Associations Between Matrix Metalloproteinase-8 and -14 and Myeloperoxidase in Gingival Crevicular Fluid From Subjects With Progressive Chronic Periodontitis: A Longitudinal Study

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Background: Matrix metalloproteinase (MMP)-8 is a central mediator in chronic periodontitis. MMP-8 can be activated by the cooperative action of other MMPs such as MMP-14, reactive oxygen species, and microbial proteases. The aim of this study is to associate the levels, molecular forms, isoenzyme distribution, and degree of activation of MMP-8 and -14, myeloperoxidase (MPO), and tissue inhibitor of MMP (TIMP)-1 in gingival crevicular fluid (GCF) from patients with progressive periodontitis at baseline and after periodontal therapy.

Methods: In this longitudinal study, GCF samples from active (n = 25) and inactive (n = 25) sites of subjects with periodontitis were screened at baseline for GCF levels of MMP-8 by immunofluorometric assay, of MMP-14 by specific activity assay, and of MPO and TIMP-1 by enzyme-linked immunosorbent assay. MMP-8 and MPO were also measured after periodontal treatment. Molecular forms were determined by immuno-Western blot analyses and subjected to densitometric scanning and statistical analyses.

Results: High MMP-8 and MPO levels and a strong MPO/MMP-8 positive correlation were found in active and inactive sites at baseline. After treatment, decreases in MPO and MMP-8 were seen, except for active sites in which MMP-8 differences were not significant ($P<0.05$).

Conclusions: We present initial data that show that GCF levels and associations between MPO and MMP-8 are related to progression episodes and treatment responses in patients with chronic periodontitis. Our results suggest an interaction between the MPO oxidative pathway and MMP-8 activation, and this cascade might be useful as a potential biomarker for treatment outcomes. *J Periodontol 2010;81:1644-1652.*

KEY WORDS

Gingival crevicular fluid; matrix metalloproteinase; periodontal disease.

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Chronic periodontitis is characterized by the occurrence of an irreversible destruction of periodontal supporting tissue. Disease appears to result from a complex interaction between the periodontopathogenic bacteria and the host immunoinflammatory response. Periodontal loss is considered to occur as cycles of acute-activity episodes that alternate with prolonged periods of quiescence.[1] The disease progression involves a network of interacting molecular pathways including proinflammatory mediators, reactive oxygen species, matrix metalloproteinases (MMPs), and their MMP inhibitors and regulators.[2,3] Type I collagen is the main extracellular matrix component of periodontal tissues, and thus, collagen degradation is regarded as one of the key factors in uncontrolled destructive lesions.[4,5] The major collagenolytic MMPs associated with the severity of periodontal disease are MMP-8 and -13.[3-14] Previous studies[13,15] demonstrated increased gingival crevicular fluid (GCF) levels of MMP-8 in association with the severity of chronic periodontitis, which decreased after successful periodontal treatment, and repeatedly high MMP-8

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levels in unstable sites, which showed a risk of further destruction. In contrast, the recent generation of MMP-8–deficient mice provided preliminary evidence for an MMP-8 role in the immune/inflammatory–response modulation and protection against alveolar bone resorption in experimentally induced periodontitis.[16]

During periodontal inflammation, latent MMP-8 can be activated by the cooperative action of other inflammatory mediators, such as MMP-14, reactive oxygen species, and microbial proteases.[17-20] Neutrophil-derived myeloperoxidase (MPO) is found in primary granules from neutrophils, and these cells are regarded as the main source of MMP-8 and -9.[21] MPO GCF levels were associated with the severity of periodontitis.[22] Besides its antimicrobial effects, MPO was reported to oxidatively activate latent proMMP-8 and -9 in vitro.[23,24] Consecutively, we propose that MPO and MMP-14 activities and/or levels associate with the levels of active MMP-8 in GCF from patients with progressive periodontitis and a reduction of these enzymes in response to periodontal treatment. The aim of this study was to associate the levels, molecular forms, isoenzyme distributions and degrees of activation of MMP-8 and -14, MPO, and tissue inhibitor of MMP (TIMP)-1 in GCF from subjects with periodontitis progressive at baseline and after periodontal therapy.

