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ORIGINAL RESEARCH PAPER

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Abstract
Objective We have recently reported that experimental periodontitis (EP) reduced methacholine-induced submandibular gland (SMG) salivary secretion. The aim of the present study was to determine whether histamine could prevent SMG impairment produced by EP.
Materials and Methods Bilateral EP was induced for 2 weeks and histamine treatment (0.1 mg/kg subcutaneously) was started 5 days before the end of the experimental period in male rats. The histamine effects on periodontitis-altered functional and histological parameters of SMG and on periodontal bone loss were evaluated.
Results Histamine treatment partially reversed the methacholine-induced salivation reduction produced by EP while preventing SMG histological damage. Histamine's effect on SMG was associated with an increased proliferation rate (2.2 ± 0.3 vs. 0.2 ± 0.2 proliferative cells per field, $P < 0.001$). Furthermore, histamine completely prevented enhanced EP-induced apoptosis (1.0 ± 0.4 vs. 60.9 ± 4.6 apoptotic cells per field, $P < 0.001$). The protective effect exerted by histamine on SMG functionality is associated with attenuation of lingual and vestibular bone loss (0.66 ± 0.04 vs. 0.97 ± 0.06 mm; $P < 0.001$).
Conclusions Histamine is able to reduce periodontitis-induced damage to SMG and bone structure.

Keywords Histamine · Salivary secretion · Submandibular gland · Apoptosis · Periodontitis

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J. P. Prestifilippo (✉)
Physiopathology Department, School of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956, 5th, 1113 Buenos Aires, Argentina
e-mail: jprestifilippo@yahoo.com.ar

E. Carabajal · E. S. Rivera · V. A. Medina
Laboratory of Radioisotopes, School of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956 PB, 1113 Buenos Aires, Argentina

M. Croci
Institute of Immunooncology, Av. Córdoba 3200, 1187 Buenos Aires, Argentina

J. Fernández-Solari · J. C. Elverdin
Physiology Department, School of Dentistry, University of Buenos Aires, 1122 Buenos Aires, Argentina

J. Fernández-Solari · V. A. Medina
National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina

Introduction

Saliva is an important protective factor, playing a key role in the local and systemic defense of the oral cavity, the oropharyngeal region, and the upper gastrointestinal tract [1–3]. It has lubricating, cleansing, antibacterial, buffering, and remineralizing effects. Therefore, disturbances in salivary gland function and a diminished secretion of saliva accompany inflammation development in the oral cavity [4–6].

Periodontitis is a chronic disease caused by oral bacterial infection and persistent local inflammation, which is responsible for its pathogenesis; it destroys the periodontal tissues, resulting in alveolar bone resorption and soft tissue attachment loss. If left untreated, the disease continues to progressive bone destruction, leading to tooth mobility and subsequent tooth loss [7–10]. Epidemiological studies have demonstrated a relationship between periodontitis and salivary dysfunction. Various studies suggest that changes in volume and/or the rate of saliva secretion and the

composition of salivary electrolytes and proteins promote the development of microorganisms associated with gingivitis and periodontitis. Saliva is the first barrier of defense against the entry of these agents in the digestive tract and inhibition of salivary secretion promotes the accumulation of plaque biofilm, increasing the risk of gingival inflammation and periodontitis [11, 12].

The submandibular gland (SMG) is one of the major salivary glands, together with the sublingual and the parotid glands, being the major contributor to the volume of whole saliva. End secretory units, called acini, are continuous with a ductal system that leads the saliva to the oral cavity [13, 14].

Recently, we have reported that experimental periodontitis (EP) reduced methacholine-induced SMG salivary secretion while increasing prostaglandin E content [15]. Furthermore, treatment with lipopolysaccharide, a microbial component of the plaque biofilm, reduced SMG salivation [16, 17]. In addition, vacuolization and apoptosis of SMG in the rat periodontitis model was observed [18]. However, the exact mechanism of periodontitis-induced salivary gland dysfunction is not clearly understood.

Salivary secretion is not only altered in different pathological states such as in autoimmune diseases but also after ionizing radiation exposure [19–25]. In this light, we have recently demonstrated that histamine prevents morphological and functional radiation-induced damage to SMG [26]. Histamine [2-(4-imidazolyl)-ethylamine] is an endogenous biogenic amine widely distributed throughout the organism and it has long been known to be a pleiotropic mediator in different (patho)physiological conditions [27, 28]. It has been previously reported that histamine could act as a stimulator of salivary secretion in SMG of cats and dogs [29, 30], and, furthermore, histamine treatment induces the translocation of aquaporin-5, which modulates water secretion to produce primary saliva, to the plasma membrane in human SMG cells [31].

