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The hydrolysis of dentin and cementum glycosaminoglycans following fluoride immersion

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Abstract

The roots of human premolar teeth were analyzed biochemically for total glycosaminoglycan after fluoride treatment and enzymatic digestion. Base levels of dentin and cementum fluoride (780 ± 105 ppm) and controls showed a statistically significant difference from the fluoride-treated teeth (1250 ± 120, 1675 ± 105, 2150 ± 145 ppm) for the 1-, 8-, and 24-hour periods respectively. The amounts of soluble hexosamine were 77% (0.51 μg/mg), 50% (0.34 μg/mg), and 25% (0.15 μg/mg) when compared to control soluble hexosamine values (0.68 μg/mg) after fluoride immersion for 1-, 8-, and 24-hours as well. It is apparent that fluoride treatment limited the degree of loss within the ground substance of root dentin and cementum.

Fluoride immersion of teeth has been reported to inhibit the resorption process during tooth replantations.[1-4] The clinical success of fluoride in inhibiting mineral loss during the caries process is also well documented.[5]

The glycosaminoglycans constitute a part of the organic matrix of dentin and cementum[6-10] which is lost during physiological resorption. Previous studies have shown that this resorptive process consists in part of glycosaminoglycan degrading enzymes derived from the pulp and periodontal tissues of resorbing teeth.[11-13] Since the glycosaminoglycans are degraded during resorption, it was the purpose of this investigation to determine the effect of fluoride immersions upon the release of glycosaminoglycans from the roots of human teeth. Specifically, it aimed to test the hypothesis that fluoride immersion results in decreased loss of organic matrix. The study also investigated dentin and cementum fluoride uptake as a function of time.

Methods and Materials

Sixty first premolars were extracted for orthodontic reasons in patients ranging in age from ten years to thirteen years, five months. The permanent dentition was chosen as the experimental model since decreases in glycosaminoglycan concentration are clearly evident in deciduous teeth.[11] These teeth were all derived from a population maintained on systemic fluorides from birth and professionally administered biannual topical applications.

Prior to fixation, the crowns were removed with a high speed dental bur and discarded. The periodontal ligaments and pulp tissues were disposed of as well. All specimens were fixed in 10% formalin for 48 hours. Following fixation, the roots were sectioned in half longitudinally with a diamond disc. These sections were then randomly placed into two groups with one group designated as control. Roots from half of the experimental and control samples were then crushed separately in a steel mortar and ground in a Wiley Mill using a 40-mesh sieve. The dentin and cementum powder (1600 mg) was then ashed in nickel crucibles at 575°C and fluoride determined by microdiffusion from a polypropylene cell[14] followed by colorimetric analysis.[15]

After initial fluoride analysis of the specimens, the second half of the experimental group was immersed in a 1.2% fluoride solution[a] for 1, 8, and 24 hours. Control teeth were routinely placed in neutral saline or phosphate buffer (pH 3.2) for the same time period.

After the immersions, fluoride content from one-half of the treated samples was analyzed as described previously. Following fluoride analysis, the glycosaminoglycan content of dentin and cementum was determined via degradation by bovine testicular hyaluronidase.[b] Roots were ground in a Wiley Mill using a 40-mesh sieve and extracted in ten volumes of cold distilled water containing 100 mg of Pronase (Sigma) for two 24-hour periods. The extracts were filtered in a glass sinter and centrifuged at 90,000 g

[a]Luride, 1.2% fluoride from sodium fluoride and hydrogen fluoride in an M/10 phosphoric acid solution, pH 3.2; Hoyt Laboratories, Needham, MA.

[b]Sigma Chemical Company, Saint Louis, MO.

for 30 minutes at 4°C. The supernatant was separated from the precipitate by decantation. The supernatant was added to one volume 0.2 M sodium chloride and was then diluted with three volumes of 95% ethanol. The precipitates were collected, washed with ethanol, recentrifuged, dissolved in water, and dialyzed against running distilled water for 24 hours. The soluble component was lyophilized and weighed. Incubations to determine the amounts of tissue polysaccharide were performed at 37°C for eight hours with testicular hyaluronidase (Sigma). Enzyme was dissolved in 0.1 M phosphate buffer at pH 6.5 in a concentration of 0.5 mg/ml and added to test tubes containing 20 mg per 0.25 ml of the glycosaminoglycan preparation.

