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Human β-defensin 2 and protease activated receptor-2 expression in patients with chronic periodontitis

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ABSTRACT

Objective: Some previous studies have shown that gingipains, trypsin-like proteases produced by *Porphyromonas gingivalis*, up-regulate human β defensin-2 (HBD-2) mRNA expression through protease-activated receptor-2 (PAR_2) in gingival epithelial cells. This study aimed at investigating salivary HBD-2 levels and crevicular PAR_2 mRNA expression in human chronic periodontitis and evaluating whether periodontal treatment affected this process.

Methods: Salivary and gingival crevicular fluid (GCF) samples were collected from periodontally healthy (control) and chronic periodontitis patients at baseline and 50 days after non-surgical periodontal treatment. Salivary HBD-2, and GCF TNF-α levels were analysed by ELISA, and PAR_2 mRNA at the GCF was evaluated by RT-PCR.

Results: *P. gingivalis* was significantly ($p < 0.05$) more prevalent in patients with chronic periodontitis when compared to controls. This prevalence decreased after periodontal therapy ($p < 0.0001$). The control group showed statistically significant lower levels of HBD-2, TNF-α, and PAR_2 expression when compared to the chronic periodontitis group. In addition, periodontal treatment significantly reduced PAR_2 expression and HBD-2 levels in chronic periodontitis patients ($p < 0.001$).

Conclusions: Our results suggest that salivary HBD-2 levels and PAR_2 mRNA expression from GCF are higher in subjects with chronic periodontitis than in healthy subjects, and that periodontal treatment decreases both HBD-2 levels and PAR_2 expression.

1. Introduction

Human β-defensins (HBDs) are small cationic peptides produced throughout the body, mainly by epithelial cells, that play an important role in the oral cavity as a first-line defence against gram-negative and gram-positive bacteria, as they are able to create pores into the bacterial membranes, killing the bacteria. Epithelial cells in the oral cavity constitutively express HBDs: HBD-1, HBD-2, and HBD-3.[1,2] However, in the presence of inflammation, a different expression of these peptides might occur.[2–5] Dommisch et al.[2] showed that in healthy gingival tissues there is a similar expression among HBD-1 and -2 mRNA. In contrast, the expression of HBD-2 is statistically higher than human b defensin-1 in both gingivitis and chronic periodontitis subjects.

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Abbreviations: PAR_2, protease-activated receptor-2; HBD-2, human β defensin-2; GCF, gingival crevicular fluid; PI, plaque index; GI, gingival index; PD, probing depth; CAL, clinical attachament level.

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A recent study by Vardar-Sengul et al.[4] showed that the expression of HBD-1 and -2 mRNA was significantly higher in chronic periodontitis subjects than in the healthy control group. In addition, in a study by Kuula et al.,[5] HBD-2 expression was found to be lower in periodontally healthy tissues than in inflamed periodontal and peri-implant tissues. Taken together, these studies suggest a potentially important role for defensins in the host response to infection by periodontal pathogens.

The modulation of the β-defensins expression in the oral cavity can be orchestrated by receptors present in the cell membrane that recognize certain molecular patterns associated to periodontal pathogens, including *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum*. Previous in vitro studies[6,7] have shown that gingipains, trypsin-like proteases produced by *P. gingivalis*, up-regulate HBD-2 mRNA expression through protease-activated receptor-2 (PAR_2) in gingival epithelial cells. PAR_2 belongs to the family of G-protein-coupled, seven-transmembrane-domain receptors. Its activation occurs through the proteolytic cleavage of the N-terminal domain by serine proteinases such as trypsin, mast cell tryptase, neutrophil proteinase 3, tissue factor/factor VIIa/factor Xa, membrane-tethered serine proteinase-1, and gingipains.[8,9] A recent study by our group[10] compared chronic periodontitis patients to healthy controls and showed that PAR_2 is up-regulated in this first group. We also showed that the presence of *P. gingivalis* in the periodontal pocket is associated with this upregulation of PAR_2 gene expression and that a higher pro-inflammatory profile is related to advanced periodontal destruction.[11] In the present study, we hypothesized that HBD-2 levels as well as the expression of PAR_2 are elevated in the saliva of chronic periodontitis subjects. As to assess this hypothesis, the salivary HBD-2 levels and the PAR_2 mRNA expression from GCF were investigated in chronic periodontitis and in healthy subjects. In addition we also evaluated whether periodontal treatment may affect these levels.

