Hydroxyproline titers in gingival crevicular fluid


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Inflammation and increased degradation of extracellular matrix resulting in a net loss of collagen are characteristic features of the onset and progression of periodontal diseases. The accumulation of leukocytes and an increased production of hydrolytic enzymes results in a 60 to 70% loss of collagen at the advancing front of the periodontal lesion (1).

Most of the breakdown products are washed out of the tissue by the inflammatory exudate from the gingival vasculature (2) to appear in the gingival crevicular fluid (GCF). Therefore, the quantification of the breakdown products in the GCF should be a useful tool to monitor loss of extracellular matrix in the gingival connective tissue.

Degradation of collagen can be monitored by the release of hydroxyproline (Hyp) which accounts for about 10% of this protein. Clq, a subcomponent of the first complement factor, is the only other significant source of Hyp. It contains 4.3% Hyp by weight (3) and is found coating bacteria in the gingival crevices of patients with chronic periodontitis (4). Consequently, an assay for collagen-derived Hyp in GCF requires the elimination of Clq-derived Hyp.

An assay for collagen-derived Hyp in 1 to 2 μl volumes of GCF has been established (5) and was used for analysis of samples during the onset and progression of experimental periodontal disease in the beagle dog.

Experimental gingivitis was initiated in 3 female beagle dogs with a healthy periodontium by refraining from all oral hygiene measures. Approximately 1.5 μl volumes of GCF were collected from 10 mandibular premolars with microcapillary tubes once every week. After 8 wk, cotton floss was applied around the neck of the teeth for 9 d, there was a significant (p<0.0001; paired t-test) increase to $(16.2±3.45 \text{ ng/μl (x±SD)})$. During the subsequent 4-wk period, when no ligatures were present to exacerbate the disease process, GCF from all sites showed decreasing concentrations of Hyp (Fig. 1).

The application of cotton floss ligatures around teeth has been shown to convert stable established gingivitis into destructive periodontitis, the major tissue destruction most likely occurring within the first 4 wk after placement of the ligatures (7). In the present study, GCF samples taken 4 d after ligature removal had increased concentrations of Hyp, suggesting that they were collected during a phase of increased degradation of extracellular matrix with loss of mature collagen.

Inflamed gingival tissues have increased levels of cAMP (8) that induce enhanced intracellular degradation of newly synthesized collagen (9). It therefore cannot be ruled out that some of the increased concentrations of Hyp in GCF, observed during the destructive phase of experimental periodontal disease, may have been contributed by this process.

Hyp in serum is found in three forms: protein-bound, peptide and free. The protein-bound fraction of serum Hyp in healthy adults shows only minor variations regardless of the diet (10), but 50% is lost in the precipitation procedure with 0.02 M sodium acetate (11). In order to determine collagen-derived Hyp in GCF, serum Hyp concentration must be subtracted from total Hyp in GCF. Consequently, since Clq was precipitated out of the GCF specimens with 0.02 M sodium acetate, the same procedure had to be applied to the serum samples.

In contrast to protein-bound Hyp, the plasma levels of peptide-Hyp and free Hyp in healthy adults are significantly increased by the intake of Hyp-
containing food but return to baseline values 8 h (peptide Hyp) and 24 h (free Hyp) after such food intake (12). Since data on the dietary effects on serum Hyp in the beagle dog are not available, it was assumed that keeping the dogs on a 24-h dietary Hyp restriction would be adequate to establish a reasonably reproducible serum Hyp baseline at the time of sample collection.

Line plots of total Hyp in GCF and collagen-derived Hyp in GCF (total Hyp in GCF minus serum Hyp) show that both graphs follow the same pattern for all sites (Fig. 2). Therefore, total Hyp in GCF can be used to monitor changes in extracellular and intracellular degradation of collagen if dietary Hyp restrictions are implemented. However, determination of collagen-derived Hyp in GCF (total Hyp in GCF minus serum Hyp) would appear to give a more accurate estimate of collagen loss in the periodontal lesion.

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References


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