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Hydroxyproline and Total Protein Levels in Gingiva and Gingival Crevicular Fluid in Periodontally Healthy Human Subjects

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Abstract

Age is known to be one of the factors which affect the rate of collagen and protein turnover in the connective tissues of the periodontium. The aim of the present study was to determine the levels of hydroxyproline (Hyp) and total protein in both the gingiva and gingival crevicular fluid (GCF) of periodontally healthy human subjects of two different age groups. The subjects of the young group were selected from among patients scheduled for extraction of upper and lower first or second premolars for orthodontic reasons. The second (older) group included individuals whose teeth were to be extracted for endodontic reasons. GCF was obtained before gingival sampling. The tissues surrounding the sockets were harvested immediately after extraction of the indicated teeth. All samples were analyzed biochemically. No significant difference was found in gingival and GCF levels of Hyp (which is unique to collagen) between the groups. Total protein levels in gingiva were significantly higher in the young group than in the older group. GCF total protein levels showed no significant difference between the groups. The higher gingival protein levels in the younger group seem to conform to previous findings.

Introduction

The bulk of gingival and other periodontal connective tissues consists of collagen fibrils, but a non-collagenous matrix is also present, including proteoglycans (PG), glycoproteins (GP), glycosaminoglycans (GAG) and small amounts of elastin[1-5].

Although extensive studies assessing the rate of collagen and protein turnover in periodontal tissues have been performed[6-10], relatively little is known about the non-collagenous matrix of the periodontium[3]. The turnover rate of collagens and non-collagenous proteins in the periodontium has been shown to be high[6-10].

Connective tissues undergo significant alterations during aging. These changes are generally considered to be a consequence, not a cause, of the aging process[11]. Age is considered to be one of the factors affecting the synthesis of collagen[11-15]

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and protein[11] in connective tissues. A marked reduction in protein and collagen production with increasing donor age has been shown in cultures established from normal human gingiva[11].

Alterations in GAG metabolism and an overall decrease in the total amount of GAGs with age have been demonstrated[16,17], and a reduction of intracellular processing and the rate of release of proteoglycans has been found in aged cells[18].

Gingival crevicular fluid (GCF) carries most of the breakdown products out of tissues, and these subsequently appear in the gingival sulcus[19]. There should be a small amount of GCF even in healthy gingiva, according to the osmotic gradient theory of ALFANO[20,21]. The GCF in healthy gingiva may contain breakdown products which appear as a result of normal turnover of collagen and non-collagenous proteins.

Quantitative studies of collagen biosynthesis in organ or explant cultures often rely on measurments of the relatively collagen-specific imino acid, hydroxyproline (Hyp), and it is generally accepted that Hyp is unique to collagen[1,4]. A more specific sign of gingival connective tissue breakdown, therefore, would be the concentration of Hyp in GCF, by which the breakdown of collagen in the connective tissue could be measured[22].

The aim of the present study was to determine and compare the levels of Hyp and total protein in both the gingiva and GCF from periodontally healthy human subjects of different age groups.

Materials and Methods

Clinical Studies

The study was performed on 15 patients, 9 females and 6 males, aged 13-29 yr (mean age 17.9 yr), who were referred to Department of Orthodontics or Endodontics Faculty of Dentistry, Hacettepe University, for dental treatment. The patients had no systemic disease or periodontal problems, and had received no antibioitcs or other medications over the previous 6 months. In order to avoid the possible effect of vitamin C, they had ingested no additional vitamin C except that in their normal diet and had been given no periodontal treatment during the same period. No periodontal treatment was performed prior to the sampling procedures, and no oral hygiene instructions were given to the patients before sampling which might have caused them to change their homecare habits.

The subjects were divided into two groups. The first group was selected from among patients scheduled for attraction of upper and lower first or second premolars for orthodontic reasons. This group included 4 females and 3 males, aged 13-17 yr (mean age 14.2 yr). The second group included 5 females and 3 males aged 17-29 yr (mean age 21.1 yr) whose teeth were to be extracted for endodontic reasons.

Clinical measurements were performed in all subjects. Pocket depths (PD) (Williams periodontal probe), and gingival index (GI)[23], sulcular bleeding index (SBI)[24], plaque index (PI)[23], and periodontal index (RPI)[23] scores were recorded. In all individuals, the areas selected for sampling were the teeth to be extracted for the reasons mentioned above.

Sampling Procedures

GCF: This was obtained from each subject before gingival sampling. BRILL's[25] deep intracrevicular technique was used for GCF sampling. Filter papers contaminated with blood were discarded. The filter papers obtained were weighed with electronic scales (Shimadzu Libror EB-50) before and after sampling, and the weights of the fluids were calculated. The filter papers were then placed in aluminum foil and stored in closed tubes at −30°C.

Gingival Tissues: The gingiva surrounding the socket was excised in small pieces immediately after extraction of the indicated teeth. All the gingival tissues were washed in saline solution after excision, and dried on filter papers. The tissues were weighed on electronic scales and stored at −30°C in closed tubes.

