Arch Toxicol (1988) 62: 328–330

Archives of Toxicology
© Springer-Verlag 1988

Fluoride-induced changes in the tooth glycosaminoglycans: an in vivo study in the rabbit

A. K. Susheela and Kamal Sharma

Fluoride and Fluorosis Research Laboratories, Department of Anatomy, All India Institute of Medical Sciences, New Delhi-110 029, India

Abstract. Effect of high fluoride ingestion on the tooth matrix glycosaminoglycans was studied in rabbits administered 10 mg NaF/kg body weight orally at 24-h intervals for a period of 9 months. Fluoride-treated tooth showed a significant reduction in glycosaminoglycan content as compared to the normal tooth. Sephadex G-75 chromatography and DEAE-cellulose ion exchange chromatography revealed the presence of small molecular weight glycosaminoglycan molecules and an increase in the charge density heterogeneity in the sulphated glycosaminoglycans of the fluoride-treated rabbit tooth as compared to the controls. These changes may be related to the dedifferentiated tooth matrix and an increase in the dermatan sulphate content in the fluoride-treated tooth matrix reported earlier.

Key words: Fluoride – Tooth – Glycosaminoglycans – Rabbit

Introduction

Fluoride when ingested in excess is known to accumulate in calcified tissues (Susheela et al. 1982). High fluoride content in drinking water and food items leads to pathological changes in the tooth, a condition termed dental fluorosis. This is characterized by chalky white texture of enamel in the early stages to a dark brown, black, pitted tooth in the advanced stages. Although it is certain that fluoride is the causative factor for this disease, as yet the mechanism underlying this disease is not well understood (Susheela et al. 1987).

Glycosaminoglycan and its sulphated isomers are important constituents of the organic matrix of tooth and are known to play a significant role in the calcification of the tissues. The function(s) of these highly sulphated molecules have been suggested to be dependent on their macromolecular (molecular size) and polyelectrolyte (charge density) characteristics (Comper and Laurent 1978). This report is an attempt to assess the action of fluoride on the sulphated isomers of tooth glycosaminoglycans.

Materials and methods

Ten, age matched rabbits were randomly divided into two groups of five animals each. One group of animals was given 10* mg NaF/kg body weight orally every 24 h for a period of 9 months. All the animals were given a standard diet (Hindustan Lever, Bombay, India) and drinking water ad lib.

Animals were sacrificed after 9 months' treatment along with age matched controls. Molars were removed and cleaned mechanically to remove the adhering tissues and dental pulp. Cleaned teeth were cut into pieces, defatted in an ether:acetone mixture (1:1, v/v) for 72 h, and dried in acetone for 24 h. Glycosaminoglycans were extracted from dry defatted tooth powder according to Hjertquist and Vejlens (1968). Glycosaminoglycans thus purified were weighed and further analyzed for molecular weight and charge density.

Molecular weight determination. Gel filteration chromatography was done on a Sephadex G-75 column (1.5×70 cm) equilibrated with 0.2 M NaCl at a flow rate of 30 ml/h; 4 ml fractions were collected and analyzed for uronic acid. Blue dextran was used to find the void volume.

Charge density heterogeneity. Ion exchange chromatography was done on DEAE-cellulose column (1.5×20 cm). Gel was equilibrated with 0.1 M sodium acetate, pH 5.0. Sample was applied in the same buffer and the column washed with 3 bed volumes of the buffer. Bound glycosaminoglycans were eluted with a linear gradient (total volume 200 ml) of 0.1–2.5 M sodium acetate, pH 5.0 at a flow rate of 30 ml/h. All the fractions were analyzed for uronic acid.

Uronic acid was estimated by the modified tetraborate-carbazole reaction with D-glucuronolactone as standard (Bitter and Muir 1962).

Serum fluoride was estimated by an ion-selective electrode using a PHM-84 Ion Meter, Radiometer, Copenhagen (Hall et al. 1972).

