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Role of systemic and local administration of selective inhibitors of cyclo-oxygenase 1 and 2 in an experimental model of periodontal disease in rats

C. M. Queiroz-Junior[1], C. M. F. Pacheco[1], K. L. M. Maltos[1], M. V. Caliari[2], I. D. G. Duarte[1], J. N. Francischi[1]

Departments of [1]Pharmacology and [2]Pathology, Institute of Biological Sciences, Federal University of Minas Gerais (UFMG), Belo Horizonte, MG, Brazil

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Abstract Background and Objective: Periodontal disease is an inflammatory condition of tooth-supporting tissues. Arachidonic acid metabolites have been implicated in development of periodontal disease, especially those derived from the cyclo-oxygenase (COX) pathway. This study investigated the role of inhibitors of cyclo-oxygenases (COX-1 and COX-2) in a model of periodontal disease in rats.

Material and Methods: A ligature was placed around the molar of rats. Losses of fiber attachment and of alveolar bone were measured morphometrically in histologically prepared sections. Infiltration of cells into gingival tissue surrounding the ligated tooth was also determined.

Results: Systemic and local administration of non-selective and selective COX-2 inhibitors, preventively, resulted in significant reduction of the losses of fiber attachment and alveolar bone, as well as decreased leukocyte numbers in gingival tissue. Preventive selective inhibition of COX-1 was as effective as COX-2 inhibition in reducing local fiber attachment loss and cell migration, but did not prevent alveolar bone loss.

Conclusion: Our results provide evidence for participation of COX-1 and COX-2 in early stages of periodontal disease in rats. Furthermore, local administration of COX inhibitors reduced the signs of periodontal disease to the same extent as systemic treatment. Therapeutic approaches incorporating locally delivered anti-inflammatory drugs could be of benefit for patients suffering from periodontal disease.

Janetti Nogueira de Francischi, PhD, Avenida Antônio Carlos, 6627, Campus Pampulha, Belo Horizonte, Minas Gerais, Brazil, CEP: 31270-901
Tel/Fax: 0055 31 3409 2695
e-mail: janettif@icb.ufmg.br

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Periodontal disease is an inflammatory condition of the tooth-supporting tissues, the periodontium, caused by subgingival accumulation of anaerobic gram-negative bacteria, and is characterized by a progressive destruction of periodontal tissues (1). Although periodontal disease has identifiable etiologic agents, such as *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Actinobacillus actinomycetemcomitans*, it is becoming clear that the host response to micro-organisms located in periodontium plays a major role in the outcome of the disease (2). The interaction of bacteria and their products with periodontal tissues triggers release of a wide range of endogenous chemical

mediators, which regulate the traffic and activation of leukocytes. Amongst these mediators, arachidonic acid metabolites, such as prostaglandins (PGs), have a recognized role in the pathogenesis of periodontal disease (3).

Prostaglandins are produced by the activity of cyclo-oxygenases (COX) on arachidonic acid, a polyunsaturated fatty acid found in the plasma membrane phospholipids of most cells. According to current knowledge, there are two important COX isoforms, COX-1 and COX-2 (4). Cyclo-oxygenase-1, the constitutive isoform, is usually responsible for the production of PGs needed for normal physiological functions, such as preservation of the stomach lining and maintenance of platelet function. The second isoform, COX-2, is predominantly expressed during inflammatory reactions and produces various types of PGs responsible for the increased local blood flow, vascular permeability, cell migration and pain characteristic of inflammation (5–7).

Some studies in the literature have demonstrated the involvement of COX-2 in periodontitis. Pouliot *et al*. (8) observed that crevicular fluids from periodontal disease patients contained elevated PGE_2 levels and presented upregulated COX-2 expression in the infiltrating leukocytes. There is also evidence that therapies using either selective (COX-2) or traditional non-steroidal anti-inflammatory drugs (NSAIDs) can modify the progression of periodontal disease in rats (9–11). Using a model of ligature-induced periodontal disease in rats, Bezerra *et al*. (9) and Holzhausen *et al*. (11) showed that systemic administration of such drugs could prevent alveolar bone loss as evaluated by a stereoscope loupe or by radiographs, respectively. Nevertheless, chronic treatment with the non-selective compound indomethacin induced gastric damage. Lohinai *et al*. (10) showed that administration of a selective inhibitor of COX-2 (NS-398) in rats with periodontal disease reduced plasma extravasation in the gingivomucosal tissue of diseased animals. These data suggested the participation of COX-1 and/or COX-2 in the pathophysiology of periodontal disease. However, the role played by selective COX-1 inhibitors in experimental periodontal disease is still lacking in the literature. Some studies have explored the use of anti-inflammatory drugs topically, i.e. they were applied locally at the site of injury (12,13). Such an approach presents advantages over systemic administration of anti-inflammatory drugs because lower doses are used, which prevent or at least decrease the outcome of their well-known side-effects.