Based on the evidence described, the aim of the present study was to determine whether histamine could prevent SMG impairment produced by experimental periodontitis. For that purpose, histamine's effect on periodontitis-altered functional and morphological parameters of SMG was evaluated. We also analyzed proliferation and apoptosis by immunohistochemistry. Finally, we investigated the effect of histamine on periodontal bone loss.

Materials and methods

Animals, treatments and periodontal disease

Forty-eight adult male Sprague–Dawley rats with an initial body weight of 230–250 g were randomly divided into four groups ($n = 12$ each) and were maintained on standard chow pellets and tap water ad libitum. Housing conditions were as follows: galvanized wire cages; five animals per cage; temperature: 22–24°C; humidity: 52%–56%; and 12-h light/dark cycles.

Group 1 (control) received saline starting 5 days before being killed and continuing until the end of the experimental period. Group 2 (histamine) received a daily subcutaneous histamine injection (0.1 mg/kg) starting 5 days before being killed and continued until the end of the experimental period. Group 3 (experimental periodontitis) exhibited bilateral EP that was induced for two weeks and received saline treatment starting 5 days before the end of the experimental period. Group 4 (histamine–experimental periodontitis) exhibited bilateral EP that was induced for two weeks and received a daily subcutaneous histamine injection (0.1 mg/kg) starting 5 days before being killed and continuing until the end of the experimental period. This concentration of histamine was chosen because it is the one that produces a marked radioprotective effect on rat salivary glands [26]. The rats were killed 5 h after histamine treatment.

In groups 3 and 4, EP was induced by placing a cotton thread ligature around the neck of both first lower molars (bilateral EP) in animals that were anesthetized with intraperitoneal administration of 2% xylazine hydrochloride (König Laboratories, Buenos Aires, Argentina) (5 mg/kg of body weight) and 5% ketamine hydrochloride (Holliday-Scott, Buenos Aires, Argentina) (50 mg/kg of body weight). The cotton thread ligature was pushed into the gingival sulcus and was left in place for two weeks until death [32].

Salivary secretion was determined by methacholine induction ($n = 6$ each group) or animals were killed by cervical dislocation and SMG were removed and weighed, and histological and histochemical characteristics were evaluated ($n = 6$ each group). Hemi-mandibles, once extracted and defleshed, were stained with 1% aqueous methylene blue for microscopic determination of vestibular and lingual bone loss ($n = 6$ each group, 12 hemi-mandibles per group).

Animal procedures were performed in accordance with the recommendations from the Guide for the Care and Use of Laboratory Animals of the National Research Council, USA, 1996.

Salivary secretion

Salivation was assessed in anesthetized rats (chloralose 100 mg/kg of body weight, 0.5 ml NaCl 0.9%). The right femoral vein was cannulated with a polyethylene catheter (P40 catheter, Rivera & Cia, Argentina) to administer the sialagogic agonist, methacholine, used in the study.

Through a midline incision in the neck, the trachea was intubated and the right SMG ducts were exposed and cannulated with a fine glass cannula to collect saliva samples. No basal salivation was observed from the glands. Salivation was induced by the administration of different concentrations of methacholine ranging from the threshold dose to the dose that exerts the maximum stimulus (0.3, 1, 3 and 10 μg/kg, in saline) (FLUKA, Berlin, Germany), sequentially injected via the right femoral vein. Salivary samples were collected for 3 min in pre-weighed aluminum foil and the quantity of saliva was determined by weighing, as previously described [33]. Three additional minutes were allowed until the administration of the next dose. Results were expressed as mg of saliva/gland.

Histopathological studies

Salivary glands were removed and fixed with 10% neutral buffered formalin. Tissue samples were embedded in paraffin and cut into 3-μm-thick serial sections. SMG morphology and histopathological characteristics were examined on tissue sections after hematoxylin–eosin staining. Light microscopy was performed on an Axiolab Carl Zeiss microscope (Göttingen, Germany). All photographs were taken at 630× magnification using a Canon PowerShot G5 camera (Tokyo, Japan).

Immunohistochemical staining

After deparaffinization, specimens were placed in citrate buffer (10 mM, pH 6.0) and heated in a microwave oven twice for 2 min at boiling temperature for antigen retrieval. Endogenous peroxidase activity was blocked with 3% H_2O_2 in distillated water. After blocking, tissues were incubated with primary mouse anti-PCNA (1:100, Dako-Cytomation, Glostrup, Denmark) antibodies overnight in a humidified chamber at 4°C. Immunoreactivity was detected by using horseradish peroxidase-conjugated anti-mouse (1:100) and visualized by diamino-benzidine staining (Sigma Chemical Co., St. Louis, MO, USA). To evaluate subcellular localization, nuclei were stained with hematoxylin. Light microscopy was performed on an Axiolab Carl Zeiss microscope. All photographs were taken at 630× magnification using a Canon PowerShot G5 camera. To control the signal specificity, serial sections were made from five selected positive cases which were subjected to the same staining procedure, with either a normal mouse or phosphate-buffered saline (PBS) to replace the first antibody. This control staining did not give rise to a signal. Proliferation was evaluated by assessing PCNA expression and results were expressed as the number of PCNA-positive cells per field of at least 15 fields examined.

