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Plasminogen activators and plasminogen activator inhibitors in gingival crevicular fluid of cyclosporin A-treated patients

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Abstract

Background: The plasminogen activator (PA) system plays many roles in the inflammatory process and tissue remodelling and repair and is considered to play a significant role in periodontal tissue destruction and healing. The aim of this study was to evaluate the role of the PA system in cyclosporin A (CsA)-induced gingival overgrowth in renal transplant patients.

Methods: Eighteen renal transplant patients exhibiting moderate to severe CsA-induced gingival overgrowth, 10 other renal transplant patients receiving CsA therapy but showing no sign of CsA-induced gingival overgrowth (CsA-H), 16 chronic gingivitis patients (CG) and 16 systemically and periodontally healthy control subjects (H) were included in the study. Gingival crevicular fluid (GCF) samples were obtained from four randomly selected sites in each subject with the exception of the CsA-induced gingival overgrowth group, where four GCF samples were harvested from sites with severe overgrowth (CsA GO+) and from four sites without any gingival overgrowth (CsA GO−). The GCF levels of albumin, tissue-type plasminogen activator (t-PA), urokinase-type plasminogen activator (u-PA), plasminogen activator inhibitor 1 (PAI-1) and plasminogen activator inhibitor 2 (PAI-2) were analysed by enzyme-linked immunosorbent assay. The results were tested for statistical differences.

Results: In CsA GO+ sites t-PA levels were significantly elevated in comparison with gingivitis and healthy sites, while PAI-2 levels in these sites showed statistically significant differences only with CsA-H and gingivitis sites ($p<0.05$). The levels of t-PA and PAI-2 were significantly higher in CsA GO− sites compared with those of CsA-H, gingivitis and healthy sites ($p<0.05$). The levels of u-PA and PAI-1 failed to show significant differences between the study groups.

Conclusions: The findings of the present study indicate alterations in GCF t-PA and PAI-2 levels in CsA-induced gingival overgrowth and might suggest involvement of the plasminogen activating system in the pathogenesis of this side-effect of CsA therapy. However, to what extent these molecules contribute to the pathogenesis of CsA-induced gingival overgrowth remains to be determined.

Key words: cyclosporin-induced gingival overgrowth; enzyme-linked immunosorbent assay; gingival crevicular fluid; human; plasminogen activator; plasminogen activator inhibitor

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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 (PAs) are serine proteases that form part of the complex enzyme cascade involved in fibrinolysis and convert the proenzyme plasminogen into the broad-spectrum proteinase, plasmin. The resultant activated 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 PAs are of two types: the tissue/blood vessel-type plasminogen activator (t-PA) and the urokinase-type plasminogen activator (u-PA) (Dano et al. 1985, Saksela 1985). The activity of the PAs in turn are regulated by two different, specifically acting plasminogen activator inhibitors (PAIs); one produced by endothelial cells as well as various normal and malignant cells; plasminogen activator inhibitor 1 (PAI-1) (Dano et al. 1999), and the other plasminogen activator inhibitor 2 (PAI-2) is produced in macrophages, epithelial cells, in the placenta 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.

Cyclosporin A (CsA) is clearly successful as a truly selective immunosuppressant drug, yet, CsA therapy is associated with several adverse effects including gingival overgrowth (Rateitschak-Plüss et al. 1983, Wysocki et al. 1983, Pernu et al. 1992). The incidence of CsA-induced gingival overgrowth differs from 8% to 85% among the studies depending on the criteria used (Hassell & Hefti 1991, Pernu et al. 1992, King et al. 1993). The exact mechanism whereby CsA induces gingival overgrowth remains largely obscure. CsA-induced gingival overgrowth is characterized by thickening of the oral gingival epithelium as well as a marked increase in the ECM of gingival connective tissue (Mariani et al. 1993). CsA has been suggested to affect epidermal growth factor receptor (EGF-r) metabolism in gingival keratinocytes resulting in an increased number of cell surface receptors, which may eventually play a role in the pathogenesis of gingival tissue alterations (Buduneli et al. 2001a). Another growth factor, transforming growth factor-beta 1 (TGF-β1) has been reported to be associated with alterations in matrix deposition and matrix degradation through its effect on PAI-1 in chronic CsA nephrotoxicity (Shihab et al. 2000). In a previous study, we have shown significant increases in GCF TGF-β1 levels in CsA-treated patients in comparison with the healthy control subjects (Buduneli et al. 2001b). Substantial impairment of fibrinolytic activity by CsA has been reported in renal transplant patients (van den Dorpel et al. 1999). Furthermore, TGF-β1 has been found to play a role in CsA-induced tubulointerstitial fibrosis by stimulating ECM protein synthesis and inhibiting ECM degradation through increasing the synthesis of PAI-1 (Shihab et al. 1997). It is likely that an alteration in PA system acts in the pathogenesis of CsA-induced gingival overgrowth. However, the PA system has not been investigated before in GCF of CsA-treated patients. Therefore, the purpose of the present study was to evaluate the possible role of the PA system in CsA-induced gingival overgrowth in renal transplant patients.

