Activity of Platelet Activating Factor Acetylhydrolase Following Phase I Periodontal Therapy

GC Keles[1], BO Cetinkaya[1], F Pamuk[2], U Balli[1]

ABSTRACT

Objective: *Elevated levels of platelet activating factor (PAF), a potent inflammatory mediator, in periodontal disease and decreased PAF levels following periodontal surgical therapy have been previously detected in gingival tissues and gingival crevicular fluid (GCF). Platelet activating factor acetylhydrolase (PAF-AH) is a calcium-independent phospholipase A_2 that catalyses the hydrolysis of PAF, thereby inactivating this mediator. The hypothesis, a relationship between activity of PAF-AH and healing following periodontal therapy, was tested by detecting activity of PAF-AH in GCF samples collected from sites that had undergone phase I periodontal therapy with generalized chronic periodontitis.*

Methods: *Twenty patients with generalized chronic periodontitis were divided into two groups (n = 10): group 1 with probing pocket depth (PPD) 4–5 mm and group 2 with PPD ≥ 6–8 mm. Clinical parameters were recorded and GCF was sampled before phase I periodontal therapy and at the 2nd, 7th, 14th, 21st and 28th day follow-up evaluation visits. Activity of PAF-AH in GCF was analysed by enzyme-linked immunosorbent assay (ELISA).*

Results: *Probing pocket depth at the 21st and 28th day in group 1, and PPD at the 14th, 21st and 28th day in group 2 were significantly decreased when compared to the baseline values (*$p < 0.001$*). Activity of PAF-AH (µmol/ml) was significantly decreased at the 7th, 14th, 21st and 28th day following phase I periodontal therapy in both groups 1 and 2 compared to the baseline values (*$p < 0.05$*).*

Conclusion: *Platelet activating factor acetylhydrolase is detectable in GCF by ELISA and showed a continuous decrease following phase I periodontal therapy. Changes in the PAF-AH activity would be a progressive marker of periodontal healing to evaluate the success of periodontal therapies.*

Keywords: Chronic periodontitis, gingival crevicular fluid, periodontal therapy, platelet activating factor acetylhydrolase

Actividad de la Acetilhidrolasa del Factor Activador de las Plaquetas Tras la Fase I de la Terapia Periodontal

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RESUMEN

Objetivo: *Niveles elevados del factor activador de las plaquetas (PAF) – un potente mediador inflamatorio en la enfermedad periodontal – y niveles disminuidos de PAF tras la terapia quirúrgica periodontal, han sido detectados previamente en los tejidos gingivales y el fluido crevicular gingival (FCG). La acetilhidrolasa del factor activador de las plaquetas (PAF-AH) es una fosfolipasa A_2 independiente del calcio, que cataliza la hidrólisis de PAF, inactivando así este mediador. La hipótesis – la existencia de una relación entre la actividad de PAF-AH y la curación tras la terapia periodontal – fue sometida a comprobación mediante la detección de la actividad de PAF-AH en muestras de FCG recogidas de sitios que pasaron* por la fase I de la terapia periodontal por periodontitis crónica generalizada.

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Métodos: Veinte pacientes con periodontitis crónica generalizada fueron divididos en dos grupos (n = 10): grupo 1 con una profundidad de bolsa al sondeo (PPD) de 4–5 mm, y grupo 2 con PPD = 6–8 mm. Se registraron los parámetros clínicos, y se obtuvieron muestras de FCG antes de la fase I de la terapia periodontal, y en las visitas de evaluación de seguimiento los días 2, 7, 14, 21 y 28. La actividad de PAF-AH en FCG se analizó mediante ensayo por inmunoabsorción ligada a enzimas (ELISA).
Resultados: La profundidad de bolsa al sondeo los días 21 y 28 en el grupo 1, y PPD los días 14, 21 y 28 en el grupo 2 se vieron disminuidas significativamente cuando se les comparó con los valores iniciales ($p < 0.001$). La actividad de PAF-AH (µmol/ml) disminuyó significativamente los días 7, 14, 21 y 28 tras la fase I de la terapia periodontal en ambos grupos 1 y 2 en comparación con los valores al inicio del estudio ($p < 0.05$).
Conclusión: La acetilhidrolasa del factor activador de las plaquetas es detectable en FCG mediante ELISA, y mostró una disminución continua tras la fase I de la terapia periodontal. Los cambios en la actividad de la PAF-AH sería un marcador progresivo de la curación periodontal para evaluar el éxito de las terapias periodontales.

