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Selective cyclooxygenase-2 inhibition prevents bone resorption

Inibidor seletivo de cicloxigenase-2 prevenindo reabsorção óssea

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ABSTRACT: The aim of the present work was to evaluate the effect of a selective cyclooxygenase-2 (COX-2) inhibitor (meloxicam) on the alveolar bone loss progression in experimentally induced periodontitis. Forty (40) Wistar rats were separated into 8 experimental groups (n = 5). Cotton ligatures were placed at the gingival margin level of the lower right first molars of some rats. Four groups were treated for 5 or 15 days with an oral dose of 15 mg/kg of body weight/day of the selective COX-2 inhibitor. The other groups were used as positive control (sham) or negative control in each experimental period. Standardized digital radiographs were taken after sacrifice at 5 and 15 days to measure the amount of bone loss at the mesial root surface of the first molar tooth in each rat. The treatment with meloxicam did not induce weight alteration or other visible systemic manifestations. One way analysis of variance (ANOVA) indicated that groups treated with meloxicam, after 5 days, had significantly less alveolar bone loss ($p < 0.05$) when compared with control groups. On the other hand, no significant differences in bone loss were observed after 15 days of treatment with meloxicam. These data provide evidence that systemic therapy with meloxicam can modify the progression of experimentally induced periodontitis in rats during the initial experimental period.

DESCRIPTORS: Alveolar bone loss; Anti-inflammatory agents, non-steroidal; Cyclooxygenase inhibitors; Periodontitis; Radiography, dental.

RESUMO: O objetivo deste trabalho foi avaliar o efeito de um inibidor seletivo da cicloxigenase-2 (COX-2) (meloxicam) na progressão da perda óssea alveolar durante o desenvolvimento da doença periodontal experimental induzida. Quarenta (40) ratos Wistar foram separados em 8 grupos experimentais (n = 5). Ligaduras de fio de algodão foram colocadas na margem gengival do primeiro molar inferior direito de alguns ratos. Quatro grupos foram tratados por 5 ou 15 dias com uma dose oral de 15 mg/kg de peso corporal/dia do inibidor seletivo de COX-2. Os outros grupos foram usados como controle positivo (sham) e controle negativo dentro de cada período experimental. Radiografias digitais padronizadas foram realizadas para medir a perda óssea na região mesial do primeiro molar inferior de cada rato. O efeito do tratamento com meloxicam não induziu alteração de peso ou outras manifestações sistêmicas visíveis. A Análise de Variância (ANOVA) indicou que os grupos tratados com meloxicam após 5 dias apresentaram perda óssea alveolar significativamente menor ($p < 0,05$). Por outro lado, a perda óssea não foi significativa após 15 dias de tratamento com meloxicam. Os dados apresentados no presente trabalho sugerem que o tratamento sistêmico com meloxicam pode modificar a progressão da periodontite experimental em ratos no período experimental inicial.

DESCRITORES: Perda óssea alveolar; Antiinflamatórios não esteróides; Inibidores de ciclooxigenase; Periodontite; Radiografia dentária.

INTRODUCTION

Cyclooxygenase (COX) catalyzes the conversion of arachidonic acid to prostaglandin H_2, which serves as the common precursor for the synthesis of prostaglandins, prostacyclins and thromboxanes. There are two isoforms of cyclooxygenases, which exhibit similar catalytic properties but differ in terms of regulation of expression[4,5,12]. COX-1 is the major isoform present in healthy tissues and generates prostanoids for different functions such as gastric cytoprotection, maintenance of

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renal homeostasis and platelet aggregation. The second isoform, COX-2, is also present at basal level in certain tissues, but its expression is up-regulated in response to inflammatory or mitogenic stimuli.

Prostaglandins, formed by cyclooxygenase, are important mediators for a number of physiological processes and pathophysiological conditions, including inflammation[18].

Prostaglandin E_2 (PGE_2) and other arachidonic acid metabolites have a recognized role in the pathogenesis of periodontal disease as important proinflammatory mediators in gingivitis and alveolar bone resorption. Elevated levels of PGE_2 are detected in the crevicular fluid of periodontitis patients, a finding that has been related to the increased severity of the disease, because the cyclooxygenase product PGE_2 is, potentially, the most important mediator of alveolar bone loss. Non-steroidal anti-inflammatory drugs (NSAIDs) are cyclooxygenase selective inhibitors and prevent the synthesis of prostaglandins, thromboxanes and prostacyclins[2]. The use of non-steroidal anti-inflammatory drugs (NSAIDs) can lead to the inhibition of PGE_2 synthesis by the direct competitive or non-competitive inhibition of the cyclooxygenase enzyme. Retrospective human studies have shown less periodontal destruction in subjects chronically ingesting various anti-inflammatory drugs in comparison with controls[3,19]. Additionally, longitudinal studies, extending from 3 months to 12 months, have shown that the administration of NSAIDs significantly reduces alveolar bone loss[6,9,10,17].