MATERIALS AND METHODS

Patients and Clinical Measurements

In this longitudinal clinical study developed at the dependencies from the University of Chile, Santiago, and the University of Helsinki, patients with moderate to severe chronic periodontitis were followed during a 9-month period from August 2008 to May 2009 until they developed clinical periodontitis progression. Patients were selected from the Center of Diagnostics and Treatment of Northern Metropolitan Health Services, Santiago, Chile. As described previously,[11] the criteria for entry in the study were patients with ≥14 natural teeth, excluding third molars and including ≥10 posterior teeth, with at least five to six teeth with sites of probing depths (PDs) ≥5 mm with attachment loss (AL) ≥3 mm and detectable bone loss in radiographs (>50% of supporting tissues were involved, according to a classification system of periodontal disease severity based on the location of the alveolar crest), and who had never received previous periodontal treatment at the time of examination.

The patients included in our study fulfilled the following criteria: they had no history of a systemic disorder, such as diabetes mellitus, osteoporosis, or use of medications known to influence periodontal tissues, were not pregnant or lactating females, and had not received antibiotic, anti-inflammatory, anticoagulant, or hormonal drugs within the 6-month period before the study.

Clinical parameters evaluated included PD, clinical AL, and dichotomous measurements of supragingival plaque accumulation (plaque index) and bleeding on probing (BOP)[11] from the base of the crevice and examined at six sites per tooth (i.e., mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual, and mesio-lingual) using a North Carolina manual probe.‖ One calibrated examiner (OR) monitored the patients and collected the clinical reports.

Disease activity was defined clinically by the tolerance method.[25] At site level, active sites were considered if they exhibited AL ≥2 mm during a 2-month period. Inactive sites were defined as those sites with similar PD and BOP but without clinical AL during the same period. At the patient level, at least two active sites were needed for the patient to be considered as undergoing disease progression.

At baseline, GCF samples from active and inactive sites were immediately taken when disease progression was detected from 25 subjects, (eight male and 17 female; age range: 35 to 62 years, mean age 47.31 ± 7.5 years) as previously described.[10,11,26] To monitor the effect of periodontal therapy over mediators of interest, GCF collections were also performed 2 months after completion of periodontal therapy in 10 subjects by evaluating the sites selected at the beginning of the study. Periodontal therapy consisted of scaling and root planing and oral hygiene instructions. The protocol was clearly explained to patients and control subjects, and all study subjects signed informed consent forms that were approved by the institutional review board from the Faculty of Dentistry, University of Chile. The protocol stated that all patients would immediately enter the treatment phase upon detection of disease progression.

Collection of GCF

After isolating the tooth with a cotton roll, supragingival plaque was removed with curets,¶ without touching the marginal gingiva. The crevicular site was gently dried with an air syringe. GCF samples were collected with paper strips# placed into the pocket until mild resistance was sensed and left in place for 30 seconds.[5] Strips contaminated by saliva or blood were discarded. GCF was extracted from the strips by centrifugation at 12,000 rpm for 5 minutes at 4°C in 40 μl elution buffer containing 0.9% NaCl. The elution procedure was repeated twice, and eluted samples were stored at –80°C until further analyses.

‖ Hu-Friedy, Chicago, IL.
¶ Hu-Friedy.
# ProFlow, Amityville, NY.

