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MMP-13 and TIMP-1 determinations in progressive chronic periodontitis

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Abstract

Matrix metalloproteinase (MMP)-13 is a collagenase involved in extracellular matrix degradation either by its direct degradative effects or by processing bioactive substrates. The aim of this study was to determine the levels of MMP-13 and tissue inhibitor of metalloproteinase (TIMP)-1 in gingival crevicular fluid (GCF) and gingival biopsies obtained from active and inactive sites during chronic periodontitis progression.

Materials and Methods: This was a longitudinal study in which chronic periodontitis patients with moderate to severe disease were included and followed until they developed progression determined by the tolerance method. GCF samples were obtained from periodontitis, active, inactive and healthy sites and additional gingival biopsies were taken from active and inactive sites. MMP-13 and TIMP-1 determinations were carried out by immunodot blots and immunowestern blots.

Results: In progressive periodontitis, MMP-13 and TIMP-1 remained unchanged between active and inactive sites, but as the TIMP-1 relative levels increased together with MMP-13 elevation in inactive samples, an inverse correlation was observed in active sites. Besides, MMP-13 was undetectable in healthy controls.

Conclusion: Chronic periodontitis is characterized by increased MMP-13 expression. During disease progression, active sites tended to decrease TIMP-1 levels in association with MMP-13 elevation.

Key words: gingival crevicular fluid; MMP-13; progressive chronic periodontitis; TIMP-1

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Chronic periodontitis is an infectious disease characterized by gingival inflammation and loss of periodontal support tissues, comprising periodontal ligament, cementum and alveolar bone (Choi et al. 2001, Armitage 2004). Bacterial products release and penetration to periodontal tissues activate host immunoinflammatory responses and consequent secretion of cytokines, eicosanoid mediators and matrix metalloproteinases (MMPs) (Salvi & Lang 2005) that will cause extracellular matrix destruction of the periodontum (Ashley 1999). It is generally accepted that periodontal lesions progress during acute periods of disease activity, followed by long periods of quiescence (Goodson et al. 1984, Jepsen et al. 2003, Teng 2003, Armitage 2004, Borrell & Papapanou 2005).

MMPs are a family of zinc- and calcium-dependent endopeptidases that work at neutral pH and are expressed by several cellular types that include keratinocytes, mesenchymal cells, endothelial cells and leucocytes (Ryan & Golub 2000). Historically, the MMPs were divided into several subgroups: collagenases (MMP-1, -8 and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10 and -11) and membrane-type associated MMPs (Dahan et al. 2001). However, as the list of MMP substrates has increased, a sequential numbering system for the MMPs has been adapted and the MMPs are now grouped according to their structure (Folgueras et al. 2004).

Initial cleavage of interstitial collagens by collagenases (MMPs -1, -8 and -13) is believed to represent a key step in periodontal lesion progression, while the tissue inhibitor of metalloproteinases-1 (TIMP-1) is related to connective tissue regeneration, by

Conflict of interest and source of funding statement

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inhibiting the collagenolytic activity of MMPs (Nomura et al. 1993).

MMP-13 was first cloned from breast carcinoma (Freije et al. 1994) and has been considered to have an important role in skeletal biology in view of its exclusive presence in the skeleton during embryonic development in cartilaginous growth plates and primary centres of ossification (Mattot et al. 1995, Stähle-Backdahl et al. 1997, Inada et al. 2004). The substrate specificity of MMP-13 includes naive interstitial collagens I, II and III (Leeman et al. 2002) and increasing interest has developed over its proteolytic processing properties of bioactive substrates such as pro-TNF-α, stromal cell-derived factor-1 (SDF-1), monocytic chemo attractant protein-3 (MCP-3), endostatin, plasminogen activator inhibitor-2 (PAI-2) and α-2 macroglobulin (Leeman et al. 2002, Folgueras et al. 2004). Expression of MMP-13 in inflamed gingival tissue and increased levels in gingival crevicular fluid (GCF) from periodontitis patients related to healthy sites have been demonstrated (Golub et al. 1997, Uitto et al. 1998, Ejeil et al. 2003; Ilenli et al. 2006). It has been proposed that MMP-13 levels could reflect alveolar bone loss during periodontitis and peri-implantitis (Golub et al. 1997, Ma et al. 2000, Kiili et al. 2002), but as bone and soft tissue loss of periodontum occurs mainly during disease progression, longitudinal studies are required to demonstrate the role of MMP-13 and TIMP-1 in the pathogenesis of periodontal tissue destruction during activity episodes. The aim of this study was to determine the levels of MMP-13 and TIMP-1 in GCF and gingival biopsies obtained from active and inactive sites during chronic periodontitis progression.