2. Materials and methods

2.1. Patients

All subjects were between 20 and 69 years and in good overall health. Patients who reported history of tobacco usage within six months of screening; use of orthodontic appliances; need for premedication with antibiotics for dental treatment; usage of antibiotics, phenytoin, calcium antagonists, cyclosporine, or anti-inflammatory drugs within one month of initial appointment; history of any disease known to compromise immune functions; pregnancy or lactation; immunosuppressive chemotherapy, and/or periodontal treatment within the last 6 months, were not included in the present study.

The study protocol was approved by the Institutional Committee on Research of the University of Taubate (protocol #385/08) in accordance with the Helsinki Declaration of 1975, as revised in 2000. All patients were instructed in the nature and objectives of the study and signed a consent form agreeing to their participation.

2.2. Clinical examination

Subjects were clinically evaluated with regards to the probing pocket depths, clinical attachment loss and bleeding upon probing recorded at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual) using a manual periodontal probe (PCPUNC 15 – Hu-Friedy, Chicago, IL, USA). Patients were then divided in two groups: control (i), formed by those showing healthy sites with probing depths of $\leq$3 mm, no attachment loss, no bleeding on probing, or no signs of inflammation (10 subjects); and chronic periodontitis (ii), formed by patients with at least four teeth with one or more sites with probing depth $\geq$ 4 mm and clinical attachment loss $\geq$ 3 mm, and bleeding on probing. For each patient in the chronic periodontitis group the periodontal site showing the deepest probing depth in each oral quadrant was selected for the collection of microbial and GCF samples.

2.3. Periodontal therapy

After the clinical evaluation, bacterial and saliva samples were taken. Each subject received oral hygiene instructions and a standard kit for mechanical supragingival plaque control. The kit contained fluoride dentifrice, a regular toothbrush, interdental toothbrushes, and dental floss. Subjects in the healthy group were instructed about personal daily oral hygiene care. Periodontitis subjects underwent scaling and root planning under local anaesthesia, in a total of four clinical visits.

Clinical data, microbial, crevicular fluid and salivary samples were taken from the same sites at baseline and 50 days after initial therapy.

2.4. Microbial sampling and P. gingivalis analysis

The periodontal sites selected were isolated and the supragingival plaque was carefully removed. One fine paper point (number 30 – Tanari – Tanariman Industrial Ltd., Manacapuru, Brazil) was inserted into the gingival sulcus/periodontal pocket and left in place for 10 s. Samples collected were stored in 1 mL of reduced Ringer's solution (0.9 g sodium chloride, 0.042 g potassium chloride, 0.025 g calcium chloride, 100 mL distilled water) at −80 °C. Bacterial suspensions were thawed, centrifuged at 12,000 × *g* for 1 min. The presence of *P. gingivalis* was assessed by polymerase chain reaction (PCR) using specific primers: sense 5′AGGCAGCTTGCCATACTGCGG3′, and antisense: 5′-ACTGTTAGCAACTACCGATGT-3′ (product size: 404 bp) under standard conditions. DNA was extracted using PureLink® Genomic DNA Kit (Invitrogen, Carlsbad, CA, USA). PCR was performed in a Mastercycler Gradient thermocycler (Eppendorf, Hamburg, Germany) as follows: one cycle 94 °C for 5 min, 35 cycles 94 °C for 30 s, 57 °C for 30 s, 72 °C for 1 min, and a final extension of 72 °C for 5 min.

After electrophoresis in 1.5% agarose gel, DNA fragments were stained with SYBR SafeTM (Invitrogen®, Carlsbad, CA, USA) and visualized by UV illumination. PCR amplifications were compared with both positive and negative controls. Molecular weight marker (Ladder 100, Invitrogen) was added in each set.