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Original Article

Presence of mast cells and the expression of metalloproteinase 9 in the gingiva of ovariectomized rats with periodontal disease

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ABSTRACT

Background: The host's answer has an important role in periodontal disease, and the mast cells have a prime role. Such cells seem to be influenced by estrogen deficiency. The objective was to evaluate the mast cells and the expression of metalloproteinase(MMP)-9 in periodontal disease induced in ovariectomized rats.
Methods: For that purpose, 36 rats were used; 18 ovariectomized (OVX) and another 18 Sham-operated (SHAM). After 60 days the periodontal disease was induced by a ligature around the first lower right molars (group P). The opposite side was the control group (group C). The euthanasia occurred 3, 7 and 14 days after the placement of the ligature. The gingiva was removed and analyzed histochemically and immunohistochemically to quantify the mast cells and to analyze MMP 9 expression.
Results: By comparing the groups SHAM-P and C and groups OVX-C and P, it was noted that mast cells from group C were higher than P in all experimental periods. When comparing groups SHAM-C and OVX-C, significant factors were not found. When comparing groups SHAM-P and OVX-P, there was an inclination for mast cells reduction with time. The MMP-9 expression was related to the presence of periodontitis.
Conclusions: It was concluded that periodontitis led to mast cells reduction and MMP-9 increase. The ovariectomy itself did not alter the MMP-9 expression and did not influence the presence of mast cells in rat papilla, however, when associated to inflammation led to a reduction of mast cells.

1. Introduction

The mast cells are widely spread over the tissues, next to blood vessels and nerves, and also in subepithelial area. The mast cells cytoplasm contains membrane-linked granules that have several biological functions. The mast cells play a part in many events during the inflammation process[1] and have been widely studied due to their contribution in tumor angiogenesis[2] and participation on periodontal disease pathogenesis.[1,3]

The mast cells are important inflammatory cells because of their cytoplasmic granules that contain histamine, slow-reacting substance of anaphylaxis (SRS-A), heparin, eosinophil chemotactic factor of anaphylaxis and bradykinin, that are released to the gingival tissue in the periodontal disease. Besides that, the mast cells interleukins increase the collagenase activity and may increase the bone resorption.[4]

Jeffcoat et al.[5] demonstrated that the treatment with degranulation inhibitor from mast cells delayed the periodontal disease progression in dogs. Thus, the anti-histaminic abuse can modulate the inflammatory process and host's response to periodontal infection.

After being activated, mast cells may produce fibroblastic growing factor (FGF), nitric oxide, interleukins, acid phosphatases and some cytokines like interferon, alpha tumoral necrosis factor (TNF-α) and metalloproteinases (MMPs).[3]

Considering its participation in inflammation and tissue repair, presence near the surfaces in bone remodeling process and its capacity to supply chemical mediators[6]; it is reasonable to suggest that those cells disclose a relationship with the osteoporosis pathogenesis.[7] Further, mast cells may exacerbate bone resorption.[8]

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Studies demonstrated a possible relationship between the presence of mast cells and the post ovariectomy estrogenic deficiency.[9] Mast cells accumulate in the tibia bone marrow one month post ovariectomy.[9] Chemical induction of mast cell degranulation produces marked changes in the activity of MMPs, among other changes in ovariectomized rats.[10]

The MMPs belong to a family of enzymes that depends on zinc to act. They have several classes, among them: collagenases, gelatinases, stromelysins, matrilisins and membrane-type MMPs. Colagen IV e V are the main subtracts for the gelatinases MMP-2 and -9[11] that have been widely studied due to their participation in tumor progression.[12]

The MMPs have been found in cases of inflammatory periodontal disease and are related to the progress of the periodontal disease.[3] Some authors have investigated the role of the MMPs 2[13,14] and 9[13–15] in the progress of periodontitis,[16] but the participation of those enzymes in the process has not been totally established.

Since mast cells play an important role in the periodontal disease in humans and are related to estrogens and MMPs, the objective of this study was to evaluate the effect of estrogenic deficiency on mast cells and in MMP 9 expression on gingivae of ovariectomized rats with experimental induction of periodontal disease.

2. Methods

The present study was performed according to the Ethical Principles for Animal Experiments and approved by the Ethics Committee in Animal Experiment of the Faculty of Pindamonhangaba under protocol 005/2007. Thirty six adult female rats (*Rattus norvegicus,* variation *albinus, Wistar*), 90 days old, weighing about 300 g supplied by the Faculdade de Pindamonhangaba – FAPI were used.

