Omega-3 Fatty Acids Reduce Inflammation in Rat Apical Periodontitis

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Abstract

Introduction: The effects of omega-3 polyunsaturated fatty acids (ω-3 PUFAs) on pro- and anti-inflammatory mediators were evaluated in a rat model of pulp exposure–induced apical periodontitis (AP). **Methods:** Twenty-eight male Wistar rats were divided into 4 groups: control, untreated rats (group C); control rats treated with ω-3 PUFAs (group C-O); rats with pulp exposure–induced AP (group AP); and rats with pulp exposure–induced AP treated with ω-3 PUFAs (group AP-O). Omega-3 PUFAs were administered orally once a day for 15 days before pulp exposure; this treatment was continued for 30 days after pulp exposure. The rats were sacrificed 30 days after pulp exposure, and their dissected jaws were subjected to immunohistochemical analysis to detect immunoreactivity for tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, IL-1β, IL-17, and IL-10 on the periapical bone surface. The results were statistically evaluated using analysis of variance and the Tukey post-test. The significance level was set at 5%. **Results:** Immunoreactivity for the proinflammatory cytokines TNF-α, IL-6, IL-1β, and IL-17 was higher in the AP group than in the AP-O, C, and C-O groups ($P < .05$). Immunoreactivity for the anti-inflammatory cytokine IL-10 was lower in the AP group than in the AP-O group ($P < .05$). **Conclusions:** Supplementation with ω-3 PUFAs can modulate the inflammatory response in rat AP, decreasing levels of TNF-α, IL-6, IL-1β, and IL-17 but increasing levels of IL-10. *(J Endod 2018;■:1–5)*

Key Words

Apical periodontitis, cytokine, endodontic infection, omega-3 fatty acid

Significance

Our findings indicate that systemic oral administration with ω-3 PUFAs can modulate the inflammation in rat apical periodontitis, decreasing TNF-α, IL-6, IL- 1β, and IL-17 and increasing IL-10 expression.

Apical periodontitis (AP) is characterized by the presence of fibrous and granulated tissue as well as by infiltrates of various inflammatory cells (T lymphocytes, B lymphocytes, and macrophages) (1). This periapical inflammation is an immune response against bacterial infection in necrotic pulp. When host defense mechanisms cannot eradicate the infection over time, chronic AP ensues, leading to destruction of the bone structures in the periapical area (2).

In AP, several proinflammatory cytokines are produced locally to mediate the immune response, and these promote bone resorption (3, 4). In particular, proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, IL-1β, and IL-17 may stimulate osteoclastogenesis and bone resorption (5). Such "cytokine storms" are related to the development of AP (4, 5).

Conversely, some studies have suggested that IL-10, which can suppress the production of proinflammatory cytokines such as IL-1, IL-6, and TNF-α (6, 7), slows AP development (5). This suggests that, to reduce bone loss during AP, researchers must develop therapeutic regimens that can down-regulate the proinflammatory response and increase the anti-inflammatory response.

Omega-3 polyunsaturated fatty acids (ω-3 PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been studied as an adjuvant therapy to treat oral diseases, including gingivitis (8), periodontitis (9–11), AP (12), and recurrent aphthous stomatitis (13). Related findings have indicated that diets supplemented with ω-3 PUFAs inhibit the production of arachidonic acid metabolites via the cyclooxygenase 2 and lipoxygenase pathways, thus reducing the proinflammatory response (14) and decreasing inflammatory symptoms (13). Another study involving pulp exposure–induced AP in rats showed that ω-3 PUFAs reduce bone resorption and promote bone generation, leading to a decrease in tartrate-resistant acid phosphatase-positive cells and an increase in osteocalcin-positive cells (12). However, to our knowledge, no study has ever investigated the effects of ω-3 PUFAs on cytokine expression in either human AP patients or animal models of endodontic infection.

Therefore, the present study aimed to evaluate the effects of an ω-3 PUFA–supplemented diet on pathogenic bone resorption in a rat model of pulp exposure–induced