Results and discussion

Serum fluoride levels and tooth GAG content are reported in Table 1. The increase in serum fluoride on NaF-treated animals is statistically significant ($p < 0.001$). The NaF-

Offprint requests to: A. K. Susheela

* This dose was arrived at while standardizing the rabbit model. This dose is not lethal; doses up to 50 mg NaF/kg body weight have been tried without any acute toxicity symptoms or mortality. Moreover, in many states in India, fluoride levels in drinking water are very high, viz. 38.5 ppm. These high fluoride levels and additional intake of fluoride through food are comparable to the levels in the present study

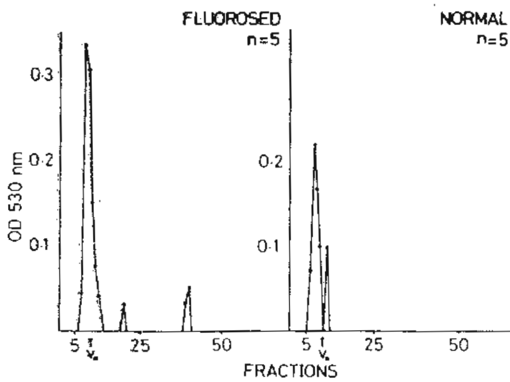
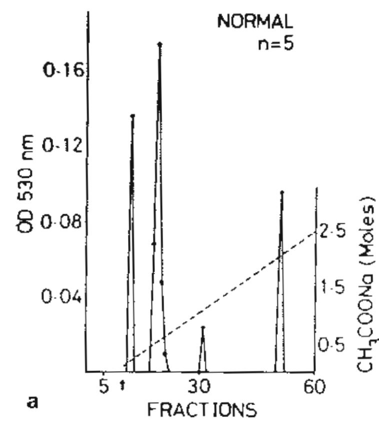


Fig. 1. Sephadex G-75 chromatograms of the fluoride-treated and normal tooth glycosaminoglycans. *Arrows* mark the void volume (*Vo*) as determined by dextran blue



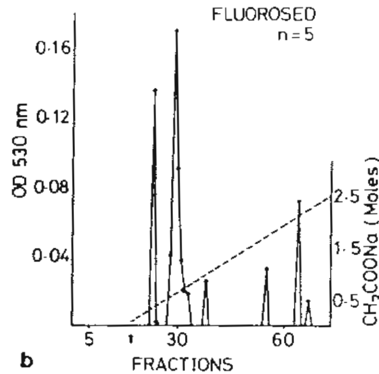


Fig. 2. a DEAE-cellulose ion exchange chromatogram of the normal tooth glycosaminoglycans. *Arrows* marks the start of the linear gradient. b DEAE-cellulose ion exchange chromatogram of the fluoride treated tooth glycosaminoglycans. *Arrows* marks the start of the linear gradient

treated animals show a significant reduction in the amount of tooth glycosaminoglycans as compared to the controls.

The Sephadex G-75 chromatograms of the NaF-treated tooth glycosaminoglycans are strikingly different from glycosaminoglycans of normal tooth (Fig. 1 and Table 2). The fluorosed tooth glycosaminoglycans eluted in three peaks, one at the void volume, representing 89.13% of the total uronic acid and two later, representing 10.87% of the total uronic acid.

The DEAE-cellulose ion exchange chromatograms of the glycosaminoglycans of fluoride-treated and normal tooth glycosaminoglycans are shown in Figs. 2a and 2b and Table 3. The fluorosed tooth glycosaminoglycans are heterogenous in their charge density and elute in six peaks between 0.36 and 2.23 M sodium acetate, as compared to the normal tooth glycosaminoglycans which eluted in four peaks between 0.28 and 2.06 M sodium acetate. Two peaks eluting at 1.73 M and 2.23 M are seen in the fluorosed tooth glycosaminoglycans only.