Meloxicam is a non-steroidal anti-inflammatory drug, a derivative from oxicam, and may be used as a selective COX-2 inhibitor[1]. Because of its selective action[16], meloxicam has some advantages over the conventional anti-inflammatory drugs that act similarly on the COX-1 and COX-2 isoenzymes, because it reduces the damage in the gastric mucosa and does not change the platelet aggregation process[1]. The aim of the present study was to evaluate the effect of meloxicam, an agent that selectively inhibits COX-2, on the progression of alveolar bone loss in an experimental model of periodontitis in rats using radiographic analysis.

MATERIAL AND METHODS

Animals

Forty male Wistar (*Novergicus albinus*) rats were housed in polypropylene cages in groups of 5 per cage and treated with standard laboratory chow (Labina, Purina, SP, Brazil) and water *ad libitum.* All protocols described below were approved by the Institutional Experimentation Committee of the School of Dentistry of Cascavel, State University of Paraná.

Experimental protocols

The rats were randomly divided into 8 experimental groups with 5 rats each. Two groups were treated with saline solution (0.9% NaCl) and used as controls. Two groups were treated with meloxicam (Neo Química, São Paulo, Brazil) PO, in a dose of 15 mg/kg of body weight/day. Two groups received a cotton ligature (Coats Corrente Ltda., SP, Brazil) around the lower right first molar in a sub marginal position to induce experimental periodontitis, according to the methods proposed by Johnson[11] (1975). The anesthesia was obtained by intramuscular administration of 0.08 ml/100 g of body weight of Ketamine (Francotar, Virbac do Brasil Ind. e Com. Ltda., São Paulo, SP, Brazil). The other 2 groups received the cotton ligature and were treated with meloxicam. The rats were sacrificed 5 or 15 days after commencement of the experimental protocol. The distribution of the animals is summarized in Table 1.

Radiographic analysis

The rat mandibles were removed to determine the degree of bone loss. Standardized digital radiographic images were obtained with the use of a computerized imaging system (Sens-A-Ray 3.11, London, UK) that utilizes an electronic sensor instead of an X-ray film. Electronic sensors were exposed at 70 kV and 8 mA with the time of expo-

TABLE 1 - Distribution of the groups of rats (n = 5), according to the treatment and time of the experiment (days). Groups I and V were considered control groups.

Groups	Treatment	Time of treatment (days)
I	Control	5
II	Meloxicam	5
III	Ligature	5
IV	Ligature and meloxicam	5
V	Control	15
VI	Meloxicam	15
VII	Ligature	15
VIII	Ligature and meloxicam	15

sition at 0.3 impulses/second. The source-to-film distance was always set at 50 cm, and an aluminum wedge was incorporated into the electronic sensor to provide a radiographic density reference. The distance between the cemento-enamel junction and the alveolar crest was determined at the mesial root surfaces of the lower right first molars with the aid of a software. Millimeters of bone loss on each radiograph were measured 3 times by the same examiner, on different days, in order to reduce variation in the data[8].

Digital images were analyzed using the SigmaScan 2.0 software (London, UK). Records of the distance between the cemento-enamel junction and the alveolar crest at the mesial surfaces of the mandibular first molars of the rats were taken. Data were expressed as means and standard deviations. ANOVA was used for statistical evaluation. Tukey's test was used to compare differences among groups. The level of significance $p < 0.05$ was adopted.

RESULTS

Clinical observations

No relevant clinical manifestations were observed in the rats treated with meloxicam.

Radiographic aspects

The satisfactory outcome of the experimental periodontitis model was confirmed, as increasing bone loss over the 15 days was evident. After 5 days, meloxicam-treated rats with periodontitis presented significant lower alveolar bone loss compared with the control group ($p < 0.05$). However, after 15 days, alveolar bone loss was not statistically different between the meloxicam-treated group and the untreated group ($p < 0.05$).

The distance between the cemento-enamel junction and the alveolar crest is reported in Tables 2 and 3.

Considering the radiographs of the mandibles of the experimental groups after 5 days, it may be observed that alveolar bone loss is greater for group III ($p < 0.05$) than for groups I, II and IV ($p < 0.05$).

The mandibles of the groups observed after 15 days did not show statistically significant differences between groups VII and VIII ($p > 0.05$), but it may be observed that alveolar bone loss in these groups is greater than that observed in groups V and VI ($p < 0.05$).

TABLE 2 - Measurements (means ± standard deviations) of the distance between the cemento-enamel junction and the alveolar bone crest (pixels) on the mesial surface of the mandibular first molars at 5 days.

Groups (n = 5)	Treatment	Means ± standard deviations
I	Control	19.2 ± 1.4 A
II	Meloxicam	20.9 ± 1.0 A
III	Ligature	30.0 ± 3.8 B
IV	Ligature and meloxicam	25.7 ± 0.3 C

Different letters represent statistically significant differences ($p < 0.05$).