In the present study, non-selective and selective COX-1 and COX-2 inhibitors were administered to rats with periodontal disease to elucidate the role played by COX isoforms in an experimental model of periodontitis. To that end, drugs were administered either systemically or locally.

Material and methods

Animals

Male Holtzman rats weighing 260–300 g from the Animal Facilities of the Institute of Biological Sciences, Federal University of Minas Gerais, Brazil, were used throughout the experiments. Animals were maintained under a light–dark cycle of 12 h–12 h (lights on 7:00 AM to 7:00 PM) at 23–25°C with water and food *ad libitum*. The handling of the animals was approved by the local Animal Ethics Committee.

Induction of periodontal disease

A model for experimental periodontal disease in rats was used as described previously (14). Briefly, rats were anaesthetized with a mixture of ketamine and xylazine (90 and 15 mg/kg, respectively, intramuscular). A sterile silk ligature (1.5 metric) was tied around the cervix of the second left maxillary molar and served as a retention device for subgingival oral micro-organisms. The contralateral right side was used as the non-ligated control. In order to avoid the impaction of material other than indigenous flora in rat periodontium, animals were kept in individual hanging cages after ligature fixation.

Measurements of alveolar bone and fiber attachment loss by morphometric analysis

At the end of the experiments, animals were killed by cervical dislocation, and the left and right maxillae halves were excised and prepared for histological evaluation as described by Pacheco *et al*. (15). They were fixed in 10% buffered formalin solution (pH 7.2 for 48 h) and demineralised by immersion in 10% ethylenediaminetetraacetic acid (EDTA) for a mean of 30 days. At the end of this period, the hemi-maxillae were alcohol-dehydrated, cleared with xylene and embedded in paraffin with the axis of the teeth parallel to the cutting direction. The blocks were then cut in serial sections of 4 μm thickness in a mesio-distal direction. The most central section from each tooth, i.e. the one where the center of the dental pulp could be observed under a light microscope, was stained with hematoxylin and eosin and further analyzed.

Images from experimental (ligated) and control (unligated) sites were obtained at 10-fold magnification through a JVC TK-1270/RGB microcamera (Victor Company of Japan, Yokohama, Japan) adapted to a microscope. The distances from the cemento-enamel junction (CEJ) to the most coronal fiber attachment (FA) and alveolar bone crest (AB) were measured using KS300 software (CarlZeiss, Oberkochen, Germany) running in a Kontron Elektronick/Carl Zeiss image analyzer (15,16). The alveolar bone loss (ABL) and the loss of fiber attachment (FAL) were obtained from the difference between the experimental and control sites, as shown in Fig. 1, and were expressed in millimeters (mm). All image analysis was carried out without knowledge of the treatments.

Assessment of cell numbers in gingival tissue

After excision of the maxillae, gingivomucosal tissue samples were removed from the buccal surface adjacent to the ligated tooth and processed in the same manner as the hemi-maxillae. An automatic macro-recorder assembler (an algorithm of the KS300 software) was

