Omega 3 Fatty Acids Reduce Bone Resorption While Promoting Bone Generation in Rat Apical Periodontitis

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Abstract

Introduction: This study evaluated the effects of the dietary supplement omega 3 polyunsaturated fatty acids (ω-3 PUFAs) on pulp exposure–induced apical periodontitis (AP) in rats. **Methods:** Twenty-eight male rats were divided into groups: control untreated rats (C), control rats treated with ω-3 PUFAs alone (C-O), rats with pulp exposure–induced AP, and rats with pulp exposure–induced AP treated with ω-3 PUFAs (AP-O). The ω-3 PUFAs were administered orally, once a day, for 15 days before pulp exposure and, subsequently, 30 days after pulp exposure. Rats were killed 30 days after pulp exposure, and jaws were subjected to histologic and immunohistochemical analyses. Immunohistochemical analyses were performed to detect tartrate-resistant acid phosphatase–positive osteoclasts and osteocalcin-positive osteoblasts on the bone surface of periapical area. Results were statistically evaluated by using analysis of variance and Tukey honestly significant difference, and $P < .05$ was considered statistically significant. **Results:** The bone resorption lesion was significantly larger in the AP group compared with AP-O, C, and C-O groups ($P < .05$). The level of inflammatory cell infiltration was significantly elevated, and the number of tartrate-resistant acid phosphatase–positive osteoclasts was significantly higher in the periapical lesions of the AP group compared with AP-O, C, and C-O groups ($P < .05$). The number of osteocalcin-positive osteoblasts was significantly increased in the AP-O group compared with the AP group ($P > .05$). **Conclusions:** Supplementation with ω-3 PUFAs not only suppresses bone resorption but also promotes new bone formation in the periapical area of rats with AP in conjunction with downregulation of inflammatory cell infiltration into the lesion. *(J Endod 2017;■:1–7)*

Key Words

Apical periodontitis, endodontic infection, omega 3 fatty acids, osteocalcin

Significance

Our findings indicate that supplementation with ω-3 PUFAs not only suppresses bone resorption but also promotes new bone formation in the periapical area of rats with apical periodontitis in conjunction with downregulation of inflammatory cell infiltration into the lesion.

Apical periodontitis (AP) is an inflammatory disease characterized by inflammatory bone destruction in response to intracanal bacterial infection (1). Bone remodeling processes, which are mediated by specific bone cells including osteocytes, osteoblasts, and osteoclasts, appear to be dysregulated in the apical periodontitis lesion (2). Currently, mechanical removal of infected dentin of root canals, accompanied by chemical disinfection, has been the standard procedure to treat apical periodontitis to reduce the level of inflammation at the affected site. However, to date, alternative therapies able to reduce bone resorption in apical periodontitis are not available.

Omega 3 polyunsaturated fatty acids (ω-3 PUFAs), as represented by eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been accepted as an adjuvant therapy in the treatment of chronic inflammatory diseases such as rheumatoid arthritis (3), cardiovascular disease (4), and diabetes (5). In addition, ω-3 PUFAs and lipid mediators derived from ω-3 PUFAs are reported to have important roles in the prevention of pathogenic bone resorption (6). These findings indicate that dietary supplement with ω-3 PUFAs inhibits the activation of proinflammatory arachidonic acid cascade, downmodulates acute inflammatory response by polymorphonuclear (PMN) leukocytes, and suppresses proliferation of lymphocytes and their production of proinflammatory cytokines (7, 8). The production of proinflammatory factors, such as prostaglandin E_2, interleukin-1β, and tumor necrosis factor-alpha, as a consequence of arachidonic acid cascade, acute inflammatory response by PMN, and lymphocyte activation, is known to promote osteoclastogenesis while suppressing osteoblastogenesis (9, 10). Many studies have reported the positive effects of ω-3 PUFAs as an adjunct therapy for periodontal disease (11–15). However, to the best of our knowledge, none of these studies has ever investigated the effect of ω-3 PUFAs on periapical bone

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0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2017.01.006

resorption processes in either human patients with apical periodontitis or animal models of endodontic infection.

Osteocalcin (OCN) plays a key role as a calcium-binding protein and is one of the most abundant non-collagenous proteins in bone tissue (16). The expression of OCN is considered to be a biomarker of bone formation (17). Especially, OCN is produced by osteoblast cells in their late stage of differentiation (18). Although it has been reported that ω-3 PUFAs can promote homeostatic osteoblastogenesis-mediated new bone formation (19), the effects of ω-3 PUFAs on the expression of OCN, as well as on pathogenic bone remodeling, remain unclear.