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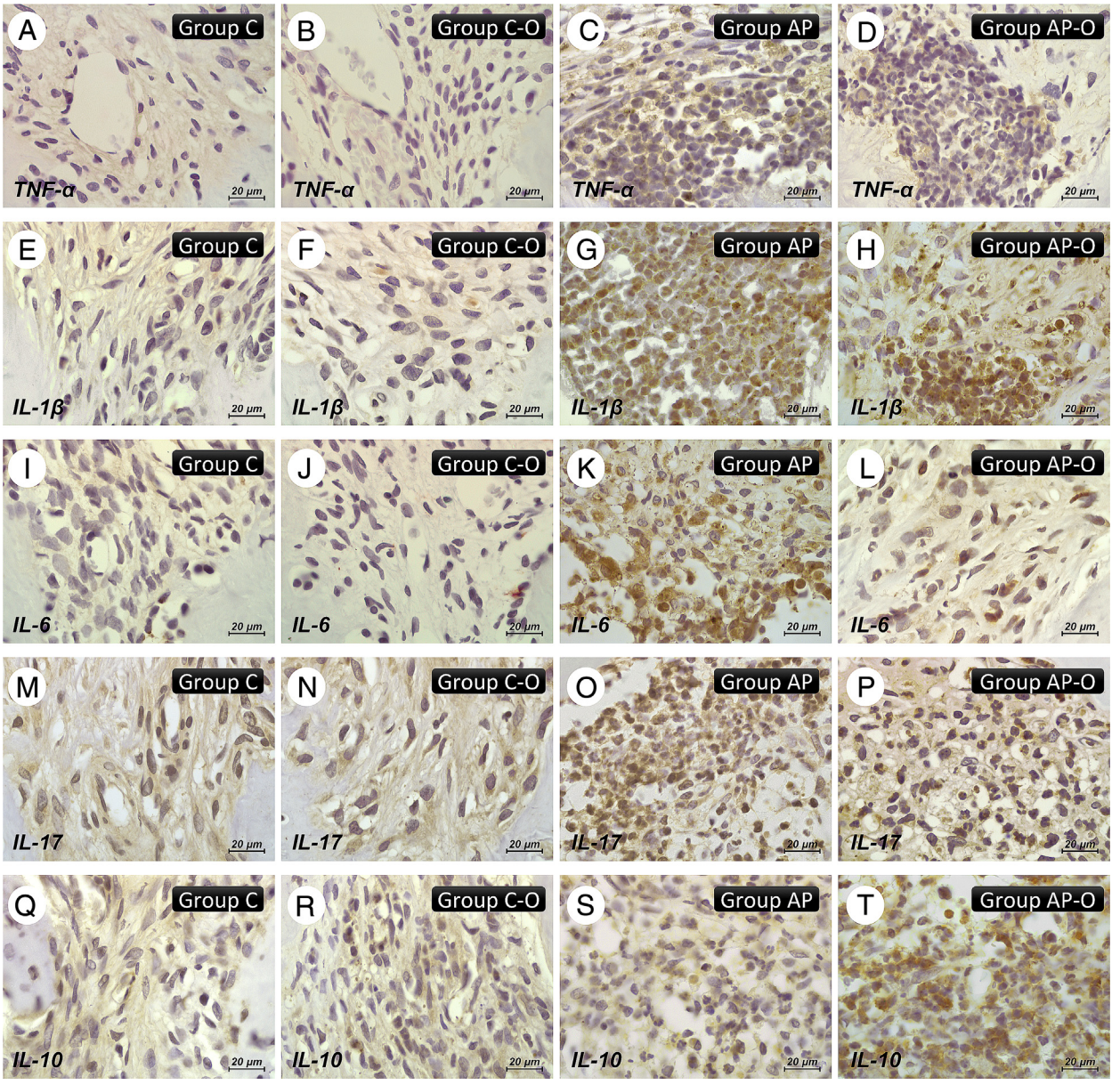

Figure 1. Immunoreactivity of TNF-α, IL-1β, IL-6, IL-17, and IL-10 in the periapical bone 30 days after pulp exposure. Representative images of the TNF-α immunoreaction among the groups: (*A*) group C, (*B*) group C-O, (*C*) group AP, and (*D*) group AP-O. Representative images of the IL-1β immunoreaction among the groups: (*E*) group C, (*F*) group C-O, (*G*) group AP, and (*H*) group AP-O. Representative images of the IL-6 immunoreaction among the groups: (*I*) group C, (*J*) group C-O, (*K*) group AP, and (*L*) group AP-O. Representative images of the IL-17 immunoreaction among the groups: (*M*) group C, (*N*) group C-O, (*O*) group AP, and (*P*) group AP-O. Representative images of IL-10 immunoreaction among the groups: (*Q*) group C, (*R*) group C-O, (*S*) group AP, and (*T*) group AP-O. Original magnification: 400×.

AP. To accomplish this, we used immunohistochemical analysis to monitor the effects of ω-3 PUFAs on the production of the proinflammatory cytokines TNF-α, IL-1β, IL-6, and IL-17 and the anti-inflammatory cytokine IL-10 in rat periapical tissues.

Materials and Methods

Experimental Animals

The experimental protocol was approved by the Institutional Ethics Committee (CEUA 2014-00550) of the Universidade Estadual Paulista, São Paulo, Brazil, and it was conducted in accordance with relevant guidelines. Six-week-old male Wistar rats (*Rattus norvegicus albinus*) (n = 28), weighing 200–250 g each, were used in this study. The rats were housed in mini-isolators equipped with the Ventilife system (Alesco, São Paulo, SP, Brazil), which injects air at a continuous, low-speed flow. In this way, the system maintains ideal air exchange and internal pressure, isolating the animals from external influences. The rats were maintained in temperature-controlled rooms and given ad libitum access to water and food.

The rats were randomly assigned to 4 groups of 7 animals each: control, untreated rats (group C); control rats treated with ω-3 PUFAs (group C-O); rats with pulp exposure–induced AP (group AP); and rats with pulp exposure–induced AP treated with ω-3 PUFAs (group AP-O).

Supplementation with ω-3 PUFAs

For 15 days before AP induction (prophylactic administration) and 30 days afterward (therapeutic administration), rats in the C-O