J Periodont Res 2008; 43: 14–21

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2007.00989.x

Protective effects of baicalin on ligature-induced periodontitis in rats

X. Cai, C. Li, G. Du, Z. Cao
Department of Periodontology, School and Hospital of Stomatology, Key Laboratory for Oral Biomedical Engineering of Ministry of Education, Wuhan University, Wuhan, China

Cai X, Li C, Du G, Cao Z. Protective effects of baicalin on the ligature-induced periodontitis in rats. J Periodont Res 2008; 43: 14–21. © 2007 The Authors. Journal compilation © 2007 Blackwell Munksgaard

Background and Objective: Baicalin is a flavonoid compound purified from the medicinal plant, *Scutellaria baicalensis* Georgi, and has been reported to possess anti-inflammatory and antioxidant activities. The purpose of this study was to test the ability of baicalin to influence the progression of experimental periodontitis in rats, as well as the expression of cyclooxygenase-2 and inducible nitric oxide synthase.

Material and Methods: Adult male Sprague–Dawley rats were subjected to placement of a nylon thread around the bilateral lower first molars and killed after 7 d. Baicalin (50, 100 or 200 mg/kg) was supplied to the animals by oral gavage, starting 1 d before the induction of periodontitis. The ligature group consisted of rats subjected to periodontitis and receiving vehicle (0.5% carboxymethylcellulose) alone. The alveolar bone loss and the area fraction occupied by collagen fibers were assessed. The expression of cyclooxygenase-2 and inducible nitric oxide synthase protein in the gingiva were detected by immunohistochemistry and western blotting.

Results: Baicalin-treated groups presented with lower alveolar bone loss than that of the ligature group, reaching statistical significance at the dose of 200 mg/kg ($p = 0.009$). The area fraction of collagen fibers was significantly higher in the baicalin (200 mg/kg)-treated group than in the ligature group ($p = 0.047$). Baicalin treatment significantly down-regulated the protein expression for cyclooxygenase-2 ($p = 0.000$) and inducible nitric oxide synthase ($p = 0.003$), compared with the ligature group.

Conclusion: Baicalin protects against tissue damage in ligature-induced periodontitis in rats, which might be mediated, in part, by its inhibitory effect on the expression of cyclooxygenase-2 and inducible nitric oxide synthase. These activities could support the continued investigation of baicalin as a potential therapeutic agent in periodontal disease.

Dr Chengzhang Li, Department of Periodontology, School and Hospital of Stomatology, Wuhan University, 237 Luo Yu Road, Hongshan District, Wuhan 430079, China
Tel: +86 27 87646304
Fax: +86 27 87646697
e-mail: l56cz@hotmail.com

Key words: baicalin; cylooxygenase-2; inducible nitric oxide synthase; periodontitis

Accepted for publication December 19, 2006

Periodontitis is an inflammatory disorder of the periodontium, characterized by the destruction of supporting connective tissue and bone loss and, in severe cases, the exfoliation of teeth. As in other inflammatory processes, periodontitis is mediated by the up-regulation of the synthesis and release of a variety of pro-inflammatory mediators, such as cyclooxygenase products, nitrogen metabolites, reactive oxygen and cytokines, thus leading to excessive injury (1).

Prostaglandin E_2, a vasoactive eicosanoid produced by activated macrophages and fibroblasts, is considered to be the major inflammatory mediator of alveolar bone destruction (2). Cyclooxygenase is the critical enzyme in the formation of prostaglandin E_2 from arachidonic acid. Currently, there are two known isoforms of the enzyme. Cyclooxygenase-1 is constitutively expressed in various

tissues, whereas cyclooxygenase-2 is an inducible enzyme believed to be responsible for prostaglandin E_2 synthesis at sites of inflammation (3,4). Studies have shown that cyclooxygenase-2 expression is significantly up-regulated in inflamed periodontal tissues (5) and that selective cyclooxygenase-2 inhibition can modify the progression of experimentally induced periodontitis in rats (6,7). These findings strongly support an inflammatory role for cyclooxygenase-2 in human periodontitis and make it the target for treatment of periodontitis.

