Initial Periodontal Therapy Reduced Systemic and Local 25-Hydroxy Vitamin D_3 and Interleukin-1β in Patients With Aggressive Periodontitis

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Background: 25-hydroxy vitamin D_3 is the major circulating metabolite of vitamin D. Elevated plasma 25-hydroxy vitamin D_3 levels were verified to be associated with generalized aggressive periodontitis (GAgP). In the present study, the influence of initial periodontal therapy on systemic and local levels of 25-hydroxy vitamin D_3 and three related elements (osteocalcin and interleukin-1β and -6) in patients with GAgP was investigated.

Methods: Nineteen patients with GAgP were enrolled. All patients received initial periodontal therapy. Gingival crevicular fluid at two sites of each subject were obtained before therapy and 2 and 6 months after therapy. Plasma was obtained before and 2 months after therapy from 12 of 19 subjects. Systemic and local levels of 25-hydroxy vitamin D_3, osteocalcin, and interleukin-1β and -6 before and after therapy were measured using radioimmunoassay or enzyme-linked immunosorbent assay kits and compared.

Results: The respective systemic 25-hydroxy vitamin D_3 and interleukin-1β levels significantly dropped from baseline to 2 months after therapy (29.28 nmol/l versus 22.50 nmol/l, $P = 0.001$, and 6.71 ng/l versus 3.23 ng/l, $P < 0.001$, respectively). The respective local 25-hydroxy vitamin D_3 and interleukin-1β levels significantly decreased from baseline to 2 and 6 months after therapy (8,950 nmol/l versus 5,650 nmol/l versus 3,438 nmol/l, $P < 0.001$, and 10,595 ng/l versus 5,495 ng/l versus 3,960 ng/l, $P < 0.001$, respectively). Systemic and local 25-hydroxy vitamin D_3 concentrations were positively correlated at baseline ($r = 0.877$; $P = 0.022$), as was osteocalcin ($r = 0.939$; $P = 0.005$).

Conclusions: 25-hydroxy vitamin D_3 and interleukin-1β levels were systemically and locally reduced in patients with GAgP by initial periodontal therapy. 25-hydroxy vitamin D_3 might be involved in periodontal inflammation. *J Periodontol 2010;81:260-266.*

KEY WORDS

Aggressive periodontitis; gingival crevicular fluid; plasma.

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The major circulating metabolite of vitamin D is 25-hydroxy vitamin D_3. In a previous study,[1] we showed that levels of plasma 25-hydroxy vitamin D_3 of patients with generalized aggressive periodontitis (GAgP) were significantly higher than those of healthy controls (29.28 ± 17.18 nmol/l versus 21.60 ± 14.38 nmol/l; $P < 0.05$) and positively correlated with the bleeding index (BI)[2] ($r = 0.321$; $P < 0.05$). Thus, it was hypothesized that circulating 25-hydroxy vitamin D_3 levels might be associated with periodontal inflammation, and the influence of initial periodontal therapy on 25-hydroxy vitamin D_3 levels in plasma and gingival crevicular fluid (GCF) warrants further investigation.

Vitamin D is a major component in the regulation of bone metabolism and expression of bone-related biomarkers (e.g., osteocalcin).[3] Circulating osteocalcin was considered a valid marker of bone turnover when resorption and formation were balanced and a specific marker of bone formation when formation and resorption were unbalanced.[4] Furthermore, osteocalcin levels in the GCF of patients with periodontitis were reported to reflect inflammation at diseased sites.[5] Calcitriol (the active metabolite of vitamin D) and some of its analogs can upregulate osteocalcin expression via the vitamin D responsive

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element in vitro.[6,7] In our previous study,[1] higher systemic levels of osteocalcin were detected in patients with GAgP than in healthy controls (0.90 ng/ml versus 0.70 ng/ml; $P < 0.05$). Therefore, we questioned whether initial periodontal therapy could influence osteocalcin levels in the plasma and GCF of patients with GAgP.

