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Plasminogen activator system in smokers and non-smokers with and without periodontal disease

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Abstract

Background: The present study assessed levels of plasminogen activator (PA) system proteins in gingival crevicular fluid (GCF) and serum of chronic gingivitis, chronic periodontitis patients and periodontally healthy subjects and evaluated how smoking influenced these levels.

Methods: Twenty chronic gingivitis; 20 chronic periodontitis patients and 20 periodontally healthy volunteers were consecutively recruited according to the inclusion criteria so that exactly half of the subjects in each category were smokers. GCF samples from four sites together with serum samples were obtained from each subject. GCF levels of tissue type PA (t-PA), urokinase type PA (u-PA), PA inhibitor-1 (PAI-1) and PA inhibitor-2 (PAI-2) and serum concentrations of cotinine, u-PA and PAI-1 were analysed by enzyme-linked immunosorbent assay.

Results: The only statistically significant difference between smokers and non-smokers was a lower GCF PAI-2 concentrations in healthy smokers compared with healthy non-smokers ($p < 0.01$). Gingivitis and periodontitis patients had higher GCF concentrations of PAI-2 than healthy subjects ($p < 0.002$ and $p < 0.02$ respectively). The ratio of u-PA:PAI-1 and t-PA:PAI-1 were significantly higher in GCF of smokers with periodontitis compared with ''healthy'' smokers, whereas the ratio of t-PA:PAI-2 was significantly lower in smokers with periodontal disease ($p < 0.05$).

Conclusions: GCF levels of the PA system proteins are increased in chronic gingivitis and periodontitis compared with healthy gingiva. Smoking had only subtle effects on the GCF PA system proteins with the exception of PAI-2, and the balance of activators and inhibitors. These findings suggest one mechanism whereby smoking may exert detrimental effects on the periodontal tissues.

Key words: ELISA; plasminogen activator; plasminogen activator inhibitor; gingival crevicular fluid; gingivitis; periodontitis; serum; smoking

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The periodontal diseases are a group of infectious/inflammatory diseases involving Gram-negative, anaerobic, and microaerophilic bacteria that colonize the subgingival area and cause local and systemic elevations of pro-inflammatory prostaglandins and cytokines resulting in tissue destruction. Risk factors including tobacco smoking modify the periodontal response to the microbial challenge. Smokers have been reported to be more susceptible to advanced and aggressive forms of periodontal disease than non-smokers (Haber et al. 1993, Ketabi & Hirsch 1997, Calsina et al. 2002). Smokers tend to respond less favourably to periodontal treatment procedures (Ah et al. 1994, Boström et al. 1998) although smoker and non-smoker patients exhibit more or less the same periodontal pathogens (Preber et al. 1992, Renvert et al. 1998, Buduneli et al. 2005). It has been suggested that smoking has an influence on host cytokine levels (Tappia et al. 1995, Boström et al. 1999, Rawlinson et al. 2003), however, the exact mechanisms by which smoking exerts detrimental effects on the periodontal tissues remain unclear.

The plasminogen-activating system is of central importance in extracellular proteolysis and acts in both physiological and pathological processes, such as tissue repair, tissue remodelling, local inflammatory reactions and neoplastic growth and invasion (Ossowski & Reich 1983). Plasminogen activators (PA) are serine proteases and of two types: the tissue/blood vessel type PA (t-PA) and the urokinase type PA (u-PA) (Dano et al. 1985, Saksela 1985). t-PA and u-PA

form part of the complex enzyme cascade involved in fibrinolysis and convert the proenzyme plasminogen into the broad-spectrum proteinase, plasmin. Plasmin is not only responsible for the degradation of fibrin, but also contributes directly and indirectly, via activation of latent collagenase, to the degradation and turnover of the extracellular matrix (ECM) (Kruithof 1988). Plasmin can be formed locally at sites of inflammation by limited proteolysis of its inactive precursor, plasminogen, which circulates in plasma and interstitial fluids (Deutsch & Mertz 1970). The activity of the PAs in turn are regulated by two different PA inhibitors (PAI); one produced by endothelial cells as well as various normal and malignant cells; PAI-1, (Dano et al. 1999), and the other; PAI-2 is produced by macrophages, epithelial cells and other cells (Astedt et al. 1986, Kruithof et al. 1995).