Nitric oxide is a signaling molecule that regulates various physiological and pathophysiological responses in the human body, including vasodilation, neural communication and host defense (8). The association of nitric oxide with periodontitis is supported by the increased levels of nitric oxide reported in human gingival fibroblasts induced by pro-inflammatory cytokines (9) and in ligature-induced periodontitis in rats (10). It is now recognized that inducible nitric oxide synthase (the inducible isoform of nitric oxide synthase) is responsible for the overproduction of nitric oxide activated by lipopolysaccharides or pro-inflammatory cytokines (11) and is up-regulated in inflamed gingival tissue (12). Early work found that administration of the nitric oxide synthase inhibitor, aminoguanadine, ameliorated tissue damage associated with periodontitis (13). Therefore, the inducible nitric oxide synthase expression level may reflect the degree of inflammation and provide a measure to assess the effect of drugs on the inflammatory process.

Baicalin (7-glucuronic acid, 5,6-dihydroxy-flavone) is a flavonoid compound purified from the medicinal plant, *Scutellaria baicalensis* Georgi, which has been used as an anti-inflammatory agent in the treatment of a variety of inflammatory diseases, such as bronchitis, nephritis, hepatitis and asthma (14,15). Baicalin has been found to exert several biological activities, which are mainly related to its antioxidant properties and to its ability to inhibit enzymes and regulate the immune response (16–19). These activities may explain the beneficial effects that baicalin intake exerts in different pathologies, including hepatotoxicity, cancer, infectious diseases, etc. (17,20). Recently, baicalin has been found to inhibit cyclooxygenase-2 activity in a castor-oil-induced diarrhea model, as well as inducible nitric oxide synthase expression in lipopolysaccharide-treated RAW 264.7 macrophages (21,22), which suggests that cyclooxygenase-2 and inducible nitric oxide synthase pathways might be involved in the anti-inflammatory mechanisms of baicalin.

So far, few studies have investigated the effect of baicalin on periodontal disease. The present study was undertaken to determine whether baicalin could inhibit alveolar bone loss and connective tissue breakdown in ligature-induced periodontitis in rats. To investigate, in greater detail, the anti-inflammatory mechanisms involved, the effect of baicalin on the expression levels of cyclooxygenase-2 and inducible nitric oxide synthase was also evaluated *in vivo*.

Material and methods

Animals

Forty-five male Sprague–Dawley rats at the age of 2 mo (180–230 g body weight) were purchased from the Laboratory Animal Center of Wuhan University. They were housed, four to five per cage, under a 12-h light/dark cycle with temperature at 24 ± 2°C and humidity at 40–60%. The rats had free access to tap water and standard rat pellets. The experiment was approved by the Institutional Animal Care and Use Committee of Wuhan University.

Drug treatment

The rats were distributed into five experimental groups of nine rats each. Baicalin (Sichuan Jinxiu Huafuning Pharmaceutical Co., Ltd, Sichuan, China) was suspended in 0.5% carboxymethylcellulose. The groups were treated daily with baicalin (50, 100 or 200 mg/kg) by gavage for 8 d, starting 1 d prior to the surgical procedure. The control group did not receive the induction of periodontitis and was given vehicle (carboxymethylcellulose) alone. The ligature group consisted of rats subjected to periodontitis that received vehicle alone.

Experimental periodontal disease

Animals were anesthetized with Su-Mian-Xin, composed of dihydroetorphine hydrochloride, dimethylaniline thiazole, EDTA and haloperidol (0.8 ml/kg body weight, intramuscularly; Veterinary Institute of Military Supplies University, Changchun, China). Sterile, 3–0 black braided nylon thread (surgilon; USS/DG, Norwalk, CT, USA) was placed around the cervical margins of the bilateral lower first molars and knotted mesially. Rats were killed under anesthesia 7 d after ligature. One side of the mandible was used for routine histological processing to paraffin wax and the other stored at −80°C and subsequently used for the measurement of alveolar bone loss. Before storage at −80°C, the gingival tissues encircling the first molar were excised and immediately stored separately at −80°C for western blotting analysis.