Vitamin D plays an important role in a number of inflammatory diseases by influencing the expression of inflammation related cytokines, such as interleukin (IL)-1 and -6.[8,9] IL-1β is a marker of active inflammation and directly relates to periodontal attachment loss (AL) and bone demineralization.[10,11] Plasma IL-1β concentrations of patients with GAgP were found to be higher than those of healthy controls (7.23 ng/l versus 3.52 ng/l; $P = 0.003$).[12] Recently, Toker et al.[13] reported that IL-1β levels in GCF were significantly reduced in moderate and deep pocket sites 6 weeks after initial periodontal therapy ($P < 0.05$) in patients with GAgP. However, the relatively long-term effect of initial periodontal therapy on IL-1β levels in GCF was not reported in the study by Toker et al.[13] IL-6 is a key proinflammatory and immunemodulatory cytokine. It is a significant activator of C-reactive protein production and also affects glucose metabolism.[14,15] Thus, IL-6 might play an important role in the contribution of periodontal infections to cardiovascular diseases and diabetes. In a previous study,[16] we demonstrated that plasma levels of IL-6 of patients with GAgP were significantly higher than those of the healthy controls. A study by Pischon et al.[17] indicated that plasma IL-6 levels of patients with AgP did not significantly change after periodontal therapy. To our knowledge, the impact of periodontal therapy on IL-6 levels in the GCF of patients with GAgP has not been reported and needs further study.

In an attempt to quantify the kinetics of 25-hydroxy vitamin D_3, osteocalcin, and IL-1β and -6 in plasma and GCF of patients with GAgP, the influence of initial periodontal therapy on the systemic and local levels of 25-hydroxy vitamin D_3 and the three related elements (osteocalcin and IL-1β and -6) was investigated.

MATERIALS AND METHODS

Study Population

Nineteen patients with GAgP (eight males and 11 females) were recruited from the Clinic of the Department of Periodontology, Peking University School and Hospital of Stomatology, from November 2006 to February 2007. All subjects resided in Beijing (latitude 40° north). The subjects ranged in age from 16 to 34 years (mean age: 24.5 ± 4.1 years).The diagnostic criteria for GAgP were defined according to the classification proposed at the International Workshop for the Classification of Periodontal Diseases and Conditions in 1999.[18] The details were as follows: 1) the onset of the periodontal disease occurred at <35 years of age; and 2) at least eight teeth had a probing depth (PD) >6 mm and radiographic evidence of alveolar bone loss, and at least three of them were not first molars or incisors. Exclusion criteria included: 1) known systemic diseases, such as cardiovascular, respiratory, or renal diseases and malignancy; 2) pregnant or lactating females; 3) periodontal therapy during the preceding 6 months; 4) intake of antibiotics in the previous 3 months; 5) smokers; 6) a history of orthodontic treatment or noteworthy occlusal disharmony; and 7) having <25 remaining teeth. The study protocol was approved by the Ethics Committee of Peking University Health Center, and written informed consent was obtained from each participant in accordance with the Declaration of Helsinki.

Study Outline

A baseline visit was conducted by a periodontist (RL), and then all subjects underwent an initial periodontal therapy consisting of oral hygiene instructions and scaling and root planing. The treatments were completed in 1 month by the same periodontist. All subjects were reexamined at 2 and 6 months after therapy, and oral hygiene instructions were reinforced at each visit.

At the three study visits (baseline and 2 and 6 months after therapy), full-mouth periodontal examinations of each subject were conducted using a William's periodontal probe. PD and AL were recorded at six sites per tooth other than wisdom teeth. The BI[2] was recorded for each tooth. A total of 10 non-study subjects were recruited and used for the calibration of the examiner. The examiner was judged to be reproducible after meeting a percentage of agreement within ±1 mm between repeated measurements of at least 95%.

GCF samples were obtained at the three visits from all 19 subjects. Plasma samples were obtained at baseline and 2 months after therapy from 12 of 19 subjects. Because seasonal changes have been reported in serum 25-hydroxy vitamin D_3 and osteocalcin,[19] the plasma samples before and after therapy were obtained in the winter or spring.

GCF Collection and Processing

After the initial examination, two sites were selected in different quadrants in the mouth of each patient (one site in upper quadrants and one site in lower quadrants). All sites were chosen from moderate (4 mm <PD ≤6 mm) or deep (PD >6 mm) pockets at posterior teeth. Each sample site was carefully isolated using cotton rolls to avoid saliva contamination. A paper strip† was placed in the pocket until mild resistance

† Whatman, Maidstone, U.K.

was felt and then left in place for 30 seconds. In the case of visible contamination with blood, the strip was discarded.