The study was conducted on total molar tooth because enamel is known to contain very little or no glycosaminoglycans (Embery et al. 1985). Therefore in reality the glycosaminoglycans studied represent mostly the dentine organic matrix. Although the enamel compartment is the first one to show fluoride toxicity symptoms, the underlying dentine organic matrix is likely to play an important role in the pathophysiology of fluorosis, especially in the case of systemic ingestion of fluoride as in the present study. A reduction in the glycosaminoglycan content in the fluorosed tooth is reported for the first time. This clearly shows altered glycosaminoglycan metabolism in the fluoride-treated tooth. Whether the reduction is because of inhibition of synthesis or enhanced catabolism is not clear.

Small molecular weight glycosaminoglycans are associated with uncalcified tissues e.g. cartilage of dog, whereas the normal calcified dog bone matrix is known to have high molecular weight glycosaminoglycans (Hjertquist and Vejlens 1968).

Table 1. Serum fluoride and tooth glycosaminoglycan levels in normal and 9-month NaF-treated rabbits

	Normal ($n = 5$)	Fluoride-treated ($n = 5$)	p value
Serum fluoride [ppm]	0.07 ± 0.01	0.81 ± 0.03	<0.001
Tooth glycosaminoglycans [mg %]	0.28 ± 0.06	0.06 ± 0.004	<0.001

Table 2. Percentage uronic acid eluting in different peaks following gel filteration chromatography on sephadex G-75

	At void volume			After void volume		
	Peak I	Peak II	Total	Peak I	Peak II	Total
Normal	85.02	14.98	100.00	–	–	–
NaF-treated	89.13	–	89.13	3.32	7.55	10.87

Table 3. Percentage uronic acid eluting at different ionic strength of the elution buffer on DEAE-cellulose

Normal	Peak eluting at different molar sodium acetate					
% uronic acid content	0.28 M 24.86	0.53 M 53.04	1.1 M 4.60	– –	2.06 M 17.49	– –
NaF-treated	Peak eluting at different molar sodium acetate					
% uronic acid content	0.36 M 40.27	0.56 M 41.83	1.0 M 3.58	1.73 M 4.03	2.1 M 8.05	2.23 M 2.24
					14.32	

Smalley and Embery (1980) and Embery et al. (1985) showed a tailing in the fluorosed tooth glycosaminoglycan peak at void volume following gel filteration chromatography on Sepharose 2B, suggesting a reduction in the molecular weight. Using Sephadex G-75 (with exclusion limit 50000) the results presented clearly show that the fluoride-treated tooth glycosaminoglycans have small molecular weight molecules which constitute 10.87% (3.32% and 7.55% in two peaks) of the total glycosaminoglycans (Table 2). Small molecular weight glycosaminoglycan molecules are not seen in the normal tooth glycosaminoglycans. This suggests the presence of low molecular weight glycosaminoglycans in the fluoride-treated tooth, which is characteristic of uncalcified tissue (Hjertquist and Vejlens 1968). Whether the small molecular weight molecules represent the product of a dedifferentiated tissue matrix as seen in the fluoride-treated cancellous bone (Jha and Susheela 1982; Susheela and Jha 1983) or altered metabolism within the tooth matrix needs to be ascertained.

The ion exchange chromatograms (Figs. 2a and 2b) show that along with the four peaks in the normal tooth glycosaminoglycans eluting at the buffer strengths of 0.28, 0.53, 1.1 and 2.06 M, the fluorosed tooth glycosaminoglycans have two additional peaks at 1.73 and 2.23 M. Although the glycosaminoglycans eluting at high buffer strength show three peaks in the fluoride-treated group, namely, at 1.73, 2.1 and 2.23 M, the total uronic acid eluting in these peaks is same as the uronic acid eluting in the single peak of 2.06 M in the case of normal group. The splitting of peaks indicates marked changes in the charge density of the highly sulphated glycosaminoglycans. This might be related to the presence of higher amounts of dermatan sulphate in the fluorosed teeth as reported in rats (Embery et al. 1985) and in humans (Susheela et al. 1988).