Microscopic examination of periodontal bone loss

The jaws were thawed, defleshed by 2 N NaOH for 10 min, then washed and air dried. Each jaw was oriented so that the buccal and lingual cusps were superimposed prior to measurement. The distance from the amelocemental junction to the alveolar crest was measured by the method of Crawford *et al*. (23), using a stereoscope (Stemi SV 11; Zeiss, Oberkochen, Germany) equipped with a videocamera (AxioCam HRc; Zeiss) and a TV monitor, which displayed the distance digitally. The photos were analyzed by SPOT RT software, v3.5 (Spot Diagnostic Instruments, Sterling Heights, MI, USA). Recordings were made in the long axis of both buccal and lingual root surfaces of the first molars (Fig. 1). There were six recordings per tooth. The mean of the recordings for each tooth (expressed in mm) was used

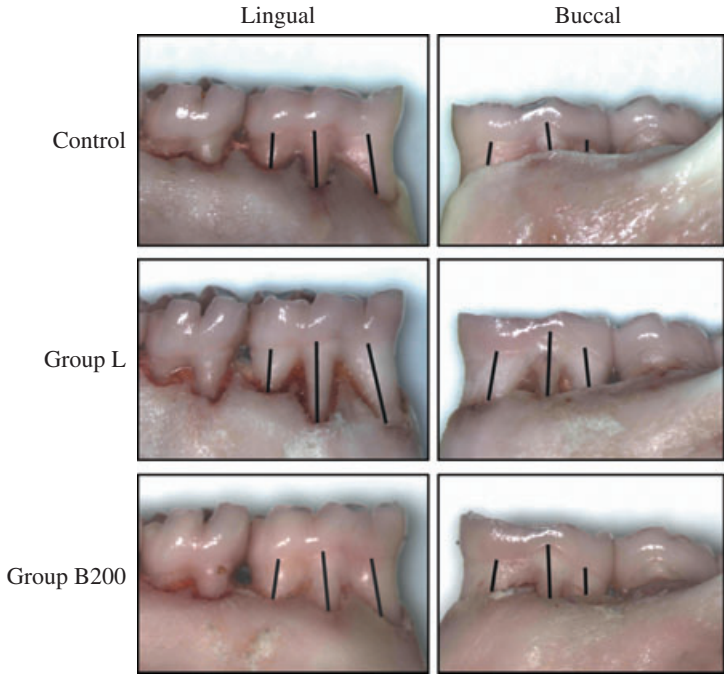

Fig. 1. Macroscopic aspects (Lingual and Buccal view) of the mandibles observed in Group C, Group L and Group B200. The black lines show the distance from the amelocemental junction to the alveolar crest.

as a measurement of bone loss. Calibrations were made using commercial standards. The sensitivity of the measurement was ≈10 μm. These measurements were performed by an independent investigator, who was unaware of the treatment regimens, and were repeated three times with an interval of 1 wk between each set of readings. The average coefficient of variation for the measurements was 1.67%.

Histological examination

The mandibles from the control group, the ligature group and the group receiving 200 mg/kg of baicalin were fixed in 4% phosphate-buffered formalin (pH 7.4, at 4°C) for 48 h and decalcified in a 10% EDTA solution for 4 wk at 4°C. Decalcified specimens were routinely processed to paraffin wax, and mesio-distal sections (6 μm) were obtained. Sections from each group were stained with hematoxylin and eosin and used for morphological examinations of histological changes induced by periodontitis.

Alveolar bone loss in the interseptal area between the first and second mandibular molars was measured on stained sections by SPOT RT software, v3.5 (Spot Diagnostic Instruments), in accordance with previously published methods (24). The distance between the level of alveolar bone crest and the amelocemental junction was measured on three random sections at the distal side of the mandibular first molar. The mean amelocemental junction to alveolar crest height was calculated. The mean amelocemental junction to alveolar crest height height was then calculated for each group of animals.

Collagen fiber staining and quantitative determination of collagen fibers

Staining of collagen fibers was performed with 0.1% sirius red F3Ba (Direct Red 80; Fluka, Buchs, Switzerland), according to Junqueira *et al*. (25), on 6-μm tissue sections.

The evaluation of the area fraction of collagen fibers in the gingival connective tissue (between the first and second molars) was determined in sections stained with sirius red. Histological sections were observed using a polarizing microscope (Olympus BHSP; Tokyo, Japan) equipped with a video camera (AxioCam HRc; Zeiss). The images were analyzed by Leica QWIN software (Leica Qwin V3; Leica Microsystems Imaging Solutions Ltd, Cambridge, UK), which calculated the area fraction occupied by the collagen fibers. For each sample, six randomly selected fields (0.01 mm^2) per tissue section were analyzed. Thus, the area fraction of collagen fibers for each specimen represented the mean of the six determinations.