Previous studies have indicated a high concentration of t-PA and PAI-2 in gingival crevicular fluid (GCF) and suggested that they may be involved in the aggravation of gingival inflammation (Kinnby et al. 1996). Furthermore, Xiao et al. (2000) have stated that t-PA and PAI-2 may play a significant role in periodontal tissue destruction and tissue remodelling and that t-PA and PAI-2 in GCF may be used as clinical markers to evaluate the periodontal diseases and assess treatment. In a recent study, we have found significantly elevated GCF levels of t-PA and PAI-2 in CsA-induced gingival overgrowth patients suggesting involvement of the PA system in the pathogenesis of this side effect of CsA therapy (Buduneli et al. 2004).

As yet, the relationship between smoking and the PA system in periodontal disease has not been evaluated. Possible alterations in the PA system components may influence periodontal tissue destruction in smokers. Thus, the aim of the present study was two-fold: (1) to evaluate the possible effects of smoking on the GCF and serum levels of the PA system and (2) to compare chronic gingivitis, chronic periodontitis patients and periodontally healthy subjects with regard to smoking and levels of the PA system constituents.

Materials and Methods

Study population

A total of 60 subjects in three periodontal health status categories were consecutively recruited into the present study. Twenty chronic gingivitis patients (11 males and nine females, with an age range of 32–48 years); 20 chronic periodontitis patients (14 males and six females, with an age range of 44–50 years) and 20 periodontally healthy volunteer subjects (six males and 14 females, with an age range of 26–41 years) were selected according to the inclusion criteria. Exactly half of the subjects in each category were smokers. Inclusion criteria were that the subjects would be free of any systemic disease, were not taking any medication and had not received antibiotics or periodontal treatment in the previous 6 months. Smoking status of each subject was ascertained by questioning the subjects and those who had never smoked were recruited into the non-smoker groups and subjects who have been smoking $\geqslant 10$ cigarettes per day for more than 5 years were selected for the smoker groups. Former smokers who had quit smoking were excluded from the study. Subjects who smoke $\geqslant 10$ cigarettes/day for less than 5 years, and those who smoke < 10 cigarettes/day for more than 5 years were also excluded in an attempt to make a clear discrimination in this cross-sectional study between smokers and non-smokers. The volunteer subjects drawn from students and staff of the dental school were all systemically healthy and had no history of periodontal disease, i.e., probing depths < 3 mm and no attachment loss, obvious clinical inflammation, sulcular bleeding, or radiographic evidence of bone loss.

GCF sampling

GCF samples were obtained from four different preselected inter-proximal sites (one site in each quadrant) in each subject and a total of 240 GCF samples were collected from 60 people in six groups as follows:

Group 1: 10 healthy non-smoker (NSH) subjects: four clinically uninflamed sites from each periodontally healthy non-smoker subject.

Group 2: 10 healthy smoker (SH) subjects: four clinically uninflamed sites from each periodontally healthy smoker subject

Group 3: 10 non-smoker gingivitis (NSG) subjects: four sites with the most prominent signs of gingival inflammation in each non-smoker chronic gingivitis patient

Group 4: 10 smoker gingivitis (SG) subjects: four sites with the most prominent signs of gingival inflammation in each smoker chronic gingivitis patient

Group 5: 10 non-smoker periodontitis (NSP) subjects: four sites with the deepest probing pocket depth in each non-smoker chronic periodontitis patient

Group 6: 10 smoker periodontitis (SP) subjects: 4 sites with the deepest probing pocket depth in each smoker chronic periodontitis patient

Sampling sites were selected from buccal aspects of the mesial and distal surfaces at the inter-proximal sites. Prior to GCF sampling, dichotomous plaque recording was performed as present or absent and supragingival plaque was then removed by sterile curettes and the surfaces were dried and isolated by cotton rolls. Filter paper strips (Periopaper, ProFlow Inc., Amityville, NY, USA) were placed in the orifices of gingival sulcus/pocket for 30 s. Care was taken to avoid mechanical trauma and strips contaminated with blood were discarded. The absorbed GCF volume was estimated by a calibrated instrument (Periotron 8000, Oraflow Inc., Plainview, NY, USA). Then, the strips were sealed into polypropylene tubes before freezing at -40°C. The readings were converted to an actual volume (μl) by reference to the standard curve. All the samples were lyophilized and stored at -20°C until the laboratory procedures.