The volume of GCF collected was determined by weighing using modifications to the method of Griffiths et al.[20] Briefly, the paper strip was placed into an Eppendorf tube and weighed prior to sample collection. After sample collection, the strip was placed in the Eppendorf tube and reweighed before being stored at –70°C. The difference between the weights was used to calculate the volume of GCF. To generate a standard curve to determine the GCF volume, 0.1, 0.3, 0.5, 1.0, 1.5, and 2.5 μl human serum were dropped to a paper strip respectively. Each strip placed in an Eppendorf tube was weighed by an electronic scale‡ before and after the serum was dropped, and the difference of the weights was calculated. A linear regression model between weight and volume was established ($r^2 = 0.993$; $P < 0.001$).

Measurement of 25-Hydroxy Vitamin D_3 and Vitamin D Related Elements

On the day of the assay, 180 μl phosphate buffered solution was added to the tubes containing the sample strips. The tubes were gently shaken at 4°C for 20 minutes and centrifuged at 13,000 rpm (r = 5.5 cm) for 10 minutes. The GCF eluates and plasma were used for the measurement.

25-hydroxy vitamin D_3 levels were detected using a commercially available radioimmunoassay kit.§ Osteocalcin levels were measured using a commercially available radioimmunoassay kit.‖ The levels of IL-1β and -6 were measured using enzyme-linked immunosorbent assay kits.¶ These assays were performed according to the manufacturers' protocols. The sensitivities of detection of 25-hydroxy vitamin D_3, osteocalcin, and IL-1β and -6 were 3.75 nmol/l, 0.35 μg/l and 0.22 and 0.16 ng/l, respectively. To avoid bias, the analyzers (KL and ZC) did not know the origin of the samples.

The mean concentrations of 25-hydroxy vitamin D_3 and the three related elements in the GCF of each subject were calculated.

Statistical Analyses

Continuous normally distributed variables were reported as mean ± SD, whereas the median (lower to upper quartile) was used to describe non-normally distributed data. Variables not normally distributed were logarithmic transformed before being used in the parametric comparative analysis. Systemic biochemical targets before and 2 months after therapy were compared using the paired-samples t test. Local clinical and biochemical parameters at the three visits were compared using the Freidman test.[21] Spearman correlation analysis was conducted between local clinical and biochemical variables at the sample sites. Associations were analyzed between systemic concentrations and average local concentrations using Pearson correlation analysis.

Statistical analyses were carried out using a software package.# A P value <0.05 was considered statistically significant.

RESULTS

Systemic levels of 25-hydroxy vitamin D_3 and IL-1β significantly drop 2 months after initial periodontal therapy (Table 1). The levels of IL-6 and osteocalcin in plasma did not change significantly after therapy.

Concentrations of 25-hydroxy vitamin D_3 and IL-1β in GCF as well as PD and BI were significantly reduced 2 and 6 months after therapy (Table 2). AL did not drop significantly until 6 months after therapy.

Table 3 presents the correlation coefficients between local clinical and biochemical parameters of the sample sites at baseline. The concentrations of IL-6 in GCF were negatively correlated with PD (r = –0.374; $P = 0.021$) and BI (r = –0.404; $P = 0.012$). Local osteocalcin levels were negatively correlated with PD (r = –0.385; $P = 0.017$) and AL (r = –0.456; $P = 0.004$). Furthermore, there was a trend that concentrations of 25-hydroxy vitamin D_3 in GCF were positively correlated with BI (r = 0.310; $P = 0.058$).

As shown in Table 4, the average 25-hydroxy vitamin D_3 concentrations in GCF were strongly positively correlated with plasma 25-hydroxy vitamin D_3 concentrations at baseline ($P = 0.022$), as was osteocalcin ($P = 0.005$).

DISCUSSION

This study focuses on the changes of systemic and local levels of 25-hydroxy vitamin D_3 and three related elements, osteocalcin and IL-1β and -6, in patients with GAgP undergoing initial periodontal therapy. It was revealed that 25-hydroxy vitamin D_3 and IL-1β levels in plasma and GCF markedly decreased after initial periodontal therapy. Furthermore, local levels of 25-hydroxy vitamin D_3 and the three related elements were 100 to several thousand times higher than the systemic levels.