Immunohistochemical analysis of cyclooxygenase-2 and inducible nitric oxide synthase

Sections were dewaxed and rehydrated into phosphate-buffered saline (pH 7.2). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 15 min. After rinsing in phosphate-buffered saline, sections were covered with normal rabbit serum (1 : 10 dilution), for cyclooxygenase-2, and with goat serum (1 : 10 dilution), for inducible nitric oxide synthase (SP kit; Vector Laboratories, Burlingame, CA, USA), for 15 min before incubation with primary antibodies overnight at 4°C in a humid atmosphere. The cyclooxygenase-2 polyclonal antibody (1 : 80 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and inducible nitric oxide synthase polyclonal antibody (1 : 100 dilution; Santa Cruz Biotechnology, Inc.) were diluted in phosphate-buffered saline. After being rinsed, the sections were incubated with biotinylated rabbit-antigoat (cyclooxygenase-2) or goat-antirabbit (inducible nitric oxide synthase) immunoglobulin (SP kit; Vector Laboratories) for 15 min at 37°C. The sections were rinsed with phosphate-buffered saline, incubated with the avidin–biotin–peroxidase complex (SP kit; Vector Laboratories), for 15 min at 37°C, and rinsed again. The immunostaining was visualized by developing in diaminobenzidine, and the sections were counterstained with Mayer's haematoxylin. Negative

controls consisted of sections processed in the absence of primary antibodies.

Quantitative evaluation of the results was carried out by two independent investigators who were blind to the histological results. Immunolabeled cells were counted in nine randomly selected microscopic fields in the gingival connective tissue between the first and second molars per section (number of cells per 0.006 mm^2), with a microscope (Olympus Optical Co. Ltd, Tokyo, Japan) using a 40× objective, and the data for each specimen represented the mean number of cell/field.

Western blotting for cyclooxygenase-2 and inducible nitric oxide synthase

Gingival tissues were homogenized in a proteinase inhibitor buffer (50 mmol/L Tris HCl, pH 8.0, 150 mmol/L NaCl, 1% Triton X-100, 100 μg/mL phenylmethanesulfonyl fluoride) on ice and then centrifuged at 14,811 ***g*** for 15 min at 4°C. The supernatant was collected and the protein content determined using the bicinchoninic acid method. The tissue homogenates (40 μg of protein per lane) were electrophoresed in a 10% sodium dodecyl sulfate-polyacrylamide gel and then transferred onto nitrocellulose membranes (Millipore Corporation, Bedford, MA, USA). Prestained protein molecular weight markers (Fermentas AB, Vilnius, Lithuania) were used for molecular weight determinations. Membranes were pretreated, for 1 h at room temperature. with blocking buffer containing 5% fat-free milk powder, 10 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl and 0.05% Tween 20, and then incubated overnight at 4°C with a 1 : 250 dilution of polycolonal antibodies against inducible nitric oxide synthase (Santa Cruz Biotechnology, Inc.) and a 1 : 1500 dilution of polyclonal antibodies against cyclooxygenase-2 (Cayman Chemicals Co., Ann Arbour, MI, USA). After washing in buffer for 30 min, the membranes were treated with horseradish peroxidase-conjugated secondary antibody (1 : 5000 dilution for inducible nitric oxide synthase, 1 : 10,000 dilution for cyclooxygenase-2; Pierce, Rockford, IL, USA) for 1 h at room temperature followed by another 30 min of washing. The ECL Western blotting system (Santa Cruz Biotechnology, Inc.) was used, in accordance with the manufacturer's instructions, for chemiluminescence of proteins, and the blots were then exposed to photographic films (KODAK, Eastman Kodak Company, Rochester, NY, USA).

Statistical analysis

All values in the figures and text are presented as the mean ± standard deviation. Univariate analysis of variance, followed by Turkey's test, was used to compare the means. The null hypothesis was rejected at $p < 0.05$.

Results

At the start of the study, there was no significant difference among the five groups in body weight ($p = 0.352$). The rats in the ligature group lost 8.4% of their basal weight on the first day after induction of periodontitis. This weight loss was sustained until day 7 when the animals weighed 235.5 ± 10.6 g, whereas control rats weighed 257.9 ± 12.4 g after the same time period ($p = 0.012$). The body weights of baicalin-treated animals (50 mg/kg: 234.1 ± 17.1 g; 100 mg/kg: 236.6 ± 16.7 g; 200 mg/kg: 232.9 ± 11.0 g) at the end of the experiment were not significantly different from those of the ligature group ($p = 0.980$).

