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Omega 3 Fatty Acids Reduce the Triglyceride Levels in Rats with Apical Periodontitis

Mariane Maffei Azuma[1], João Eduardo Gomes-Filho[1], Carolina de Barros Morais Cardoso[1], Camila Barbosa Pipa[1], Luis Gustavo Narciso[2], Suely Regina Mogami Bomfim[2], Rogério de Castilho Jacinto[1], Luciano Tavares Angelo Cintra[1]

[1]Endodontics, Araçatuba Dental School, UNESP - Universidade Estadual Paulista, São Paulo, SP, Brazil
[2]Clinic and Surgery and Animal Reproduction, Araçatuba School of Veterinary Medicine, UNESP - Universidade Estadual Paulista, São Paulo, SP, Brazil

Correspondence: Dr. Luciano Tavares Angelo Cintra, R. José Bonifácio, 1193, Vila Mendonca, 16015-050 Araçatuba, SP,, Araçatuba, SP, Brazil. Tel: +55-18-3636-2867. Fax: +55-18-3636-3253. e-mail: lucianocintra@foa.unesp.br

The aim of this study was to evaluate the influence of the prophylactic and therapeutic supplementation with omega 3 polyunsaturated fatty acids (ω-3 PUFAs) on the lipid profile and periapical bone resorption in rats with apical periodontitis. Forty male rats were divided into groups: control rats (C), rats treated with ω-3 PUFAs (C+O), rats with pulp exposure-induced apical periodontitis (AP), and rats with AP treated with ω-3 PUFAs (AP+O). The administration of ω-3 PUFAs was carried out orally once a day for 15 days before pulp exposure and, subsequently, for an additional 30 days after pulp exposure. AP was induced by exposing pulpal tissues to the oral environment. The samples were collected after 30 days. Triglycerides and cholesterol levels were enzymatically measured using the Trinder method. The jaws were collected and submitted for histological analysis. Two-way analysis of variance and Kruskal-Wallis tests were used for statistical analysis, and the significance was set at $p<0.05$. The triglyceride levels of the AP group were significantly higher than those of the C, C+O and AP+O groups ($p<0.05$). However, the difference in the cholesterol levels among the groups was not significant ($p>0.05$). Rats with AP showed larger areas of bone resorption as well as higher inflammatory intensity compared with rats with AP supplemented with ω-3 PUFAs. It may be concluded that the presence of multiple AP foci increased the triglyceride levels. In addition, omega 3 supplementation might reduce these levels in rats with AP, as well as the bone resorption areas of periapical tissues.

Key Words: apical periodontitis, lipid profile, omega 3 fatty acids, triglyceride levels, cholesterol levels.

Introduction

Apical periodontitis (AP) is an infectious disease characterized by pathologic bone destruction, which is mediated by various inflammatory mediators secreted from immunocompetent cells that have infiltrated the periapical tissues in response to intracanal bacterial infections (1). The influence of AP in systemic health has been studied, e.g., alterations in inflammatory cells, cytokines and lipid profiles in the blood (2-4). In this context, it has been observed that the presence of 1 focus of AP might enhance the levels of triglycerides in diabetic rats with periodontal disease (2). On the other hand, the presence of 1 focus of AP did not alter the cholesterol and triglyceride levels in normoglycemic rats (2). A systematic review using many cross-sectional studies indicated that the overall prevalence of perirradicular pathology in various patient populations is very high (5). Moreover, AP has occurred with an average of more than 2 lesions per patient (6). Thus, it would be very intriguing to study the impact of multiple AP foci on systemic health, with regard to the triglyceride and cholesterol levels. Also, alternative therapies that may reduce the lipid plasma levels that AP induces should be investigated.

Supplementation with omega 3 polyunsaturated fatty acids (ω-3 PUFAs)—mainly docosahexaenoic acid (DHA) and eicosapentaenoic (EPA)—can attenuate chronic inflammatory diseases via various mechanisms (7). Thus, ω-3 PUFAs have protected mice against infections, regulated serum triglycerides and cholesterol levels, inhibited the synthesis of lipid mediators and altered the cellular functions of inflammatory cells (8,9). Moreover, previous studies have demonstrated that ω-3 PUFAs serve as a substrate for enzymatic conversion to a novel series of lipid mediators called resolvins and protectins (10). The production of resolvins and protectins, which are mediators of the resolution of inflammation, is very important clinically because they are related to the induction of bone regeneration, modulating osteoclast and osteoblast cells functions (11). Because of all of these properties, the use of ω-3 PUFAs has been shown to have therapeutic value along with anti-inflammatory and protective actions in rheumatoid arthritis, cystic fibrosis, ulcerative colitis,

asthma, atherosclerosis, cancer, cardiovascular disease and periodontitis (11). A previous study demonstrated that ω-3 PUFAs not only decreased bone resorption but also promoted bone generation in the context of AP, decreasing osteoclasts and increasing osteoblast cells (12).