Materials and Methods

Patients

A group of 76 patients were selected from the Center of Diagnostic and Treatment of Northern Metropolitan Health Services, Santiago, Chile, and consecutively enrolled with a diagnosis of moderate to severe chronic periodontitis. The criteria for entry were a minimum of 14 natural teeth, excluding third molars and including at least 10 posterior teeth where five to six had sites with probing depth (PD) $\geqslant$5 mm with attachment loss $\geqslant$3 mm and extensive bone loss in radiography, according to a classification of the severity of periodontal disease based on the location of the alveolar crest. Subjects had received no periodontal treatment at the time of clinical examination, they did not suffer from systemic illness and had not received antibiotics or non-steroid anti-inflammatory therapy during the 6-month period before the study. All subjects received supragingival prophylaxis to remove gross calculus to allow the measurement of PD. Besides, nine healthy subjects without clinical attachment loss (CAL) or periodontal pockets were selected and GCF samples were taken.

Patients were followed longitudinally until they developed periodontitis progression. The protocol was clearly explained to all patients and controls, and Institutional Reviews Board-approved informed consents were signed. The protocol stated that, within 2 weeks of the detection of disease activity, all patients would be provided with periodontal treatment. Periodontal therapy consisted of scaling, root planing and oral hygiene instructions.

Clinical measurement

Clinical parameters were evaluated in all teeth, excluding third molars, and included PD, CAL and dichotomous measurements of supragingival plaque accumulation (PI) and bleeding on probing (BOP) to the base of the crevice. Six sites were examined for each tooth: mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual. An automated disc probe (Florida Probe Corporation, Gainesville, FL, USA) was used for attachment level and PDs that were taken twice separated for a week. One calibrated examiner monitored the patients and collected the clinical reports.

Disease activity was defined by the tolerance method (Haffajee et al. 1983). At the site level, active sites were considered if they exhibited attachment loss $\geqslant$2.0 mm during the following 2-month period. Inactive sites were defined as those sites with PD and BOP equivalent to active sites, but without attachment loss during the same period. At the patient level, at least two active sites were needed to consider the patient as undergoing disease progression. Clinical parameter measurements were monitored at baseline and at 2 and 4 months. When a patient was diagnosed as undergoing disease progression, GCF collections were performed from one active ($n = 7$) and one inactive site ($n = 7$), simultaneously. Additionally, gingival biopsies corresponding to active ($n = 7$) and inactive ($n = 7$) sites were obtained.

From the group of 76 patients examined, a total of 20 subjects with chronic periodontitis and seven who underwent disease progression were included in this study. Upon detection of disease activity, subjects were entered into the treatment phase. Other nine GCF samples from healthy patients were included as controls.

Collection of GCF

After isolating the tooth with a cotton roll, supragingival plaque was removed with curettes (Hu Friedy, Gracey, IL, USA), without touching the marginal gingiva. The crevicular site was then dried gently with an air syringe. GCF was collected with paper strips [ProFlow, Amityville, NY, USA (Pierce, IL, USA)]. Strips were placed into the sulcus/pocket until mild resistance was sensed and left in place for 30 s. Strips contaminated by saliva or blood were excluded from the sampled group. Following the GCF collection, the volume of the sample on the paper strips was measured using a calibrated Periotron 8000 (ProFlow). The readings from the Periotron 8000 were converted to an actual volume (μl) by reference to the standard curve. After GCF collection, strips were placed in Eppendorf vials and kept under -80°C. GCF was extracted by centrifugation at $15{,}000 \times g$ for 5 min. at 4°C in 50 μl of elution buffer containing 50 mM Tris HCl, pH 7.5, 0.2 M NaCl, 5 mM $CaCl_2$ and 0.01% Tritón X-100. The elution procedure was repeated twice and samples were stored at -80°C until further analysis.