Along with the high charge density glycosaminoglycans, the low charge density molecules also show a change in the distribution of glycosaminoglycans in the first three peaks (Table 3). Fluorosed tooth glycosaminoglycans have substantially greater amount of glycosaminoglycans eluting in the first peak as compared to the controls.

Changes in the amount of glycosaminoglycans and their biochemical characteristics have been reported to be associated with the mineralization of the organic matrix in calcified tissues (Engfeldt and Hjerpe 1972; Lohmander and Hjerpe 1975). The reduced glycosaminoglycans content and the altered molecular weight and charge density profiles as observed in the present study are therefore likely to affect the mineralization of the dental organic matrix. However, whether the changes in the glycosaminoglycans of the tooth matrix are adequate to explain the abnormalities of mineralization needs to be ascertained. This would require further studies on the fluorosed tooth as well as on the mechanism of dental matrix mineralization.

In conclusion, the present report reveals that prolonged exposure to fluoride leads to substantial quantitative and qualitative changes in the tooth glycosaminoglycans. These changes include a reduction in the amount of glycosaminoglycans, presence of small molecular weight molecules and altered charge density, especially in the highly sulphated isomers.

Acknowledgements. One of the authors (A. K. S.) acknowledges Ministry of Environment and Forests, Government of India for grants-in-aid. K. S. acknowledges the All India Institute of Medical Sciences, New Delhi, for the fellowship awarded under the PhD programme.

References

Bitter T, Muir HM (1962) A modified uronic acid carbazole reaction. Anal Biochem 4: 330–334

Comper WD, Laurent TC (1978) Physiological function of connective tissue polysaccharides. Physiol Rev 58: 255–315

Embery G, Stanbury JB, Smalley JW (1985) The metabolism of proteoglycans and glycosaminoglycans in dental fluorosis. In: Fluoride toxicity. Susheela AK (ed) Proceedings of the 13th Conference of International Society for Fluoride Research, New Delhi, pp 65–77

Engfeldt B, Hjerpe A (1972) Glycosaminoglycans of dentine and predentine. Calc Tissue Res 10: 152–159

Hall LL, Smith FA, Lopez OH, Gardner DE (1972) Direct potentiometric determination of total ionic fluoride in biological fluids. Clin Chem 18: 1455–1458

Hjertquist SO, Vejlens L (1968) The GAG of dog compact bone and epiphyseal cartilage in the normal state and in experimental hyperparathyroidism. Calc Tissue Res 2: 314–334

Jha M, Susheela AK (1982) In vivo chondrogenesis and histochemical appearance of dermatan sulphate in rabbit cancellous bone. Differentiation 22: 235–236

Lohmander S, Hjerpe A (1975) Proteoglycans of mineralizing rib and epiphyseal cartilage. Biochem Biophys Acta 404: 93–109

Smalley JW, Embery G (1980) The influence of fluoride administration on the structure of proteoglycans in developing rat incisor. Biochem J 190: 263–272

Susheela AK, Jha M (1983) Cellular and histochemical characteristics of osteoid formed in experimental fluoride poisoning. Toxicol Lett 16: 35–40

Susheela AK, Sharma YD, Jha M, Singh M, Jagannath B, Jain SK (1982) Fate of fluoride following its administration into a biological system – an in vivo study. Fluoride 15: 173–177

Susheela AK, Sharma K, Rajan BP, Gnanasundaram N (1987) Biochemical studies on fluoride action on glycosaminoglycans of human and animal teeth. In: Rugg Gunn AJ, Rahmatulla M (eds) New frontiers in fluoride studies for health. COSTED

Susheela AK, Sharma K, Rajan BP, Gnanasundaram N (1988) Human dental fluorosis: the status of sulphated isomers of glycosaminoglycans. Arch Oral Biol (in press)

Received April 18, 1988/Accepted June 30, 1988