Determinations of MMP-8 and -14, MPO, and TIMP-1 in GCF

GCF MMP-8 and -14 and MPO immunoreactivities were determined by immuno-Western blotting using a specific rabbit polyclonal antihuman MMP-8 antibody (1:500 dilution), as previously described,[3,27-29] rabbit polyclonal antihuman MMP-14 antibody** (1:500 dilution), and monoclonal antihuman MPO antibody†† (1:1,000 dilution). MPO recombinant protein was added as positive control.‡‡ Quantification was carried out with an imaging densitometer.||||

GCF samples of periodontitis progression from active and inactive sites were screened for MMP-8 by a time-resolved immunofluorometric assay (IFMA), as described by Hanemaaijer et al.,[29] for MPO by using an MPO enzyme-linked immunosorbent assay (ELISA) kit¶¶ and for TIMP-1 by using an ELISA system## following manufacturer's recommendations. Protein levels were obtained from a standard curve and expressed as nanograms per milliliter of eluted GCF.

MMP-14 Activity Assay

MMP-14 was measured by using an MMP-14 activity assay system*** according to the manufacturer's recommendations. Briefly, standards that corresponded to proMMP-14 and the samples were added to a microplate precoated with immobilized specific antibody, and any MMP-14 was bound. The assay used the pro-form of a detection enzyme with an artificial sequence that was activated by captured active MMP-14. The detection enzyme was then measured by using a specific chromogenic peptide substrate and read at 405 nm in a microplate spectrophotometer. Concentrations of MMP-14 in the samples were determined by interpolation from a standard curve and expressed as nanograms per milliliter. Total enzyme and endogenous levels of free active MMP-14 in the samples were detected with and without adding aminophenylmercuric acetate (APMA),††† respectively.

Statistical Analyses

Differences regarding dichotomic measurements were analyzed by using the χ^2 test, comparisons between two related groups were analyzed by using the paired *t* test or Wilcoxon test depending on data distribution, and comparisons between two unrelated groups were analyzed with the unpaired *t* test (normal distribution). Spearman correlation was applied to determine association between variables. $P < 0.05$ was considered statistically significant. Statistical analyses were performed with software.‡‡‡

RESULTS

Clinical parameters from subjects with progressive periodontitis are presented in Table 1. Periodontal parameters showed no differences between active and inactive sites at baseline, whereas both groups demonstrated significant improvements after periodontal treatment ($P < 0.01$).

To resolve the different isotypes and/or molecular forms of MMP-8 and -14 and MPO in GCF from patients with untreated progressive chronic periodontitis, immuno-Western blot analyses in non-reductant conditions were carried out. Analyses of GCF at baseline demonstrated the presence of MMP-8 and -14 and MPO immunoreactivities in active and inactive sites. Molecular forms associated with disease progression are shown in Figure 1. MMP-8 immunoreactivities (Fig. 1A) demonstrated proenzyme and active forms of neutrophil (polymorphonuclear leukocyte [PMN]) (85 and 64 kDa, respectively) and mesenchymal (55 and 48 kDa, respectively) isoforms. Additional bands corresponding to MMP-8 complexes (≥100 kDa) and fragments (<46 kDa) were frequently seen. MMP-14 soluble forms were also identified as complexes (≥100 kDa), bands of ≈50 kDa, and fragments (37 ≤ kDa) (Fig. 1B). Finally, MPO immunoreactivities (Fig. 1C) were identified at ≈75, 90, 130, and 160 kDa in GCF from active and inactive sites.

Densitometric analyses were carried out for MMP-8 and -14 immunoreactivities (Table 2). Regarding MMP-8, only active forms of PMN and mesenchymal types and their respective percentages of activation (percentages of active from total MMP-8) increased in active sites compared to inactive sites ($P > 0.05$). Among MMP-8 isotypes, the neutrophil type predominated over the mesenchymal type in active and inactive sites ($P = 0.005$); conversely, pro-forms and total MMP-8 (determined as the sum of all detected bands) increased in inactive sites, but none of these differences were significant ($P > 0.05$). Regarding soluble forms of MMP-14, total levels were higher in active sites, as were 55-kDa bands and fragments, but differences were insignificant ($P > 0.05$).

Baseline GCF levels of MMP-8 that were determined by IFMA, of MMP-14 that were determined by a specific-activity assay system, and of MPO and TIMP-1 that were determined by ELISA demonstrated no significant variations among untreated active and inactive sites (Table 3).