Palabras claves: Periodontitis crónica, fluido crevicular gingival, terapia periodontal, acetilhidrolasa del factor activador de las plaquetas

INTRODUCTION

Periodontitis, an oral infectious disease, is characterized by clinical attachment loss, alveolar bone resorption, periodontal pocketing and gingival inflammation (1). Although the main cause of periodontal disease is the presence of periodontal micro-organisms, subsequent progression and disease severity are considered to be determined by the host-immune response (2). The goals of periodontal therapy are the elimination of the infection, arrest the disease progression, and regeneration of the periodontium (3, 4). Phase I periodontal therapy is aimed at alteration or elimination of the microbial aetiology and contributing factors for periodontal diseases by effective pocket/root debridement [nonsurgical scaling and root planing (SRP)] and the establishment of a proper self-performed supragingival plaque control (5, 6).

Platelet activating factor (PAF) is a potent phospholipid mediator which is synthesized by the thrombocytes, neutrophils, macrophages, eosinophils and epithelial cells (7–14). This mediator is linked to many inflammatory and immune responses, including platelet stimulation, neutrophil and monocyte activation, increased vascular permeability, smooth muscle contraction, and bone resorption by osteoclasts (11–16). Elevated levels of PAF in gingival tissue (8, 12) gingival crevicular fluid (12, 17) and blood (13) in periodontal disease, and also higher concentrations of PAF in gingival tissue in peri-implantitis (16) were previously observed. A significant decrease in PAF levels of whole mixed saliva in subjects with chronic periodontitis after initial periodontal therapy was demonstrated (18). Data suggest that PAF levels were decreased in gingival crevicular fluid (GCF) continuously by 24 weeks and also in gingival tissue at six months after flap surgery plus guided tissue regeneration (GTR) and flap surgery alone (14, 19). Platelet activating factor acetylhydrolase (PAF-AH) is a calcium-independent phospholipase A_2 that catalyses the hydrolysis of PAF, thereby inactivating this mediator (20–23). This enzyme plays a major role in the regulation of PAF levels, was detected in gingival tissue and in GCF as well as in serum and plasma (8, 23–25). There is evidence that no significant difference was found in PAF-AH activity between inflamed and healthy gingiva (8). Contrary to this finding, the levels of PAF-AH in GCF were significantly increased in subjects with experimental gingivitis; also a negative correlation was detected between PAF and PAF-AH activity at the end of experimental gingivitis (24). Data also suggest that treatment of periodontitis significantly reduced the PAF-AH activity in GCF and serum (24, 25).

To the best of our knowledge nothing is known about PAF-AH activity in the periods (2^{nd} to 28^{th} day) following periodontal therapy in generalized chronic periodontitis patients. The hypothesis, a relationship between activity of PAF-AH and healing following phase I periodontal therapy, was tested by detecting activity of PAF-AH in GCF samples collected from sites with generalized chronic periodontitis that had undergone phase I periodontal therapy.

SUBJECTS AND METHODS

Subject selection

Twenty generalized chronic periodontitis patients (nine males and 11 females) with the age range 36–48 years were recruited for the study. All patients fulfilled the diagnostic criteria defined by the International Workshop for the Classification of Periodontal Diseases and Conditions for chronic periodontitis (26). Generalized chronic periodontitis was diagnosed according to the number of disease sites with probing pocket depth (PPD) ≥ 4 mm. Patients exhibiting > 30% of the sites with PPD ≥ 4 mm were considered to have generalized chronic periodontitis (26).

The patients were divided into two equal groups (n = 10): group one with PPD = 4–5 mm (mean age 40.32 ± 6.65 years), group 2 with PPD ≥ 6–8 mm (mean age 42.36 ± 8.02 years).

The patients signed informed consent forms. The study protocol and consent forms were approved by the University Institutional Review Board.

The exclusion criteria were (i) medical history of cancer, rheumatoid arthritis, diabetes mellitus, cardiovascular diseases (ii) compromised immune system (iii) pregnancy and lactation (iv) ongoing drug therapy that may affect the clinical features of periodontitis (v) systemic antimicrobials during the six weeks preceding the baseline examination (vi) any dental treatment during the past six months and (vii) smoking.