Determination of apoptosis

Apoptotic cells were determined by terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate biotin nick end labeling (TUNEL) assay. Fragmented DNA in cells undergoing apoptosis was detected using an ApopTag® Plus Peroxidase *In-Situ* Apoptosis Kit (Chemicon International, Temecula, CA, USA) according to the manufacturer's instructions. Tissues were visualized using an Axiolab Carl Zeiss microscope. All photographs were taken at 630× magnification using a Canon PowerShot G5 camera. Negative control sections were incubated in the absence of TdT. Results were expressed as the number of TUNEL-positive cells per field of at least 15 fields examined.

Microscopic examination of periodontal bone loss: distance method

Immediately after death, hemi-mandibles were resected, defleshed and stained with 1% aqueous methylene blue to delineate the cemento-enamel junction (CEJ) and the alveolar crest (AC). A stereomicroscope (Stemi DV4 Stereomicroscope, Carl Zeiss MicroImaging, Göttingen, Germany) and a digital caliper (Digimess, Geneva, Switzerland) were used to measure three lingual and three vestibular distances (mesial, central and distal) from the CEJ to the most apical area of theca [34]. The mean of the six recordings (three lingual and three vestibular) on each molar was used as a measure of the total alveolar bone loss in mm.

Determination of PGE content by specific radioimmunoassay

Measurement of prostaglandin E (PGE) content was performed as previously described [15]. Briefly, gingiva were homogenized in 1 ml ice-cold ethanol (100%), centrifuged at 10,000*g* for 15 min at 4°C, and the supernatants were collected and evaporated in a Speed-Vac. The residues were resuspended with radioimmunoassay buffer. The cross-reactivity of PGE_2 was 100% with PGE_1 and lower than 0.1% with other prostaglandins. The intra-assay and inter-assay coefficients of variation for PGE_2 were 8.2% and 12%, respectively. Studies were performed using six rats per group. The PGE content was expressed as picograms (pg) per mg of tissue.

Statistical analysis

Data shown are mean ± standard error of the mean (SEM). Statistical evaluations were made by analysis of variance (ANOVA) followed by the Newman–Keuls

multiple comparison test. P values < 0.05 were considered significant. All statistical analyses were performed with GraphPad Prism Version 5.00 software (San Diego, CA, USA).

Results

Effect of histamine and EP on SMG functionality and morphology

In order to further explore the effect of EP on SMG physiology, we first evaluated its role in SMG functionality and its possible modulation by histamine. The present results demonstrate that periodontal disease significantly decreased salivation by approximately 50% at all concentrations of methacholine tested, in comparison to untreated rats (Table 1). Interestingly, histamine treatment partially reversed the decrease in EP-induced salivation, since the reduction of saliva secretion was approximately 30% in histamine-treated and EP-induced rats (Table 1). In addition, EP produced alterations in SMG morphology, including partial loss of secretor granular material and periductal edema and a slightly increased vacuolization of acinar cells compared to the control group. Histamine treatment seemed to counteract the histopathological changes (Fig. 1).

Effect of histamine and EP on proliferation and apoptosis of SMG

Proliferating cell nuclear antigen (PCNA) is a well-documented indicator of active proliferation, being an essential component of the DNA replication machinery [35]. PCNA expression was scarce and preferentially restricted to the ductal cells and no significant difference was observed after histamine treatment. Induction of EP markedly reduced the PCNA immunoreactivity, indicating a reduction of proliferation. Conversely, histamine treatment significantly increased PCNA-positive cells per field (2.2 ± 0.3 vs. 0.2 ± 0.2, $P < 0.01$) in the EP group (Fig. 2; Table 2).

Enhanced apoptosis of SMG acinar cells is observed in periodontal disease induced in rats [18]. In order to investigate whether histamine's effect could be associated with a reduction in glandular cell death, we evaluated the number of apoptotic cells in SMG by the TUNEL assay. SMG from control animals revealed a small number of TUNEL-positive cells, primarily in ducts, that was not modified by histamine treatment. The EP group showed increased apoptotic cells in SMG in acini and principally in the ducts. In contrast, histamine completely prevented the EP-induced enhanced apoptosis (1.0 ± 0.4 vs. 60.9 ± 4.6, $P < 0.001$). (Figure 3; Table 2).