Subsequent to GCF sampling, clinical periodontal recordings including probing pocket depth (PPD), gingival index (GI) (Silness & Löe 1964) and papilla bleeding index (PBI) (Saxer & Mühlemann 1975) were performed at the sampling sites. The Williams probe was used for clinical periodontal recordings, which were performed by one examiner who was blinded to the smoking status of the subject. Radiographic examination was also carried out to detect alveolar bone destruction. Two millilitres of peripheral venous

blood was taken from the antecubital vein of each subject, left to clot, centrifuged at 230.5 *g* for 10 min and then approximately 1 ml of serum was transferred to clean polypropylene tubes and frozen immediately at -40°C.

Enzyme immunoassay

PAI-1 and u-PA ELISA kits were purchased from American Diagnostica Inc. (500 West Avenue, Stanford, CT, USA) the PAI-2 kit was purchased from Abraxis (Warminster, PA, USA) and the t-PA ELISA kit was obtained from Innovative Research (Southfield, MN, USA). Human albumin, goat anti-human albumin, rabbit anti-human albumin, horseradish peroxidase labelled anti rabbit Ig (goat) Extravidin peroxidase were purchased from Sigma (Poole, Dorset UK). Levels of albumin, t-PA, u-PA, PAI-1 and PAI-2 were analyzed by enzyme-linked immunosorbent assay (ELISA). After a pilot experiment, it was decided to pool the four GCF samples for each subject in the six study groups, and the study unit was selected as the subject rather than the site. The four absorbed GCF samples were eluted together into two volumes of 500 µl PBS, which were then pooled. The pooled GCF samples were aliquoted and stored at -70°C until further laboratory procedures were performed. For u-PA, PAI-1, and PAI-2 analyses, GCF samples were diluted 1:4 according to the manufacturer's recommendations. For the t-PA assay the GCF sample was used neat. For the albumin assay, the pooled GCF samples were diluted 1/250 for pooled GCF volumes <0.5 µl, and 1/500 for pooled GCF volumes >0.5 µl. The ELISA assay was carried out according to the manufacturer's recommendations or as described before (Adonogianaki et al. 1995). Briefly, the immunolon-4 plates were coated with trapping antibody o/n at 4°C. The plates were washed and the non-specific binding sites were blocked. Samples and the standards were added to duplicate wells and incubated for 90 min at room temperature. The plates were washed and detection antibody was added and the plates were incubated for 60 min at room temperature. After washing, conjugated antibody was added and incubated for 30 min at 37°C. The plates were washed again and streptavidin peroxidase was added and incubated for 30 min at 37°C. Later on the substrate was added and incubated to develop colour change. Finally, the optical densities were read and the samples were compared with the standards. The results for albumin were expressed as ng for total amount calculations, and as ng/µl for GCF concentrations. The results for PAs and PAIs were expressed as pg for total amount calculations, as pg/µl when adjusted for GCF volume, and as pg/mg albumin when adjusted for 1 mg albumin. Serum cotinine levels were also evaluated by using the relevant EIA kit (Cozart, Oxford, UK) and smoking status was confirmed (smokers $\geqslant 100$ ng/ml cotinine; non-smokers <50 ng/ml cotinine).

Statistical analyses

Where appropriate, parametric tests and distribution free tests were used for statistical analysis. Analysis of variance and post hoc Bonferroni tests were used for the clinical data. For the protein levels and concentrations in the GCF, medians were employed as a measure of central tendency as the data were mostly non-normally distributed. The Kruskall–Wallis test followed by Dunn's post test was used for the group comparisons of the protein levels, concentrations in the GCF and the ratios of PAs and PAIs. Mann–Whitney *U*-test and Bonferroni correction was employed for comparisons in protein levels and concentrations between smokers and non-smokers with the same disease category i.e. healthy, gingivitis or periodontitis. The differences in the clinical data within the same disease category were analyzed by a *t*-test with Bonferroni correction applied. Pearson correlations were utilized to look at the relationships between GCF volume and albumin concentration. Spearman's rank correlations were used to look at the relationships between the measurements of the GCF proteins and the GCF volume, albumin concentration and probing pocket depth.

Results

Patient groups, demographic variables and mean values of clinical measurements are outlined in Table 1. The smoking status of the patients was confirmed by the cotinine EIA. The serum cotinine levels in the smoker subjects ranged from 312 to 456 ng/ml and the non-smoker groups exhibited extremely low levels of serum cotinine.