Gingival tissue biopsies

An incision was made with a surgical blade No11 at 1–2 mm through the gingival crevice to the alveolar crest, following major tooth axis taking both epithelial and connective tissues. The specimens were washed with sterile NaCl 0.15 M to eliminate detritus and blood clots and then were kept under -80°C. To prepare tissue homogenates, samples were thawed on ice and were homogenized with a ''Potter Elvejhem'' in 0.15 M NaCl with a proteinase

inhibitor cocktail (Roche Molecular Biochemicals, Mannheim, Germany), centrifuged at 13,000 × *g* for 6 min. at 4°C and kept under −80° until analysed.

Immunoblots

To determine the molecular forms associated with chronic periodontitis and the specificity of antibodies, immunowestern blots were performed for GCF and tissue homogenizates. After boiling for 5 min., samples were run on 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) at 200 V for 1 h under reductive conditions (Laemmli 1970). Separated proteins were transferred onto a PVDF membrane (Immobilon, Millipore, Bedford, MA, USA) and unspecific interactions were blocked using 1% casein in TBST (TBS with 0.5% Tween 20 for 1 h). Anti-human MMP-13 and TIMP-1 monoclonal antibodies (Chemicon, Temecula, CA, USA) diluted 1:200 were incubated with membranes overnight at 4°C in the same blocking solution. After washing the membranes with TBST 0.5%, a second anti-mouse peroxidase-conjugated antibody (Chemicon) diluted 1:100,000 was incubated for 1 h and a positive reaction was identified using the chemiluminescence method (Biotrack ELISA system, Pierce Biotechnology, USA). Negative controls were made following a similar procedure, but without incubating the primary antibody.

After confirming the mono specificity of antibodies, immunodot blots were carried out to determine the relative levels of MMP-13 and TIMP-1 in GCF and gingival tissue from patients and healthy volunteers. Aliquots of GCF samples were applied in duplicate on PVDF membranes (Immobilon), while the following steps were carried out as described for immunodot blots.

Results were expressed as arbitrary units per site in GCF and as arbitrary units per milligram of total protein in tissue homogenizates.

Data analysis

The clinical parameters, total protein concentrations, GCF volumes as well as MMP-13 and TIMP-1 levels at the site level were expressed as means ± standard deviation. Because of MMP-13 and TIMP-1 relative levels were not normally distributed, the Wilcoxon test was applied to analyse differences between active and inactive sites from progression patients. Pearson's correlation was used to determine the relationship between MMP-13 and TIMP-1, and significance was determined. Differences in protein concentration and GCF volumes between healthy, active and inactive sites were analysed by ANOVA and Tukey's test. A statistical significance was considered when $p < 0.05$.

Table 1. Clinical parameters of periodontitis patients and controls

	Periodontitis ($n = 27$)	Controls ($n = 9$)
Age (years)	45.86 ± 7.69	45.38 ± 7.74
Females	72.72	66.66
Probing depth (mm)	3.44 ± 0.68*	1.98 ± 0.41*
Attachment level (mm)	3.98 ± 0.50†	0.62 ± 0.35†
% sites with plaque	60.40‡	18.10‡
% sites with bleeding on probing	45.40§	8.44§

Values are expressed as means ± SD.
*Mean probing depth. Periodontitis *versus* control: $p = 0.002$.
†Mean attachment level. Periodontitis *versus* control: $p = 0.001$.
‡% Sites with plaque. Periodontitis *versus* control: $p = 0.0005$.
§% Sites with bleeding on probing. Periodontitis *versus* control: $p = 0.007$.