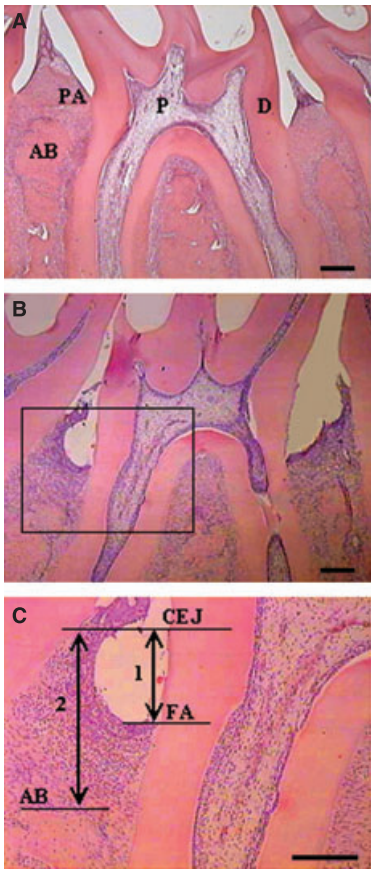

Fig. 1. Photomicrographs from transverse histological sections of maxillary rat molar teeth. (A) Main structures of a healthy tooth and its supporting tissues: dental pulp (P), dentin (D), alveolar bone crest (AB) and periodontal attachment (PA; fourfold magnification). (B) An example of a molar tooth presenting signs of periodontal disease: fiber attachment loss and reduction of the alveolar bone crest (fourfold magnification). (C) The square in (B) is shown at higher magnification (10-fold magnification) to illustrate the distance: (1) from the cemento-enamel junction (CEJ) to the most coronal fiber attachment (FA); and (2) from the CEJ to the alveolar bone crest (AB). Scale bars represent 300 μm (A, B) and 200 μm (C).

elaborated for capture, image processing and segmentation, definition of morphometrical conditions and counts of all the nuclei contained in each image as described previously (15,16). Image processing techniques were applied in order to highlight the nucleus of the cells. The nuclei from the cellular types usually found in the gingiva and recruited leukocytes were then counted (15,16). The measurements were made by an observer who was unaware of the nature of the tissue sample. For each gingival tissue sample, six images from different locations of the gingival connective tissue section were obtained at 40-fold magnification and were counted. The results of each field count were summed and compared with the number of nuclei from the gingival tissue from naïve animals. Results are presented as the number of migrated cells, which were obtained from the difference between the number of nuclei of the experimental animals and the number of nuclei from naïve animals.

Drugs and reagents

Indomethacin (Sigma Chemical Co., St Louis, MO, USA) was diluted in Tris buffer solution (0.1 M, pH 8.2). Celecoxib (Searle & Co., Caguas, PR, USA), a selective COX-2 inhibitor, was diluted in sterile physiological saline (NaCl; 0.9%, w/v). SC236 {4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide} and SC560 [5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethyl pyrazole], selective COX-2 and COX-1 inhibitors, respectively, were diluted in ethanol, saline and Tween 85 (10:85:5, respectively), all purchased from Sigma Chemical Co., except for SC236 acquired from Cayman (Ann Arbor, MI, USA). Ethanol, xylene, formaldehyde, EDTA and hematoxylin were obtained from Synth (Diadema, Brazil) and eosin from Vetec (Duque de Caxias, Brazil).

Experimental protocol

Previous studies in our laboratory showed that surgical placement of a ligature around the cervix of the second upper molar of the rats induced a significant fiber attachment (FAL) and alveolar bone loss (ABL) on the 5th day after induction of periodontitis, reaching its peak at day 11. Additionally, it was shown that preventive administration of drugs for three consecutive days (3rd to 5th day after ligature placement) was the most effective treatment schedule (15). Based on such findings, all drugs were administered preventively (3rd to 5th day after ligature placement), and the animals were killed by the 11th day after ligation. Indomethacin (2 mg/kg/day, $n = 5$), celecoxib (3–30 mg/kg/day, $n = 5$ per group), SC236 (12 mg/kg/day, $n = 5$) and SC560 (0.5 mg/kg/day, $n = 4$ or 5 mg/kg/day, $n = 5$) were administered by subcutaneous bolus injection into a skin fold in the dorsal region of the neck (volume of 0.1 mL per 100 g body weight). Initial doses of all drugs used in the present work were based on previous studies in the literature (17–19). Indomethacin (100 μg/site/day, $n = 5$), celecoxib (60–240 μg/site/day, $n = 5$ per group), SC236 (120 μg/site/day, $n = 5$) and SC560 (100 μg/site/day, $n = 4$ or 300 μg/site/day, $n = 5$) were also injected locally (in a total volume of 0.1 mL) into the gingival tissue surrounding the ligated tooth at the buccal side. In order to verify that the doses used for local administration did not produce systemic effects, indomethacin (100 μg/site/day, $n = 5$) and celecoxib (120 μg/site/day, $n = 5$) were contralaterally injected in another group of periodontal disease-induced animals. No volume alteration was visually observed in gingivomucosal tissue during local injection. Control animals ($n = 6$) in all experiments were injected with the vehicle in which the drugs were diluted using the same route and time of administration.

Statistical analysis

Data are presented as means ± SEM (in mm or number of cells), in groups of 4–6 animals. Difference between means was assessed by one-way ANOVA followed by Student–Newman–Keuls test. Probabilities smaller than 5% ($p < 0.05$) were considered to be statistically significant.