All enzymes showed detectable levels in GCF samples, whereas TIMP-1 was under the assay detection limit in most cases, regardless of the presence or

** BioGenesis, Poole, U.K.
†† R&D Systems, Minneapolis, MN.
‡‡ R&D Systems.
|||| Bio-Rad model GS-700 Imaging Densitometer with the Quantity-One program, Bio-Rad, Hercules, CA.
¶¶ Immundiagnostik, Bensheim, Germany.
Biotrak, GE Healthcare, Amersham, Slough, U.K.
*** Biotrak, GE Healthcare.
††† Sigma-Aldrich, St. Louis, MO.
‡‡‡ Stata V10, StataCorp, College Station, TX.

Table 1.

Clinical Parameters in Subjects With Periodontitis Progression

Parameter	Subjects at Baseline (N = 25)	Active Sites (n = 25)			Inactive Sites (n = 25)		
		Baseline	After Treatment	*P*	Baseline	After Treatment	*P*
PD (mm; mean ± SD)	3.40 ± 1.21	5.16 ± 0.97	3.96 ± 0.76	<0.001	5.24 ± 0.83	3.96 ± 0.80	<0.001
Clinical AL (mm; mean ± SD)	4.10 ± 1.21	5.68 ± 0.69	4.62 ± 0.69	<0.001	5.36 ± 0.64	4.0 ± 0.58	<0.001
BOP (%)	59	100	28	–	100	16	–
PI (%)	63	100	24	–	100	24	–

PI = plaque index; – = not applicable.
Comparisons between active and inactive sites at baseline and after treatment, (P >0.05).

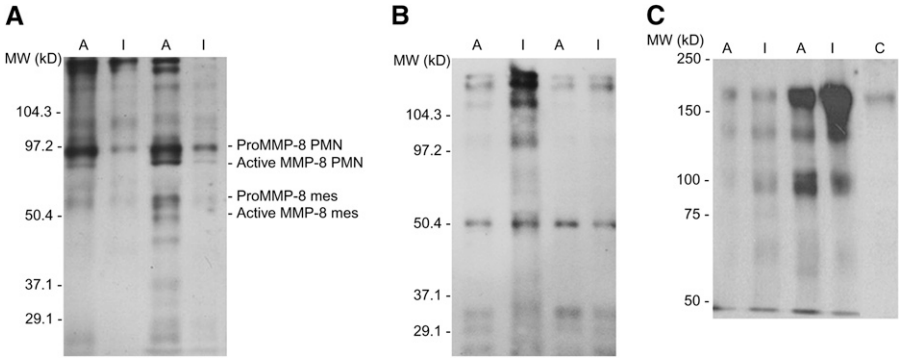

Figure 1.
Representative MMP-8 (A), MPO (B), and soluble MMP-14 (C) immunoreactivities in GCF from active and inactive sites at baseline. Mobilities of the molecular weight (MW) markers are indicated on the left side of each panels. A = active sites; I = Inactive sites; C = positive control; Mes = mesenchymal.

absence of disease activity. Total levels of MMP-8 and MPO slightly decreased in active sites compared to inactive sites, but differences were insignificant. MMP-14 measurements were expressed as endogenous active enzyme (without APMA addition) and as the indirect measurement of total enzyme levels when APMA was added. Both measurements showed a tendency to increase in active sites, but none of these results showed statistical significance (Table 3). TIMP-1 levels were only detected in 18% of inactive sites and 14% of active sites (P >0.05; Table 3).

With the aim to target potential associations between these enzymes or their molecular forms in GCF samples, Spearman correlation analysis was done at baseline (n = 18). As shown in Table 4, a significant strong positive correlation was found in both active and inactive sites between MMP-8 levels detected by IFMA and MPO, whereas MMP-14 total levels were positively correlated with active MMP-14. Active and total MMP-14 levels were inversely correlated with MPO and MMP-8, but this association was only significant in active sites. As an attempt to clarify the nature of the association found between MPO and MMP-14 and -8, active forms of PMN and mesenchymal MMP-8 isoforms were correlated with MPO and MMP-14 molecular forms (Table 5). Again, MPO levels and total MMP-8 determined by immuno-Western blot and densitometric analyses showed a significant positive correlation (P <0.05). Both MMP-8 active forms corresponding to mesenchymal and PMN isotypes displayed positive correlations with MPO (P<0.05), and the association with the PMN type was the strongest (r = 0.77). In contrast, none of the MMP-14 forms correlated with active MMP-8, regardless of the isotype.