Results

The clinical features of periodontitis patients *versus* controls are shown in Table 1. Significant differences between diseased group and controls were observed only with regard to periodontal clinical parameters.

The mean extracted volume from each ''periopaper™'' and total protein concentrations are presented in Table 2. The volumes obtained from healthy sites were significantly lower than periodontitis sites and similar between active and inactive sites. Interestingly, protein concentration showed significant differences, being about seven- and three-fold higher in active and inactive sites, respectively, compared with healthy controls.

MMP-13 molecular forms were observed in progression subjects as bands of 60 kDa for the proenzyme, 56 kDa intermediate forms and 48 kDa fully active enzyme (Fig. 1). An MMP-13 predominant band was seen in all samples at 56 kDa, but in active sites 48 kDa faint bands were detected in both tissue and fluid, whereas in GCF, additional degraded forms near 35 and 25 kDa were also seen. The presence of TIMP-1 in diseased GCF was demonstrated as a single band of 34 kDa (Fig. 2).

Table 2. Comparison of gingival crevicular fluid (GCF) volumes extracted per ''periopaper'' and total protein concentrations between active, inactive and healthy sites

Sites	GCF mean volumes (μl)	Mean protein concentration (mg/ml)
Active	0.94 ± 0.21*	0.68 ± 0.059†,‡
Inactive	0.92 ± 0.18§	0.37 ± 0.059‡,¶
Healthy	0.22 ± 0.13*,§	0.11 ± 0.081†,¶

Values are expressed as means ± SD.
*GCF mean volumes active *versus* healthy: $p < 0.05$.
†Mean protein concentration active *versus* healthy: $p < 0.05$.
‡Mean protein concentration active *versus* inactive: $p < 0.05$.
§GCF mean volumes inactive *versus* healthy: $p < 0.05$.
¶Mean protein concentration inactive *versus* healthy: $p < 0.05$.

After demonstrating the monospecificity of all antibodies by immunowestern blot, MMP-13 levels were determined by immunodot blot in GCF from healthy ($n = 9$) and chronic periodontitis patients ($n = 20$). All diseased samples had MMP-13 detectable signals, but not in control samples (data not shown). Similarly, MMP-13 could not be observed by immunowestern blot in healthy subjects (data not shown).

In progressive periodontitis patients, MMP-13 and TIMP-1 levels by immunodot blot in GCF did not show significant differences (Fig. 3a). Similar results were observed in tissue homogenizates (Fig. 3b). Interestingly, we observed in GCF from inactive sites that TIMP-1 relative levels increased together with MMP-13 levels. Conversely, in active sites, TIMP-1 decreased when MMP-13 levels increased (Fig. 4). Nevertheless, in spite of regular to good correlations having

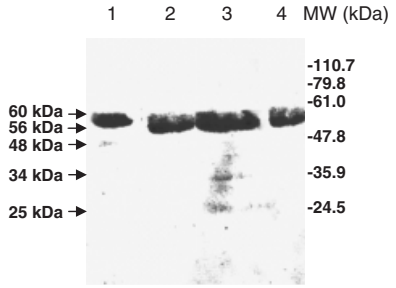

Fig. 1. Identification of matrix metalloproteinase-13 in gingival tissue and gingival crevicular fluid (GCF). Lane 1, active site gingival tissue homogenizates; Lane 2, inactive site gingival tissue homogenizates; Lane 3, active site GCF; Lane 4, inactive site GCF; MW, molecular weight standard.

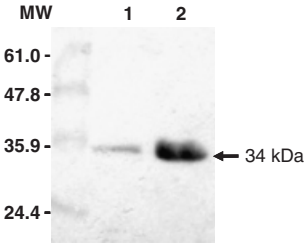

Fig. 2. Immunowestern blot for tissue inhibitor of metalloproteinase-1 in gingival crevicular fluid (GCF) and gingival tissue in chronic periodontitis. MW, molecular weight standard; Lane 1, GCF from chronic periodontitis patients; Lane2, gingival tissue homogenizates from chronic periodontitis patients.

