 85.5	91.7 ± 58.9†	88.1 ± 44.7*†
Total IL-1β	122.6 ± 53.5	27.9 ± 16.5†	10.2 ± 7.7†	119.8 ± 66.5	36 ± 24.2†	36.3 ± 13.2*†
Gingival crevicular fluid volume IL-10	0.593 ± 0.13	0.332 ± 0.09†	0.204 ± 0.06†	0.601 ± 0.14	0.423 ± 0.09*†	0.433 ± 0.09*†
IL-10 concentration	33.7 ± 11.7	35.6 ± 13.2	38.6 ± 13.9†	34.3 ± 15.1	35 ± 13.3	35.2 ± 13.6
Total IL-10	20.5 ± 9.6	11.9 ± 5.6†	7.9 ± 3.7†	20.6 ± 10.3	14.6 ± 5.7†	15.1 ± 6.2*†

Values are mean ± standard deviation.
IL, interleukin.
*Significant difference between the two groups at $p \leq 0.05$.
†Significant change by time compared with the baseline at $p \leq 0.05$.

raised some debate regarding its use alone in periodontal regeneration (44,45). This has urged many researchers to focus on the development of new therapeutic approaches to improve the clinical performance of the material and promote the regenerative outcomes.

In the present study, 1.35 g of DHA/EPA in a fish oil dietary supplement plus 75 mg of aspirin were administered daily for 6 mo. Evaluation of the clinical improvement following 6 mo of this treatment regimen plus DFDBA in the experimental group revealed a statistically significant reduction in the gingival index and gingival bleeding index mean scores compared with the control group. Notably, these scores started to show some increase at 6 mo following DFDBA plus placebo. Also, by the end of the study, the experimental intervention resulted in an improved mean probing pocket depth and gain in attachment (2.3 and 1.5 mm, respectively). The demonstrated significant improvement in clinical parameters in the ω-3 PUFA + aspirin group was continuous over the whole observation period, suggesting that a continuation of the experimental protocol may have resulted in further clinical improvement. This significant and sustainable enhancement of the regenerative process around the grafted material may be attributed to the beneficial effects of the combined host-modulating agents, being a source of stable, pro-resolving lipid mediators. These bioactive lipid mediators were found to display potent pro-resolution and anti-fibrotic activities (46) that could overcome the reported limitations in outcome following use of DFDBA as a result of bone loss around the graft material (10) and fibrous tissue encapsulation (47).

In the present study, the significant improvement in gingival inflammation, as well as in pocket depth and attachment level measurements achieved at 3 and 6 mo following ω-3 PUFA + aspirin adjunctive therapy, are in accordance with previous studies that have demonstrated the therapeutic efficacy of dietary supplementation of ω-3 PUFAs in reducing alveolar bone resorption through the reduction of osteoclastic activity and dampening of gingival inflammation through their anti-inflammatory properties (48). Moreover, the demonstrated clinical improvement is in agreement with previous evidence supporting the role of aspirin and aspirin-triggered lipoxins in reducing gingival inflammation, pocket depth and attachment loss, as well as modulating specific cytokines involved in periodontal wound healing (49,50). Also, it reveals the potential synergy of action reported in previous studies between ω-3 PUFAs and aspirin (29). It is interesting to note that, in the present study, the impact of the adjunctive therapy on periodontal treatment outcome is in agreement with the recently demonstrated beneficial effects of incorporating 900 mg of EPA/DHA in a fish oil dietary supplement given daily, in conjunction with 81-mg aspirin tablets for 6 mo, into a periodontal therapy protocol of scaling and root planing for periodontitis patients (30). The authors reported that the experimental protocol achieved statistically significant improvement of the tested clinical parameters, including reduction of the mean pocket depth and gain in attachment, compared with the control protocol. Moreover, they noted that this improvement was correlated with a significant reduction in the levels of specific biochemical markers of periodontal tissue destruction. Interestingly, in the current study, recovery of periodontal tissues at the treated sites in both groups was accompanied by a reduction in the levels of IL-1β that are known to influence connective tissue repair and bone healing (8,9). However, 6 mo following therapy, quantitative analysis of the IL-1β concentration in gingival crevicular fluid samples from the control sites revealed statistically significant higher levels of this cytokine than at the experimental sites. This reflected the down-regulatory actions of ω-3 PUFAs + aspirin on the synthesis of specific cytokines with a known detrimental effect on periodontal wound healing (51,52). The demonstrated significant reduction of IL-1β levels following the

ω-3 PUFA + aspirin regimen is supported by the findings of Alexander *et al*. (53), who reported a reduction in the concentration of IL-1β at 3 and 6 mo following surgical periodontal therapy (flap debridement or regenerative therapy). However, they noted that this reduction became significant only after 6 mo into the maintenance therapy.

On the other hand, gingival crevicular fluid sampling at different assessment intervals did not recover appreciable amounts of IL-10. Also, quantitative analysis of the cytokine concentration did not demonstrate significant variations in IL-10 levels between the two study groups at any assessment point. These findings are consistent with previous studies that failed to demonstrate significant effects of dietary supplementation of ω-3 fatty acids on the levels of IL-10 (54). In the meantime, the reduction in the mean total amounts of IL-10 following combined therapy in the experimental group was not surprising and did not contradict the interpretation of the results because it can be attributed to the reduction of mean gingival crevicular fluid volumes used for the calculation.

Studies have provided evidence that the anti-inflammatory and resolving effects of these fatty acids are dose- and time-dependent (55). However, the dose and time required to prevent or treat inflammatory conditions are not clear. Although several studies have suggested that dietary supplementation of a dose of about 1.5 g/d of EPA/DHA is sufficient to control inflammatory processes (56), others recommended higher doses (3.5 g/d) for longer durations (3–12 mo) (57).

As with all other medications, high doses of ω-3 fatty acids may carry potential risks. The American Heart Association has suggested that a dose of 0.5–1.8 g/d of EPA + DHA is generally regarded as safe in healthy people and significantly reduces death from heart disease. In addition, the American Heart Association guidelines recommend monitoring, by a physician, of people consuming high doses of EPA + DHA (> 3 g/d) because of the potential complication of excessive bleeding (58).

Conclusions

The current study suggests that a regimen of ω-3 fatty acids plus low-dose aspirin may provide therapeutic beneficial effects to augment the results following DFDBA regenerative therapy. In addition, the present results may suggest a combined potential of these agents to modulate the expression of specific cytokines known to play a role in periodontal regeneration.

Recommendations

Further human research is warranted to evaluate the effects of using this combination therapy on both clinical outcomes of other regenerative therapeutic modalities of periodontal osseous defects, as well as other cellular and molecular components of periodontal tissues involved in regeneration.

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