Because of the strong association found between MMP-8 and MPO, we compared both GCF enzyme levels at baseline and after conventional periodontal treatment in active and inactive sites (Fig. 2). Although MPO levels were significantly reduced in inactive and active sites (P = 0.02 and 0.03, respectively; Fig. 2A), MMP-8 levels were significantly reduced only in inactive sites (P = 0.02 for inactive sites and P = 0.50 for active sites; Fig. 2B). A positive correlation between MPO and MMP-8 still remained after treatment, but for inactive sites it was borderline significant (active sites: r = 0.65, P = 0.04, and n = 10; inactive sites: r = 0.65, P = 0.058, and n = 9).

DISCUSSION

Because periodontal loss is regarded to occur as cycles of acute-activity episodes,[1] in this study we analyzed GCF levels of MMP-8 and -14, MPO, and TIMP-1 in active and inactive sites from patients with progressive chronic periodontitis, which was assessed clinically. Additionally, we analyzed the association among markers of interest and compared them at

Table 2.

Densitometric Analyses of MMP-8 and Soluble MMP-14 Molecular Forms in GCF From Active and Inactive Sites at Baseline

Enzyme Form	Inactive (n = 14)	Active (n = 14)
PMN MMP-8 pro (median [interquartile range])	1.17 (1.38)	1.12 (1.29)
PMN MMP-8 active (median [interquartile range])	0.39 (0.92)	0.52 (0.80)
PMN MMP-8 (% activation)	7.40 ± 5.21	8.86 ± 6.69
Mesenchymal MMP-8 pro (median [interquartile range])	0.22 (0.76)	0.15 (0.60)
Mesenchymal MMP-8 active (median [interquartile range])	0.15 (0.55)	0.45 (0.36)
Mesenchymal MMP-8 (% activation; mean ± SD)	3.52 ± 2.76	4.38 ± 1.20
MMP-8 complexes (≥100 kDa) (median [interquartile range])	3.92 (4.48)	1.89 (5.87)
MMP-8 fragments (≤46 kDa)	0.71 (1.67)	0.13 (1.23)
MMP-8 total (median [interquartile range])	7.50 (10.10)	5.30 (7.48)
MMP-14 complexes (≥100 kDa) (median [interquartile range])	0.36 (3.76)	0.28 (1.52)
MMP-14 (≈50 kDa) (median [interquartile range])	0.02 (0.20)	0.07 (0.14)
MMP-14 fragments (≤37 kDa) (median [interquartile range])	0.13 (0.30)	0.25 (0.22)
MMP-14 total (median [interquartile range])	0.053 (4.27)	1.07 (1.20)

P >0.05 for all comparisons between active and inactive sites.

Table 3.

Levels of MMP-8, MPO, MMP-14, and TIMP-1 in GCF from Active and Inactive Sites at Baseline

Enzyme	Inactive Sites (n = 25)	Active Sites (n = 25)
MMP-8 (ng/mL; median [interquartile range])	153.86 (185.52)	119.35 (196.92)
MPO (ng/mL; median [interquartile range])	660.05 (789.81)	287.94 (675.79)
MMP-14 active enzyme (ng/mL; median [interquartile range])	5.57 (7.55)	6.89 (3.31)
MMP-14 total (ng/mL; median [interquartile range])	16.82 (15.22)	18.80 (33.07)
TIMP-1 (% detection)*	18.18	14.29

P >0.05 for all comparisons.
* Expressed as the relative frequency of positive detection.