As a first step, the three non-smoker groups were compared with each other (Table 1) (Fig. 1). Total amounts but not concentrations of u-PA in GCF samples showed statistically significant differences between the NSH subjects and NSP patients ($p<0.01$) (Fig. 1a). Total amount of t-PA in the NSH subjects was significantly lower than that of the NSG patients ($p<0.05$) (Fig. 1b). t-PA GCF

Table 1. Clinical characteristics of the study groups

	Group 1, Non-smoker healthy	Group 2, Smoker healthy	Group 3, Non-smoker gingivitis	Group 4, Smoker gingivitis	Group 5, Non-smoker periodontitis	Group 6, Smoker periodontitis
n	10	10	10	10	10	10
Male:female	1:9	5:5	6:4	5:5	7:3	7:3
Age	34.2 ± 8.1	32.1 ± 7.9	39.1 ± 7.6	38.9 ± 9.7	46.6 ± 7.7	45.4 ± 7.8
Probing pocket depth (mm)*	1.68 ± 0.41	1.65 ± 0.44	3.25 ± 0.44[1,5]	3.13 ± 0.52[2,6]	5.28 ± 0.89[6]	5.45 ± 1.06[2,4]
Gingival index*	0.00 ± 0.00	0.00 ± 0.00	1.60 ± 0.21[1,5]	1.60 ± 0.39[2]	2.40 ± 0.60[1]	1.98 ± 0.67[2]
Papilla bleeding index*	0.00 ± 0.00	0.00 ± 0.00	1.33 ± 0.68[1,5]	1.18 ± 0.74[2]	2.23 ± 0.78[1]	1.40 ± 0.88[2]
Plaque index*	0.00 ± 0.00	0.00 ± 0.00	0.78 ± 0.45	0.85 ± 0.33	0.86 ± 0.34	0.81 ± 0.34
Gingival crevicular fluid volume (µl)*	0.99 ± 0.37	0.60 ± 0.18[1]	1.64 ± 0.47[1]	1.41 ± 0.35[2]	2.03 ± 0.42[1]	1.36 ± 0.35[2,5]

*Results were calculated from the averages of the four sampling sites and the GCF volume is the pooled volume of 4 samples for each subject.
[1,2,4,5,6]Indicates statistically significant differences between the study groups ($p<0.05$).
GCF, gingival crevicular fluid.