To our knowledge, 25-hydroxy vitamin D_3 levels have not been reported in GCF samples. In our previous study,[1] higher systemic 25-hydroxy vitamin D_3 levels were detected in patients with GAgP than those in healthy controls (29.28 ± 17.18 nmol/l versus 21.60 ± 14.38 nmol/l, respectively; $P < 0.05$), and the systemic levels were found to be correlated with BI (r = 0.321; $P < 0.05$). In the current study, the reduction

‡ AE240S, Mettler, Zurich, Switzerland.
§ DiaSorin, Stillwater, MN.
‖ HTA Co. Ltd., Beijing, China.
¶ R&D Systems, Minneapolis, MN.
# SPSS 11.5, SPSS, Chicago, IL.

of systemic (Table 1) and local (Table 2) 25-hydroxy vitamin D_3 levels after initial therapy provided more reliable evidence for the association between 25-hydroxy vitamin D_3 and periodontal inflammation in patients with GAgP. In addition, the trend of correlation between GCF 25-hydroxy vitamin D_3 concentrations and BI further illustrated the role that 25-hydroxy vitamin D_3 played in periodontal inflammation.

Traditionally, the conversion of vitamin D to 25-hydroxy vitamin D_3 by vitamin D_3 25-hydroxylase occurs principally in the liver.[3,22] However, it can be hypothesized that 25-hydroxy vitamin D_3 might also be generated by inflammatory periodontal tissues. This hypothesis is based upon the following results from the present study: 1) local 25-hydroxy vitamin D_3 levels were much higher than systemic levels (300 times different); 2) systemic and local 25-hydroxy vitamin D_3 levels were positively correlated; and 3) initial periodontal therapy reduced both systemic and local levels of 25-hydroxy vitamin D_3. Thus, to further verify this hypothesis, the expression of vitamin D_3 25-hydroxylase in inflammatory periodontal tissues needs to be tested in future studies.

As the most abundant non-collagenous protein of mineralized tissues, osteocalcin is predominantly synthesized by osteoblasts,[23,24] and osteocalcin was produced by periodontal ligament cells.[25,26] Additionally, putative epithelial rests of Malassez cells expressed osteocalcin due to epithelial-mesenchymal interactions.[26] In the present study, systemic and local osteocalcin levels did not vary significantly after therapy. Because the initial periodontal therapy mainly focused on the elimination of periodontal inflammation, it might not have had a significant impact on the bone turnover activity. However, the osteocalcin production in all of the aforementioned cells might not have been influenced by the initial periodontal therapy.

In the study by Toker et al.,[13] IL-1β concentrations in GCF in moderate and deep pocket sites of patients with AgP significantly decreased 6 weeks after therapy. In the present study, the decrease of IL-1β concentrations in GCF continued until 6 months after therapy. Additionally, no correlation was found between local IL-1 levels and clinical parameters at the same sites in this study. This result was the same as reported by Toker et al.[13] but different from

Table 1.

Comparison of Systemic Biochemical Parameters Before and After Therapy

Biochemical Parameters	Baseline	2 Months
25-hydroxy vitamin D_3 (nmol/l; mean ± SD)	29.28 ± 4.90	22.50 ± 3.93*
Osteocalcin (μg/l; mean ± SD)	1.03 ± 0.46	1.01 ± 0.37
IL-1β (ng/l ; median [range])	6.71 (5.42 to 8.55)	3.23 (2.60 to 4.92)*
IL-6 (ng/l; median [range])	1.00 (0.82 to 1.10)	1.06 (0.61 to 1.61)

n = 12.
* Significantly different compared to baseline (P <0.05).

Table 2.

Comparison of Local Biochemical and Clinical Parameters Before and After Therapy

Biochemical and Clinical Parameters	Baseline	2 Months	6 Months
25-hydroxy vitamin D_3 (nmol/l; median [range])	8,946.80 (5,755.27 to 20,058.96)	5,655.27 (2,057.53 to 11,006.48)*	3,444.78 (594.64 to 8,472.86)*
Osteocalcin (μg/l; median [range])	108.78 (56.35 to 176.73)	80.60 (48.80 to 150.00)	97.99 (70.43 to 172.34)
IL-1β (ng/l; median [range])	10,592.24 (4,289.09 to 29,728.65)	5,498.59 (2,423.74 to 11,271.50)*	3,962.50 (1,583.69 to 12,785.78)*
IL-6 (ng/l; median [range])	3,798.21 (2,647.02 to 4,896.71)	3,730.24 (2,333.27 to 6,885.85)	4,531.38 (2,764.77 to 5,839.50)
PD (mm)	6 (5 to 8)	4 (3 to 5)*	4 (3 to 5)*
AL (mm)	6 (4 to 7)	6 (4 to 7)	5 (2 to 7)*†
BI	4 (3 to 4)	2 (2 to 3)*	2 (1 to 2)*

n = 19.
* Significantly different compared to baseline (P <0.05).
† Significantly different compared to 2 months (P <0.05).