Material and Methods

Study population

A total of 60 subjects were included in the present study. Eighteen renal transplant patients (eleven males and seven females; mean age 32.3 ± 7.2 years) receiving CsA therapy and exhibiting moderate to severe CsA-induced gingival overgrowth and 10 other patients (five males and five females; mean age 32.6 ± 7.1 years) with renal transplantations and using CsA, but exhibiting no sign of CsA-induced gingival overgrowth were monitored. None of the renal transplant patients exhibited any sign of periodontitis, while gingivitis was evident. These patients had been taking CsA for more than 6 months and the dose had been adjusted to maintain stable serum levels of 80–300 ng/ml. No sign of graft rejection was detected in these renal transplant patients. Sixteen patients with chronic gingivitis (seven males and nine females; mean age 32.8 ± 9.4 years) and no history of treatment with drugs known to induce gingival overgrowth were also included in the study. There were 16 volunteers in the healthy control group (seven males and nine females; mean age 29.6 ± 6.1 years) drawn from students and staff of the dental school. These volunteers were all systemically healthy and had no history of periodontal disease, that is, probing depths < 3 mm and no attachment loss, clinical inflammation, sulcular bleeding, or radiographic evidence of bone loss.

Subsequent to GCF sampling, clinical periodontal recordings including plaque index (PI), papilla bleeding index (PBI) (Saxer & Mühlemann 1975) and hyperplastic index (HI) (Pernu et al. 1992) were performed prior to any periodontal intervention. The degree of gingival overgrowth was classified into four categories based on the criteria of Angelopoulos and Goaz as modified by Pernu et al. (1992). Radiographic examination was also carried out to detect alveolar bone destruction. CsA-treated patients without any sign of alveolar bone loss were selected for the present study. Serum CsA levels of the CsA-treated patients were obtained from their medical records.

Gingival crevicular fluid sampling

GCF samples were obtained from a total of 304 sites from 60 people in five groups. Group 1: CsA GO+ sites (four sites with CsA-induced gingival overgrowth in each CsA-treated patient); group 2: CsA GO− sites (four sites without CsA-induced gingival overgrowth in each CsA-treated patient); group 3: CsA-H sites (four sites in each CsA-treated but gingival overgrowth free patient); group 4: gingivitis sites (four inflamed sites in each gingivitis patient); and group 5: healthy sites (four clinically uninflamed sites from each periodontally healthy subject). Sampling of GCF from CsA GO− sites could be performed in 16 out of the 18 renal transplant patients with CsA-induced gingival overgrowth, since there was no site without gingival overgrowth in the remaining two patients.

Sampling sites were selected from buccal aspects of the mesial and distal surfaces at the interproximal sites. All GCF samples were collected from randomly selected sites of each subject. Prior to GCF sampling, supragingival plaque was removed by sterile curets and, after air drying, the surfaces were 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 6000 ProFlow Inc., Amityville, NY). 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.

Enzyme immunoassay

PAI-1 and u-PA enzyme-linked immunosorbent assay (ELISA) kits were purchased from Dakocytomation (Cambridge, Cambridgeshire, UK). The PAI-2 kit was purchased from Abraxis (Warminster PA, USA) and the t-PA ELISA development reagents were obtained from Affinity Biologicals (Ontario, Canada). 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 analysed by ELISA. After a pilot experiment, it was decided to pool the four GCF samples for each subject in five 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 $<0.5\,\mu$l, and 1/500 for pooled GCF volumes $>0.5\,\mu$l. 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/ml 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.

Statistical analyses

Where appropriate, parametric tests and distribution free tests were used for statistical analysis. Analysis of variance and post hoc Bonferroni's tests were used for the clinical data. The Kruskal–Wallis test followed by Dunn's post-test was used for the group comparisons of the protein levels and concentrations in the GCF. Since the samples in group 1 and group 2 were taken from the same subjects, a paired *t*-test was used for comparisons of the clinical data and the paired Wilcoxon signed-rank test was employed for the comparisons of protein levels in GCF samples. Pearson's 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 pocket depth.