All clinical measurements were assessed at six different sites around each tooth by the same calibrated examiner (intra-examiner calibration). Probing pocket depth and clinical attachment level (CAL) were measured by a Florida Probe (Florida Probe Corp, Gaineswille, FL, USA) before phase I periodontal therapy and at the 14th, 21st and 28th day follow-up evaluation visits. The plaque index (27) and gingival index (28) were assessed and GCF samples were collected before periodontal therapy and at the 2nd, 7th, 14–21st and 28th day follow-up evaluation visits.

In patients with chronic periodontitis, the site that showed the highest clinical signs of inflammation (*ie* redness, bleeding on probing and oedema) and the highest PPD along with radiographic confirmation of alveolar bone loss was selected for sampling before periodontal therapy. A GCF sample was taken from the same experimental site before and after periodontal therapy. Prior to GCF sampling, the sites were isolated with cotton rolls, saliva was removed and the supragingival plaque, if present, was also removed using a sterile curet. Gingival crevicular fluid was sampled with filter paper (Periopaper, ProFlow, Inc, Amityville, New York, USA). Paper strips were placed into the crevice until mild resistance was felt, and left in position for 30 seconds. Strips with visible sign of saliva or blood contamination were discarded. Gingival crevicular fluid volume of each strip was determined by electronic impedance (Periotron 8000, ProFlow Inc, New York, USA). Samples were placed into a sterile polypropylene tube and stored at -70 °C until analysis.

At the initial visit, clinical measurements and GCF sampling were performed. Oral hygiene instruction was given that included a demonstration of the (modified) Bass brushing technique, a demonstration on the use of dental floss and a demonstration of the use of inter-dental brushes. No use of antimicrobial mouth rinsing solutions was allowed for the duration of the study. Full-mouth scaling and root planing were performed under local anaesthesia by the same periodontist using scalers and curettes within 24 hours. Also, supragingival polishing was performed. Subgingival irrigation, mouth rinsing with chlorhexidine, and tongue brushing with chlorhexidine were not made.

A modification of the protocol described by Curtis *et al* was used for GCF elution from the periopapers (29). Each sampled strip was placed into 400 µl eppendorf centrifuge tube containing 100 µl of 2% bovine serum albumin in phosphate buffered saline and then incubated for 60 minutes at 4 ºC. This tube was placed into a 1.5 ml microcentrifuge tube and centrifugation was carried out in 10 000 g for five minutes at 4 ºC after creating a hole on the bottom of the 400 µl tube to provide elution of GCF into the microcentrifuge tube. The procedure was repeated twice, and the 200 µl samples were collected. Activity of PAF-AH in GCF samples was analysed by standard enzyme-linked immunosorbent assay (ELISA) procedures at 450 to 550 nm using a commercially available PAF-AH kit (Cayman Chemical Company, Ann Arbor, MI).

Statistical analysis was performed using a commercially available software programme (SPSS 15.0; SPSS Inc, Chicago, IL). For the statistical analysis of PPD and CAL, only the recordings representing the deepest clinical site of each tooth were used (30). The Shapiro Wilk test was used to investigate whether or not the data were normally distributed. A paired T parametric test was used for statistical comparisons of GCF volume and PAF-AH activity between different time points in groups 1 and 2. The intragroup comparisons for clinical data in study groups were performed by Wilcoxon signed ranks nonparametric test. *P*-value of < 0.05 was considered as statistically significant.

RESULTS

Probing pocket depth at the 21st and 28th day in group 1, and PPD at the 14th 21st and 28th day in group 2 were significantly decreased compared to the baseline values ($p < 0.001$). No significant difference was found between the CAL values at different time points among the groups (Table 1). The scores of plaque and gingival indices at the 7th, 14th, 21st and 28th day in both groups were significantly decreased compared to the baseline scores ($p < 0.001$) [Table 2].

Gingival crevicular fluid volume and PAF-AH activity before phase I therapy and at different time points following the therapy in the study groups are presented in Table 3. Significant decreases in GCF volume and PAF-AH activity for both groups at the 7th, 14th, 21st and 28th day were observed ($p < 0.05$).