Therefore, the aim of this study was to evaluate the influence of the prophylactic and therapeutic supplementation with omega 3 polyunsaturated fatty acids (ω-3 PUFAs) on the lipid profile and periapical bone resorption in rats with apical periodontitis.

Material and Methods

The Institutional Ethics Committee (CEUA 2014-00550) of Universidade Estadual Paulista, São Paulo, Brazil, approved the experimental protocol, which was conducted in accordance with relevant guidelines. Forty male Wistar rats (*Rattus norvegicus albinus*), 6 weeks old, weighing 200-250g each, were used in this study. The rats were housed in a mini-isolator for rats (Alesco, São Paulo, SP, Brazil) in temperature-controlled rooms and were given ad libitum access to water and food.

The rats were randomly assigned to 4 groups with 10 rats each: control untreated rats (C), rats treated with ω-3 PUFAs (C+O), rats with pulp exposure-induced apical periodontitis (AP) and rats with pulp exposure-induced AP treated with ω-3 PUFAs (AP+O). The rats of the C+O and AP+O groups were orally gavaged with omega-3 fatty acid (Omega 3 Catarinense-Laboratório Catarinense S.A, Joinville, SC, Brazil) (40 mg/kg; 60% EPA and 40% DHA), and the rats of the C and AP groups received filtrate water 15 days before AP induction (prophylactic administration) and 30 days after AP induction (therapeutic administration). This means supplementation with omega-3 fatty acid or water took place for 45 days total (13).

The rats were intramuscularly anesthetized with ketamine (87 mg/kg; Francotar; Virbac do Brazil Ind. e Com. Ltda., Roseira, SP, Brazil) and xylazine (13 mg/kg; Rompun; Bayer S. A., São Paulo, SP, Brazil). The pulps of the right maxillary and mandibular first and second molars were exposed using surgical round burs (Broca Ln Long Neck; Maillefer, Dentisply Ind e Com Ltda, Petrópolis, Brazil) (groups AP and AP+O) for the development of AP (2) and were analyzed via histological analysis.

After 30 days of pulp exposure, the animals were killed with an overdose of the anesthetic solution. To determine the serum lipid profile, venous blood samples (50 µl) were collected via cardiac puncture after the rats had fasted overnight for 8–12 h. The blood samples were centrifuged immediately after collection at 1,800×g for 15 min at 4°C to obtain the plasma. The plasma total cholesterol and triglyceride levels were measured enzymatically by using a commercial kit (Cholesterol Liquiform Labtest® and Triglicérides Liquiform Labtest®, respectively—Labtest Diagnóstica Ind. e Com. Ltda, Lagoa Santa, MG, Brazil) (2).

For histologic analysis, the right hemi-jaw of each animal was harvested and soaked for 48 h in 4% formaldehyde. After demineralization with 18% EDTA, the samples were embedded in paraffin. Serial paraffin sections that were 6 mm thick were made of the mesial-distal aspects of the whole right lower first molars and were stained with hematoxylin-eosin. Histologic analysis was conducted by using the following parameters: the nature and extension of the inflammation, the presence and extension of necrosis, the state of the vasculature, and the pattern of the cellularity of the dental and periapical tissues.

The intensity of the inflammatory infiltration was graded as follows: absent (0 to few inflammatory cells: score 1); mild (<25 inflammatory cells: score 2), moderate (25-125 inflammatory cells: score 3) or severe (>125 inflammatory cells: score 4).

For the AP and AP+O groups, the area of the periapical lesion associated with the distal root of the mandibular first molars was histometrically measured. The area was calculated by rounding up the lesion boundary, considering the outer external surface of the alveolar bone, and it was expressed in square micrometers. For each rat, 7 serial histologic sections were measured histometrically using an image processing system, which consisted of a light microscope (DM 4000 B Leica), a color camera (DFC 500, Leica, Wetzlar, Germany), a color image processor (Leica Qwin V3 software, Leica, Wetzlar, Germany) and a personal computer (Intel Corel I5, Windows 10). The apical periodontitis areas were determined for each side, and the average value (mean ± standard deviation) was calculated for each experimental group.