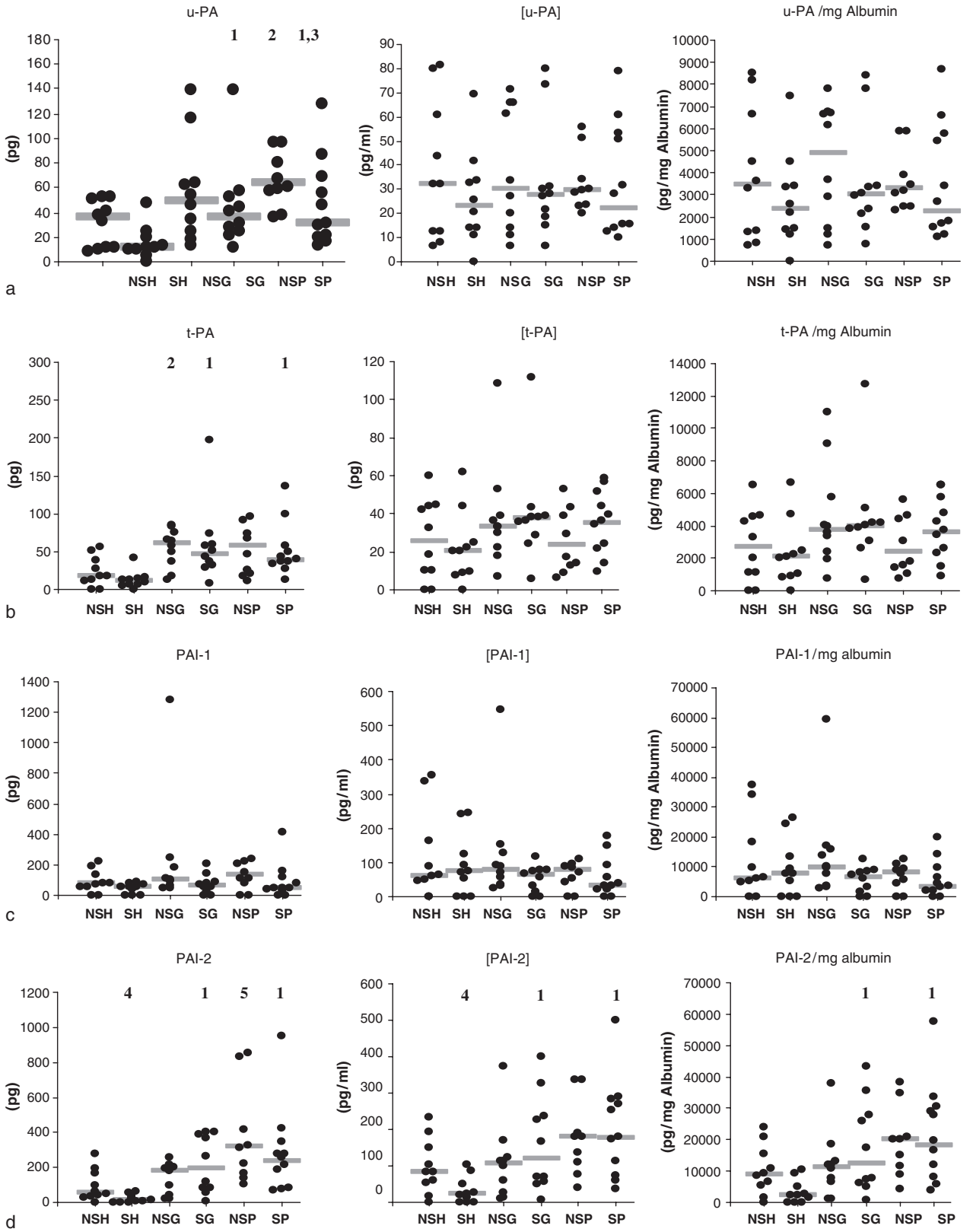

Fig. 1. GCF u-PA (a), t-PA (b), PAI-1 (c), PAI-2 (d) levels, concentrations and levels adjusted to the albumin content. 1, Significantly higher than the SH group ($p<0.05$); 2, Significantly higher than the NSH group ($p<0.05$); 3, Significantly higher than the NSP group ($p<0.05$); 4, Significantly higher than the NSH group ($p<0.05$); 5, Significantly higher than the NSG and NSH group ($p<0.05$). GCF, gingival crevicular fluid; PA, plasminogen activator; u-PA, urokinase type PA; t-PA, tissue type PA; PAI-1, PA inhibitor-1; PAI-2, PA inhibitor-2; NSH, 10 healthy non-smoker subjects; SH, 10 healthy smoker subjects; NSG, 10 non-smoker gingivitis subjects; SG, 10 smoker gingivitis subjects; NSP, 10 non-smoker periodontitis subjects; SP, 10 smoker periodontitis subjects.

Table 2. Serum u-PA, PAI-1 and cotinine levels and concentrations

	Group 1, Non-smoker healthy	Group 2, Smoker healthy	Group 3, Non-smoker gingivitis	Group 4, Smoker gingivitis	Group 5, Non-smoker periodontitis	Group 6, Smoker periodontitis
u-PA (pg)	1342 (1163–1549)	1757 (1499–2180)	1750 (1361–2203)	1655 (1305–2022)	2799† (1740–3015)	2683 (1092–2747)
u-PA (pg/μl)	1.24 (1.10–1.48)	1.58 (1.40–2.16)	1.82 (1.31–2.34)	1.56 (1.33–1.95)	2.52† (1.56–2.83)	2.36 (1.24–2.61)
PAI-1 (pg)	37955 (36198–40130)	37672 (35637–42454)	44349† (41186–46872)	42993 (35512–45050)	44087 (34382–50937)	40629 (34801–47271)
PAI-1 (pg/μl)	35.3 (33.6–37.5)	37.6 (32.1–44.5)	45.1† (40.2–48.4)	42.0 (34.6–43.9)	42.0 (32.9–45.4)	39.2 (35.3–44.9)
Cotinine (ng/ml)	23.8 (20.9–27.1)	402.5* (345.9–430.2)	25.5 (23.6–26.2)	400.9* (342.1–419.5)	26.2 (25.0–28.2)	423.0* (381.8–433.6)

All values are median (interquartile range).
u-PA, urokinase type plasminogen activator; PAI, plasminogen activator inhibitor.
*Significantly different from all the non-smoker groups ($p<0.01$);
†Significantly different from the non-smoker healthy group ($p<0.05$).

concentrations did not exhibit significant differences between the three non-smoker study groups ($p>0.05$). No significant differences were found between the non-smoker groups with regard to the total amount and concentration of PAI-1 ($p>0.05$) (Fig. 1c). The highest total amount of PAI-2 was found in the NSP patients, followed by NSG patients and NSH subjects, while the difference between the NSH and NSP groups as well as the difference between the NSG and NSP groups were statistically significant ($p<0.01$, $p<0.05$ respectively) (Fig. 1d). The differences between the three non-smoker groups were not significant when PAI-2 concentrations were compared ($p>0.05$). There were no significant differences in the ratios of PAs to the PAIs between the non-smoker groups.