Results

Systemic effects of non-selective (indomethacin), selective COX-1 (SC560) and selective COX-2 inhibitors (celecoxib and SC236) on indicators of periodontal disease in rats

Initially, an effective dose of celecoxib from a dose-range study was established for this selective COX-2 inhibitor.

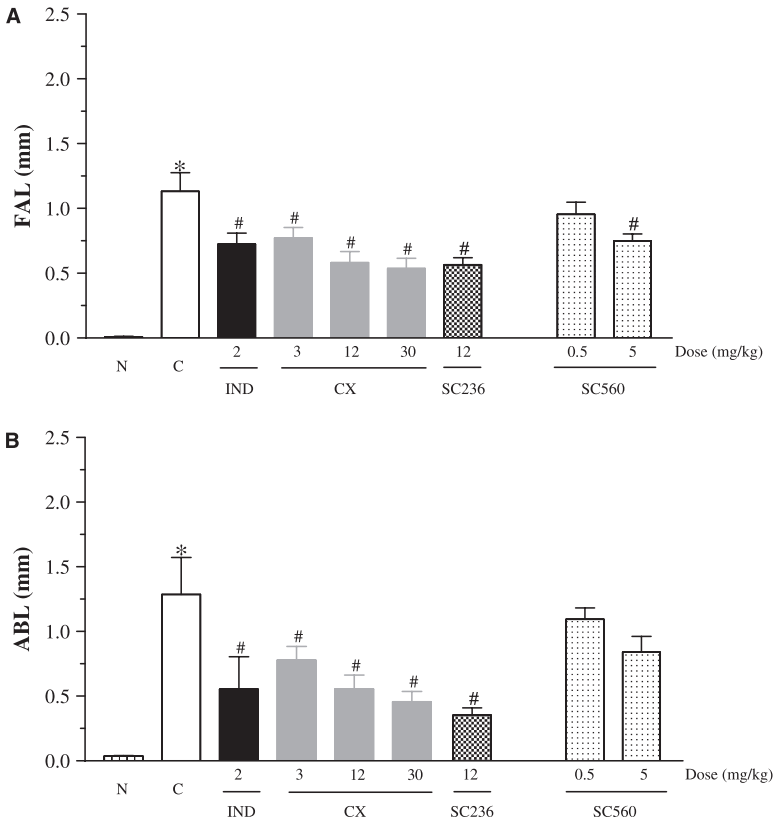

Fig. 2. Effect of systemic administration of celecoxib, SC236 and SC560, compared with indomethacin, in the development of periodontal disease in rats. Periodontal disease was induced by the placement of a silk ligature around the 2nd maxillary molar of the rats, as described in the Material and methods section (day 0). Celecoxib (CX; 3, 12 or 30 mg/kg/day), SC236 (12 mg/kg/day), SC560 (0.5 or 5 mg/kg/day) and indomethacin (IND; 2 mg/kg/day) were administered subcutaneously (0.1 mL per 100 g body weight) once a day, for three consecutive days, from the 3rd to 5th day after applying the ligature. Animals were killed on the 11th day of ligation. Sterile physiological saline or vehicle (saline + ethanol + Tween 85) was given in the same volume and route of administration in control animals (C). Naïve (N) animals were left unligated. The symbols * and # indicate a significant difference ($p < 0.05$) in relation to groups N and C, respectively, using one-way ANOVA followed by Student–Newman–Keuls test; 4–6 animals per group.

As shown in Fig. 2, celecoxib (3–30 mg/kg) administered systemically (subcutaneously), reduced the loss of both fiber attachment (FAL; Fig. 2A) and alveolar bone crest (ABL; Fig. 2B) in a dose-dependent manner in diseased animals compared with control (diseased and vehicle-treated) animals. Animals that did not have a ligated tooth (group N in Fig. 2) did not show any sign of disease. In addition, the increased number of cells in the adjacent gingival tissue of the ligated tooth showed a dose-dependent reduction in celecoxib-treated animals (Fig. 3).

Figures 2 and 3 also show that animals treated systemically with the experimental compound SC236 (12 mg/kg/day), a selective COX-2 inhibitor, or with indomethacin (2 mg/kg/day), a non-selective COX inhibitor, exhibited a significant ($p < 0.05$) decrease in FAL and ABL as well as in leukocyte number present in gingival tissue when compared with control groups. Loss of alveolar bone crest was, however, more sensitive to drug treatment than fiber attachment loss.