Results

Patient groups, demographic variables and mean values of clinical measurements are outlined in Table 1. No significant differences were observed in the mean age and sex distribution between the study groups ($p>0.05$). When the clinical periodontal indices were considered, the probing pocket depths (PPD) in CsA GO+ sites were significantly higher than those of CsA GO−, CsA-H and healthy control group ($p<0.05$) and no other significant difference was found between any two groups with regard to PPD values. Statistically significant differences in GCF volumes were detected only between CsA GO+ sites and healthy sites ($p<0.05$) (Table 1).

Albumin levels showed statistically significant differences only in total amounts within GCF and no significant differences were observed when concentrations were analysed (Table 2). The total amount of albumin in CsA GO+ sites was significantly higher than those of CsA-H and healthy sites ($p<0.05$). Chronic gingivitis sites also exhibited significantly elevated total amounts of albumin when compared with the healthy control sites ($p<0.05$).

u-PA levels exhibited significant differences only when total amount in GCF were considered, that is, CsA GO+ sites showed significantly elevated

Table 1. Clinical characteristics of the study groups

	Group 1 CsA GO+sites	Group 2 CsA GO-sites	Group 3 CsA healthy sites	Group 4 gingivitis sites	Group 5 healthy sites
n	18	16	10	16	16
M:F	11:7	10:6	5:5	7:9	7:9
Age	32.3 ± 7.2	32.6 ± 7.1	32.6 ± 7.1	32.8 ± 9.4	29.6 ± 6.1
PPD (mm)*	4.96 ± 1.47	2.38 ± 0.76	1.98 ± 0.83	2.99 ± 0.71	1.41 ± 0.41
PBI*	1.13 ± 1.11	0.30 ± 0.40	0.13 ± 0.32	1.36 ± 0.63	0.00 ± 0.00
PI*	2.1 ± 0.90	1.6 ± 0.60	1.5 ± 1.00	1.5 ± 1.00	0.2 ± 0.40
HI*	2.22 ± 0.80	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
GCF volume (μl)*	1.10 ± 0.62	0.83 ± 0.53	0.34 ± 0.26	0.82 ± 0.30	0.40 ± 0.37

No significant differences were found between the groups with regard to the age of the subjects and gender distributions.
*Results were calculated from the averages of the four sampling sites and the GCF volume is the pooled volume of four samples for each subject.

u-PA total amounts in comparison with the healthy control sites ($p<0.05$), and chronic gingivitis sites showed significantly increased total amounts in comparison with the healthy control sites ($p<0.05$) (Table 2).

Statistically significant differences in all three t-PA values (total amount in GCF, concentration in GCF and concentration per 1 mg of albumin) were observed between the study groups (Table 2). The highest total amount of t-PA was detected in CsA GO+ sites and it was significantly different from CsA-H, chronic gingivitis and healthy control sites ($p<0.05$). When t-PA values were adjusted for GCF volume, CsA GO+ sites exhibited significantly elevated values in comparison with chronic gingivitis and healthy control sites ($p<0.05$). t-PA concentrations in CsA-H sites were significantly higher than in chronic gingivitis sites ($p<0.05$). When t-PA values were adjusted per albumin levels, significant differences were found between CsA GO+ sites and chronic gingivitis, healthy control sites, between CsA GO− and chronic gingivitis sites, and also between CsA-H and gingivitis, healthy control sites ($p<0.05$).

PAI-1 values in healthy control sites were similar to the values in CsA-H sites. When the total amount values were considered, the differences between healthy sites and CsA GO+, CsA GO− and gingivitis sites were all statistically significant ($p<0.05$) (Table 3). When adjusted for GCF volume, the only significant difference was found between CsA GO− sites and healthy control sites ($p<0.05$).

Statistically significant differences between the study groups were similar for both total amount and GCF concentration values of PAI-2. Namely, CsA GO+ sites exhibited significantly higher values in comparison with gingivitis and healthy control sites ($p<0.05$), and CsA-H sites exhibited no significant difference with any of the study groups ($p>0.05$) (Table 2).

