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Study of the participation of MMP-7, EMMPRIN and cyclophilin A in the pathogenesis of periodontal disease

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ABSTRACT

Background and objective: Periodontal disease is an infectious disease resulting from the immunoinflammatory response of the host to microorganisms present in the dental biofilm which causes tissue destruction. The objective of this study was to evaluate the immunohistochemical expression of matrix metalloproteinase 7 (MMP-7), extracellular matrix metalloproteinase inducer (EMMPRIN) and cyclophilin A (CypA) in periodontal disease.
Design: Gingival tissue samples were divided as follows: clinically healthy gingiva (n = 32), biofilm-induced gingivitis (n = 28), and chronic periodontitis (n = 30). Histological sections of 3 μm were submitted to immunoperoxidase method and undergone quantitative analysis. The results were analyzed statistically by the Mann-Whitney and Spearman correlation tests, with the level of significance set at 0.05 ($\alpha = 0.05$).
Results: Immunopositivity for MMP-7, EMMPRIN and CypA differed significantly between the three groups, with higher percentages of staining in chronic periodontitis specimens, followed by chronic gingivitis and healthy gingiva specimens ($p < 0.05$). Immunoexpression of CypA and MMP-7 was higher in the intense inflammatory infiltrate observed mainly in cases of periodontitis ($p < 0.05$). CypA expression was positively correlated with MMP-7 ($r = 0.831$; $p < 0.001$) and EMMPRIN ($r = 0.289$; $p = 0.006$). In addition, there was a significant positive correlation between probing depth and expression of MMP-7 ($r = 0.726$; $p < 0.001$), EMMPRIN ($r = 0.345$; $p = 0.001$), and CypA ($r = 0.803$; $p < 0.001$).
Conclusion: These results suggest that MMP-7, EMMPRIN and CypA are associated with the pathogenesis and progression of periodontal disease.

1. Introduction

Periodontal disease is characterized by periodontal attachment loss and bone destruction (Dong, Xiang, Li, Cao, & Huang, 2009; Wang, Yang, Li, Shang, & Xiang, 2014). Although the onset of periodontitis is associated with the specific subgingival microbiota, matrix metalloproteinases (MMPs) play an important role in the progression of this periodontal condition (Dentino, Lee, Mailhot, & Hefti, 2013; Dong et al., 2009; Kinane, 2001). Several members of the MMP family are involved in the destruction of periodontal tissue, including MMP-7 (matrilysin 1). Unlike other MMPs, which are produced and released only upon response to injury, MMP-7 is mainly produced in many non-injured exocrine and mucosal epithelia rather than connective tissue cells (Beklen et al., 2007; Emingil et al., 2006; Tervahartiala et al., 2000). In gingival crevicular fluid (GCF), it was shown that patients with periodontal disease had comparable GCF MMP-7 levels to the healthy ones. The lack of elevated GCF MMP-7 levels in diseased groups might provide evidence that this MMP is preferentially released into the GCF for early defensive purposes. In addition to its activity on the extracellular matrix proteins, MMP7-is associated with the mucosal antimicrobial defense, contributing to the innate host defense (Emingil et al., 2006).

The activity of MMPs is controlled by different mechanisms, including inducer-mediated positive regulation of expression (Beklen et al., 2007). The extracellular MMP inducer (EMMPRIN,

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also known as CD147 or basigin), a member of the immunoglobulin family, is a cell surface glycoprotein which is found in large amounts on normal, inflammatory and tumor cells (Biswas et al., 1995; Dong et al., 2009; Major, Liang, Lu, Rosebury, & Bocan, 2002; Noguchi et al., 2003; Yurchenko, Constant, Eisenmesser, & Bukrinsky, 2010). This protein is involved in the pathogenesis of different diseases such as periodontitis and its action is mainly due to its inductive effect on MMPs and its ability to mediate chemoattractant activity by serving as the signaling receptor for extracellular cyclophilins (Feldman, La, Lombardo Bedran, Palomari Spolidorio, & Grenier, 2011). The expression of EMMPRIN in the GCF tends to be increased according to the severity of the disease, suggesting that this molecule may participate in regulating progression of periodontal disease (Emingil et al., 2006).

Cyclophilin A (CypA) is an intracellular protein which is expressed abundantly in all types of mammalian cells (Seizer, Gawaz, & May, 2014). CypA is secreted by different cell types after stimulation, for example, macrophages activated by LPS and smooth muscle cells or released after cell death (Seizer et al., 2010, 2014). When released into the extracellular space, CypA binds to its surface receptor EMMPRIN and thus triggers a cascade of inflammatory processes (Gürkan et al., 2009; Yurchenko et al., 2002), acting as a potent chemotactic factor for leukocytes (T cells, monocytes, neutrophils, and eosinophil granulocytes) (Seizer et al., 2014).