TABLE 3 - Measurements (means ± standard deviations) of the distance between the cemento-enamel junction and the alveolar bone crest (pixels) on the mesial surface of the mandibular first molars at 15 days.

Groups (n = 5)	Treatment	Means ± standard deviations
V	Control	20.1 ± 2.3 A
VI	Meloxicam	21.1 ± 3.0 A
VII	Ligature	27.7 ± 2.1 B
VIII	Ligature and meloxicam	26.8 ± 4.2 B

Different letters represent statistically significant differences ($p < 0.05$).

DISCUSSION

There is evidence that patients who are treated with various anti-inflammatory drugs show less periodontal destruction. Rats have been extensively used to study the effects of these drugs in the periodontium. The rat model is very convenient because rats are small, not expensive and easy to handle. The experimental model of periodontitis in rats was characterized by accumulation of biofilm, flattening and displacement of the gingival crest, increasing proliferation of the epithelium into underlying connective tissue and infiltration of mainly mononuclear inflammatory cells caused by the presence of a ligature around the tooth[15]. The establishment of the size of the sample was based in the relative standard error. On the other hand, there was no dispersion of the results.

According to Klausen *et al.*[13] (1989), the radiographic method to diagnose and measure bone loss is comparable to the morphometric method with respect to reproducibility and ability to discriminate among various experimental groups. Our

study demonstrates that cotton ligatures around the lower right first molar teeth resulted in progressive bone loss ($p < 0.05$).

During the initial experimental period, body weight loss was observed in animals with periodontal disease compared with baseline. However, in the final experimental periods, no weight alterations were observed. The administration of meloxicam seemed to prevent the onset of significant bone loss at 5 days (groups III and IV were significantly different – $p < 0.05$). In the subsequent period (15 days), no significant further exacerbation of mean bone loss was found, and groups VII and VIII were not significantly different ($p > 0.05$).

The efficacy of NSAIDs in the treatment of periodontal disease in humans and animals has been analyzed considering many clinical or radiographic parameters, like the presence of edema, gingival erythema, exudates of the crevicular gingival fluid, the presence of PGE_2 in the gingival sulcus fluid, probing depth, level of clinic insertion, alveolar bone height, bone loss or gain rate and bone metabolism[1].

Analyzing the therapeutic effect of non-steroidal anti-inflammatory drugs in the experimental periodontal disease, it may be observed that neutrophils and macrophages were present in the inflamed periodontal tissues and they were responsible for the PGE_2 and eicosanoid release. Prostaglandin seems to be an important factor in causing alveolar bone destruction[12]. The administration of non-steroidal anti-inflammatory drugs inhibits cyclooxygenase, reducing the eicosanoid level, and has proven beneficial to the treatment of periodontitis.

The groups with ligature around the mandibular right first molar[11] had an initial gingival lesion, and this fact provoked the dilatation of the microvascular net of the region, with an increase in the hydrostatic pressure and fluid permeability, and also in the displacement of the leucocytes to the crevicular gingival fluid; cell response had been well established between two and four days. In this study, the group treated with meloxicam did not show results statistically different from the group not treated with meloxicam when the treatment was performed for 15 days (groups VII and VIII ($p > 0.05$)).

The leucocytes move around the conjunctive tissue and most of them seem to accumulate in the junctional epithelium region and in the gingival sulk[14]. Meloxicam causes COX-2 inhibition and then there is a reduction of the inflammatory process in the acute stage, as demonstrated in the groups treated for 5 days (I, II, III and IV ($p < 0.05$)), with statistically significant differences between groups III, IV and I-II ($p < 0.05$); this fact shows that a quenching of the acute inflammatory reaction in the first days might have occurred[7].

The beneficial effects following NSAID therapy are mainly a result of the inhibition of the metabolic transformation of arachidonic acid into prostanoids, especially prostaglandins of the E series, via the COX pathway[8]. In this investigation, the decrease in bone loss progression may be due to the inhibition of the COX-2 enzyme which, according to Morton, Dongari-Bagtzoglou[15] (2001), is up-regulated in highly inflamed periodontal tissues. These authors also demonstrated that both inflammatory cytokines such as interleukin-1β and bacterial constituents might be responsible for the enhanced COX-2 expression and PGE_2 synthesis *in vivo*.

The reduction of meloxicam efficacy to fight inflammation after long periods can be explained due to the bacterial accumulation (biofilm) and, consequently, due to an increase in the immune-inflammatory stimulus that might occurr. Thus, the activation of other inflammatory mediators can be explained by the absence of the inhibition of bone resorption found in the groups treated with meloxicam in the available periods[11]. Meloxicam and other NSAIDs may be considered promising agents in the treatment of periodontal disease, but other studies may be necessary and should be idealized to explore the various aspects that are not yet elucidated.

CONCLUSION

The groups treated with meloxicam for a short period after induced periodontal disease by ligature showed small alveolar bone loss, suggesting that this drug acts in the acute stage, quenching the inflammatory reaction, preventing alveolar bone loss in the first days.

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