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Protective effects of etoricoxib, a selective inhibitor of cyclooxygenase-2, in experimental periodontitis in rats

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Background: The purpose of the present study was to evaluate the effect of a potent selective cyclooxygenase-2 (COX-2) inhibitor, etoricoxib, on the prevention of alveolar bone loss in experimental periodontitis induced in rats.

Methods: Ninety Wistar rats were separated into three experimental groups. Cotton ligatures were placed at the gingival margin level of lower right first molars. The rats were randomly assigned to one of the following groups: control received a daily oral dose of 1 ml/kg of saline solution; Eto1 received 6 mg/kg of etoricoxib; Eto2 received 12 mg/kg of etoricoxib. Serum levels of etoricoxib and white blood cells were determined. Standardized digital radiographs were taken after death at 3, 5, 10, 18 and 30 days to measure the amount of bone loss at the mesial root surface of the first molar tooth in each rat.

Results: One-way analysis of variance (ANOVA) indicated that groups treated with both doses of etoricoxib had significantly ($p < 0.05$) less alveolar bone loss when compared to controls. Furthermore, etoricoxib treatment significantly inhibited the leukocytosis observed 3 days after the induction of periodontitis.

Conclusion: These data provide evidence that systemic therapy with etoricoxib can retard alveolar bone loss in a ligature-induced periodontitis model in rats.

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Periodontitis is a chronic inflammatory disease initiated and perpetuated by a small group of anaerobic Gram-negative bacteria that colonize the subgingival area. Although it is considered that bacteria and their products can cause direct pathologic effects on the periodontal tissues, damage to the periodontium, characterized by destruction of connective tissues including the alveolar bone, occurs mainly through indirect mechanisms involving the activation of a number of host-mediated destructive processes (1–3).

The cylooxygenase-2 (COX-2) product prostaglandin E_2 (PGE_2), a vasoactive eicosanoid produced by activated macrophages and fibroblasts, is found in the crevicular fluid of patients with periodontitis, and is considered the major inflammatory mediator of alveolar bone destruction (4, 5). In addition, COX-2 expression is significantly up-regulated in inflamed periodontal tissues (6), thus making COX-2 inhibition an attractive pharmacological target in order to reduce the production of PGE_2 at sites of inflammation and tissue destruction.

Several studies have shown that the use of COX-2 inhibitors, or coxibs, leads to fewer adverse effects than those observed with non-selective non-steroidal anti-inflammatory drugs (NSAIDs) (7). Some studies showed that systemic therapy with coxibs could modify the progression of experimentally induced periodontitis in rats (8, 9).

Etoricoxib [5-chloro-2-(6-methylpyridin-3-yl)-3-(4-methylsulfonylphenyl) pyridine] is a novel COX-2 inhibitor clinically indicated for treatment of post-operative pain, rheumatoid arthritis, gout and osteoarthritis. Compared to other COX-2 inhibitors, etoricoxib shows higher selectivity towards this COX isoform, with basis on the COX-1/COX-2 IC_{50} ratios obtained by the *ex-vivo* human whole blood assay (etoricoxib: 106, rofecoxib: 35, valdecoxib: 30, celecoxib: 7.6, nimesulide: 7.3, etodolac: 2.4, meloxicam: 2.0) (10).

The main scope of the present study was to evaluate whether the higher selectivity of etoricoxib would confer therapeutic effects when used in a pathological situation such as periodontitis. In this way, we studied the effects of chronic etoricoxib administration on the progression of alveolar bone loss in an experimental model of periodontitis in rats.

Materials and methods

Animals

Ninety 5-week-old male Wistar rats (*Norvergicus albinus*), of approximately 90 g, were housed in polypropylene cages in groups of five per cage, and received standard laboratory chow and tap water *ad libitum*. All protocols described below were approved by the local Ethics Committee from the School of Dentistry of Araraquara, São Paulo, Brazil.

Experimental protocol

Anesthesia was obtained by intramuscular administration of 0.08 ml/100 g body weight of ketamine (Francotar, Virbac do Brazil Ind. e Com. Ltda., São Paulo, SP, Brazil).

A cotton ligature was placed in a submarginal position around the lower right first molar of each rat to induce experimental periodontitis (8). Twenty-four hours later, the rats were randomly divided into three groups of 30 animals each, according to the following treatments: Eto1 group received etoricoxib (Arcoxia, Merk do Brazil, São Paulo, Brazil) at a dose of 6 mg/(kg day) (p.o.), Eto2 group received etoricoxib at a dose of 12 mg/(kg day) (p.o.), and the control group received 1 ml/(kg day) of saline solution (0.9% NaCl). The etoricoxib was administered at 7.00 AM. At days 3, 5, 10, 18 and 30 after the beginning of the pharmacological treatments, six animals from each group were submitted to the experimental protocols described below.

