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Collagenolytic Activity of Crevicular Fluid and of Adjacent Gingival Tissue

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The fluid in and gingival tissue lining periodontal pockets were collected from male patients undergoing periodontal therapy. The collagenolytic activity of the crevicular fluid, and the ability of the gingiva in culture to degrade (a) an exogenous collagen substrate, and (b) endogenous collagen newly synthesized and labeled with H³-hydroxyproline, were related to the severity of gingival inflammation. Although inflammation appeared to have only a slight effect on gingival collagenolytic activity and on the turnover of collagen newly synthesized in culture, a marked effect was observed on the collagenase activity in the crevicular fluid. This study suggests that the collagen destructive activity of the periodontal lesion can be assessed by monitoring crevicular fluid collagenolytic activity.

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Introduction

Endogenous collagenase has been implicated in collagen breakdown during periodontal disease since reports appeared that explants of inflamed gingiva produced more collagenolytic activity in tissue culture than clinically normal gingiva.¹⁻³ However, Robertson and Grupe⁴ observed, using a different culture system than that used in the earlier studies, that inflamed gingival fragments released lower amounts of active collagenase into culture media than normal tissue. They subsequently reported that much of the collagenase originating from the inflamed tissue was present in an inactive or latent form, whereas the enzyme from normal tissue seemed fully active.⁵ Thus, the relationship between the severity of gingival disease and collagenase remains undefined.

Recently, collagenase activity has been detected in the fluid of human gingival crevices and pockets.⁶⁻¹¹ Our investigations indicated that this activity increased in the presence of inflammation;^{6,9,10} originated primarily from the gingival tissue, not from subgingival bacteria,¹⁰ and was present in both latent and active forms.^{9,10} One of our objectives has been to determine whether the activity of this crevicular fluid enzyme reflects ongoing collagen breakdown in the adjacent gingival tissue, and, thus, could provide diagnostically useful information on periodontal disease activity. In the present study, we attempted to relate human crevicular fluid collagen-degrading activity to the collagenolytic activity in the adjacent gingival tissue.

Materials and methods.

Gingival crevicular fluid (GCF) and gingival tissue samples were obtained from seven male subjects, 43-50 years of age, who were undergoing periodontal therapy and already had several appointments for scaling and oral hygiene instruction. An additional four males, 42-55 years old, with clinically normal gingiva were included in the study to obtain GCF samples from relatively normal crevices. Tissue samples were not obtained from the latter group of subjects. GCF was collected on sterile filter paper strips[†] that were inserted for one minute into proximal crevices and pockets around maxillary posterior teeth. GCF volume was determined with an electronic meter (Periotron[®]) and the sample immediately placed into small plastic microfuge tubes[§] on dry ice. The gingival index¹² and pocket depth were then recorded. The area was anesthetized, care being taken not to infiltrate the gingiva. Using an inverse bevel incision, the wedge of papilla lining each donor pocket (referring to the site from which GCF was

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[†]Harco Electronics, Ltd., Winnipeg, Canada

[§]Beckman Instruments, Inc., Mountainside, NJ

collected) was dissected and the tissue placed into Geys balanced salt solution[‡] containing penicillin, streptomycin and mycostatin, each at a final concentration of 100 units/ml. A full thickness mucoperiosteal flap was then raised for therapeutic reasons.

One and a half hours later, the GCF was assayed for collagenolytic activity using C¹⁴-glycine labeled collagen fibrils as substrate,¹⁰ and the excised gingivae were incubated in tissue culture as described below. Using an aseptic technique, the tissues were diced into small fragments, and two such pieces, weighing between 4-6 mg, wet weight, were implanted on a gel of reconstituted collagen saturated with Tyrode's media containing antibiotics.¹³ Fifty μ l of Tyrode's containing L-proline-3,4-³H[#] (50 μ ci/ml) was added to each culture, and the tissues were incubated at 37°C in a humidified atmosphere containing 95% air and 5% CO₂. After three days, the cultures were centrifuged at 8,000 g for 1 h at 20°C. Samples of the supernatant and residue were hydrolyzed in 6 N HCl, 105°C, for 24 hours and the hydrolysates applied to a 0.9 cm x 10.0 cm column containing aminex AG 50 W-X12 (15-20 micron spherical; sodium form),[¶] using a modification of the technique of Stern *et al.*¹⁴ to separate hydroxyproline (hyp) and proline. Hyp was measured colorimetrically¹³ and H³-hyp was counted in a liquid scintillation spectrometer (isocap/300 system).^{**} The ability of the gingival fragments to lyse the collagen gel and to release hyp into the supernatant media was taken as a measure of tissue collagenolytic activity.¹³ Both the radioactive collagen used for the GCF assay and the unlabeled collagen used for the gingival collagenolytic assay were resistant to lysis by the non-specific protease, trypsin (typically, less than 8% of the C¹⁴-labeled gel, or hyp measured chemically, was released from the reconstituted collagen fibrils).