The three smoker groups were also compared with each other by Kruskall–Wallis test. (Table 1) (Fig. 1). Total u-PA and t-PA amounts but not concentrations in the SH subjects was significantly lower than those in the smoker patients ($p<0.01$) (Figs 1a, b). The total t-PA levels in the SG and SP groups were similar ($p=0.899$) (Fig. 1b). No statistically significant differences were found in total amount or concentration of PAI-1 between the three smoker groups ($p>0.05$) (Fig. 1c). The total PAI-2 level and concentration in the SH group was significantly lower than the patient groups ($p<0.01$), while the difference between the SG and SP groups was not statistically significant ($p=1.00$) (Fig. 1d). The ratios of u-PA:PAI-1 and t-PA:PAI-1 were significantly higher in smoker gingivitis and periodontitis patients compared with healthy smokers ($p<0.05$) (data not shown). The ratio of t-PA:PAI-2 in smoker periodontitis patients was significantly lower than healthy smokers ($p<0.05$). These ratios did not exhibit significant differences between smoker gingivitis and smoker periodontitis patients ($p>0.05$).

Statistical comparisons were also made between the smoker and non-smoker groups of each periodontal health status category by Bonferroni corrected *t*-test or Mann–Whitney *U*-test. Total GCF albumin amount in the SH was significantly lower than that of the NSH group (6161 and 9272 ng respectively, $p<0.05$). There was no significant difference between total albumin levels of SG and NSG groups (13,802 and 15,900 ng, respectively, $p=0.488$). SP group revealed significantly lower total albumin levels compared with the NSP group (12,858 and 16,466 ng, respectively, $p<0.01$). GCF albumin concentrations did not show any significant differences between the study groups ($p>0.05$). None of the calculations for t-PA and PAI-1 exhibited statistically significant differences between the smoker and non-smoker groups within each category ($p>0.05$) (Figs 1b, c). u-PA level in the SP group was significantly lower than the NSP group ($p<0.05$) (Fig. 1a). PAI-2 levels and concentrations were significantly higher in the NSH group compared with the SH group ($p<0.05$) (Fig. 1d). Comparisons between the smokers and non smokers in each disease category revealed no significant differences in the ratios of PAs to PAIs in GCF.

Among the three non-smoker groups, the highest serum total amount and concentration of u-PA and PAI-1 was observed in the NSP and NSG groups, respectively and both were significantly higher than the NSH group ($p<0.05$) (Table 2). The three smoker groups did not show any statistically significant differences with regard to the serum u-PA and PAI-1 levels. Comparisons of smoker and non-smoker groups did not reveal any significant differences, although the smoker groups showed a tendency for lower levels.

Pearson correlation analysis revealed statistically significant positive correlations between GCF volumes and PPD, PBI, GI values as well as with the amount of albumin ($p<0.01$) (data not shown). Spearman correlation analysis revealed significant positive correlations between the amounts of u-PA, t-PA, PAI-1 and PAI-2 and GCF volumes, as well as the amount of albumin ($p<0.01$) (Table 3). Probing pocket depth, papilla bleeding index and gingival index values showed significant positive correlations with u-PA, t-PA, PAI-2 ($p<0.01$). Albumin concentrations correlated closely with the concentrations of t-PA, u-PA, PAI-1 and PAI-2 in the GCF and the concentrations of these proteins showed significant correlation with each other. GCF volume, GCF u-PA, t-PA, and PAI-1 levels showed statistically significant negative correlation with serum cotinine levels ($p<0.05$).