Effect of histamine on EP-induced bone loss

The measures of lingual and vestibular bone loss, as distances between the CEJ and the AC, demonstrated an enhanced bone loss in the EP group (Table 3; Fig. 4). Histamine treatment during the last 5 days of EP induction diminished the lingual and the vestibular bone loss (0.66 ± 0.04 vs. 0.97 ± 0.06 mm; $P < 0.001$) (Table 3; Fig. 4). PGE_2 is one of the most extensively studied mediators of periodontal disease activity [36]. We therefore further investigated PGE_2 content in gingival tissue by radioimmunoassay. Results demonstrated that periodontitis significantly increased PGE_2 levels as compared to controls while histamine treatment reduced its content in gingiva of rats with EP (Fig. 5).

Discussion

The objective of periodontal treatment should ultimately be to regenerate the periodontal tissue using non-surgical or surgical techniques, biomaterials for guided tissue regeneration, bone substitutes, growth factors, and so forth. Appropriate experimental animal models are therefore required for testing new therapies for damaged periodontal tissues [37]. Rodents and rats, in particular, are ethically relevant models for experimental periodontal research, since the structure of the dental gingival area is quite similar to that observed in humans. One experimental

Table 1 Histamine prevents salivary secretion decrease induced by experimental periodontitis

Methacholine dose (μg/kg)	Control	Experimental periodontitis	Histamine–experimental periodontitis	Histamine
0.3	4.2 ± 0.3	$2.0 \pm 0.1^{***}$	$3.2 \pm 0.1^{***,\#\#\#}$	$5.0 \pm 0.1^{**}$
1	14.2 ± 1.2	$7.0 \pm 0.5^{*}$	10.0 ± 1.5	18.5 ± 3.0
3	40.5 ± 0.5	$19.5 \pm 1.5^{***}$	$27.9 \pm 1.1^{***,\#\#\#}$	42.5 ± 1.5
10	95.0 ± 8.0	$42.0 \pm 2.0^{***}$	$57.0 \pm 2.1^{***}$	88.0 ± 7.0

Mean methacholine-stimulated salivary secretion in rat experimental groups expressed as mg of saliva/gland ($n = 6$ per group). Data represent means ± SEM. * $P < 0.05$, *** $P < 0.001$ vs. control. ### $P < 0.001$ vs. experimental periodontitis

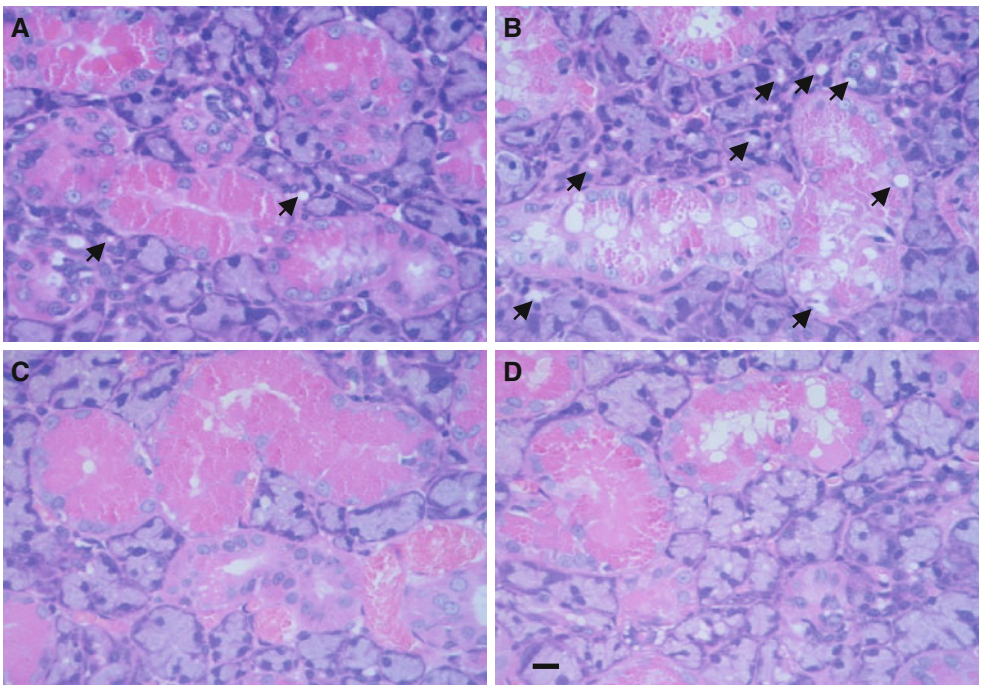

Fig. 1 Histamine prevented SMG morphological alterations produced by periodontitis. **a** Histological appearance of control SMG showing few vacuoles (*arrows*) and normal structural organization of the gland. **b** SMG of EP group showing alteration of the epithelial architecture with partial loss of secretory granular material and enhanced vacuolization of acinar cells (*arrows*). **c** SMG of histamine-treated and EP-induced animals showing preserved structure organization of secretory granules, with homogeneity of the granule diameter and normal appearance of granular convoluted ducts. **d** SMG of histamine-treated rats exhibiting the same histological features of control group. Pictures were taken at 630× magnification. *Scale bar* 20 μm