Effect of baicalin on alveolar bone loss

Macroscopic examination of the mandibles revealed severe bone matrix resorption in the ligature group (Fig. 1). Baicalin reduced the mean amelocemental junction to alveolar crest height distance in the lower first molar at all doses tested (Figs 1 and 2A). However, only baicalin at a dose of 200 mg/kg resulted in a significant reduction of the amelocemental junction

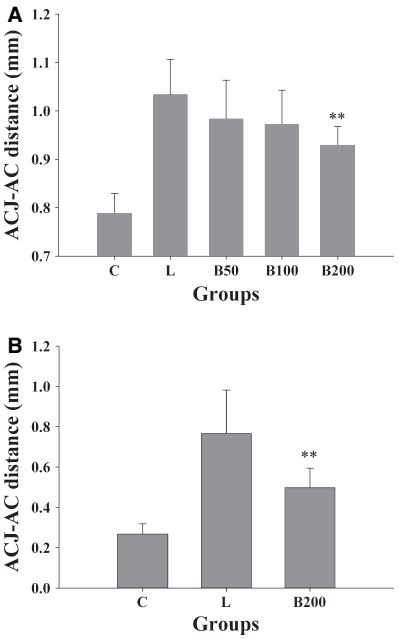

Fig. 2. Effect of baicalin treatment on alveolar bone loss. Data shown are mean ± SD of $n = 9$ rats for each group. (A) Microscopic examination of ACJ-AC distance on defeshed mandibles. $^{**}p = 0.009$ using ANOVA, compared to the Group L. No differences were seen within baicalin-treated groups. (B) Quantitative analysis of the change in ACJ-AC distance using H&E stained sections, measured from alveolar bone crest to the amelocemental junction at the distal side of the mandibular first molar. $^{**}p = 0.003$ compared to the Group L.

to alveolar crest height distance compared with the ligature group ($p = 0.009$). There were no significant differences between baicalin-treated groups (Fig. 2A), but all demonstrated significantly higher amelocemental junction to alveolar crest height distances compared with the control group ($p = 0.000$). Measurements taken from hematoxylin and eosin-stained sections showed essentially similar differences in amelocemental junction to alveolar crest height distances among the control group, the ligature group and the group receiving 200 mg/kg of baicalin ($p = 0.000$, $p = 0.003$, Fig. 2B).

Histological appearance

The periodontium of the animals subjected to experimental periodontitis

(the ligature group) showed accentuated inflammatory cellular infiltration, as well as destruction of alveolar bone. Tissues from baicalin-treated animals displayed reduced cell infiltration, coupled with preservation of the alveolar process, compared with the ligature group (Fig. 3).

Effect of baicalin on the area fraction of collagen fibers

Figure 4 illustrates collagen bundles from each group. The area fraction of collagen fibers was 31.08 ± 14.85% in the ligature group compared with 86.66 ± 6.62% in the control group (Fig. 5). The group treated with 200 mg/kg baicalin had a significantly higher area fraction of collagen fibers (48.13 ± 18.69%) than the ligature group ($p = 0.047$, Fig. 5). However, the area fraction of collagen fibers in the group receiving 200 mg/kg of baicalin was significantly lower compared with the control group ($p = 0.000$, Fig. 5).

Effect of baicalin on cyclooxygenase-2 and inducible nitric oxide synthase protein expression

Both cyclooxygenase-2 and inducible nitric oxide synthase immunoreactivi-

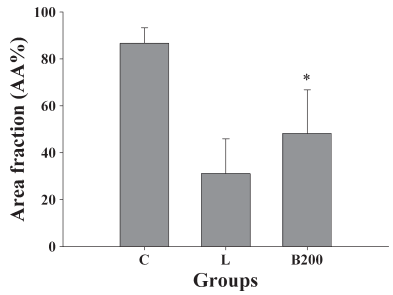

Fig. 5. Quantitative analysis of the change in area fraction (AA%) of collagen fibers in gingival tissue between the first and second molars. Data are mean ± SD of $n = 9$ rats for each group. *$p = 0.047$ compared to the Group L.