Laboratory Studies

Gingival Tissues

Preparation of Tissue Homogenates: The gingival tissue samples were homogenized in a Potter-Elvejhem tissue homogenizer at 4°C for 3 min using saline solution and approximately 1 ml of 50 mM potassium phosphate buffer (pH 7.0) per 100 mg tissue. The homogenates were centrifuged at 500 rpm for 10 min at 4°C, and then used for determination of total protein levels.

GCF: The GCF samples absorbed on filter paper strips were put into glass tubes containing 0.5 ml of distilled water for 30 min; they were then vortexed (Vortex, Geniee Scientific Industries Inc., Bohemia, NY 11716, Model K-550-6) for 15 s. Soluble extracts were next transferred to different tubes. This procedure for washing the filter papers was repeated, and the two 0.5-ml washes from each tube were combined.

Hydrolysis: The tissue homogenates and fluid extracts were placed in hydrolysis tubes after their exact volumes had been recorded. Concentrated HCl equal in volume to the homogenates and extracts was added to the tubes, followed by hydrolysis at 110°C for 16 h. The extracts were neutralized with NaOH (pH 7.0), then centrifuged, and the clear supernatant fractions were used for spectrophotometric determination of Hyp[26] and total protein[27] levels.

Statistical Analysis:

The differences between the groups were tested by ANOVA (Analysis of Variance).

Results

The mean PD of the subjects was 1.992±0.10 mm. The mean index scores were 0.558±0.13 GI, 0.630±0.15 SBI, 0.795±0.08 PI and 0.489±0.14 RPI.

The individual levels of Hyp and total protein in gingiva and GCF of the first (young) and second (older) groups are given in Tables 1 and 2, respectively. The mean levels of Hyp and total protein in gingiva and GCF of the two groups are shown in Table 3. Hyp levels in gingiva and GCF were higher in the young group than in the older group, but not to a significant extent.

Total protein levels in gingiva were significantly higher in the young group than in the older group ($p<0.01$).

Table 1 Individual values of Hyp and total protein levels in gingiva and GCF in Group I (Young)

Age (yr)	Hyp Gingiva (μg/ml)	Hyp GCF (μg/ml)	Total Protein Gingiva (mg/ml)	Total Protein GCF (mg/ml)
15	1.71	1.10	2.83	0.53
14	4.19	1.83	1.38	0.26
13	4.52	3.16	1.17	0.09
17	3.80	2.38	1.02	0.09
13	1.81	0.83	2.78	1.11
15	1.44	1.75	1.35	0.59
15	3.96	1.64	4.05	0.81

Table 2 Individual values of Hyp and total protein levels in gingiva and GCF in Group II (Older)

Age (yr)	Hyp Gingiva (μg/ml)	Hyp GCF (μg/ml)	Total Protein Gingiva (mg/ml)	Total Protein GCF (mg/ml)
19	2.38	2.38	0.81	1.21
18	2.38	1.72	1.36	0.75
18	0.71	0.41	1.37	0.20
22	2.85	2.71	2.84	0.66
18	2.85	0.77	0.45	0.09
29	2.83	2.45	0.57	0.33
27	2.38	2.61	0.45	0.49
17	3.33	0.81	0.74	0.05

Table 3 Mean $\pm$ SE values of Hyp and total protein levels in gingiva and GCF in both groups

	Gingival Hyp (μg/ml)	GCF Hyp (μg/ml)	Gingival Total Protein (mg/ml)	GCF Total Protein (mg/ml)
Group I (Young)	3.06$\pm$0.51	1.81$\pm$0.29	2.08$\pm$0.42*	0.49$\pm$0.14
Group II (Older)	2.40$\pm$0.27	1.73$\pm$0.33	1.07$\pm$0.28	0.47$\pm$0.14

* Significantly higher than that of group II $p<0.01$

Discussion

The study individuals, with a mean age of 17.9 yr, were divided into two groups of the younger and the older patients. Although Hyp levels in gingiva and GCF were higher in the young group, the difference did not reach statistical significance. Gingival total protein levels were significantly higher in the young group than in the older group, similar to the findings reported previously[11]. Levels of GCF Hyp and total protein were also higher in the young group, but not to a significant extent. The mean ages were 14.2 yr in the young group and 21.1 yr in the older group. In spite of the 7-year difference between the two groups, all the

individuals were young. According to the present findings, it might be considered that if the age difference between the groups had been greater, the difference in gingival Hyp levels between the groups might have been more marked, since it has been reported that the rate of collagen synthesis is higher in younger individuals and decreases with age[11–15]. Gingival Hyp and total protein levels seem to be reflected in GCF, and no significant difference could be found between the groups.

A marked and surprisingly uniform decrease in protein and collagen production with increasing donor age was shown in cultures established from normal human gingiva[11]. It has been suggested that this decreased protein and collagen production could have resulted from decreased levels of synthesis rather than enhanced levels of intra- or extracellular protein degradation[11]. The higher gingival protein levels in the young group found in this study seem to support these findings.

Insufficient attention has been paid to the role of age in events occurring in the peridontium, and further research is needed in this area.

Conclusions

1) Although gingival and GCF Hyp levels were higher in the younger group, the differences between the two groups were not statistically significant.
2) Gingival total protein levels were significantly higher in the young group than in the older group ($p<0.01$).
3) The difference in GCF total protein levels between the groups was not statistically significant.

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