model widely used to study periodontal disease is by fixing ligatures around the teeth. This animal model has characteristics for the disease process that are similar to those encountered in humans [38–40]. In severe degrees of periodontitis, the lesions affect the inter-radicular and inter-dental spaces with extensive alveolar bone resorption and denudations of the molar roots [41]. In the present study we demonstrated that 15 days of EP induced in rats produced a considerable decrease in SMG salivary secretion, indicating that periodontitis severely altered the SMG functionality. In agreement with these results, we have recently reported that 7 days of EP caused a drastic reduction in the capacity of SMG to secrete saliva in response to cholinergic stimulation [15]. The inhibitory effect on salivation induced by EP was partially prevented by treatment with a low concentration of histamine administered subcutaneously. In line with this observation, we have previously demonstrated that histamine treatment completely reversed ionizing radiation-induced salivary secretion impairment [26].

The altered functionality of SMG was associated with changes in glandular morphology, which mainly include a reduction in secretor granular material, and also an enhanced vacuolization of acini. Furthermore, EP not only significantly increased ductal but also enhanced acinar cell apoptosis while decreasing the immunoreactivity of the proliferation marker PCNA. In accordance with this, other studies suggest that vacuolization and apoptosis of the acinar cells in the SMG induced by periodontitis may possibly affect salivary properties [18]. Furthermore, vacuolization and apoptosis of the acinar cells are observed after irradiation [26, 42, 43], and lead to diminished secretion of saliva. These findings support the hypothesis that histopathological changes and apoptosis of SMG cells could be involved in the EP-induced salivary secretion defect. Histamine treatment prevented both morphological and functionality changes in the SMG and significantly decreased the enhanced apoptosis and partially reversed the decreased proliferation produced by EP. Similar results were obtained in irradiated SMG, in which histamine treatment prevented the apoptosis and the ionizing radiation-induced reduced proliferation [26]. The inhibitory effect of histamine on apoptosis has also been described in intestinal mucosal apoptosis induced by ischemia–reperfusion [44, 45] and ionizing radiation [46]. This result might be partly supported by the fact that histamine, working as a growth factor, accelerated repair of damaged mucosa in the rat small intestine [44, 45].

The secretagogue properties of histamine on the salivary gland that seemed to be mediated via H2 receptors have

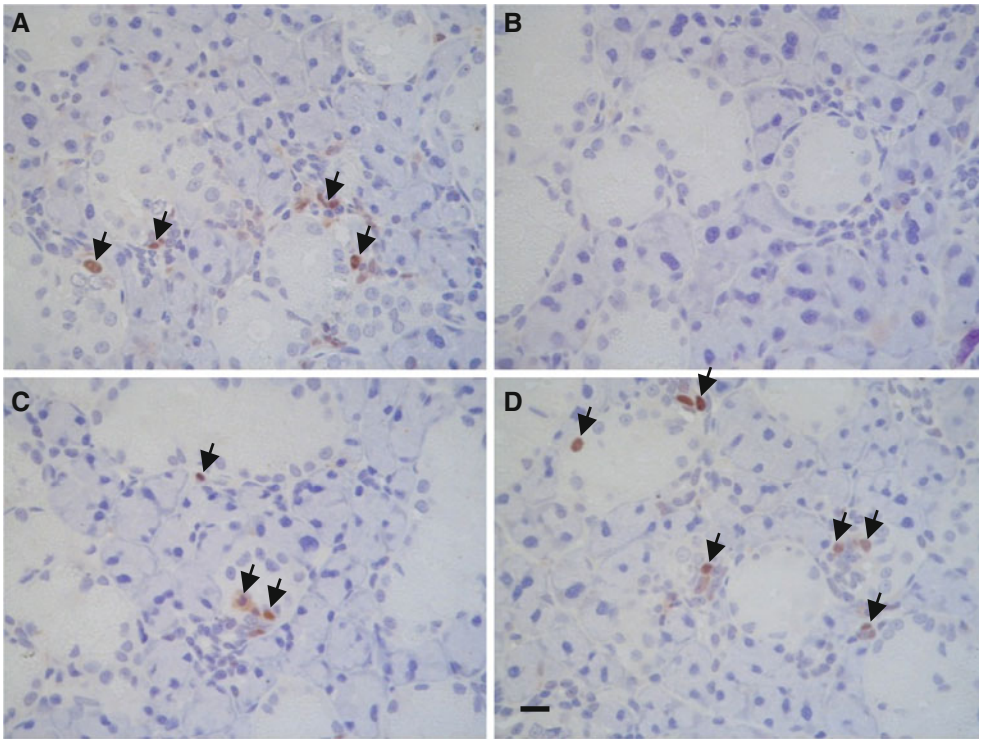

Fig. 2 Histamine enhanced PCNA expression in SMG of EP group. **a**, **d** Similar PCNA immunoreactivity in SMG from control and histamine-treated rats. **b** Almost total absence of PCNA immunoreactivity in SMG of EP rats. **c** Partial preservation of PCNA-positive cells in SMG of histamine-treated and EP rats. *Arrows* indicate PCNA-positive cells. Pictures were taken at 630× magnification. *Scale bar* 20 μm