Discussion

To our knowledge, this is the first study to investigate the role of smoking on the

Table 3. Spearman correlations (group size; $n = 60$)

	u-PA (pg)	u-PA (pg/μl)	t-PA (pg)	t-PA (pg/μl)	PAI-1 (pg)	PAI-1 (pg/μl)	PAI-2 (pg)	PAI-2 (pg/μl)	Serum cotinine (ng/ml)
GCF (μl)	0.484†	−0.113	0.553†	0.004	0.354†	−0.055	0.494†	0.214	−0.264*
PPD (mm)	0.435†	0.061	0.463†	0.204	0.122	−0.078	0.580†	0.445†	0.134
GI	0.451†	0.016	0.547†	0.207	0.143	−0.125	0.609†	0.409†	0.024
PBI	0.545†	0.094	0.528†	0.163	0.153	−0.136	0.530†	0.316*	−0.031
Albumin (ng)	0.476†	−0.096†	0.532	0.004	0.336†	−0.052	0.501†	0.232	−0.245
Albumin (ng/μl)	−0.173	0.061	−0.173	−0.048	−0.042	0.104	−0.295*	−0.200	0.012
u-PA (pg)		0.776†	0.580†	0.360†	0.468†	0.253	0.524†	0.442†	−0.286*
u-PA (pg/μl)			0.292*	0.458†	0.344†	0.412†	0.228	0.322*	−0.138
t-PA (pg)				0.783†	0.415†	0.15	0.583†	0.491†	−0.294*
t-PA (pg/μl)					0.278*	0.266*	0.365	0.440	−0.129
PAI-1 (pg)						0.864†	0.195	0.134	−0.367†
PAI-1 (pg/μl)							−0.020	0.031†	−0.174
PAI-2 (pg)								0.936	−0.171
PAI-2 (pg/μl)									−0.187

Correlation is significant at
†$p < 0.01$ level (2-tailed) and at
*$p < 0.05$ level (2-tailed).
GCF, gingival crevicular fluid; PPD, probing pocket depth; GI, gingival index; PBI, papilla bleeding index; u-PA, urokinase type plasminogen activator; t-PA, tissue type plasminogen activator; PAI, plasminogen activator inhibitor.

GCF levels of albumin and the plasminogen-activating system constituents in various periodontal disease categories and health. Self-reporting by the patients of their smoking status turned out to be accurate, there was 100% match with the cotinine test results and we can claim that there was a very clear discrimination between the smoker and non-smoker groups enabling us to evaluate the possible effects of smoking.

The destructive process of periodontal tissues is likely to be associated with increased levels of proteolytic enzymes in GCF (Curtis et al. 1988, Page 1992). Thus, GCF is considered to be a good medium for reflecting periodontal breakdown. In an attempt to overcome the possible fluctuations arising from GCF volume changes, we have expressed our results in three ways: as total amounts in GCF samples per 30 s sampling; as concentrations in GCF; and finally as concentrations per mg of albumin.

The plasminogen-activating system is known to be associated with fibrinolysis and thrombolysis and, thereby it is of central importance in ECM degradation and remodelling. Plasmin acts directly on the ECM by cleaving non-collagenous ECM proteins and also indirectly by activating a whole range of other enzymes including matrix metalloproteinases. Therefore, strict control of its activation is important for maintaining the integrity of tissues. Previously, local production of t-PA, PAI-2 and u-PA in the gingival tissue has been suggested (Kinnby et al. 1991, 1993, 1999). Our results would agree with this hypothesis even though the levels of all the PA system proteins except t-PA correlated closely to the albumin content of the GCF indicating that some of the protein is derived from plasma.

In previous studies investigating the GCF levels of the PA system in relation to the periodontal disease status, no comparisons were made between the smoker and non-smoker subjects (Kinnby et al. 1991, 1994, 1996, Brown et al. 1995, Xiao et al. 2000, Olofsson et al. 2002). Xiao et al. (2000) have reported significantly high levels of t-PA, PAI-2 in GCF in the gingivitis and periodontitis patients. They found significant decreases in GCF levels of these proteins by initial phase of periodontal treatment indicating a good correlation between t-PA and PAI-2 and the severity of periodontal conditions. The increases in the amounts of t-PA, u-PA, PAI-1 suggest an increase in local production of these proteins but do not prove it. In contrast, a significant increase in the amount of PAI-2 and the adjusted concentration of PAI-2 indicate that local production of these proteins had increased in patients with periodontal disease. The proportional increase in the gingivitis and the periodontitis groups of the amounts of the PA system proteins over the healthy control levels is greater in the smokers than in the non-smokers. In periodontal disease any effects smoking may have on the PA system protein levels appear to be masked. However, our results show a perturbation in the balance of PAI-1 and PAI-2 to t-PA and of PAI-1 to u-PA in the GCF of smokers and this may indicate a potential deficiency in the control of PA activity in smokers with periodontal disease. Such a potential imbalance may contribute to increased tissue turnover. Thus, the balance between PAs and PAIs reflects the net plasminogen-activating capacity. The ratio of t-PA:PAI-2 levels in GCF from deteriorating and stable periodontal pockets was compared in a previous study (Olofsson et al. 2002) and the authors have failed to find significant differences between the patients showing progression or no progression in the maintenance phase. However, the deteriorating sites in the progression group exhibited a higher t-PA:PAI-2 ratio in comparison with the stable sites in the same individuals and this finding has been suggested to indicate an involvement of the PA system in the proteolytic events leading to breakdown of the periodontal tissues.