In contrast, systemic (0.5 or 5 mg/kg/day) administration of SC560, a selective COX-1 inhibitor, was effective in reducing FAL (Fig. 2A) but did not reduce ABL (Fig. 2B), whereas it was as effective as COX-2 inhibitors in decreasing cell migration into gingival tissue of animals with periodontal disease (Fig. 3).

Effects of local (gingival) administration of non-selective (indomethacin), selective COX-1 (SC560) and selective COX-2 inhibitors (celecoxib and SC236) in experimental periodontal disease

Local injection of celecoxib at 60, 120 or 240 μg/site/day, SC236 at 120 μg/site/day, or indomethacin at 100 μg/site/day, preventively (from 3rd to 5th day), significantly reduced fiber attachment loss (Fig. 4A) and alveolar bone loss (Fig. 4B) to an extent similar to systemic treatment. In addition, gingival administration of celecoxib (highest doses), SC236 or indomethacin significantly decreased the leukocyte recruitment to gingival tissue compared with control animals (Fig. 5). Local administration of SC560 (100 or 300 μg/site/day) showed similar results to that of systemic administration, i.e. it was effective in reducing FAL (Fig 4A) but did not reduce ABL (Fig. 4B), and it decreased cell migration into the gingival tissue of animals with periodontal disease (Fig. 5).

Histopathological evaluation of the effect of selective COX-1 and COX-2 inhibitors in leukocyte recruitment to gingival tissue of rats with periodontal disease

Histopathological analysis revealed that gingivomucosal tissue from animals with periodontal disease treated with non-selective or selective COX-1 and COX-2 inhibitors had a similar number of cells to those observed in the gingiva of non-ligated teeth, with a predominance of resident cells, while control (vehicle-treated) animals with periodontal disease presented a mixed polymorpho-mononuclear inflammatory infiltrate, with a predominance of neutrophils compared with tissues from non-ligated animals (Fig. 6).

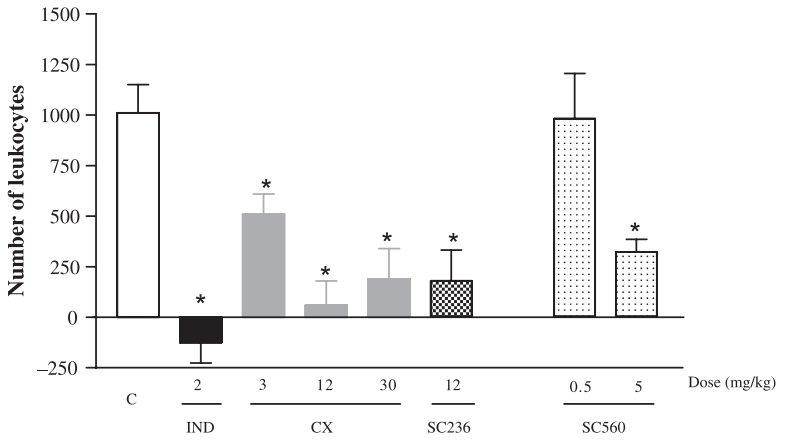

Fig. 3. Effect of systemic administration of celecoxib, SC236 and SC560, compared with indomethacin, on leukocyte recruitment to gingival tissue of rats with periodontal disease. Subcutaneous administration of celecoxib (CX; 3, 12 or 30 mg/kg/day), SC236 (12 mg/kg/day), SC560 (0.5 or 5 mg/kg/day) or indomethacin (IND; 2 mg/kg/day) followed the same protocol as described in the legend to Fig. 2. The negative value shown in this figure for the IND group indicates that this sample had a number of cells smaller than the naïve group in the evaluated gingival tissue. An asterisk indicates a significant difference ($p < 0.05$) in relation to group C, using one-way ANOVA followed by Student–Newman–Keuls test; 4–6 animals per group.

Effects on periodontal disease following contralateral administration of celecoxib or indomethacin

Celecoxib (120 μg/site/day; 0.1 mL) or indomethacin (100 μg/site/day; 0.1 mL) was administered in two different groups, either near the ligated (ipsilateral) or near the non-ligated (contralateral) tooth, and the evaluation of periodontal tissue loss was made in the ligated tooth. Only the ipsilateral administration of celecoxib and indomethacin reduced the loss of both fiber attachment and alveolar bone crest when compared with administration of the same drugs in the contralateral side (Table 1). Similarly, preventive contralateral treatment was not able to reduce the leukocyte recruitment to the gingival tissue surrounding the ligated tooth (Table 1), confirming a local effect of both celecoxib and indomethacin in periodontal disease, at least for the drug concentrations used in our experiments.