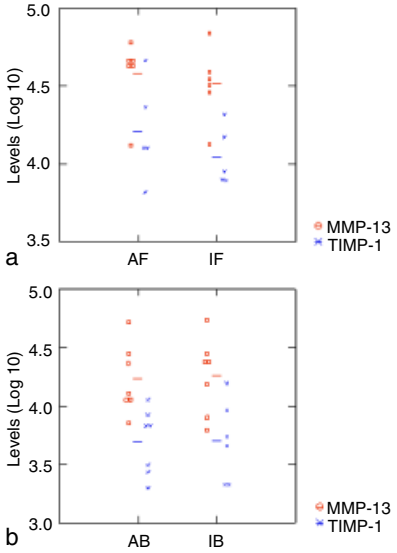

Fig. 3. Matrix metalloproteinase (MMP)-13 and tissue inhibitor of metalloproteinase (TIMP)-1 levels in progressive chronic periodontitis. (a) MMP-13 and TIMP-1 levels in gingival crevicular fluid (GCF). Aliquots of GCF were applied in duplicate on nitrocellulose membranes and relative TIMP-1 and MMP-13 were determined by immunodot blot and computed densitometry. Results are expressed as absolute arbitrary units. AF, GCF from active sites; IF, GCF from inactive sites; $p > 0.05$. Horizontal bars represent means for MMP-13 and TIMP-1 in the study groups. (b) MMP-13 and TIMP-1 levels in gingival tissue homogenizates. Aliquots of gingival tissue homogenizates were applied in duplicate on nitrocellulose membranes and relative TIMP-1 and MMP-13 were determined by immunodot blot and computed densitometry. Results are expressed as arbitrary units per milligram of total protein. AB, gingival tissue homogenizates from active sites; IB, gingival tissue homogenizates from inactive sites. $p > 0.05$. Horizontal bars represent means for MMP-13 and TIMP-1 in study groups.

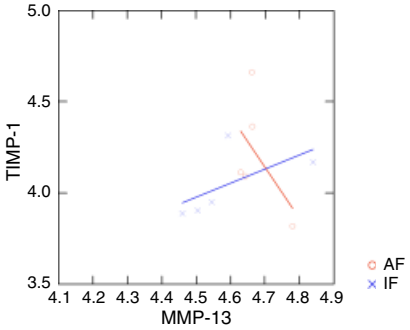

Fig. 4. Relationship between matrix metalloproteinase (MMP)-13 and tissue inhibitor of metalloproteinase (TIMP-1) relative levels in gingival crevicular fluid (GCF) from progressive periodontitis patients. Relative levels determined by immunodot blot were analysed by Pearson's correlation. AF, GCF from active sites, $r = -0.532$, $p = 0.357$. IF, GCF from inactive sites, $r = 0.609$, $p = 0.276$.

been found, they were not significant ($p > 0.05$).

Discussion

Periodontal diseases are a group of multifactorial pathologies that involve alveolar bone and periodontal soft tissue destruction, mainly as a result of host immunoinflammatory response activation to bacterial infection (Salvi & Lang 2005). Periodontal support tissue destruction is the hallmark of chronic periodontitis that progresses during disease activity, which occurs as acute recurrent episodes, followed by intermittent periods of remission (Jepsen et al. 2003).

The results of this study support our hypothesis that MMP-13 has an important role in tissue destruction associated with periodontitis progression. We demonstrated that MMP-13 expression is significantly elevated in periodontitis patients compared with healthy subjects. Additionally, we show a tendency to decrease TIMP-1 levels as MMP-13 increased in active sites.

