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Collagenolytic Activity of Periodontal Ligament During Human Deciduous Root Resorption

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Abstract

Human periodontal ligament from resorbing deciduous teeth demonstrated collagenolytic activity when incubated on collagen gels. This activity was directly proportional to the size of the explant and length of time of incubation. No activity was observed in ligaments obtained from completely formed premolar teeth.

Introduction

Collagen is the predominant organic component of dentin, cementum and periodontal ligament.[1,2] Since these structures are degraded during physiological resorption, it follows that collagenolytic activity should be present during this process. Such activity has been demonstrated in bovine deciduous teeth.[3] Bovine dental sac collagenase[4] has also been characterized and related to tooth resorption. Collagenolytic activity has also been observed in rabbit periodontal ligament during tooth movement.[5] However, human collagenolytic activity during tooth resorption has not yet been reported. The purpose of this investigation is to demonstrate collagenolytic activity within the periodontal ligament of resorbing teeth and to describe the histological changes during this process.

Methods and Materials

Thirty children were included in this study, 12 females and 18 males, ranging in age from eight year four months to 12 years two months. A total of 46 deciduous resorbing canines, first molars, and second molars were examined, while 15 fully formed first premolars were used as controls. All procedures in this study were performed under sterile conditions. Using a standard surgical technique, the teeth were removed prior to orthodontic therapy. Root resorption of deciduous teeth was judged clinically and radiographically to be between 50% and 75% completed in all cases. This was based upon measurements of these teeth with a Boley millimeter gauge and comparison with averages given by Kramer and Ireland.[6] The roots from premolar controls were judged similarly to be fully formed. Using a high speed handpiece with water coolant, 5 mm of the remaining apical third of the root was removed with a diamond disc on all specimens. From the apically sectioned root apex, periodontal ligaments were removed with a scalpel and washed for three hours at 37°C in mammalian Tyrode's solution containing 0.5 mg/ml streptomycin, 1000 units/ml penicillin, 40 ug/ml amphotericin B, and 75 ug/ml gentamycin. After washing, the tissue was cut into sections ranging in size from 1 to 4 mm.[2]

The detection of collagenolytic activity was accomplished by incubation of periodontal ligament fragments for seven days on reconstituted collagen gels as described by Gross and Lapiere.[7]

Periodontal ligament cultures were incubated at 37°C in a moist atmosphere containing 90% O_2 and 10% CO_2, and were observed daily for seven days for lytic activity. Areas of explant and lysis were measured with an ocular micrometer. Resorption products that occurred in the area of lysis were collected with a micropipette and analyzed biochemically for hydroxyproline according to the methods of Stegemann.[8] Control chambers were incubated in the same manner as above, but without tissue explants. Bacterial contamination occurred infrequently and, when present, was visible as discrete colonies on the gel. Although none of these cases produced observable lysis of the gel, these specimens were discarded. Immediately after incubation, periodontal ligaments were fixed in 10% buffered formaline and routinely processed for light microscopy. A second group of ligaments from experimental and control specimens was fixed immediately after re-