Standards containing known amounts of chondroitin-4-sulphate, chondroitin-6-sulphate, and hyaluronic acid (Sigma) were incubated under identical conditions. N-acetylhexosamine was assayed according to the method of Reissig, Strominger, and LeLoir.[16]

Results

Average levels of dentin and cementum fluoride from teeth maintained in systemic and topical applications was 780 ± 105 ppm. This value was similar to dentin fluoride concentrations of primary teeth from individuals living in an area where the fluoride content of the drinking water was between 1.9 and 2.2 ppm.[17] Fluoride immersion for the 1-, 8-, and 24-hour periods resulted in increased uptake over the time period. This uptake was significantly different from the base fluoride levels and control values (Table 1). Differences in fluoride levels between the fluoride-treated groups were also statistically significant over the 24-hour period.

Table 1. Fluoride uptake by dentin and cementum over a 24-hour period.

Experimental Group (fluoride-Treated)		
	Immersion Time	F ppm** (ash) Mean ± S.D.
	1 Hour	1250 ± 120*
	8 Hours	1675 ± 105*
	24 Hours	2150 ± 145*
Control Group		
	Immersion Time	F ppm** (ash) Mean ± S.D.
Base Level	—	780 ± 105
Saline Control	1 Hour	740 ± 88
	8 Hours	765 ± 100
	24 Hours	748 ± 78
Phosphate Control	1 Hour	751 ± 77
	8 Hours	762 ± 85
	24 Hours	745 ± 75

* Significant; $p < 0.01$

** Fluoride ppm is based upon five samples for each determination.

The water soluble glycosaminoglycan acetylhexosamine content of dentin and cementum combinations after fluoride therapy is shown in Table 2. Glycosaminoglycans extracted from control teeth correspond to amounts reported elsewhere.[6,7,11] Experimental groups for the 1-, 8-, and 24-hour periods yielded soluble N-acetylhexosamine means of 0.51, 0.34, and 0.15 ug/mg respectively. An inverse relationship existed between the release of N-acetylhexosamine and the period of fluoride treatment which resulted in a 77%, 50%, and 25% decrease in soluble hexosamine released after the 1-, 8-, and 24-hour immersions.This comparison showed that an increase in the fluoride immersion period resulted in a net decrease of extractable organic material from the premolar roots. Statistical analysis revealed a significant difference between the means for each of the groups.

Discussion

A relationship exists between fluoride treatment and mineralized tissue resorption. Reports by Goldhaber[18] indicate that *in vitro* bone resorption was inhibited by immersion for one minute in sodium fluoride. Treatment of avulsed teeth with fluoride prior to their replantation into the oral cavity has also been shown to inhibit resorptive activities.[1-4] Similarly, the resorption remodeling of extraction sockets was limited following the insertion of fluoride solutions into the freshly wounded socket.[19]

Our evidence suggests that fluoride will also affect the degree of hydrolysis of the extracellular matrix because of its tightly bound existence within the crystal structure of the mineralized tissues.[6-8] The mechanism for this activity is unknown. It has been shown that fluoride is bound by extracellular matrix proteins *in vivo,*[20] and may therefore modify the electrochemical and physicochemical properties of this tissue. This modification may ultimately affect the tissue solubility during hydrolysis. Interestingly, fluoride concentration of the dentin of primary teeth has been observed to fall at the pulpal interface resulting from osteoclastic activity.[17] No measurement of this activity, however, has been reported in fluoride-treated and fluoride-free populations. Although it appears that fluoride partially inhibits both mineral and organic resorption *in vitro,* we are aware of no data indicating increased exfoliation times within a population maintained on systemic and topical applications of fluoride. The examination

Table 2. Relative amounts of water soluble glycosaminoglycan in dentin and cementum after fluoride immersion and enzyme digestions.

Specimen	*Time*	*Mean Glycosaminoglycan** (ug/mg of Lyophilized Tissue)	*Standard Deviation*	*% Decrease in Water-Soluble Hexosamine When Compared With Controls*
Saline Control	1 H	0.68	0.06	—
	8 H	0.68	0.06	—
	24 H	0.68	0.03	—
Phosphate Control	1 H	0.62	0.03	—
	8 H	0.61	0.03	—
	24 H	0.61	0.03	—
Fluoride Immersed	1 H	0.51**	0.04	77%
	8 H	0.34**	0.05	50%
	24 H	0.15**	0.02	25%

* Glycosaminoglycans are represented as micrograms of N-acetylhexosamine per mg of lyophilized tissue based upon seven samples for each determination.

** Significant; $p < 0.01$ (t-test)

of such data is anticipated shortly. We conclude, however, that fluoride treatment inhibits mineralized tissue resorption and delays the loss of the organic matrix as well.

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