GCF constituents are derived from serum, connective tissue, epithelia, inflammatory leucocytes and bacteria from periodontal tissues. With the onset of infection, GCF displays a shift from a transudate to an inflammatory exudate that derivates from increases in vascular and epithelial barriers permeability (Oringer et al. 2002) that permits extravasation of high molecular proteins from general circulation (Curtis et al. 1990, Adonogianaki et al. 1996). In healthy subjects and under resting conditions, the flow rate corresponds approximately to 0.1 μl/min./tooth, but it increases by up to five-fold in subjects affected by gingivitis and periodontitis (Pisano et al. 2005). This fact could explain the differences in GCF volume and protein concentration between healthy and periodontitis sites, whereas the marked increment in protein concentration between inactive and active sites from progression patients could reflect local insertion tissue destruction during disease activity. These activity bursts have been characterized by fast activation of catabolic processes in affected sites that leads to uncontrolled destruction of the extracellular matrix of the periodontum (Alpagot et al. 2001), where collagens type I and III represent the main constituents of both periodontal ligament and alveolar bone (Ma et al. 2000), permitting the release and detection of the resultant peptides in GCF.

Additionally, our results agree with previous studies of Golub et al. (1997) and Lamster et al. (1986), in which they state that the more sensitive method for demonstrating specific protein changes in GCF is their expression as absolute values under standardized periods of fluid collection (30 s).

MMP-13 in progressive periodontitis was mainly detected as a band of 56 kDa that could represent a partially activated form, derived from 60 kDa pro-enzyme (Knäuper et al. 1996; Golub et al. 1997; Hernández et al. 2006). In active sites, we could detect faint bands of 48 kDa corresponding to fully active forms and

partially degraded fragments with a molecular weight between 35 and 25 kDa (Ilgenli et al. 2006). Pro-MMP-13 activation requires direct pro-peptide cleavage by active MMP-14, MMP-3 and/or MMP-2 (Cowell et al. 1998). When pro-MMP-13 is activated by MMP-14 in the presence of TIMP-1, there is a first cleavage in the Gly^{35}-Ile^{36} site that generates an intermediate fragment of 56 kDa and a second cleavage in N-terminal Tyr^{85} (Knäuper et al. 1996). It is not known whether this last proteolytic processing is due to further MMP-14 activity or due to auto proteolysis that generates fully active 48 kDa collagenase (Knäuper et al. 1996). Once MMP-13 reaches its active form, it becomes highly unstable, generating degraded fragments of low molecular weight (Knäuper et al. 1997). This extracellular proteolytic processing represents an important control mechanism in bone catabolism (Rifas & Arackal 2003) and is thought to be essential for inducing bone resorption during processes like tumoral invasion. Bone-resorptive mediators like interleukin-1 (IL-1) and PGE_2 induce a marked expression of MMPs that include MMP-13, -3 and -2 by osteoblasts, which have been associated with increments in bone resorption in mouse calvaria (Oshiba et al. 2003). The same authors found marked expression of MMP-13 and -14 in bone metastasis in vivo and in MMPs -2, -9 and -13 in vitro, while addition of BB94, an MMP inhibitor, did suppress resorptive activity by breast carcinoma cells. Thus, MMP-13 could participate in osteolysis during periodontitis (Nishikawa et al. 2002).

It is generally accepted that MMP-13 tissue expression is highly restricted to situations in which rapid turnover of fibrillar collagens is required (Uitto et al. 1998, Nishikawa et al. 2002). Thus, MMP-13 appears to be the primary collagenase expressed in the epithelium and it is also expressed by connective tissue cells of inflamed periodontum, where MMP-13 may play an important role in the growth of the epithelium into connective tissue during mucosal inflammation (Uitto et al. 1998). MMP-13 is also expressed in the bone, where it is subjected to a subtle balance depending on osteoblasts' exposition to many local and systemic factors (Rydziel et al. 2000). In this study, we failed to demonstrate MMP-13 in healthy subjects by immunowestern blot. Similarly, we could detect MMP-13 in all samples from periodontitis patients, but in none of the healthy subjects by immunodot blot.