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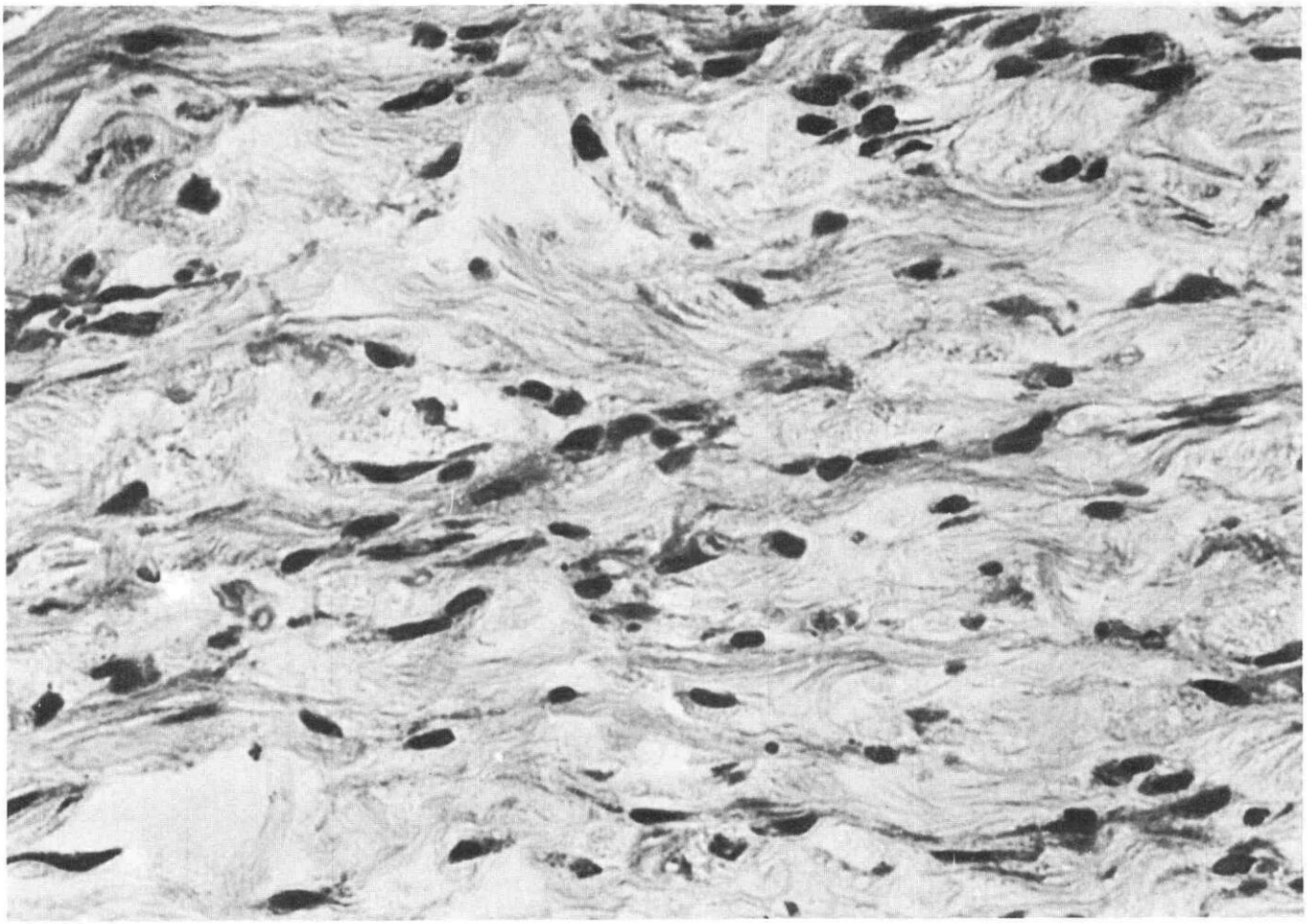

Figure 1: Periodontal ligament from the root of resorbing deciduous canine exhibiting normal morphology. [Hematoxylin and Eosin, magnification X160]

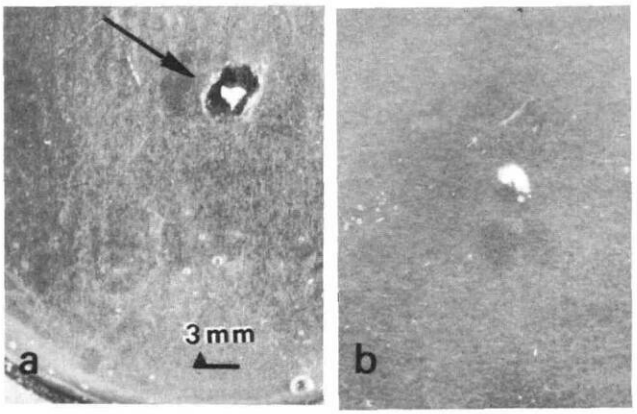

Figure 2a: Periodontal ligament from the root of a resorbing deciduous canine, incubated on a collagen substrate for four days. Note the area of lysis (arrow) surrounding the tissue indicating collagenolytic activity.

Figure 2b: Periodontal ligament from the root of a premolar, incubated on a collagen substrate for four days. Note the absence of collagenolytic activity when compared to Figure 2a.

moval from the tooth and processed for histology in the same manner described above.

Results

Histological Findings

The periodontal ligaments from both the resorbing deciduous and premolar control teeth appeared similar microscopically except for an occasional inflammatory cell present in the former (Figure 1). No osteoclasts were observed. Fibroblasts and collagen fibers appeared in a typical parallel relationship and resembled normal periodontal tissue. Ligaments fixed immediately were identical to those cultured and then fixed in formalin, except for cellular outgrowth in the latter.