Collagen formation was assessed by measuring the total amount of H³-hyp formed *in vitro*; this was calculated from the amount of H³-hyp recovered in the media and in the residue. The breakdown of newly synthesized collagen was estimated by measuring the H³-hyp in the media; more

than 95% of the H³-hyp in the media existed as dialyzable small molecular weight fragments.

Results.

The relationship between GCF flow (a measure of gingival inflammation^{15,16}) and collagenolytic activity in the donor pocket area is presented in Fig. 1. As expected, a direct relationship exists between GCF flow and gingival disease ($p < .01$), particularly when flow was correlated with gingival index. GCF collagenolytic activity was the parameter that showed the greatest correlation with GCF flow ($r = 0.85$; $p < .01$). Gingival tissue collagenolytic activity showed a positive relationship with flow that was much weaker ($r = 0.23$; not statistically significant) than that observed for the fluid. Moreover, the collagenolytic activity in the fluid and tissue were not significantly correlated to each other.

The formation of H³-hyp by the gingival explants in culture is presented in Fig. 2. The amount of H³-hyp synthesized during the incubation tended to increase with GCF flow ($r = 0.31$). This reflected an increased amount of H³-hyp recovered in the media ($r = 0.34$) because the H³-hyp in the tissue showed no increase and possibly decreased slightly ($r = -0.19$). However, the relationships between GCF flow and each of these three parameters were not statistically significant.

The effect of gingival inflammation, assessed with the Gingival Index, on: (a) GCF collagenolytic activity, (b) gingival tissue collagenolytic activity, and (c) the breakdown of the newly synthesized gingival collagen (labeled with H³-hyp in culture) is presented in the table. GCF collagenolytic activity increased with mild gingivitis (GI=1) and increased further with severe inflammation (GI=2). Severe inflammation also increased tissue collagenolytic activity and the release of H³-hyp into the media as collagen degradation products; however, the effect on GCF collagenase activity was more marked.

Discussion.

This study confirmed previous reports that GCF collagenolytic activity is elevated during inflammation of the adjacent tissue,^{6,9,10} the severity of inflammation was assessed clinically¹² and by monitoring

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[¶]Bio-Rad Laboratories, Richmond, CA

^{**}Nuclear-Chicago, Des Plaines, IL

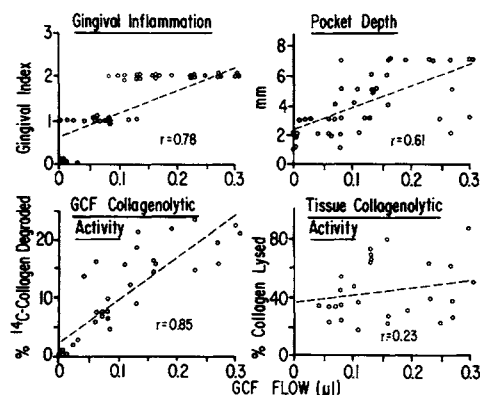


Fig. 1 - The relationship between GCF flow and (1) the severity of gingival disease, and (2) the collagenolytic activity of the tissue adjacent to, and fluid in, the gingival pocket.

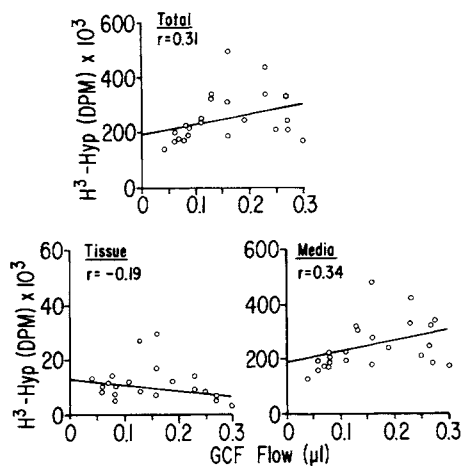


Fig. 2 - The relationship between GCF flow and the synthesis and degradation of collagen labeled with H^3 -hyp in tissue culture.