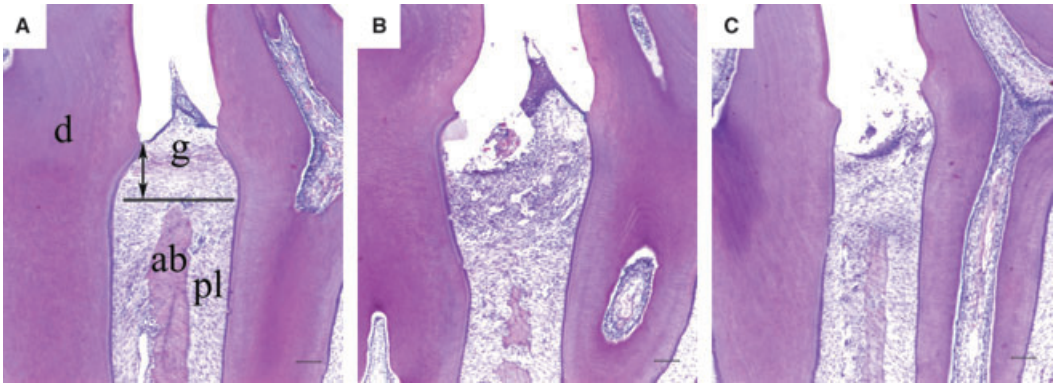

Fig. 3. Histological appearance of the region between the first and second molar roots. (A) Normal mandible from the control group, showing dentin (d), gingival (g), alveolar bone (ab), and periodontal ligament (pl). The double headed arrow shows the vertical distance from alveolar bone crest to the amelocemental junction (ACJ-AC). (B) Group L, showing accentuated inflammatory cellular infiltration and destruction of alveolar bone. (C) Baicalin-treated group (200 mg/kg), showing discrete cell influx, coupled to preservation of the alveolar process (hematoxylin and eosin stain; Bars = 100 μm).

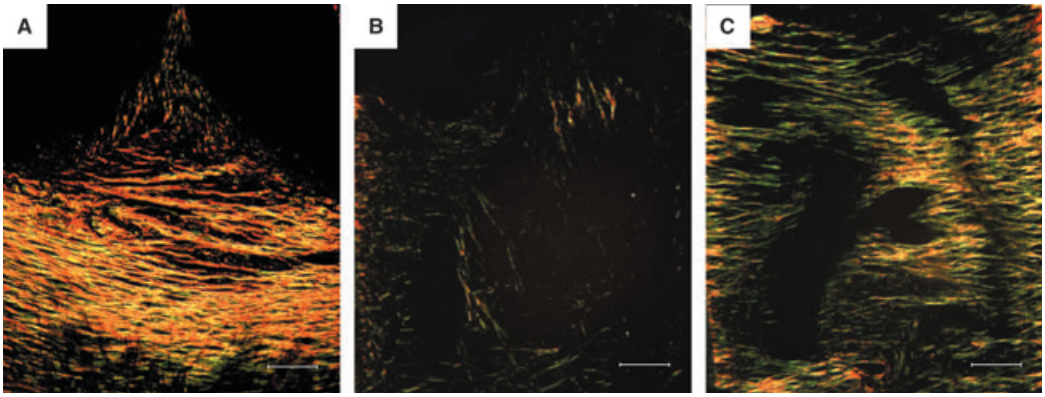

Fig. 4. Collagen fiber staining by sirius red. Tissue sections were observed using polarization microscopy, so the background appears black and collagen bundles stain yellow, green, or red according to their orientation. (A) The control group: collagen bundles are well defined and correctly organized. (B) Group L: collagen bundles are thin and intensively dissociated with large interbundle spaces. (C) Group B200: collagen fibers appears dissociated slightly, with several irregular spaces (loss of perivascular collagen) existing between these bundles. Bars = 50 μm.