Table 2 Effect of histamine on cell proliferation and apoptosis of submandibular gland in rats with experimental periodontitis

Group	PCNA-positive cells/field	TUNEL-positive cells/field
Control	4.2 ± 0.8	0.3 ± 0.2
Experimental periodontitis	0.2 ± 0.1***	60.9 ± 4.6***
Histamine–experimental periodontitis	2.2 ± 0.3$^{**,\#\#}$	1.0 ± 0.4$^{\#\#\#}$
Histamine	3.7 ± 0.4	0.4 ± 0.2

Data represent positive cells per field of at least 15 fields examined (mean ± SEM, $n = 6$ per group). ** $P < 0.01$, *** $P < 0.001$ vs. control. ## $P < 0.01$, ### $P < 0.001$ vs. experimental periodontitis

been previously reported [47]. Furthermore, previous studies have demonstrated the presence of histamine H1 and H2 receptor subtypes in rat SMG [31, 48, 49]. Histamine treatment also induces the translocation of aquaporin-5, which modulates water secretion to produce primary saliva, to the plasma membrane in human SMG cells through the histamine H1 receptor [31]. In this regard, histamine H1 receptor antagonists prescribed for allergic reactions are reported to cause xerostomia (dry mouth), which is a pathological condition characterized by decreased salivary secretion [50]. On the other hand, other studies demonstrated that H2 receptor antagonists enhanced salivary secretion [51], while preventing periodontitis when locally administered [52]. In our study, a secretory effect of histamine in the control group (non-periodontitis) was not observed, except for a very slight increase at the lowest methacholine concentration, suggesting that the preventive effect of histamine on periodontitis-induced salivary secretion dysfunction is preferentially due to its capacity to reduce the apoptotic response and to enhance the proliferative capacity of SMG.

Reduced salivary secretion can increase the numbers of pathological bacteria, and induce oral diseases such as periodontitis [11, 12]. In this regard, we have previously reported that submandibulectomy has a deleterious effect on the periodontal tissues, particularly marginal alveolar bone, indicating the importance of this gland in maintaining healthy periodontal conditions [53]. We demonstrated that histamine treatment augmented salivary secretion of SMG from rats with EP. Therefore, we subsequently investigated whether histamine could reduce the magnitude of the induced periodontitis. Bone loss was calculated as the distance between the CEJ and the AC, which is the recommended method for short (≤15 days EP) observation periods rather than the area method [54]. It is important to point out that the molars with EP exhibited significant bone loss. Our results are in agreement with previously reported