Discussion

Destruction of periodontal tissues observed in periodontal disease has been largely attributed to the action of arachidonic acid metabolites, especially prostaglandin E_2. Indeed, some studies have demonstrated a relation between increased PGE_2 levels at gingival sites and periodontal destruction (20,21). Furthermore, the beneficial effect of NSAIDs in experimental periodontitis in rats is also associated with inhibition of prostaglandin synthesis through an action on cyclo-oxygenases (COXs; 9). The efficacy of COX-2 inhibitors in reducing the signs of ligature-induced periodontal disease in rats, such as alveolar bone destruction, cell migration and plasma extravasation, is attributed to their ability to decrease PG levels via COX-2 blockade as shown in previous studies (10,11).

Since the identification of two isoforms of cyclo-oxygenase enzymes (COX-1 and COX-2), the inhibition of COX-2 has been related to the reduction of signs and symptoms of inflammation (5). Induction of COX-2 synthesis in inflammation leads to the production of PGE_2, which accounts for most of the pathophysiological events observed in periodontal disease, including vasodilatation, inflammatory cell recruitment, bone resorption and collagen destruction (3,22). Thus, selective COX-2 blockade has been a good approach to limit development of periodontal disease (10,11).

The present study showed that administration of both non-selective and selective COX-1 and COX-2 inhibitors, at early stages of the disease, was able to prevent further periodontal disease development, reducing signs of the disease in periodontal tissues. Moreover, we showed that the efficacy of selective COX-2 inhibitors in periodontal disease was comparable to a classical NSAID, indomethacin, used as a standard non-steroidal anti-inflammatory drug (23).

Most importantly, the effects on periodontal disease were achieved with both systemic and local administration of COX inhibitors. Recently, systemic side effects have been associated with use of selective COX-2 inhibitors as well as non-selective inhibitors (24), and therefore it may be preferable to treat periodontal disease with NSAIDs administered locally.

The importance of selective COX-2 inhibitors in ameliorating periodontal destruction is not only associated with their ability to decrease PGE_2 production, but this class of drugs has also been shown to control interleukin (IL)-1_β-stimulated IL-6 release by fibroblasts of patients with severe periodontitis (25). In addition, IL-6 was demonstrated to play a role in modulating the inflammatory cascade in chronic periodontitis (26).

Recent studies have demonstrated an additional benefit of nimesulide, a relatively selective COX-2 inhibitor, which decreases gingival levels of $PGF_{2\alpha}$ rather than PGE_2 (27). In contrast, $PGF_{2\alpha}$ has been demonstrated to enhance IL-6 levels at periodontal sites (28). Therefore, the beneficial effects of selective COX-2 inhibition in periodontal disease may not be exclusively ascribed to low levels of prostaglandin E_2.

Cyclo-oxygenase-1 is considered to be a constitutive isoenzyme, which regulates physiological functions through the synthesis of prostacyclin and thromboxanes (4,6,24). However, participation of COX-1 in inflammatory processes has also been described in some animal models of inflammation (29,30). In our hands, the selective