GCF flow.¹⁶ We previously concluded that this GCF activity was the result of a specific collagenase originating from the adjacent gingival tissue. This was based on the following: (1) the collagen gel lysed by the GCF was neither susceptible to degradation by non-specific proteases,^{6,9,10} nor was it degraded by plaque or saliva originating from subjects contributing the active GCF samples,⁶ (2) the GCF produced collagen breakdown products of the type generated by gingival and other mammalian collagenases.¹⁰ Furthermore, the GCF enzyme, like the collagenase from the adjacent gingival tissue,⁵ could be activated by sodium thiocyanate, indicating that the collagenase existed in both active and latent forms.^{9,10}

The data also indicate that the severely inflamed gingiva in culture degraded more endogenous collagen than mildly inflamed tissue, based on the observation of increased amounts of H^3 -hyp in the media as small molecular weight fragments. What is less clear is whether severe inflammation stimulated degradation to a greater extent than it stimulated synthesis (see Fig. 2), or whether the synthesis and degradation (turnover) of the H^3 -labeled collagen were both accelerated to a similar degree. An effect on turnover would be consistent with the results of Claycomb *et al.*,²¹ who described increased synthesis of H^3 -hyp labeled collagen *in vitro* by gingiva affected by periodontitis.

A positive correlation was observed

between gingival tissue collagenolytic activity (assayed using an exogenous collagen substrate similar to that described by Fullmer)^{1,22} and GCF flow. However, this was much weaker than the relationship between flow and GCF collagenolytic activity. Moreover, Robertson and Grupe,⁴ using a different system, actually found *less* collagenase activity in the culture media of inflamed gingiva compared to non-inflamed gingiva. Several reasons could explain why the enzyme activity in the GCF appeared to be more strongly correlated to gingival disease than collagenolytic activity in the tissue itself:

(1) In the gingiva, as in other tissues, most of the extracellular collagenase could be bound to the matrix collagen^{18,19} making the enzyme unavailable to degrade the exogenous collagen substrate used in the enzyme assay. Collagenase in the GCF is not faced with this restriction. (2) With inflammation, there is a leakage of serum proteins into the tissues and, ultimately, into the crevicular fluid. Thus, increased collagenase activity in the inflamed gingiva may not be detected, because serum proteins, particularly $\alpha 2$ -macroglobulin, can bind to and inhibit the enzyme.^{5,19} In fact, Robertson *et al.*⁵ have demonstrated that the collagenase activity in the culture fluids of inflamed gingival fragments was markedly enhanced by NaSCN, which denatures the inhibitor and activates latent enzyme.

TABLE
COLLAGENOLYTIC ACTIVITY IN THE PRESENCE OF MILD AND SEVERE
GINGIVAL INFLAMMATION

	Gingival Index			% increase from GI=1 to GI=2
	0	1	2	
GCF collagenolytic activity (% C ¹⁴ - collagen degraded)	0.5 ± 0.4 ^a	9.0 ± 1.6 ^b	18.5 ± 1.4 ^c	105.5
Tissue collagenolytic activity (% gel degraded)	--	34 ± 4 ^a	47 ± 5 ^b	38.3
H ³ -hyp labeled collagen degraded in culture (DPM × 10 ³)	--	171 ± 11 ^a	268 ± 23 ^b	56.7

a vs. b vs. c: p<.01

On the other hand, the collagenase in GCF showed an extremely strong correlation with severity of inflammation. GCF contains numerous proteolytic enzymes originating from both bacterial and tissue sources.¹⁵ These proteases could successfully compete with collagenase for binding to the inhibitors in the GCF;²⁰ alternatively, they could degrade the protein inhibitors. Thus, a greater proportion of collagenase molecules in the GCF could exist in the active rather than latent form, compared to the same enzyme in the gingival tissue. The fact that NaSCN increased collagenase activity in inflamed gingival tissue about 500%,⁵ whereas GCF activity from pockets, exhibiting a similar degree of inflammation, was only increased about 100%,¹⁰ provides evidence that a smaller proportion of the GCF enzyme molecules exist in a latent form.

Our observation that the collagenolytic activity of the periodontal lesion increases with inflammatory disease is consistent with the findings of Rudin *et al.*,¹⁷ that the amount of collagen assessed histologically in gingival biopsies was inversely related to the flow of GCF. In conclusion, monitoring the collagenolytic activity in the periodontal pocket, using the non-invasive technique of collecting crevicular fluid, could become diagnostically useful in assessing periodontal disease activity and could help clarify how gingival collagen

metabolism is altered during pathologic conditions.

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