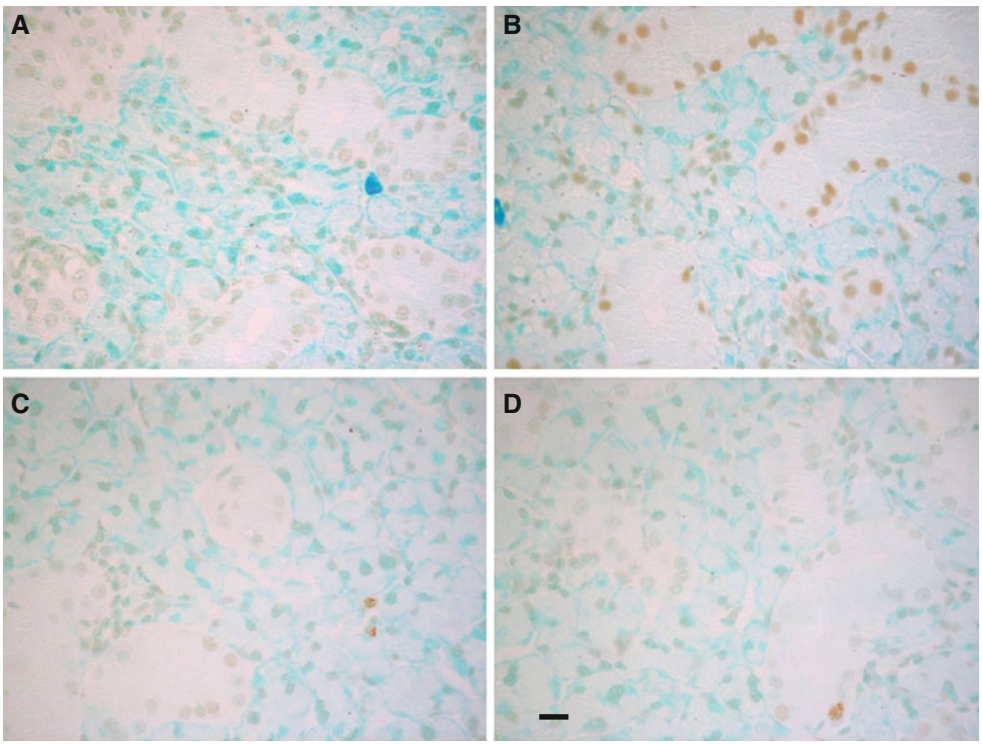

Fig. 3 Histamine prevented EP-induced apoptosis in SMG. Apoptosis was analyzed by TUNEL assay. **a** Almost total absence of TUNEL-positive cells in glandular cells in untreated rats. **b** Increased TUNEL-positive cells principally in ductal cells of the SMG of EP rats. **c** Reduction of TUNEL-positive cells in SMG of histamine-treated and EP rats. **d** Similarly, a low number of TUNEL-positive cells are observed in SMG of histamine-treated rats

Table 3 Histamine avoids bone loss induced by experimental periodontitis

Group	Distance (mm)
Control	0.42 ± 0.03
Experimental periodontitis	$0.97 \pm 0.06^{***}$
Histamine–experimental periodontitis	$0.66 \pm 0.04^{***,\#\#\#}$
Histamine	0.39 ± 0.03

Effect of histamine on bone loss induced by experimental periodontitis (distance between the cemento-enamel junction and alveolar crest at the level of the lower first molar). Data represent means ± SEM. *** $P < 0.001$ vs. control. ### $P < 0.001$ vs. experimental periodontitis

data in which EP produced a two-fold increase in alveolar bone loss [15, 53, 55]. Interestingly, this detrimental effect was diminished by histamine treatment. PGE_2 is involved in several chronic inflammatory diseases including periodontitis, which causes loss of the gingival tissue and alveolar bone supporting the teeth [36]. PGE synthases are expressed in gingival tissue of patients with periodontitis [56] and PGE_2 production is induced by pathogens involved in periodontal infections such as *Prevotella intermedia* in human periodontal ligament fibroblasts [57]. We thus explored the PGE_2 content and results indicate that it is significantly increased in gingival tissue of animals with bilateral EP, suggesting an inflammatory response that correlated well with the bone loss observed. Furthermore, histamine treatment was able to partially decrease the EP-induced incremental PGE content in gingival tissue.

In the present study, we clearly demonstrated that histamine treatment significantly prevents EP-induced SMG damage, ameliorating histological and functional injury. Histamine's effect on SMG was associated with an increased rate of proliferation, as evidenced by the enhanced PCNA protein expression. Furthermore, results show that SMG of histamine-treated rats demonstrated a considerable decrease in ductal and also acinar cell apoptosis that might represent one of the major effects of histamine on the SMG preservation. The protective effect exerted by histamine on SMG functionality was also associated with attenuation of the bone loss which is characteristic of periodontal disease. This outcome agrees with the fact that the SMG is an important organ involved in the immunoprotection of the oral tissues [1–3]. Current studies are aimed at fully determining the receptors involved in these responses by using different histamine receptor antagonists.

Histamine dihydrochloride (developed as a subcutaneous formulation known as Ceplene) is being used in