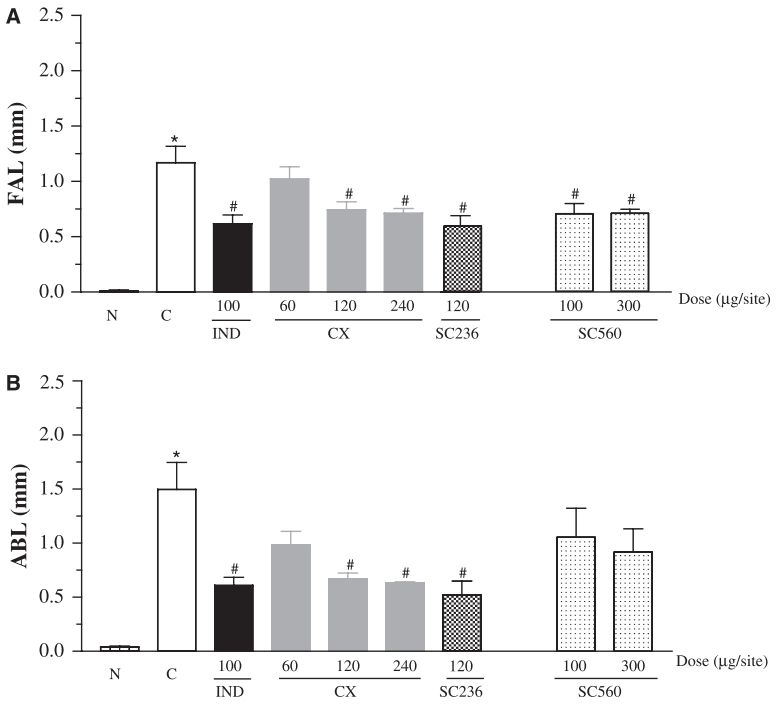

Fig. 4. Effect of local administration of celecoxib, SC236 and SC560, compared with indomethacin, on indicators of periodontal disease in rats. Local administration of celecoxib (CX; 60, 120 or 240 µg/site/day, 0.1 mL), SC236 (120 µg/site/day, 0.1 mL), SC560 (100 or 300 µg/site/day) or indomethacin (IND; 100 µg/site/d, 0.1 mL), once a day, was made for three consecutive days, from the 3rd to 5th day. Animals were killed on the 11th day after tooth ligation. The same volume of sterile physiological saline or vehicle (saline + ethanol + Tween 85) and route of administration were used in control animals (C). Naïve animals (N) were left unligated. The symbols * and # indicate a significant difference ($p < 0.05$) in relation to groups N and C, respectively, using one-way ANOVA followed by Student–Newman–Keuls test; 4–6 animals per group.

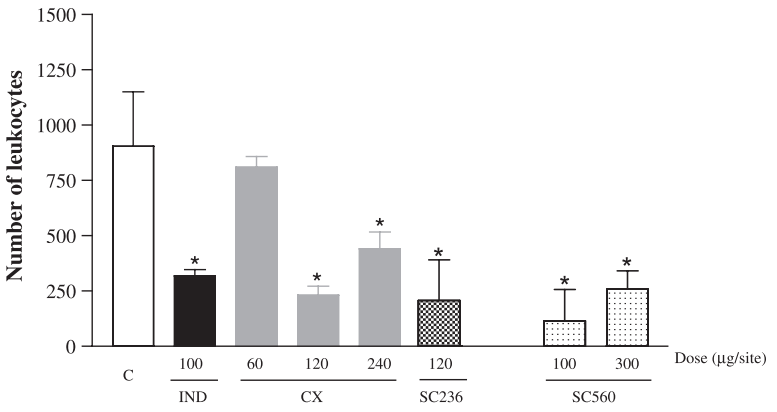

Fig. 5. Effect of local administration of celecoxib, SC236 and SC560, compared with indomethacin, on leukocyte recruitment to gingival tissue of rats with periodontal disease. Local administration of celecoxib (CX; 60, 120 or 240 µg/site/day, 0.1 mL), SC236 (120 µg/site/day, 0.1 mL), SC560 (100 or 300 µg/site/day) or indomethacin (IND; 100 µg/site/day, 0.1 mL) followed the same protocol as described in the legend to Fig. 4. An asterisk indicates a significant difference ($p < 0.05$) in relation to group C, using one-way ANOVA followed by Student–Newman–Keuls test; 4–6 animals per group.

COX-1 inhibitor SC560 reduced the signs of periodontal disease observed in the present study, particularly the fiber attachment loss and the leukocyte migration to the affected site. Such effects were similar to those of selective COX-2 inhibitors. Nevertheless, this drug did not affect the alveolar bone loss, suggesting a different profile of activity for COX-1 and COX-2 isoforms in periodontal disease. Thus, these results provide new evidence for a COX-1 involvement in the development of periodontal disease. Consistent with such findings is a recent study showing that up to 30% of PGE_2 was generated via the COX-1 pathway in synovial tissues from patients with osteoarthitis (31). Recently, however, SC560 was also shown to inhibit PGE_2 by a COX-1-independent pathway (32). Finally, our data also showed a positive effect of a selective COX-1 inhibitor in the model of periodontal disease in rats when administered locally into the gingiva. Consistently, local administration of SC560 reproduced the effects of its systemic administration, i.e. no change in the alveolar bone loss was observed. Such findings therefore clearly establish a differential profile of activity for COX-1 and COX-2 in this experimental model of periodontal disease.