Pearson's correlation analysis revealed statistically significant positive correlations between GCF volumes and the amount of albumin ($p<0.001$) (data not shown). Spearman's 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 ($p<0.001$). PPD values showed significant positive correlations with

Table 2. GCF albumin, plasminogen activator and plasminogen activator inhibitor levels, concentrations and levels of plasminogen activator and plasminogen activator inhibitor adjusted to the albumin content

	Group 1 CsA GO+sites ($n=18$)	Group 2 CsA GO-sites ($n=16$)	Group 3 CsA healthy sites ($n=10$)	Group 4 gingivitis sites ($n=16$)	Group 5 healthy sites ($n=16$)
Albumin (ng)	9241 (4153–15,833)* [3,5]	6258 (1542–12,252)[3,5]	1446 (1104–1956)[1,2,4]	5927 (4771–7445)[3,5]	1385 (1123–2604)[1,2,4]
Albumin (ng/μl)	8919 (7552–9582)	8121 (4804–9601)	6783 (4394–8239)	8238 (6145–9092)	6818 (4185–9022)
t-PA (pg)	134 (102–193)[2,3,4,5] †	87 (58–108)[1,3,4,5]	32 (24–49)[1,2]	41 (16–85)[1,2]	15 (10–27)[1,2]
t-PA (pg/μl)	137 (116–182)[4,5]	117 (77–203)[4,5]	151 (100–193)[4,5]	49 (25–79)[1,2,3]	66 (47–114)[1,2,3]
t-PA (pg/mg)	15,588 (11,693–37,968)[4,5]	15,192 (8035–41,939)[4,5]	23,999 (16,284–37,088)[4,5]	5112 (3769–12,486)[1,2,3]	9068 (6705–14,312)[1,2,3]
u-PA (pg)	58 (27–95)[3,5]	29 (14–41)[3,5]	9 (6–32)[1,2]	24 (17–41)	10 (6–14)[1,2]
u-PA (pg/μl)	56 (38–103)	32 (24–61)	46 (17–74)	34 (27–53)	47 (22–58)
u-PA (pg/mg)	6126 (4227–11,891)	5061 (3096–8281)	7149 (3199–11,247)	5933 (3555–7275)	6349 (4550–8704)
PAI-1 (pg)	98 (21–172)	71 (36–99)[5]	34 (0–107)	43 (31–61)	11 (0–35)[2]
PAI-1 (pg/μl)	81 (25–148)	113 (34–205)	59 (0–248)	56 (32–80)	23 (30–88)
PAI-1 (pg/mg)	8771 (2758–18,238)	14,371 (4104–36,045)	7135 (0–56,430)	6946 (4756–10,795)	2902 (0–18,408)
PAI-2 (pg)	3 (1–11)[4,5]	2 (1–5)[5]	1 (0–5)	1 (0–2)[1]	0 (0–1)[1,2]
PAI-2 (pg/μl)	3 (1–8)	5 (3–9)	1 (0–10)	1 (0–2)	1 (0–5)
PAI-2 (pg/mg)	512 (133–1032)[4]	685 (405–1195)[3,4,5]	104 (0–2040)[2]	128 (0–262)[1,2]	107 (0–618)[2]

*Median (interquartile range).
†The difference between groups 1 and 2 in the levels of t-PA was also significant according to the Wilcoxon test ($p<0.05$).
[1,2,3,4,5]Significant differences between groups according to the Kruskall–Wallis and Dunn's post hoc test ($p<0.05$).

Table 3. Spearman's correlations (group size; $n = 76$)

	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)
GCF(μl)	0.571*	− 0.134	0.602*	− 0.338*	0.419*	0.063	0.451*	0.119
PD (mm)	0.607*	0.216	0.620*	0.136	0.286†	0.089	0.457*	0.274†
PBI	0.491*	0.261†	0.302*	− 0.052	0.247†	0.131	0.270†	0.162
PI	0.301*	− 0.028	0.419*	0.086	0.099	− 0.023	0.192	0.076
Albumin (ng)	0.545*	− 0.106	0.575*	− 0.333*	0.432*	0.087	0.435*	0.099
Albumin (ng/μl)	0.211	0.088	0.142	− 0.125	0.185	− 0.043	0.204	0.05
u-PA (pg)		0.654*	0.573*	0.042	0.554*	0.377*	0.792*	0.592*
u-PA (pg/μl)	0.654*		0.156	0.327*	0.265†	0.341*	0.534*	0.601*
t-PA (pg)	0.573*	0.156		0.498*	0.312*	0.14	0.449*	0.280†
t-PA (pg/μl)	0.042	0.327*	0.498*		− 0.122	0.023	0.051	0.21
PAI-1 (pg)	0.554*	0.265†	0.312*	− 0.122		0.884*	0.529*	0.351*
PAI-1 (pg/μl)	0.377*	0.341*	0.14	0.023	0.884*		0.352*	0.298*
PAI-2 (pg)	0.792*	0.534*	0.449*	0.051	0.529*	0.352*		0.893*
PAI-2 (pg/μl)	0.592*	0.601*	0.280†	0.21	0.351*	0.298*	0.893*	

*Correlation is significant at $p < 0.01$ level (two-tailed).