DISCUSSION

In the present study, PAF-AH activity, which is considered to be associated with healing following phase I periodontal therapy, was evaluated in GCF before phase I therapy and at different times up to the 28th day following the therapy. The clinical findings of this study demonstrated a significant reduction in PPD from the 21st day in group 1 and from the 14th day in group 2 compared to the baseline values. Moreover, clinical measurements such as PPD and the scores of plaque and gingival indices were positively correlated with the GCF volume and PAF-AH activity. Our results clearly showed that

Table 1: Probing pocket depth and clinical attachment level values of the study groups (mm)

	Baseline	2 days	7 days	14 days	21 days	28 days
Probing on Probing						
Group 1	4.6 ± 0.16	4.6 ± 0.16[a,c]	4.2 ± 0.20[a,c]	3.6 ± 0.16[a,c]	3.3 ± 0.15[b,c]	3.1 ± 0.23[b,c]
	5.0 (4.0 – 5.0)	5.0 (4.0 – 5.0)	4.0 (3.0 – 5.0)	4.0 (3.0 – 4.0)	3.0 (3.0 – 4.0)	3.0 (2.0 – 4.0)
Group 2	7.0 ± 0.15	7.0 ± 0.15[a,c]	5.9 ± 0.35[a,c]	5.4 ± 0.22[b,c]	5.2 ± 0.20[b,c]	4.5 ± 0.22[bc]
	7.0 (6.0 – 8.0)	7.0 (6.0 – 8.0)	6.0 (4.0 – 8.0)	5.5 (4.0 – 6.0)	5.0 (4.0 – 6.0)	4.0 (4.0 – 6.0)
Clinical Attachment Level						
Group 1	5.0 ± 0.21	5.0 ± 0.21[a,c]	4.6 ± 0.31[a,c]	4.0 ± 0.30[a,c]	3.7 ± 0.30[a,c]	3.5 ± 0.37[a,c]
	5.0 (4.0 – 6.0)	5.0 (4.0 – 6.0)	4.5 (3.0 – 6.0)	4.0 (3.0 – 6.0)	3.5 (3.0 – 6.0)	3.5 (2.0 – 6.0)
Group 2	7.6 ± 0.31	7.6 ± 0.31[a,c]	6.5 ± 0.45[a,c]	6.0 ± 0.37[a,c]	5.8 ± 0.36[a,c]	5.1 ± 0.28[a,c]
	7.5 (6.0 – 9.0)	7.0 (6.0 – 9.0)	6.5 (4.0 – 9.0)	6.0 (4.0 – 8.0)	6.0 (4.0 – 8.0)	5.0 (4.0 – 6.0)

Data are expressed as the mean ± standard error of the mean and as the median (minimum–maximum)
[a]No significant difference from the baseline values among groups ($p > 0.05$)
[b]Significant difference from the baseline values among groups ($p < 0.001$)
[c]No significant difference from the value of the previous time interval among groups ($p > 0.05$)

Table 2: Plaque index and gingival index scores of the study groups

	Baseline	2 days	7 days	14 days	21 days	28 days
Plaque Index						
Group 1	2.5 ± 0.17	1.4 ± 0.22[a,c]	0.7 ± 0.21[b,c]	0.5 ± 0.17[b,c]	0.1 ± 0.10[b,c]	0.6±0.22[b,c]
	2.0 (2.0 – 3.0)	1.0 (1.0 – 3.0)	1.0 (0.0 – 2.0)	0.5 (0.0 – 1.0)	0.0 (0.0 – 1.0)	0.5 (0.0 – 2.0)
Group 2	2.1 ± 0.10	1.2 ± 0.20[a,c]	0.4 ± 0.16[b,c]	0.3 ± 0.21[b,c]	0.2 ± 0.20[b,c]	0.2 ± 0.13[b,c]
	2.0 (2.0 – 3.0)	1.0 (0.0 – 2.0)	0.0 (0.0 – 1.0)	0.0 (0.0 – 2.0)	0.0 (0.0 – 2.0)	0.0 (0.0 – 1.0)
Gingival Index						
Group 1	2.5 ± 0.17	1.5 ± 0.22 [a,c]	0.9 ± 0.18[b,c]	0.6 ± 0.16[b,c]	0.6 ± 0.16[b,c]	0.5 ± 0.17[b,c]
	2.5 (2.0 – 3.0)	1.0 (1.0 – 3.0)	1.0 (0.0 – 2.0)	1.0 (0.0 – 1.0)	1.0 (0.0 – 1.0)	0.5 (0.0 – 1.0)
Group 2	2.1 ± 0.10	1.9 ± 0.10[a,c]	1.4 ± 0.16[b,c]	1.0 ± 0.15[b,c]	0.9 ± 0.10[b,c]	0.9 ± 0.10[b,c]
	2.0 (2.0 – 3.0)	2.0 (1.0 – 2.0)	1.0 (1.0 – 2.0)	1.0 (0.0 – 2.0)	1.0 (0.0 – 1.0)	1.0 (0.0 – 1.0)