In conclusion, the present study showed the involvement of COX-1 and COX-2 isoforms in the development of periodontal disease in rats, although the role played by the COX-2 isoform was more predominant in the signs of periodontal disease, which is in agreement with previous data in the literature. Furthermore, this study demonstrated that local administration of either COX-1 or COX-2 inhibitors result in a similar activity profile to systemic administration, an important feature to be considered when taking into account the well-known systemic side effects attributed to non-steroidal anti-inflammatory drugs.

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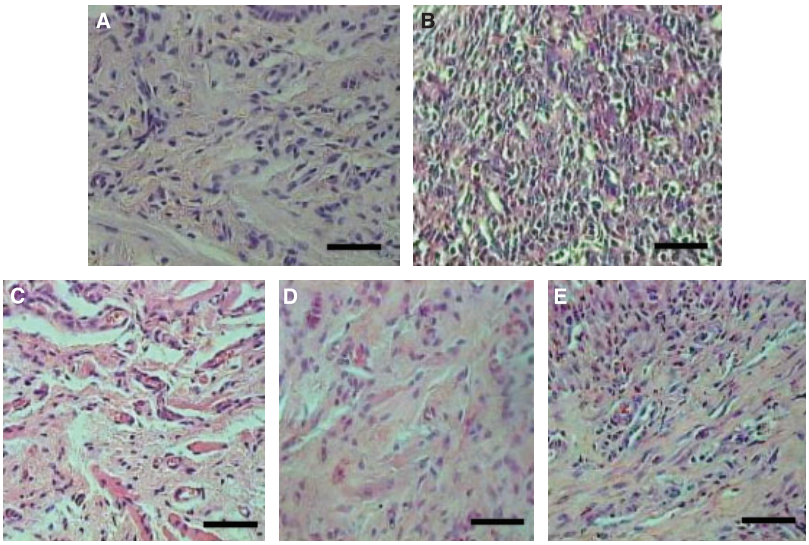

Fig. 6. Photomicrographs from histological sections of gingival connective tissue of naïve rats or rats with periodontal disease treated locally with saline, non-selective or selective COX-2 or COX-1 inhibitors. (A) Gingival connective tissue from a naïve animal (non-ligated), showing the predominance of fibroblasts. (B) Gingival tissue from a control animal (ligated and saline-treated), showing an intense inflammatory infiltrate of mixed polymorpho-mononuclear cells. (C,D,E) Experimental gingival tissue from animals treated locally with indomethacin (100 μg/site/day, 0.1 mL), SC236 (120 μg/site/day, 0.1 mL), or SC560 (100 μg/site/d, 0.1 mL), respectively. Since the drug treatments decreased cell migration to gingivomucosal tissue, a predominance of fibroblasts is seen. All photomicrographies were captured at a magnification of 40-fold. Scale bars represent 50 μm.

Table 1. Comparison between ipsi- and contralateral administration of celecoxib and indomethacin on indicators of periodontal disease in rats

	Loss (mm; means ± SEM)		
Groups (n = 5 animals/group)	Fiber attachment	Alveolar bone crest	Leukocytes (means ± SEM)
C	1.169 ± 0.15	1.313 ± 0.29	1055 ± 134
CX-IPSI	0.703 ± 0.07*	0.669 ± 0.05*	18 ± 135*
CX-CONTRA	1.002 ± 0.13	1.045 ± 0.13	954 ± 152
IND-IPSI	0.618 ± 0.08*	0.543 ± 0.08*	328 ± 28*
IND-CONTRA	1.021 ± 0.11	0.954 ± 0.24	806 ± 126

Periodontal disease was induced in rats as described in the Material and methods section. Indomethacin (IND-CONTRA; 100 μg/site/day, 0.1 mL) or celecoxib (CX-CONTRA; 120 μg/site/day, 0.1 mL) was injected locally in the gingival tissue next to the non-ligated (contralateral) tooth from the 3rd to 5th day after tooth ligature placement, while no treatment was made in the ligated side. Animals were killed on the 11th day. Control animals (C) received the same volume of sterile physiological saline in the right side. Ipsilateral groups of animals received indomethacin (IND-IPSI; 100 μg/site/day, 0.1 mL) or celecoxib (CX-IPSI; 120 μg/site/day, 0.1 mL) preventively in the gingival tissue next to the ligated tooth. Significant mean differences between means ($p < 0.05$; one-way ANOVA followed by Student–Newman–Keuls test) are indicated by *, in relation to group C.

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