Statistical Analysis

The levels of cholesterol and triglycerides were statistically determined using analysis of variance for multiple comparisons, and the Tukey test was used for pairwise comparisons at 5% significance. Lesion size was analyzed using the Student t Test at 5% significance. Histologic findings were analyzed with the Kruskal-Wallis test. Dunn's method was used for pairwise comparisons at 5% significance. Statistical analyses were performed by using SigmaPlot software (San Jose, CA, USA).

Results

Blood analysis was performed for all of the rats 30 days postoperatively. The changes in the plasma lipid concentrations of the rats of each group are shown in Table 1. No statistically significant differences were observed in the cholesterol levels among the groups ($p>0.05$).

The total triglyceride levels were statistically higher in

the AP group when compared with the C, C+O and AP+O groups ($p<0.05$). No statically significant differences were found in the triglyceride levels among the AP+O, C+O and C groups ($p>0.05$).

Representative hematoxylin-eosin–stained sections are shown in Figure 1. The histologic findings showed that all periapical tissues of teeth with pulp exposure developed apical periodontitis (Figures 1C and 1D). In addition, no inflammation was noted in the periapical region of the C and C+O groups (Figures 1A and 1B). However, in the AP and AP+O groups, the pulp showed signs of total necrosis 30 days after pulp exposure, and the AP was established and restricted exclusively to the periapical region (Figures 1C, 1D, 1G and 1H). Furthermore, the AP was composed primarily of neutrophils (polymorphonuclears) and mononuclear cells. The statistical analysis showed that the magnitude of the inflammatory reaction and bone loss was higher in the AP group compared with the AP+O, C+O and C groups ($p<0.05$) (Table 2). The inflammatory infiltrate was larger in the AP+O group when compared with the C and C+O groups ($p< 0.05$) (Table 2 and Fig. 1). The area of the periapical lesion of the mandibular first molar was larger in the AP group when compared with the AP+O group ($p<0.05$) (Table 2 and Fig. 1).

Discussion

AP was induced by creating pulpal exposure in rats' molars and then allowing subsequent bacterial infection to set in from the oral environment (2-4). This exposure was conducted over a 30-day period because this amount of time is sufficient to observe the development of AP and complete pulpal necrosis (3,4). AP induction was confirmed using histological analysis. Our results showed that ω-3 PUFAs decreased inflammatory cell infiltration as well as the AP bone resorption areas, which is in agreement with a previous study (12).

Rats received ω-3 PUFAs in both prophylactic and therapeutic administration, as both types of administration showed better outcomes in suppressing the bone resorption induced in periodontitis compared with rats that received therapeutic administration alone (13). In addition, a previous study elucidated that the concomitant prophylactic and therapeutic administration of ω-3 PUFAs decreased the bone resorption of rats with AP (12). To further explain, the prophylactic administration of omega 3 fatty acids for 2 weeks provides maximum increases in EPA and DHA levels in cell membranes (14), promoting and maintaining the membrane stability and fluidity in response to an inflammatory challenge caused by the induction of periodontitis.

Hypercholesterolemia induces pathologic manifestations, such as decreased myocardial capillary density and increased endothelial damage (15). Patients who develop periodontal diseases have shown increased total cholesterol levels, low-density lipoproteins, and triglyceride levels (16). On the other hand, a previous study performed using a rat's model showed that 1 AP focus did not alter the cholesterol levels is rats (2), which is in accordance with our results, as the presence of 4 AP foci did not alter the total cholesterol levels in rats. Further studies should be performed in order to investigate the role of AP and the supplementation with ω-3 PUFAs on the cholesterol parameters separately, as low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL).

Hypertriglyceridemia is associated with cardiovascular risk according to several epidemiological studies (17). In addition, each 1-mmol/l (88mg/dl) decrease in triglyceride levels has been associated with a decrease in the risk of coronary heart disease in 14% of men and 37% of

Table 1. Mean and standard deviation (mean±SD) values of the lipid profiles (in milligrams per deciliter) of the rats from the 3 groups

Groups	Lipid profiles (mean±SD)*	
	Cholesterol levels	Triglyceride levels
C	63.30 ± 10.10a	56.78 ± 13.89a
C+O	66±7,86 a	58,33±17,82 a
AP	58.20 ± 06.86a	73.38 ± 19.60b
AP+O	58.63 ± 03.96a	55.00 ± 08.54a

*Same letters in the columns indicate the absence of statistical differences among the groups ($p>0.05$)