A comparison of all studied mediators (i.e., MMP-8 and -14, MPO, and TIMP-1) in GCF from untreated active and inactive sites from patients with progressive periodontitis showed no differences in levels of total enzymes or specific molecular forms. MPO and MMP-8 levels, as determined by IFMA and further corroborated by immuno-Western blot and densitometric analyses, increased together, demonstrating the presence of a strong positive correlation in both sample groups. As an attempt to further understand the nature of this association, active MMP-8 forms that corresponded to PMN (≈65 kDa) and mesenchymal isotypes (≈48 kDa) were correlated with MPO levels. Both active enzyme isoforms positively correlated with MPO, but the association was even higher for the neutrophil type. Additionally, neutrophil MMP-8 predominated over mesenchymal MMP-8. Periodontal treatment improved clinical parameters and reduced MPO levels. Although MMP-8 levels were reduced in both active and inactive sites, reductions were significant only for inactive sites together with the apparent loss of the MPO/MMP-8 association. Whether this finding is clinically and biologically relevant needs further study. Given the low frequency of progression occurrence, data should be interpreted carefully considering the small size of treated progressive groups.

In accordance with our findings, previous reports[13,15] showed reductions of MMP-8 levels after SRP, whereas sites with an unstable character and progression of periodontitis had repeatedly high MMP-8 levels. Periodontal treatment complemented baseline and after conventional periodontal treatment. The present study presents initial data that support a role for an MPO/MMP-8 interaction in progression episodes of periodontal supporting tissue destruction and the potential of MPO and MMP-8 as biomarkers of treatment outcomes. Although periodontal treatment resulted in MPO and MMP-8 reductions in all sites, the decrease of MMP-8 in active sites was not significant and the MPO/MMP-8 association still persisted after periodontal treatment.

Table 4.

Spearman Correlation Matrix Among MMP-8, MPO, and MMP-14 Measurements (ng/mL) in GCF From Active (n = 9) and Inactive (n = 9) Sites at Baseline

	MMP-8		MPO		MMP-14 activity		MMP-14 levels	
Enzyme	Inactive	Active	Inactive	Active	Inactive	Active	Inactive	Active
MMP-8	1.0000	1.000	—	—	—	—	—	—
MPO	**0.7448***	**0.9167***	1.000	1.000	—	—	—	—
MMP-14 active	–0.2308	–0.2176	–0.2876	–0.3766	1.0000	1.000	—	—
MMP-14 total	–0.5000	**-0.7280***	–0.5858	**-0.8954***	**-0.7011***	**0.6639**†	1.000	1.000

* $P < 0.05$.
† $P = 0.0512$.
Bold = significant change (P values).

Table 5.

Spearman Correlation Coefficients Between MPO and MMP-14 Versus MMP-8 Molecular Forms in Progressive Patients (n = 12) at Baseline

Enzyme Form	MPO	MMP-14 (≈50 kDa)	MMP-14 (≥100 kDa)	MMP-14 (≤37 kDa)
Active PMN MMP-8	**0.7741***	0.1598	–0.0564	0.4368
Active mesenchymal MMP-8	**0.6853***	0.2900	0.1056	0.5161
Total MMP-8	**0.6014***	–0.0725	0.0916	0.2776

* $P < 0.05$.
Bold = significant change (P values).

with low-dose doxycycline resulted in collagenase reduction together with reductions in collagen breakdown fragments pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen (ICTP), reflecting a decrease in periodontal destruction.[4] Furthermore, increases in GCF MMP-8 and ICTP during periodontal maintenance were previously associated with an increased risk of future periodontal AL.[30] Based on these findings, the lack of a difference in MMP-8 levels after treatment of active sites might be interpreted as a poor host response, representing sites at risk for further loss of periodontal support. Additionally, the MPO/MMP-8 association could reflect the persistence of MMP-8 activation and, consequently, the need for further treatment and follow-up. Adjunctive therapies based on topical antimicrobials and host modulation, such as the application of subantimicrobial doxycycline, could further reduce collagenase and disease activities.[4] Although the MPO/MMP-8 correlation tended to remain after treatment, the strength of the association was reduced relative to baseline, but this fact could also have been influenced by the reduction in the number of samples analyzed. Conversely, the persistence of MMP-8 at physiologic levels after treatment could also have involved the downregulation of the inflammatory process and the onset of the reparative phase. It was previously shown that MMP-8 can regulate, in addition to collagen turnover, the inflammatory response in an MMP-8 null mice model,[31] and thus, physiologic or reduced levels of MMP-8 can participate in defensive processes by decisive processing of anti-inflammatory cytokines and chemokines during periodontitis.[16]