Data are expressed as the mean ± standard error of the mean and as the median (minimum–maximum)
[a]No significant difference from the baseline values among groups ($p > 0.05$)
[b]Significant difference from the baseline values among groups ($p < 0.001$)
[c]No significant difference from the value of the previous time interval among groups ($p > 0.05$)

Table 3: Gingival crevicular fluid volume (µl) and PAF-AH activity (µmol/ml) values of the study groups

	Baseline	2 days	7 days	14 days	21 days	28 days
Gingival Crevicular Fluid Volume (µl)						
Group 1	0.627 ± 0.010	0.611 ± 0.011[a]	0.457 ± 0.008[bc]	0.365 ± 0.009[bc]	0.293 ± 0.007[bc]	0.201 ± 0.008[bc]
Group 2	0.741 ± 0.012	0.688 ± 0.009[a]	0.513 ± 0.008[bc]	0.402 ± 0.008[bc]	0.331 ± 0.011[bc]	0.263 ± 0.010[bc]
PAF-AH Activity (µmol/ml)						
Group 1	0.204 ± 0.004	0.199 ± 0.003[a]	0.188 ± 0.003[bc]	0.180 ± 0.002[bc]	0.172 ± 0.003[bc]	0.158 ± 0.003[bc]
Group 2	0.210 ± 0.003	0.202 ± 0.001[a]	0.190 ± 0.001[bc]	0.182 ± 0.001[bc]	0.174 ± 0.002[bc]	0.163 ± 0.002[bc]

Data are expressed as the mean ± standard error of the mean
PAF-AH – platelet activating factor acetylhydrolase
[a]No significant difference from the baseline values among groups ($p > 0.05$)
[b]Significant difference from the baseline values among groups ($p < 0.05$)
[c]Significant difference from the value of the previous time interval among groups ($p < 0.05$)

phase I periodontal therapy resulted in a significant decrease in PAF-AH activity in GCF at early healing periods (to 28th day).

From a clinician's perspective, it is notable that in the periodontology literature there are a few studies related to the PAF-AH activity that analyse the enzyme activity in serum (25), gingival tissue (8), and GCF (24) using different determination methods except ELISA. However, PAF-AH activity in plasma was determined by ELISA in an experimental mice study (23). In light of present day knowledge, GCF reflects cellular activities in the surrounding periodontal tissues and the constituents of GCF are involved in tissue formation and remodelling (31–33). This is the first study investigating PAF-AH activity in GCF before and at the 7th, 14th 21st and 28th day following phase I periodontal therapy.

It is important to note that there is no information on the time the GCF samples were obtained following conventional periodontal treatment in the study reported by Baltas *et al* (24). However, results of that study are consistent with our findings that PAF-AH activity in GCF was significantly reduced following phase I periodontal therapy. Data also suggest that treatment of periodontititis significantly reduced the serum activity of PAF-AH (25). This might probably be related to the diminution of the systemic inflammatory response following successful periodontal therapy.

There is evidence that PAF-AH is predicted to suppress inflammatory signalling (22). Since the expression of plasma PAF-AH is increased by stimulation with lipopolysaccharide and other inflammatory agonists, and decreased by anti-inflammatory drugs and cytokines (34). The present study confirms these findings that GCF PAF-AH activity was higher in periodontally diseased sites than the sites at the 7th, 14–21st and 28th day following etiotropic phase (phase I) of periodontal therapy which reduce or eliminate gingival inflammation. It is therefore possible that activity of PAF-AH in GCF is decreased due to the anti-inflammatory effect of phase I therapy.

In conclusion, PAF-AH is detectable in GCF by ELISA and showed a continuous decrease following phase I periodontal therapy at early periods. In light of these findings, it is relevant to assume that changes in the PAF-AH activity in GCF might be useful for monitoring the progress of periodontal healing. This information is important to the clinician since changes in the PAF-AH activity would be a progressive marker of periodontal healing to evaluate the success of periodontal therapies. Additional studies should be undertaken to further elucidate the role of PAF-AH on wound healing mechanisms following periodontal regenerative therapies.

Author's Note

The authors declare that they have no financial relationships related to any products involved in this study.

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