Determination of serum etoricoxib concentrations and white blood cell count

Peripheral venous blood samples were collected by intracardiac puncture before 9.00 AM following an overnight fasting period. Serum samples obtained from etoricoxib-treated animals at days 3, 18 and 30 were used for the analysis of etoricoxib content by high-performance liquid chromatography (HPLC), according to the method previously described by Bräutigam *et al.* (11).

In order to evaluate the initial effects of etoricoxib on peripheral blood leukocytosis, total and differential white blood cell counting was performed at day 3 in blood samples obtained from six animals of each experimental group employing an automatic apparatus (Technicon H1, Domont, Puteaux Cedex, France).

Radiographic examination

The jaws were removed to determine the degree of bone loss. Standardized digital radiographs were obtained by means of a computerized imaging system (Computed Dental Radiography for Microsoft Windows, Schick Technologies, Inc., Dialom Dental Products, Long Island City, NY, USA) that utilizes an electronic sensor instead of X-ray film. Electronic sensors were exposed at 65 kV and 10 mA with the time of exposition at 12 impulses/s. The source-to-film distance was always set at 50 cm, and an aluminum wedge was incorporated in the electronic sensor to provide a radiographic density reference. The distance between the cemento–enamel junction and the height of alveolar bone was determined for mesial root surfaces of lower right first molars with the aid of the software. The extent of bone loss (in mm) from each radiograph was measured three times by the same examiner (unaware of the treatments) during different days, in order to avoid any bias and to reduce the data variation.

Statistical analysis

Differences in serum etoricoxib concentrations and white blood cell countings among the experimental groups were analyzed by the Student's *t*-test for unpaired samples. One-way analysis of variance (ANOVA) was used to compare mean alveolar bone loss among the groups. In case of significant differences among the groups, *post hoc* two group comparisons were assessed by the Tukey–Kramer test. *p*-values < 0.05 were considered statistically significant.

Results

Determination of serum etoricoxib concentrations

Table 1 summarizes the results on serum etoricoxib concentrations obtained from groups Eto1 and Eto2 on days 3, 18 and 30 after starting the treatment. These data confirm the oral bioavailability of etoricoxib in rats when administered orally and that treatment with the higher dose of etoricoxib [12 mg/(kg day)] resulted in significantly higher serum levels of the drug than did the treatment with the lower dose at all the studied treatment periods ($p < 0.0001$).

White blood cell counting

Table 2 summarizes the results on total and differential white blood cell data on day 3. Peripheral white blood cell count was significantly higher in the controls than in the etoricoxib-treated rats. In the Eto1 group, the percentages of neutrophils and monocytes were significantly smaller when compared to those observed in the control group ($p < 0.05$), whereas in the Eto2 group, significantly lower percentages of neutrophils, monocytes and

Table 1. Etoricoxib concentrations (ng/ml) measured by high-performance liquid chromatography in rat serum samples obtained at days 3, 18 and 30 after the induction of periodontitis. Mean ± standard error of the mean (SEM)

Groups	Periods of treatment		
	3 days	18 days	30 days
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Eto1	201.3 ± 7.6*	199.4 ± 5.9*	202.1 ± 8.1*
Eto2	274.4 ± 6.9*†	242.8 ± 8.1*†	243.9 ± 7.7*†

*$p < 0.05$ vs. control group within the same period of treatment; obtained by Student's *t*-test for unpaired samples.
†$p < 0.05$ vs. Eto1 group within the same period of treatment, obtained by Student's *t*-test for unpaired samples.

lymphocytes were observed. No significant differences were found between etoricoxib and the control groups regarding eosinophils and basophils.

Clinical adverse observation

No clinically relevant manifestations were observed in the animals treated with etoricoxib at the 6 mg/(kg day) dose. However, diarrhea and small corporal weight loss was observed in three rats receiving the 12 mg/(kg day) dose, and one animal presented with skin tissue disorders (irritant dermatitis). The remaining animals did not show any visible clinical abnormality. No alterations of either the palatal or lingual mucosa were observed in any etoricoxib-treated rat.

Effect of etoricoxib on bone loss

The linear bone loss measurements are reported in Table 3.

The satisfactory outcome of the experimental periodontitis model was confirmed, as increasing bone loss over the 30-day period was evident, with significant increases in mean bone loss from days 3–30 in all groups. Both etoricoxib-treated animal groups presented with significantly lower alveolar bone loss than that observed in the control group at all the studied treatment periods ($p < 0.05$); however, the degree of alveolar bone loss was not statistically different between the groups receiving etoricoxib at either 6 or 12 mg/(kg day).