Neutrophil-derived MPO was previously proposed to participate in the pathogenesis of periodontitis.[21,32,33] To our knowledge, we describe, for the first time, MPO molecular forms in GCF from patients with periodontitis. Immunoreactivities of ≈75 kDa are consistent with monomeric MPO, whereas immunoreactivities of ≈90, 130, and 160 kDa are consistent with the native enzyme, which corresponds to a tetramer composed of two light and two heavy polypeptide chains of about 55 to 63 kDa and 10 to 15 kDa, respectively.[34,35] MPO inactivates pathogenic microbes by generating reactive oxygen species but also oxidatively activates latent proMMP-8 and -9 in vitro and inactivates TIMPs.[23,24,36] Non-proteolytic activation of proMMP-8 can be directly induced by MPO-derived hypochlorous acid, which is likely to represent the most direct mechanism for triggered neutrophils to endogenously activate MMP-8.[23] Although our results showed a tendency for total MMP-8 to decrease

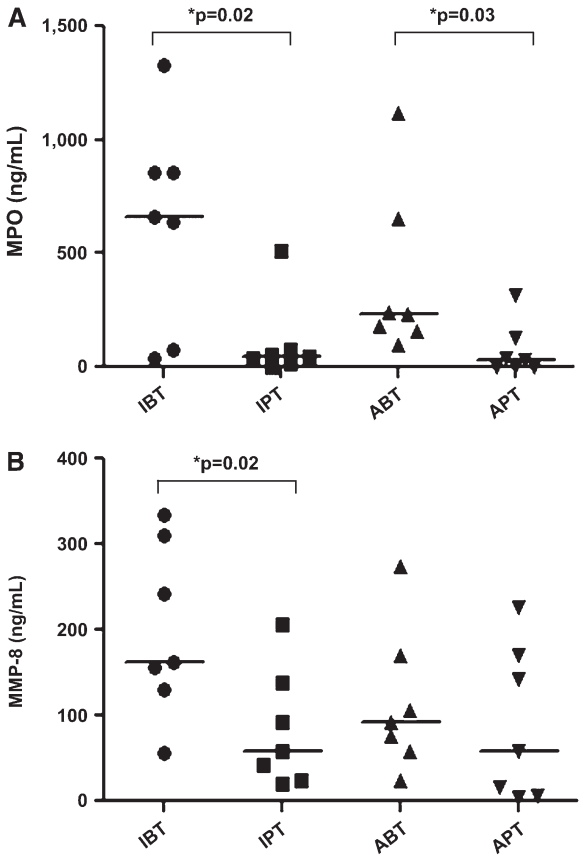

Figure 2.
*MPO **(A)** and MMP-8 **(B)** levels in GCF from active and inactive sites at baseline and after treatment. Lines represent medians (n = 7). IBT = inactive sites, baseline; IPT = inactive sites, post-treatment; ABT = active sites, baseline; APT = active sites, post-treatment.*