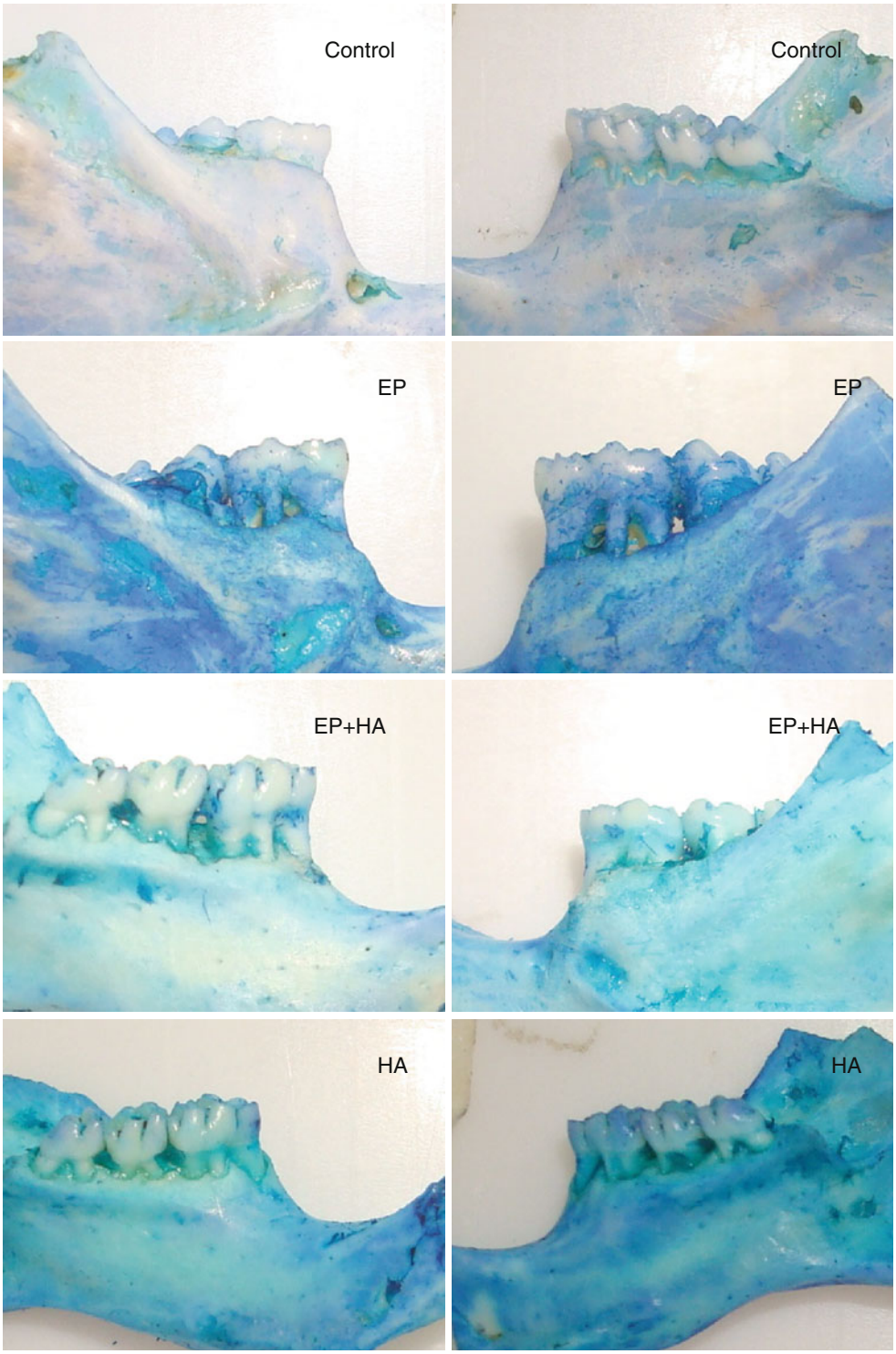

Fig. 4 Examination of periodontal bone loss by the distance method. Photographs of mandibles showing lingual (*left column*) and vestibular (*right column*) sections of lower first molars in the untreated (*control*), experimental periodontitis (*EP*), histamine-treated (*HA*), and histamine–experimental periodontitis (*EP+HA*) groups

several clinical trials as an adjuvant with interleukin-2 or interferon-alpha therapy for the potential treatment of different types of cancer such as metastatic melanoma, acute myelogenous leukemia and renal cell carcinoma. In all cases, histamine dihydrochloride was generally well tolerated and no unexpected or irreversible side effects were reported, demonstrating that histamine dihydrochloride can be safely administered [58, 59].

Based on the presented evidences, we conclude that histamine is able to reduce periodontitis-induced damage to SMG and bone structure. Although further studies are needed to fully understand histamine's role in periodontal

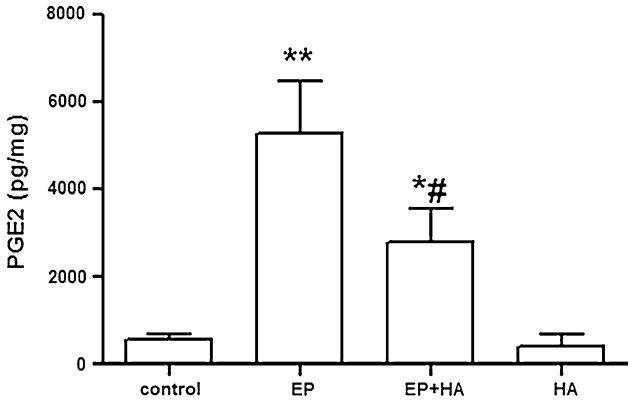

Fig. 5 Histamine partially reversed periodontitis-induced PGE levels. Mean PGE content in the untreated (*control*), experimental periodontitis (*EP*), histamine-treated (*HA*), and histamine–experimental periodontitis (*EP+HA*) groups. The results are expressed in pg PGE/mg of gingival tissue. *Error bars* represent means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control; $^{\#}P < 0.05$ vs. EP

disease, results suggest that this compound deserves to be studied prospectively as a potential agent for diminishing periodontitis.

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