Discussion

COX-2 is not only expressed during inflammatory conditions in response to pro-inflammatory cytokines and endotoxins, but also constitutively expressed in some tissues (such as brain and kidney) under non-pathological conditions. Along with COX-1, cyclooxygenases regulate a variety of physiological and pathological processes through the synthesis of prostaglandins, prostacyclin and thromboxanes from arachidonic acid (12). At sites of injury and inflammation, macrophages, and fibroblasts express COX-2, which subsequently up-regulates the production of PGE_2 involved in the inflammatory response (4). As PGE_2 is an important pro-inflammatory mediator in gingivitis and alveolar bone resorption, COX-2 expression may also have detrimental roles in periodontal disease.

Our results demonstrated that oral administration of etoricoxib reduced the degree of bone resorption during experimental periodontitis. Radiographic evidence in the present study confirmed that ligature induces significant alveolar bone resorption (13), an effect that was partially blocked by etoricoxib treatment. We have previously demonstrated that the use of celecoxib, another COX-2 inhibitor, also suppressed pathological changes in the same experimental model of periodontitis (8). The exact mechanism(s) by which coxibs inhibit alveolar bone loss still remains to be established. However, there is strong evidence to support the hypothesis that the decrease in bone resorption is mainly due to the inhibition of PGE_2 synthesis. PGE series are slow acting, but powerful, mediators of bone resorption and affect both active mature osteoclasts as well as differentiated osteoclast precursors. PGE_2 is well documented to participate in the immunoinflammatory response in periodontitis in mediating connective tissue alterations and bone resorption, and the non-steroidal anti-inflammatory drugs effectively inhibit these activities.

Several studies have demonstrated that elevated levels of PGE_2 are present in gingival crevicular fluid and periodontal tissues from patients exhibiting gingivitis, periodontitis, and peri-implantitis (14, 15). Furthermore,

Table 2. Mean total (cells/mm^3) and differential (%) white blood cell counts at day 3

Cell	Control				Eto1				Eto2				Normal range
	Mean	SEM*	Median	Range	Mean	SEM*	Median	Range	Mean	SEM*	Median	Range	
Total leukocytes	17772	4872	15986	13824–19888	11116	2289	15821	10092–13988	10990	1987	13343	11243–17587	8000–19000
Lymphocytes	65.4	7.6	58.7	53.5–66.6	53.3	6.2	45.1	35.5–56.76	42.8†	6.6	43.7	29.5–45.7	47–73
Monocytes	3.9	1	3.1	3.8–6.2	2.1†	0.9	2.7	1.2–3.9	2.2†	1.2	3	0.2–3.9	0–3
Neutrophils	35.6	2.3	24.7	22.8–36.5	24.0†	5.1	20.6	14.1–29.3	24.4†	4.1	23.9	18.5–28.2	24–27
Eosinophils	0.5	0.1	0.6	0.1–1.5	0.4	0.2	0.5	0.1–1.4	0.4	0.2	0.3	0.1–0.7	0–0.7
Basophils	0.4	0.1	0.3	0.1–1.1	0.1	0.3	0.6	0.1–0.9	0.5	0.1	0.3	0.1–0.7	0–1

*Standard error of the mean; †$p < 0.05$ compared to control group (Student's *t*-test for unpaired samples).

Table 3. Alveolar bone loss (in mm; as mean ± SEM) at days 3, 5, 10, 18 and 30 after the induction of periodontitis in rats

	Period of treatment (days)				
Groups	3	5	10	18	30
Control	0.37 ± 0.049	1.00 ± 0.05†	1.20 ± 0.05†	1.38 ± 0.05	1.62 ± 0.09†
Eto1	0.20 ± 0.007	0.74 ± 0.03†*	0.87 ± 0.02*	1.10 ± 0.01†*	1.28 ± 0.01*
Eto2	0.15 ± 0.011*	0.61 ± 0.03†*	0.83 ± 0.01†*	1.03 ± 0.01†*	1.17 ± 0.01*

*$p < 0.05$ vs. control group within the same treatment period (Tukey's test).
†$p < 0.05$ vs. the previous period within the same group of treatment (Tukey's test).

mean crevicular PGE_2 concentrations are also significantly elevated in patients exhibiting disease progression compared to periodontally stable individuals (16). In addition, other studies have demonstrated that monocytes from patients with refractory periodontitis or with aggressive forms of periodontitis are hyperresponsive to bacterial lipopolysaccharide and produce high levels of PGE_2 (17, 18). For these particular clinical situations, COX-2 inhibition may represent a potential target of intervention in order to reduce the production of pro-inflammatory PGE_2 at sites of inflammation and tissue destruction. The use and complete understanding of coxibs is still in its infancy; however, it seems likely that in the near future, drugs able to either inhibit COX-2 activity or block its expression will represent attractive alternatives as modulators of inflammatory processes.

In conclusion, our results clearly demonstrate that COX-2 plays a crucial role in the pathogenesis of periodontitis, as systemic treatment with etoricoxib prevented alveolar bone loss in a rat model of ligature-induced periodontitis.

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