in active sites at baseline, active forms and the percentage of enzyme activation tended to increase and correlated with MPO levels. These findings suggested that in vivo periodontal disease progression leads to MPO-dependent oxidative activation of MMP-8 (especially the PMN type). On the other hand, TIMP-1 levels remained unchanged but were low or even undetectable in active and inactive sites by using our method. Thus, an interesting new pathway involving MPO-mediated xidative activation of MMP-8 might operate inside the complex proteolytic network that underlies periodontitis progression. In line with our results, a recent study[37] showed that MMP-8 and MPO levels, together with MMP-9 levels, significantly increased in the GCF from patients with periodontitis compared to in healthy controls and decreased after periodontal treatment. Although the authors[37] demonstrated a positive correlation between MMP-9 and MPO, they found no correlation between MMP-8 and MPO. Whether this fact relies on the presence of disease progression and the determination of the nature of the MPO/MMP-8 association in periodontitis requires further study. Furthermore, detectable and clear differences exist among the immunoassays targeting MMP-8 in periodontitis-affected oral fluids such as GCF.[13]

Soluble forms of MMP-14 in GCF from subjects with periodontitis and in other organic fluids were previously described.[3,17,19] Immunoreactivities >100 kDa in GCF could represent soluble MMP-14 complexed with other MMPs or inhibitors,[3] but no correlation was found in the present study between MMP-14 complexes and MMP-8. Interestingly, an inverse correlation between MMP-14 versus MMP-8 and MPO was demonstrated in GCF from active sites. MMP-14 was described to activate proMMP-8 in vitro through a specific proteolytic cleavage,[17] but based on our results, it is unlikely that this proteolytic activation pathway may play an important role during the progression of chronic periodontitis in vivo. Furthermore, MMP-14 might be downregulated in the presence of oxidants, such as PMN-derived HOCl. Like MMP-8, MMP-14 belongs to the MMP family of collagenases; consecutively, increasing MMP-14 levels and decreasing MMP-8 levels in the GCF from active sites might also obey to a negative regulatory mechanism among collagenolytic MMPs. Our results show that progressive sites displayed a tendency for MMP-14 levels to be higher and for MMP-8 levels to be lower. We cannot discard that an unbalance of MMP-14 and, probably, MMP-13 over MMP-8 could favor the development of progressive episodes during chronic periodontitis. MMP-14 and -13 activities are not limited to collagen breakdown but regulate many other MMP activities,[38] and thus, slight changes could markedly potentiate MMP-mediated proteolysis during disease. MMP-14 can activate proMMP-13 in vitro, and recently, we provided preliminary evidence for a role of MMP-13 over proMMP-9 activation and periodontitis progression.[29]

Although MMP-8 has been classically and widely involved in the breakdown of supporting tissue associated with periodontitis,[6,12-14] a potentially protective role was recently supported based on the development of an experimentally induced periodontitis model in MMP-8 $^{-/-}$ mouse. Kuula et al.[16] reported more extensive alveolar bone loss in MMP-8 $^{-/-}$ mice versus wild-type mice and proposed that physiologic levels of MMP-8 could play a protective role against alveolar bone resorption. MPO can further potentiate this role by oxidatively upregulating the catalytic competence of MMP-8, inactivating TIMP-1, and killing periodontopathogens. Thus, the role and interactions among proteolytic, oxidative, and MMP networks during periodontitis in vivo requires further study. In addition to these local potential effects derived from destructive periodontitis, systemic effects have been reported, such as changes in

serum components involved in the acute-phase response[39] and lipoproteins.[16] *Porphyromonas gingivalis*–induced periodontitis was associated with systemic inflammatory and lipoprotein changes, especially in MMP-8 null allele mice. Particularly, disease progression determined by clinical tolerance method, was associated with increased serum antibodies to *P. gingivalis* and C-reactive protein, elevating the risk for atherosclerotic complications.[39]

CONCLUSIONS

We reported high MMP-8 and MPO levels in GCF from patients with progressive chronic periodontitis, the presence of a strong association between both enzymes, and reductions of both enzymes in response to treatment, except for MMP-8 in active sites. These findings suggested that MPO/MMP-8 interactions could be relevant in disease progression and treatment responses, either by potentiating supporting tissue destruction or providing a defense mechanism, and further support the usefulness of MMP-8 in chairside point-of-care diagnostics in periodontitis.

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