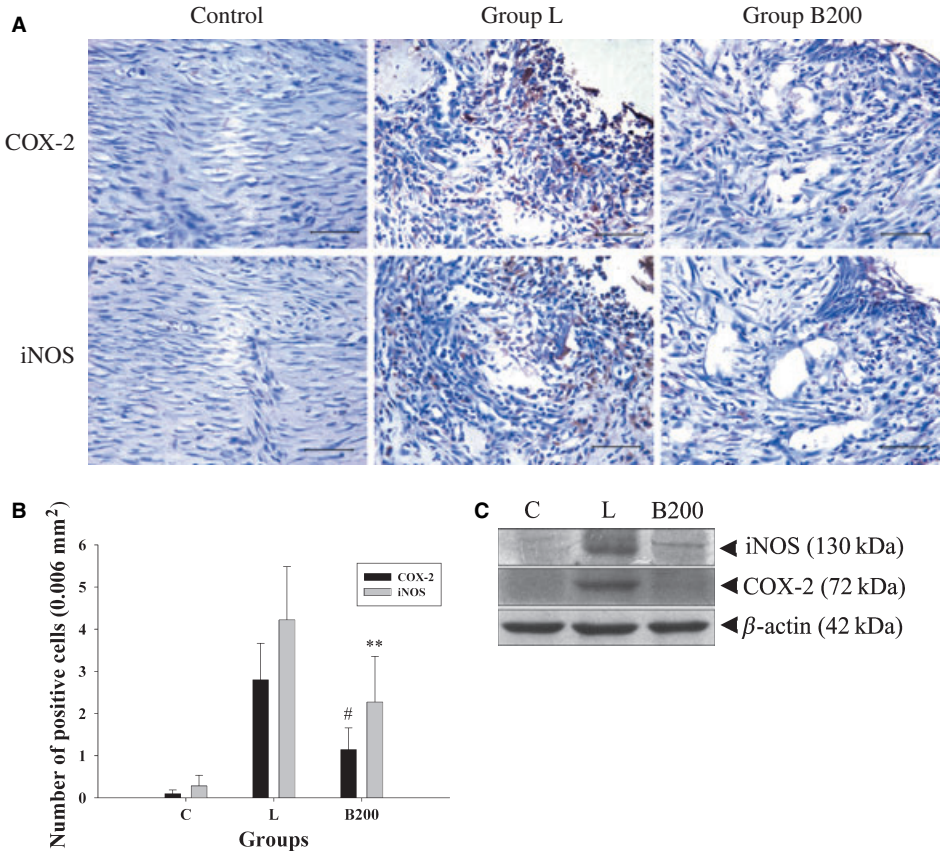

Fig. 6. Effect of baicalin on the protein expression levels of COX-2 and iNOS in the gingiva. (A) Immunohistochemical localization of COX-2 and iNOS. Bars = 50 μm. (B) Baicalin (200 mg/kg) administration significantly decreased the number of iNOS positive cells and COX-2 positive cells. Data are mean ± SD of $n = 9$ rats for each group. $^{**}p < 0.01$; $p < 0.001$ compared to the Group L. (C) Production of COX-2 and iNOS was down-regulated by the treatment of baicalin (200 mg/kg). β-actin levels were used as a loading control. This Western blot is representative of three experiments performed on different experimental days.

ties were mainly localized to macrophages, plasma cells and fibroblasts. Moreover, polymorphonuclear leukocytes were also immunoreactive for inducible nitric oxide synthase. In the control group, few cells were stained for cyclooxygenase-2 and inducible nitric oxide synthase (Fig. 6A). Positive cells were significantly increased in the ligature group compared with the control group ($p = 0.000$, Fig. 6B). Baicalin (200 mg/kg) administration significantly decreased the number of cyclooxygenase-2-positive cells ($p = 0.000$) and inducible nitric oxide synthase-positive cells ($p = 0.003$) compared with the the ligature group (Fig. 6B), but showed a significantly increased number of cells positive for cyclooxygenase-2 ($p = 0.000$) and inducible nitric oxide synthase ($p = 0.000$) compared with the control group (Fig. 6B).

Furthermore, we confirmed the effect of baicalin on inducible nitric oxide synthase and cyclooxygenase-2 protein expression in gingival tissues by western blotting. As shown in Fig. 6C, baicalin (200 mg/kg) treatment inhibited the up-regulation of inducible nitric oxide synthase (130 kDa) and cyclooxygenase-2 (72 kDa) protein expression.

Discussion

In this report, we investigated the anti-inflammatory effect of baicalin in acute ligature-induced periodontitis in rats. The current study, for the first time, demonstrated that baicalin reduces alveolar bone loss and connective tissue breakdown in this experimental periodontitis animal model, which might be explained, in part, by its inhibitory effect on cyclooxygenase-2 and inducible nitric oxide synthase expression.