†Correlation is significant at $p < 0.05$ level (two-tailed). Total number of correlations is 104.

t-PA, u-PA, PAI-2 ($p < 0.01$) and PAI-1 levels ($p < 0.05$). PBI values exhibited significant positive correlations with t-PA, u-PA, PAI-1 ($p < 0.01$) and PAI-2 levels ($p < 0.05$). PI values showed significant positive correlations only with t-PA and u-PA levels ($p < 0.01$).

Discussion

In the present study, the levels of albumin, t-PA, u-PA, PAI-1 and PAI-2 in GCF samples obtained from CsA-treated renal transplant patients were evaluated and compared with the control groups. To our knowledge, this is the first study to investigate the levels of albumin and the plasminogen activating system in GCF samples of CsA-treated patients. The renal transplant patients receiving CsA therapy were divided into two groups depending on the presence or absence of CsA-induced gingival overgrowth. Furthermore, CsA GO − sites in patients exhibiting CsA-induced gingival overgrowth were also sampled and served as a control group in order to eliminate individual modifying factors.

The quantity of various proteins may show elevations, which could be related simply to an increase in GCF volume at a site. Therefore, evaluating concentrations of protein in GCF may be more informative but may also create falsely high concentrations of proteins especially when GCF volumes are low (in less inflamed and healthy sites) and subsequently more affected by evaporation after sampling. Considering these problems we have expressed our present 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 (correcting for the amount of albumin present in GCF sample and thus the contribution by serum to the GCF).

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 (MMPs). Therefore, strict control of its activation is important for maintaining the integrity of tissues. Furthermore, Duymelinck et al. (1998) have suggested that the local increases in expression of TIMP-1 rather than a decrease of MMP expression, contributes to the development of CsA-induced focal interstitial fibrosis in the rat.

Shihab et al. (2000) have reported that CsA-induced up-regulation of expression of TGF-β1, PAI-1 and various matrix proteins and these increased expressions have been found to be associated with alterations in matrix deposition and matrix degradation through the effect on PAI-1. Elevated plasma PAI levels in CsA-treated patients have been reported to induce hypofibrinolysis (Verpooten et al. 1996). The impairment of the endogenous fibrinolysis activity was attributed either to a defective release of PA from the vessel wall or to high plasma levels of PAI-1 (Levi et al. 1992). TGF-β1 has been suggested to selectively stimulate the synthesis of connective tissue matrix components possibly by reducing the synthesis of proteinases that are involved in connective tissue degradation, such as collagenase and PAs (Laiho & Keski-Oja 1989). Shihab et al. (1996) found that the mRNA expression of PAI, a protease inhibitor stimulated by TGF-β1, followed TGF-β1 and matrix proteins, suggesting that the fibrosis of chronic CsA nephropathy likely involves the dual action of TGF-β on matrix deposition and degradation. In a previous study, CsA treatment has been reported to increase GCF TGF-β1 levels in renal transplant patients (Buduneli et al. 2001a). Accordingly, the present study revealed increased GCF PAI levels in CsA-treated patients.

The lack of significant correlation between GCF volume and the increased PPD values arising from gingival overgrowth in CsA-treated patients might suggest that the overgrown tissue is less vascular and current histopathological experiments will clarify this issue. The plasminogen activating system should also be investigated immunohistologically in CsA-induced gingival overgrowth (study in progress). The significant positive correlations found between GCF levels of t-PA, u-PA, PAI-1, PAI-2 and PPD as well as PBI support the hypothesis that the PA system is involved in deterioration of periodontal status and are in line with the previous reports (Kinnby et al. 1996, Xiao et al. 2000). Furthermore, the present finding that PI values exhibited significant positive correlations only with the enzyme levels, t-PA and u-PA may be a reflection of the possible indirect effect of plaque on periodontal tissue destruction via induction of the PAs.

In conclusion, the results of the present study indicate that CsA therapy

increases the GCF levels of the plasminogen activating system, particularly the enzymes; t-PA and u-PA. The differences in u-PA concentrations among the CsA groups were not significant, in contrast the t-PA concentrations (adjusted for the albumin content) were significantly higher in the CSA GO+ group than in the gingivitis and healthy control groups. However, the null hypothesis of no change in t-PA concentrations between the CSA-GO – and healthy control group could not be rejected even though the *p*-value in a one-tail test would be sufficiently small to support the hypothesis of increased concentrations of t-PA at CSA-GO – sites. Nevertheless, it is unlikely that the GCF plasminogen activating system levels are the sole factor responsible for the CsA-induced gingival overgrowth. To what extent the more pronounced expression of PAs and PAIs in CsA-treated patients contributes to the pathogenesis of CsA-induced gingival overgrowth remains to be determined.

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