Table 2. Scores and median of intensity of inflammatory cells and AP area (mm2) according to the groups

Intensity of inflammatory cells	Groups				Statistical analysis
	C	C+O	AP	AP+O	
1: absent	10/10	10/10	0/10	0/10	Kruskal-Wallis ($p<0.05$)
2: mild	0/10	0/10	0/10	4/10	
3: moderate	0/10	0/10	4/10	6/10	
4: severe	0/10	0/10	6/10	0/10	
AP area ($x10^4$ μm^2± SD)	13.1± 1.39a	12.53±1.07a	84.82±30.7b	46.58 ±17.7c	t- Student ($p<0.05$)

*Same letters in the columns indicate the absence of statistically significant differences among the groups ($p>0.05$).

woman (18). Previous studies showed that the presence and severity of periodontal diseases alter the triglyceride levels in humans (19,20), which is in accordance with this study, which observed an increase in the triglyceride serum levels of rats in the presence of 4 AP foci, showing that endodontic infections may influence systemic health. On the other hand, another study showed that only 1 AP focus did not alter the triglyceride levels of rats (2).

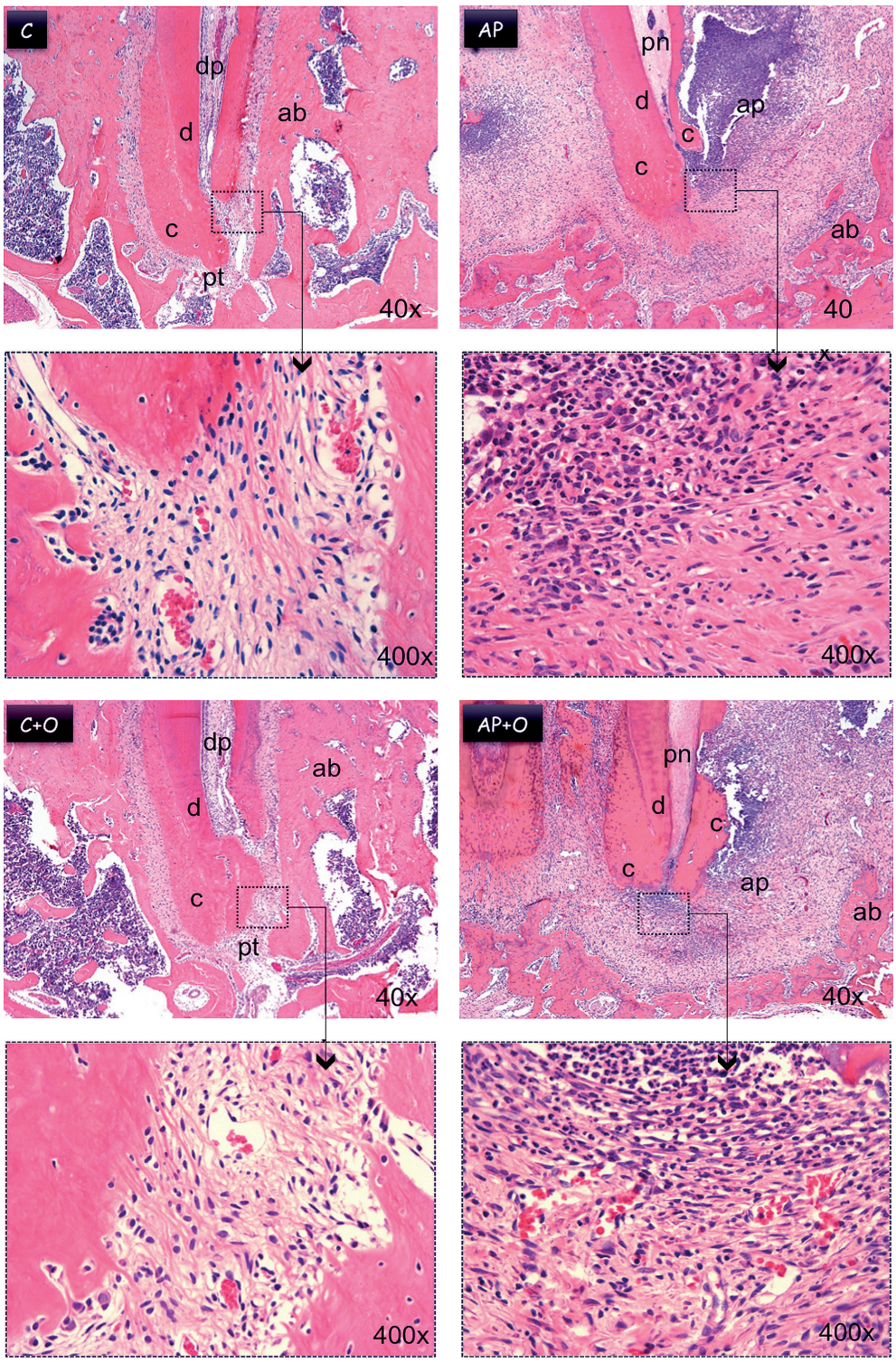

Figure 1. Representative photomicrography of healthy molars (groups C and C+O) and molars with AP after 30 days (groups AP and AP+O). C and C+O: Normal aspects of pulp tissue and periodontal insertion in the periapical region. AP and AP+O: Molars with apical periodontitis showing pulp necrosis and established periapical lesion with the presence of neutrophils (polymorphonuclears) and mononuclear cells. Hematoxylin-eosin staining, 40x and 400x original magnifications. Abbreviations: dp, dental pulp; d, dentin; c, cementum; pt, periapical tissues; ab, alveolar bone; pn, pulp necrosis; ap, apical periodontitis.

Therefore, the systemic alteration may be proportional to the amount and severity of the infection sites. An increase on triglycerides levels as a consequence of AP may be related to the presence of bacteria and its lipopolysaccharides (LPS). Previous studies have demonstrated that LPS-induced hypertriglyceridemia is due to a delay in catabolism of circulating lipids, as well as to decrease in adipose tissue lipoprotein lipase activity (21, 22). Another hypothesis is that the proinflammatory cytokines released during AP development (23), may increase the mobilization of lipids from liver and adipose tissue (24), altering theirs levels systemically.