The animals were divided in two groups: ovariectomized group (OVX) and false operated group (Sham), n = 18. Ovariectomy was performed as previous described.[8] After 60 days, to induce periodontal disease, rats were first anesthetized with an intramuscular injection of ketamine (90 mg/kg) and xilazine (10 mg/kg). A cotton ligature was placed in a subgingival position around the cervix of right first mandibular molars in each animal. The opposite side was used as control.

After the ligature placement, animals were randomly assigned to three experimental groups: 3, 7 or 14 days according to the experimental period (n = 6 animals per group).

Rats were euthanized under general anesthesia and perfusion of formaldehyde 10%. The gingiva around the first molar was removed, storage in formaldehyde 10% for at least 24 h included in paraffin and submitted to standard histological technique to HE stain, histochemistry using Toluidine Blue stain and immunohistochemical technique.

Light microscopy was used to qualitative analysis of HE slides. Ten semi-serial cuts with an average thickness 5 μm and an interval of 100 μm from each animal were obtained and toluidine blue-stained for visualization of the metachromatic mast cells. The mast cells quantification was performed in a light microscope with 400× of magnitude in the lingual papilla. The data obtained were submitted to statistical analysis.

The immunohistochemical reaction was performed by the streptavidin-biotin-peroxidase method (LSAB Systems kit DAKO Carpinteria CA, USA). The primary antibody used was the anti-MMP-9 (Santa Cruz Biotechnology) 1:200, incubated overnight at 4 °C.

The positive control was a case of epidermoid carcinoma. Negative controls, without the primary antibody were performed. Blinded qualitative analysis was performed in light microscopy.

Descriptive and inferential analyses were performed. The variance analysis test (ANOVA, two factors) and the Tukey multiple comparisons tests were applied (5%).

3. Results

In control groups, differential characteristics between groups OVX and SHAM were not seen in the experimental periods. The lining epithelium of the mucosa and gingival epithelium were orthokeratinized stratified squamous epithelium. The gingival epithelium was covered by a thin layer of keratin and the sulcus by the sulcular epithelium. The lamina propria of lingual and vestibular papillae formed by a fibrous connective tissue showed some blood vessels mixed with collagenous and fibroblasts fiber bundles. The dentogingival junction had organized bundles and collagenous fibers fan arranged. Mast cells were found around the mucosa blood vessels, deep in the specimen. Such cells were also seen permeating the lingual or vestibular papilla, but in smaller amount.

In animals with periodontal disease, the more relevant inflammatory alterations were seen in the papilla region (Fig. 1). The sulcular and junctional epithelium showed an intense exocytosis of the neutrophils and mononuclear cells besides the thickness increase. The junctional epithelium showed elongated sometimes extending deeply. In many fragments an analysis of the junctional epithelium was not possible since the tissue seemed to be torn or absent. In the lamina propria, numerous blood vessels were found, many of them exhibiting dilatation and surrounded by inflammatory cells. Edematous areas with bundles of collagenous fibers dissociated were seen in some cuts. Hyalinization areas characterized by eosinophilic and amorphous material were seen mainly in ovariectomized animals.

The inflammatory infiltrate did not show clear modifications during experimental periods. Rare mast cells were found in the papilla region, near the inflamed area. However, they were present along the mucosa subjacent to the lining epithelium and mostly around the major deep blood vessels. Sometimes microbial colonies were found on the surface.

Cytoplasmatic granules on mast cells of the tissue exhibited metachromasia to Toluidine Blue stain. Several granules dispersed in the cellular cytoplasm were seen. The mast cells present in the papilla generally showed pink colored granules easily seen, while the mast cells found in the connective tissue subjacent to the epithelium or even in the submucosa showed many cytoplasmic gathered granules, resulting in a dark purplish color. Sometimes

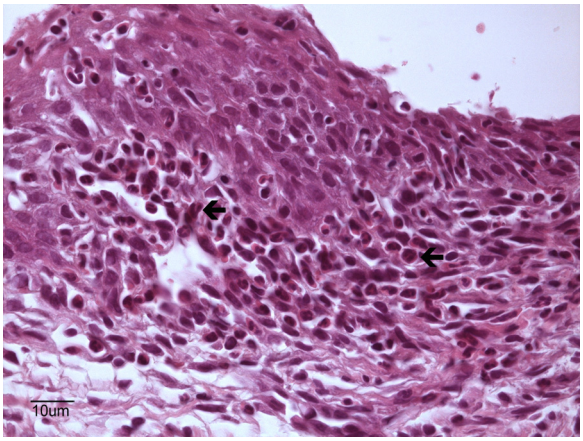

Fig. 1. Intense inflammatory infiltrate composed by neutrophils (arrows) and mononuclear cells in the junctional epithelium and in the subjacent connective tissue in SHAM-P 14 days.

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