Because total enzyme activity also depends on pro-enzyme activation and inhibition of the active collagenase by TIMP-1, we determined its levels in periodontitis progression. Previously, Pozo et al. (2005) reported TIMP-1 reduced levels in association with higher total collagenase and gelatinase activity in periodontitis patients than in controls, and a recovery of TIMP-1 levels after scaling and root planing. Other studies have detected collagenase and gelatinase in GCF and demonstrated positive correlations between the activity of these MMPs and the severity of periodontal disease. Among them, MMPs -8 and -9 are the predominant MMPs in adult periodontitis, whereas MMP-13 comprises only 3–4% of the total collagenase in GCF (Tervahartiala et al. 2000, Kiili et al. 2002) and less or no MMP-1 has been detected (Golub et al. 1997). Additionally, MMP-13 but not MMP-8 is produced by human osteoblasts in bone tissue and is capable of activating pro-MMP-9 produced by osteoclasts (Rydziel et al. 2000). Thus, MMP-13 may contribute to periodontal tissue degradation mediated by MMP-8 and -9 (Ilgenli et al. 2006) and it could reflect collagen degradation from bone tissue during periodontitis (Golub et al. 1997).

In spite of the fact that we could not detect differences in total MMP-13 and TIMP-1 determinations between active and inactive sites from periodontitis progression patients, we could observe a tendency towards an inverse correlation between MMP-13 and TIMP-1 in active samples, but more samples are needed to confirm this finding. Additionally, our previous studies report that MMP-13 activity determined by a fluorescence assay is significantly increased in active compared with inactive sites in progressive periodontitis and healthy subjects (Hernández et al. 2006), but there were no significant differences between inactive and healthy controls (unpublished data). Taken together, these results indicate that MMP-13 expression is higher in periodontitis patients than in healthy subjects. During periodontitis progression, MMP-13 levels could be counterbalanced by increments in TIMP-1, but this could not occur in active sites, where an inverse correlation was seen. Thus, an imbalance between MMP-13 and its inhibitor characterizes diseased sites; but during progression of the disease, periodontal support loss occurs mainly because of an increase of MMP-13 activity, reflecting that pro-MMP-13 activation could represent a key step in hard and soft tissue destruction, which could not be counterbalanced by TIMP-1, either inhibiting fully active enzyme or avoiding its complete activation.

Recent studies have demonstrated that the role of MMPs is not limited only to their degradative effect over the extracellular matrix, but they also target many bioactive substrates, like growth factor receptors (GFR), cellular adhesion molecules, chemokines, cytokines, apoptotic ligands and angiogenic factors (Folgueras et al. 2004, Mott & Werb 2004, Sakai et al. 2006). MMP-13 processes MCP-3, transforming the agonist form to an antagonist form, inactivates SDF, activates pro-tumour necrosis factor-α (TNF-α), inactivates the antiprotease α-1 antichemotripsin and inactivates the MMP inhibitor α-2 macroglobulin (Leeman et al. 2002, Uitto et al. 2003). Besides, MMP-13 has a central role in the activation cascade of other MMPs, generating active MMP-9 (Leeman et al. 2002, Pozo et al. 2005), and additionally, pro-MMP-13 could activate itself by autocatalysis (Pardo & Selman 2006). All these regulatory proteolytic mechanisms could indicate that minimal changes in MMP-13 levels or activity are capable of activating amplification proteolytic downstream cascades that increase significantly the whole degradative effects on periodontal tissue that characterize periodontitis activity.

As it is widely accepted that during periodontitis activity there is bone and soft tissue breakdown (Vernal et al. 2004), collagenolytic activity of MMP-13 could play a central role in regulating this process and the balance between MMP-13 and TIMP-1 expression could reflect the onset of periodontal activity.

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Clinical Relevance

Scientific rationale for the study: During periodontitis activity, there is bone and soft tissue breakdown. MMP-13 collagenolytic activity could play a central role by degrading periodontal extracellular matrix or by processing bioactive substrates. Thus, an imbalance between MMP-13 and TIMP-1 expression could reflect the onset of periodontal activity.

Principal findings: MMP-13 could play an important role in tissue destruction during periodontitis progression. In chronic periodontitis, MMP-13 expression was significantly elevated compared with healthy subjects. During disease progression, MMP-13 tended to increase, together with TIMP-1 levels, but in active sites, a tendency towards an inverse correlation was observed.

Practical implications: MMP-13 and TIMP-1 variations could represent a useful marker of disease activity and targets for new therapeutical approaches.