In this study, the dietary with ω-3 PUFAs reduced the bone resorption area and inflammation of rats with endodontic infection, which is in agreement with a previous study (12). In addition the dietary with ω-3 PUFAs fatty acids reduced the triglyceride levels in rats with endodontic infections. Since periapical periodontitis is associated with an increase in some proinflammatory cytokines in blood (23), a decrease in the AP inflammation may be associated with the reduction of serum triglycerides levels. Another hypothesis is the fact that the main mechanism of ω-3 PUFAs is the direct inhibition of triglyceride synthesis and reduction of very-low-density lipoprotein assembly and secretion (25).

Therefore, the dietary with omega 3 fatty acids proved to be an alternative to reducing the triglyceride levels in individuals with 4 AP foci. The results of our study showed that the presence of multiple AP foci increases the triglyceride levels in rats. In addition, the diet with omega 3 fatty acids may reduce the triglyceride levels that have already been altered by AP as well as the bone resorption of periapical tissues.

Resumo

O objetivo deste estudo foi avaliar a influência da suplementação profilática e terapêutica com os ácidos graxos ômega-3 no perfil lipídico e na reabsorção óssea, em ratos com periodontite apical. Quarenta ratos machos foram divididos em grupos: ratos controle (C), ratos tratados com ácidos graxos ômega-3 (C+O), ratos com periodontite apical induzida por meio de exposição pulpar (PA), ratos com PA tratados com ácidos graxos ômega-3 (PA+O). A administração do ômega-3 foi realizada oralmente, uma vez ao dia durante 15 antes da exposição pulpar e, subsequentemente, por mais 30 dias depois da exposição pulpar. A PA foi induzida por meio da exposição do tecido pulpar ao ambiente oral. Após 30 dias, os ratos foram mortos e os níveis de triglicérides e colesterol foram mensurados pelo método enzimático de Trinder. As mandíbulas foram coletadas e submetidas à análise histológica. Análise de variância de dois fatores e teste de Kruskal-Wallis foram utilizados para análise estatística, e o nível de significância foi de $p < 0,05$. Os níveis de triglicérides do grupo PA foram significativamente maiores que dos grupos C, C+O e PA+O ($p<0,05$). Entretanto, não houve diferença significativa nos níveis de colesterol entre os grupos ($p>0,05$). Ratos com PA apresentaram maior área de reabsorção óssea bem como maior intensidade no infiltrado inflamatório comparados aos ratos com PA suplementados com ômega-3. Pode-se concluir que a presença de múltiplos focos de PA aumentou os níveis de triglicérides. Além disso, a suplementação com ômega-3 pode reduzir estes níveis em ratos com PA, bem como a área de reabsorção óssea dos tecidos periapicais.

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