Therefore, this study aimed to evaluate the effect of the dietary supplement ω-3 PUFAs on the pathogenic bone resorption induced in a rat model of apical periodontitis by pulp exposure–elicited endodontic infection. To evaluate the impact of ω-3 PUFAs on dysregulated bone remodeling processes, tartrate-resistant acid phosphatase (TRAP)-positive osteoclast cells, as well as OCN-positive osteoblasts, were monitored through histologic and immunohistochemical analyses.

Materials and Methods

Experimental Animals

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

The rats were randomly assigned into 4 groups (7 rats/group):

1. Control untreated rats (C)
2. Healthy rats treated with ω-3 PUFAs (C-O)
3. Rats with pulp exposure–induced AP
4. Rats with pulp exposure–induced AP treated with ω-3 PUFAs (AP-O)

Supplementation with ω-3 PUFAs

Rats in the C-O and AP-O groups were orally gavaged with ω-3 PUFAs (Omega 3 Catarinense-Laboratório Catarinense S.A, Joinville, SC, Brazil) (water solution, 40 mg/kg; 60% EPA and 40% DHA, once a day), whereas rats in groups C and AP received control distilled water during 15 days before AP induction (prophylactic administration) and 30 days after AP induction (therapeutic administration). As a consequence, rats were treated with either ω-3 PUFAs or control water for a total period of 45 days (12, 13).

Induction of AP

Rats were 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 pulp of the right mandibular first molar was exposed by using a dental round bur (Broca Ln Long Neck; Maillefer, Dentisply Ind e Com Ltda, Petrópolis, SP, Brazil) (groups AP and AP-O) for the development of AP. The coronal pulp tissue was exposed to the oral cavity for 30 days.

Immunohistologic Analyses

Mandibles sampled from killed rats were decalcified in 10% EDTA for 30 days and submitted to conventional histologic processing for the creation of paraffin-embedded tissue sections. Semi-serial sections (4 μm) were performed in the laterolateral direction, allowing sectioning of the mandibular first molar in its longitudinal axis. Sections were stained with hematoxylin-eosin (H&E) or submitted to immunohistochemistry by using an indirect immunoperoxidase technique for TRAP (primary antibody goat anti-TRAP SC 30832; Santa Cruz Biotechnology, Santa Cruz, CA) and primary antibody rabbit anti-OCN SC-30044 for OCN, following the previously described protocol (20). Histopathologic, histometric, and immunohistochemical analyses were performed by a certified histologist (E.E.) who was blinded to the experimental groups.

Histologic analysis was conducted by using the following parameters: nature and extension of inflammation, presence and extension of necrosis, state of vasculature, and pattern of cellularity of dental and periapical tissues.

The intensity of 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 AP and AP-O groups, the area of 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 alveolar bone, and it was expressed in square micrometers. For each rat, 7 serial histologic sections were measured histometrically by using an image processing system that 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), and a personal computer (Intel Core I5, Intel Corp, Santa Clara, CA; Windows 10, Microsoft Corp, Redmond, WA). The AP areas were determined for each side, and the average value (mean $\pm$ standard deviation) was calculated for each experimental group.

The numbers of osteoclasts and osteoblasts were analyzed in the histologic section used for histometric analysis. The perimeter was calculated by contouring the boundary of the AP with the aid of Leica Microsystems software. The numbers of TRAP-positive multinucleated cells as well as OCN-positive cells were calculated in the perimeter and expressed in cells/millimeter.

Statistical Analysis

The numbers of TRAP-positive cells, OCN-positive cells, and lesion size were statistically determined by using analysis of variance for multiple comparisons, and the Tukey test was used for pairwise comparisons at 5% significance. Histologic findings were analyzed with the Kruskal-Wallis test. The Dunn method was used for pairwise comparisons at 5% significance. Statistical analyses were performed by using SigmaPlot software (San Jose, CA).

Results

Histopathologic and Histometric Analysis

To evaluate the effects of ω-3 PUFAs on cell infiltrate in the AP, histologic images of H&E-stained periapical area in the different experimental groups of rats killed at day 30 were performed and are shown in Figure 1. No sign of inflammation was noted in the periapical tissue of C or C-O groups (Fig. 1*A* and *B, E* and *F*). However, in the AP and AP-O groups, the dental pulp showed signs of total necrosis at 30 days after pulp exposure, and the enlargement of periapical space between bone and cementum of tooth apex was observed, indicating that bone surrounding apex was pathogenically resorbed in response to pulp exposure (Fig. 1*C* and *D, G* and *H*). Furthermore, these lesions