The Frequency and Extent of Collagenolytic Activity in Periodontal Ligament

The degree of collagenolytic activity was measured

TABLE 1. Collagenolytic Activity of Periodontal Ligament* in Resorbing and Non-Resorbing Teeth

Tooth	Incubation Period (days)					
	1	2	3	4	5	7
Deciduous Canine (N=24)	—	±	**	***	****	*****
Deciduous First Molar (N=10)	—	±	**	***	****	*****
Deciduous Second Molar (N=12)	—	±	**	***	****	*****
First Premolar (N=15)	—	—	—	—	—	—

*Area of ligament explants was approximately 2 mm2.

— = No lysis of collagen gel.
\+ = Lysis of gel beginning beneath the tissue.
** = Lysis of gel between 1 and 2 mm around the tissue.
*** = Lysis of gel between 2 and 3 mm around the tissue.
**** = Lysis of gel between 3 and 4 mm around the tissue.
***** = Lysis of gel between 4 and 5 mm around the tissue.

over a period of seven days, with 2 mm^2 tissue explants (Table 1). All periodontal ligament fragments from resorbing teeth showed lysis of collagen gels (Figure 2a). Control ligaments displayed no activity (Figure 2b).

The extent of substrate lysis increased proportionally when compared to the size of the tissue explant (Table 2) and length of time of incubation. An approximate three-fold size increase in mean explant area resulted in a 40% increase in gel lysis. Furthermore, the amount of collagen solubilized as measured by release of hydroxyproline was correlated with tissue size and with the area of lysis. A three-fold increase in mean explant area resulted in an increase in measurable hydroxyproline from 3.7 ± 0.4 ug to 8.8 ± 0.7 ug after seven days of incubation.

Discussion

The mechanisms involved in the breakdown of collagen during physiological processes, such as newt limb regeneration and wound repair, have been reported.[7,9-11] Collagenolytic activity has been observed in the periodontal ligament during orthodontic tooth movement over a seven day period,[5] but has not been detected in normal cultured periodontal ligament fibroblasts.[12] These reports indicate that collagenolytic activist exists within the periodontal ligament of animal models undergoing connective tissue resorption, but is either absent or undetectable in normal, homeostatic tissue. These results are consistent with our data, indicating that one source of human collagenolytic activity resides within the periodontal ligament of resorbing teeth, while no activity could be measured from the normal ligaments of premolars.

The stimulus for this activity, however, is still unknown. As previously stated, the pressures associated with orthodontic tooth movement have been observed to contribute to the collagenolytic activity observed within the periodontal ligament.[5] Similar types of forces occurring during the eruption of succedaneous teeth and prior to the exfoliation of the deciduous teeth may contribute analogously to dental collagenolysis observed in our study.

Surprisingly, little inflammatory infiltrate was observed in the ligaments of resorbing teeth, a finding which is contradictory to previously reported observations in adjacent root areas during this event.[3,13-16] In-

TABLE 2. Relationship Between Size of Periodontal Ligament Explant Area of Lysis, and Hydroxyproline Released from Collagen Gel

Number of Cultures*	Explant Area Range in mm^2	Mean Explant Area in mm^2	Mean Area of Lysis in mm^2	Hydroxyproline (ug)
6	.8 - 1.4	1.1 ± 0.3	3.2 ± 0.4	3.7 ± 0.4
8	1.9 - 3.0	2.2 ± 0.3	4.4 ± 0.3	6.1 ± 0.3
7	3.2 - 4.3	3.6 ± 0.3	5.6 ± 0.2	8.8 ± 0.7

*Incubation period was seven days for all cultures observed.

flammatory tissue, however, has not been observed when mucopolysaccharidase activity was present during resorption,[17] and has led the authors to postulate that the periodontal ligament fibroblasts were responsible for the production of this enzyme during a phase of root resorption. It would appear that a similar situation occurs during collagenolytic activity. The presence of an inflammatory infiltrate as the result of trauma and its role in the collagenolytic activity of root resorption is presently under investigation in our laboratory and will be reported in the future. The importance of inflammatory cells during physiologic resorption, however, may be overemphasized, since collagen removal as a result of phagocytosis by periodontal ligament fibroblasts has been reported,[18] and may be an additional contributing factor to tissue removal.

Conclusions

Collagenolytic activity has been demonstrated in cultures of human periodontal ligaments of resorbing deciduous teeth. Such activity was associated with normal periodontal tissues without the presence of an inflammatory infiltrate. Both the length of time of incubation and the size of the tissue explants resulted in increased lysis of the collagen gels and indicated that a healthy periodontal ligament may be responsible, in part, for deciduous root resorption.

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