Prostaglandin E_2 and nitric oxide are pleiotropic molecules with diverse effects on vasodilatation, immune responses and bone cell function (2,8). In the site of inflammation, the overproduction of prostaglandin E_2 and nitric oxide derived from cyclooxygenase-2 and inducible nitric oxide synthase, respectively, are well documented to participate in the immunoinflammatory response in mediating connective tissue alteration and bone resorption. Recent *in vivo* and *in vitro* studies have found that baicalin might have the ability to suppress cyclooxygenase-2 and inducible nitric oxide synthase pathways, thus reducing the overproduction of nitric oxide and prostaglandin E_2 (21,22,26,27). In this study, our results demonstrated that treatment with baicalin reduced alveolar bone loss

and was accompanied by a decreased expression of cyclooxygenase-2 and inducible nitric oxide synthase proteins, which suggested that one possible anti-inflammatory mechanism of baicalin in periodontitis might involve the inhibition of cyclooxygenase-2 and inducible nitric oxide synthase expression. This effect was further supported by a recent report that baicalin could inhibit the mRNA expression of receptor activator of nuclear factor-κB ligand (RANKL), a molecule that plays an important role in osteoclastogenesis from osteoclast precursors to mature osteoclasts, via the suppression of cyclooxygenase-2 expression in cultured human periodontal ligament cells induced by interleukin-1β (28).

The precise mechanisms involved in the suppressive effects of baicalin on cyclooxygenase-2 and inducible nitric oxide synthase are currently unknown, but it has been postulated that baicalin might inhibit nuclear transcription factor-κB (a redox-sensitive transcription factor known to regulate the expression of cyclooxygenase-2 and inducible nitric oxide synthase) by acting as an antioxidant (29–32). A study by Kim *et al*. showed that baicalin exhibited cytoprotective effects against cell death by peroxynitrite in an endothelial cell culture system, via suppression of nuclear factor-κB binding activity and down-regulation of the expression of inducible nitric oxide synthase and cyclooxygenase-2 (27). Furthermore, baicalin has been shown to diminish the production of reactive oxygen intermediates, a signaling molecule that modulates leukocyte endothelial adhesion, in phorbol-12-myristate-13-acetate or *N*-formyl-methionyl-leucyl-phenylalanine-induced neutrophils or monocytes (33,34). Recently, it has been reported that baicalin has the ability to bind selectively to several chemokines, such as interleukin-8, and reduces chemokine-induced neutrophil infiltration *in vivo* (35). It also attenuates the formation of pro-inflammatory cytokines in carrageenan-injected paws (26). The decreased expression levels of cyclooxygenase-2 and inducible nitric oxide synthase, observed in the present study, might be a result of the direct suppressive effect of baicalin or, alternatively, to the inhibition of inflammatory cellular infiltration. More extensive studies are needed to investigate the exact signal pathways involved in the pharmacological activities of baiclin in periodontitis.

Collagen is the major extracellular matrix component of gingiva. Collagen degradation, combined with its formation, is needed for successful connective tissue development. The present study showed that the density of collagen fibres was higher in the baicalin-treated group than in the ligature group, which provided evidence that the beneficial effect of baicalin is on collagen metabolism. It is well known that the matrix metalloproteinases (MMPs), a family of neutral endopeptidases that have Zn^{2+} at their active sites, are responsible for the degradation of collagen fibers (36). Our group has demonstrated that baicalin can inhibit the secretion of pro-MMP-1 and expression of MMP-3 in interleukin-1-induced human gingival fibroblasts and periodontal ligament cells (16). In addition, baicalin could also block MMP-8 release from polymorphonuclear leukocytes induced by IL-8 (37). In this report, the inhibitory effect of baicalin on the cyclooxygenase-2 pathway might be another explanation, as suggested from a role of prostaglandin E_2 as an enhancer of collagenase synthesis in macrophages (38). Besides, it has been reported that baicalin has the ability to stimulate the cellular activity of gingival fibroblasts, accompanied by the increase of synthesis of collagen (39). In view of these findings, the favorable effect of baicalin on collagen metabolism might be the result of a decrease in the degradation of collagen by an endogenous collagenase and/or an increase in the production of collagen.

Baicalin is a major active component of the medicinal plant, *S. baicalensis* Georgi, which has been extensively used in China and Japan as an anti-inflammatory and anti-infectious agent, without significant detrimental effect (14,15,18,22). The present study indicated that baicalin reduced periodontal tissue destruction in ligature-induced periodontitis in rats, which might be, at least in part, mediated by the inhibitory action of baicalin on the expression of cyclooxygenase-2 and inducible nitric oxide synthase. These activities could support its consideration as a valid drug in the pharmacological treatment of periodontitis. However, further studies are needed to investigate its efficiency in human periodontal disease.

Acknowledgements

This study was supported by the Key Technology R & D Program of Hubei Province in China. Grant number: 2001 AA308B07.

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