the results of studies by Engebretson et al.[27,28] and Andriankaja et al.[29] in which the mean levels of IL-1β in GCF were related to the mean periodontal clinical parameters of the subjects. Thus, the difference of analysis methodology may be the reason for these different results. In consideration of our recent findings,[12] because patients with GAgP had higher systemic IL-1β concentrations than healthy controls, the influence of initial periodontal therapy on systemic IL-1β levels was investigated. We found that local and systemic IL-1β levels were significantly reduced after therapy, which illustrated that initial periodontal therapy might alleviate the inflammation burden of the whole body.

Pischon et al.[17] detected plasma IL-6 levels before and after periodontal therapy in 21 patients with AgP, and no change was found after scaling and root planing or after antibiotic treatment. Similarly, systemic and local IL-6 levels remained relatively stable after therapy in the present study. It was observed that leukocyte count of patients with AgP dropped from 7.7×10^9/l to 5.9×10^9/l 6 months after initial periodontal therapy, and the percentage of neutrophil dropped from 65.7% to 55.4%.[30] IL-6 was reported to be secreted by IL-1–producing cells such as monocytes/macrophages, epithelial cells, and dentritic cells[31-35] and also by type 2 T-helper cells.[35] This raises the possibility that the differences in expression variation of IL-1 and -6 after therapy may be associated with T-helper cells, which warrants further investigation.

Hitherto, studies[5,36] simultaneously including systemic and local levels of 25-hydroxy vitamin D_3 or the three related elements were scarce. Prior to the present study, Wilson et al.[5] found that osteocalcin could be detected in the sera of 14 patients with periodontitis but was not detectable in GCF samples of the same subjects. However, they mentioned that the GCF samples might have been more diluted than other studies.[5] The GCF dilution rate was investigated and proved suitable in our preexperiment so that the local osteocalcin concentrations could be detected in the present study. Offenbacher et al.[36] reported that IL-6 levels in GCF (3.63 or 7.47 pg/ml) were higher than those in serum (0.47 or 1.22 pg/ml) in the same subjects. Namely, local IL-6 levels were higher than systemic levels, but the gap was less remarkable than that in the present study. However, all of the subjects enrolled in the study of Offenbacher et al.[36] were pregnant women, and the inhomogeneity of research subjects may be the reason for the difference.

Because some subjects did not agree to submit plasma samples after initial periodontal therapy, plasma samples were only obtained from 12 of the 19 subjects. The potential bias due to non-response for plasma samples is the limitation of this study.

Table 3.

Correlation Coefficients Between Local Biochemical and Clinical Parameters at Baseline

Clinical Parameters	25-Hydroxy Vitamin D_3 (nmol/l)	Osteocalcin (μg/l)	IL-1β (ng/l)	IL-6 (ng/l)
PD	–0.121	–0.385*	0.001	–0.374*
AL	–0.015	–0.456*	0.031	–0.308
BI	0.310†	–0.106	–0.1	–0.404*

n = 19.
* Statistically significant (P <0.05).
† Trend of statistical significance (P = 0.058).

CONCLUSIONS

Initial periodontal therapy reduced 25-hydroxy vitamin D_3 and IL-1β levels in plasma and GCF. 25-hydroxy vitamin D_3 might play a role in periodontal inflammation. Whether 25-hydroxy vitamin D_3 might be generated by inflammatory periodontal tissues requires further verification.

Table 4.

Correlation Coefficients Between Concentrations in Plasma and Average Concentrations in GCF

	Baseline				2 Months			
Correlation Coefficient	25-Hydroxy Vitamin D_3	Osteocalcin	IL-1β	IL-6	25-Hydroxy Vitamin D_3	Osteocalcin	IL-1β	IL-6
r	0.877*	0.939*	–0.187	–0.193	–0.241	0.564	0.224	–0.485

n = 12.
r = Pearson correlation coefficient.
* Statistically significant (P <0.05).

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