An increase in EMMPRIN levels in GCF is associated with increased inflammation and, consequently, with the severity of periodontal disease, suggesting that this molecule may participate in the regulation of disease progression (Emingil et al., 2006). However, the contribution of EMMPRIN and MMPs to extracellular matrix (ECM) destruction and the possible participation of CypA in periodontal disease remain unknown. In the present study, we investigated the expression of MMP-7, EMMPRIN and CypA in clinically healthy gingival tissue and tissues with different periodontal conditions, such as gingivitis and periodontitis, discussing the role of these proteins and determining their possible relationship with periodontal clinical parameters. The study's hypothesis was that immunoexpression of MMP-7, EMMPRIN, and CypA would be increased in periodontitis and gingivitis when compared to clinically healthy gingival tissue. We also hypothesized that the expression of these proteins correlates with clinicopathological parameters (tooth mobility, probing depth, clinical attachment loss, and intensity of inflammatory infiltrate).

2. Materials and methods

2.1. Study population

Fifty-two subjects were included in the study. All patients were recruited from the Department of Dentistry, Federal University of Rio Grande do Norte (UFRN), between August 2012 and January 2013.

Patients were not included if they had hypertension, diabetes or active systemic infection, were smokers, were taking drugs during the 3 months before tooth extraction, were currently pregnant or breast-feeding, had a history of vascular or other systemic diseases, and had oral diseases other than chronic periodontitis.

All patients received detailed information about the study and gave informed consent. The protocols were reviewed and approved by the Research Ethics Committee of UFRN (Permit No. 183.121).

2.2. Sample collection

The sample consisted of 90 gingival specimens obtained from 52 patients which were divided into three groups: clinically healthy gingiva (n = 32) – specimens exhibiting a pink color, firm consistency, scalloped contour of the gingival margin, firm papillae occupying the entire space below the contact points/surfaces, and no bleeding on probing (Lindhe, Lang, & Karring, 2010). The probing depth was less than 3 mm at all sites. Biofilm-induced gingivitis (n = 28) – specimens exhibiting clinical signs of biofilm-induced gingivitis characterized by a red color, edematous papillae, a possible change in probing depth, and spontaneous bleeding or bleeding on probing in the absence of clinical attachment loss. Chronic periodontitis (n = 30) – specimens exhibiting clinical signs of chronic periodontitis characterized by a probing depth of 4 mm or greater, spontaneous bleeding or bleeding on probing, clinical attachment loss, and radiographically apparent bone loss.

2.3. Morphologic analysis

For the morphologic analysis, 5-μm thick tissue sections were stained with hematoxylin and eosin. The intensity of the inflammatory infiltrate was evaluated according to the method adapted from Tsai et al. (2004). Grading of each specimen was recorded on the inflammatory condition in 1 microscopic field, starting from the junctional epithelium and proceeding deeper into connective tissue. Briefly, each specimen was graded at 200× magnification as: mild, inflammatory cells less than one-third; moderate, inflammatory cells between one third and two-thirds; and intense, inflammatory cells more than two-thirds.

2.4. Immunohistochemistry

For immunohistochemistry, 3-μm thick sections were mounted on organosilane-coated slides (3-aminopropyltriethoxysilane; Sigma Chemical Co., St. Louis, MO, USA). The specimens were incubated with the following primary antibodies: anti-MMP 7 (clone ID-2, Millipore; antigen retrieval with Tris-EDTA, pH 9.0, in a Pascal pressure cooker at 121 °C for 3 min; dilution 1:100; incubation for 18 h), anti-EMMPRIN (Invitrogen; antigen retrieval with EDTA, pH 8.0, in a Pascal pressure cooker at 121 °C for 3 min; dilution 1:400; incubation for 60 min), and anti-CypA (clone M-24, Santa Cruz Biotechnology; antigen retrieval with Tris-EDTA, pH 9.0, in a Pascal pressure cooker at 121 °C for 3 min; dilution 1:200; incubation for 18 h). Peroxidase activity was visualized by incubation of the specimens in diaminobenzidine as chromogen. Inflammatory fibrous hyperplasia specimens served as a positive control. The negative control consisted of replacement of the primary antibody with 1% bovine serum albumin in buffer.

2.5. Evaluation of immunoexpression

All slides were examined independently by two observers. Immunostaining for MMP-7, EMMPRIN and CypA was analyzed quantitatively under a light microscope (Olympus CH30, Olympus Japan Co., Tokyo, Japan). Five fields of highest anti-MMP-7, anti-EMMPRIN and anti-CypA immunoreactivity were selected at 200× magnification. Images of each field at 400× magnification were acquired with an Infinity 1–3C camera (Lumenera Co., Ottawa, Canada) coupled to a light microscope (Nikon Eclipse E200MV, Nikon Co., Tokyo, Japan) using the Lumenera Infinity Analyze software (version 5.0.3, Lumenera Co.). The number of inflammatory and endothelial cells (positive and negative) was counted in each field using the ImageJ® program (National Institute of Mental Health, Bethesda, MD, USA) and the percentage of positive cells was calculated for each specimen.

2.6. Statistical analysis

The data were analyzed using the SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) and submitted to specific tests, adopting a level of