Chronic smoking has been linked to an impaired fibrinolytic activity mainly because of a high plasma PAI-1 concentrations, with no clear effect on t-PA (Allen et al. 1984, Haire et al. 1989, Gleerup & Winther 1996, Simpson et al. 1997). Reduced fibrinolytic activity in vein walls of smokers, a local cellular effect of smoking, has been reported (Kjaeldgaard & Larsson 1986). According to our present findings, the only

statistically significant difference between the smokers and non-smokers was GCF concentrations of PAI-2 in the healthy control group. Smokers showed a consistent tendency for decreases in the total amount and concentrations of t-PA, u-PA and PAI-1, while a tendency for increases in PAI-2 levels was observed.

Consistent with previous reports, increased amounts of u-PA, PAI-1, and PAI-2 were observed in GCF of non-smokers parallel to the deterioration in the clinical periodontal status, whereas the highest amount of t-PA in GCF was detected in the non-smoker gingivitis group rather than in the non-smoker periodontitis group. However, in this study differences in concentration of these proteins were not significant. In contrast, the profile in the smoker groups was completely different. The smoker gingivitis patients exhibited the highest values in the enzymes and inhibitors of the PA system. This is an unexpected but interesting finding and may be considered as a sign of the complex effects of smoking on the PA system in various periodontal disease categories. In general the effects of smoking on GCF concentrations of PA system components was confined to differences in PAI-2 concentration and in subtle but significant changes in the ratio of activators to inhibitors in patients with periodontal disease. Reduced PAI-2 concentrations in healthy smokers may predispose these individuals to increased tissue damage during the early stages of periodontal disease. Once they have developed chronic disease the levels of PAI-2 have increased in response to the inflammation at which point there are no longer any differences between diseased smokers and diseased non-smokers.

Abundant cross-sectional data support the relationship between smoking and periodontitis. Comprehensive reviews on this subject have been recently published (Kinane & Chestnutt 2000, Johnson & Hill 2004). A strong dose–response relationship between the amount smoked and the severity of periodontal destruction has been shown, which further supports the role of smoking as a risk factor for periodontitis (Grossi et al. 1994, 1995, Calsina et al. 2002). Smokers are almost 4 times more likely to have severe periodontitis than non-smokers (Haber et al. 1993). However, the exact mechanisms by which smoking exerts its deleterious effects on periodontium remain unclear. It is likely that smoking damages host mechanisms rather than directly affecting the composition of subgingival plaque bacteria (Buduneli et al. 2005). Accordingly, Eggert et al. (2001) proposed a mechanism through which the molecular byproducts of smoking might influence progression of periodontitis via damage to cells that normally protect the periodontal environment. Previous studies evaluating the possible effects of smoking on the PA system have suggested that fibrinolytic factors particularly t-PA may be affected by chemical ingredients of tobacco (Kuo et al. 1989, Wang et al. 1997, Newby et al. 2001, Pellegrini et al. 2001). Furthermore, serum cotinine levels have been reported to be positively correlated with plasma t-PA levels (Lindholt et al. 2003).

The serum concentrations of u-PA and PAI-1 showed statistically significant differences only between the three non-smoker groups, and although significant differences in GCF levels of these proteins were also observed between the three smoker groups the concentrations, with the exception of PAI-2 did not differ significantly. The present results indicate that the serum PA system is not particularly affected by smoking.

In conclusion, although the PA system protein levels are increased in periodontal inflammation compared with health, the results of the present study suggest that smoking has a limited effect on the plasminogen-activating system in GCF, affecting only PAI-2 concentrations in healthy subjects and altering the balance of activators and inhibitors in the patients with periodontal disease. It remains to be investigated whether this subtle effect contributes to the increased risk of severe periodontal tissue destruction observed in smokers.

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