

SHORT COMMUNICATION

URINARY EXCRETION OF GLYCOSAMINOGLYCANS, HYDROXYPROLINE AND HYDROXYLYSINE IN RABBITS AFTER EXCESSIVE INGESTION OF FLUORIDE

Mohan Jha, Jasbeer Koacher and A. K. Susheela

Fluorosis Research Laboratory, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India

(Received 23 August 1982; revision received 21 March 1983)

SUMMARY

1. Urinary levels of glycosaminoglycans (GAG) and hydroxyproline from normal and fluoride treated rabbits were estimated. The hydroxylysine content of serum and urine of rabbits after excessive ingestion of fluoride was also investigated.

2. There was a progressive decrease in GAG content, reduction in hydroxylysine, whereas the hydroxyproline content was increased after fluoride ingestion.

3. Enhanced hydroxyproline in urinary excretion is due to collagen breakdown after fluoride ingestion.

4. The reduction in hydroxylysine content is due to reduced collagen cross-link formation.

5. The report suggests the possibility of using the urinary levels of GAG or hydroxyproline or hydroxylysine as an index of fluoride intoxication.

Key words: fluoride intoxication, rabbit, urine, serum, glycosaminoglycans, hydroxyproline, hydroxylysine.

INTRODUCTION

Fluorosis is endemic in several parts of India where the earth's crust is highly enriched in fluoride-containing mineral, polluting potable water and food. Excessive ingestion of sodium fluoride through potable water and food is known to cause skeletal and dental fluorosis. The manifestations viz: calcification of ligaments, joint pain, stiffness of the vertebral column, besides dental discolouration, pitting and chipping of the teeth are associated with the disease. Although dental fluorosis can be diagnosed from overt symptoms, skeletal fluorosis is diagnosed radiologically at

Correspondence: A. K. Susheela, Fluorosis Research Laboratory, Department of Anatomy, All India Institute of Medical Sciences, New Delhi 110029, India.

0305-1870/83/1000-0615 \$02.00 © 1983 Blackwell Scientific Publications

advanced stages of the disease. A test for the early detection of the disease does not exist as the molecular defects of the disease are still unexplored.

Recent studies conducted in our laboratory have provided evidence that in fluoride toxicity, collagen biosynthesis is significantly reduced (Susheela & Mukherjee, 1981). It has been shown that the collagen laid down is inadequately cross-linked (Susheela & Sharma, 1981). It is also known that following excess fluoride ingestion, trabecular bone is dedifferentiated to cartilage and excessive glycosaminoglycan content is known to occur in both cancellous and cortical bones (Jha & Susheela, 1982; Susheela & Jha, 1981, 1982). Collagen protein and glycosaminoglycans are associated in laying down the bone matrix. As changes in collagen and glycosaminoglycan content of bone have been observed, it was considered meaningful to investigate the status of the two amino acid residues of collagen protein: hydroxyproline and hydroxylysine in urine. The report also reveals the status of glycosaminoglycans in urinary excretion.

METHODS

Adult, healthy rabbits were maintained under laboratory conditions, fed normal pelleted diet (Hindustan Lever, Bombay) and 10 mg NaF/kg body weight through an intragastric tube, at 24 h for 60 days. The animals were maintained in metabolic cages. The cages were cleaned daily and the urine samples collected over a period of 24 h (1000 h to 1000 h) on day 15, 30, 45 and 60 were analysed. Urine samples collected in a similar manner from rabbits which were not administered with NaF were used as controls.

Estimation of glycosaminoglycan (GAG) in urine

Glycosaminoglycan content of urine was estimated according to the Alcian Blue method of Gold (1979). The results are expressed as mg GAG/24 h urine.

Estimation of hydroxyproline in urine

Urine samples were hydrolyzed in 6 N HCl at 110°C for 10 h in sealed tubes and neutralized with KOH. Hydroxyproline from the hydrolysate was estimated by method of Kivirikko, Laitinen & Prockop (1967).

Estimation of hydroxylysine content in urine and serum

Hydroxylysine content of urine was estimated after the animal had been intoxicated with NaF for a period of 10 months; 24 h urine sample was collected from normal and sodium fluoride ingested rabbits. Following acid hydrolysis, hydroxylysine estimation was carried out by thin layer chromatography and periodate oxidation according to the method described by Blumenkrantz & Prockop (1971). Hydroxylysine was also estimated in serum of rabbits intoxicated with sodium fluoride.

RESULTS AND DISCUSSION

The results obtained for urinary GAG and hydroxyproline contents show that hydroxyproline excretion is increased while GAG excretion is decreased after fluoride ingestion (Table 1). It is noteworthy that after fluoride ingestion, the total GAG content was enhanced in human and rab-

Table 1. Urinary excretion of total hydroxyproline and glycosaminoglycans from normal and fluoride treated rabbits

	Control		15 days		30 days		45 days		60 days	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Hydroxyproline (5)	4.36	1.04	6.70	1.52*	14.11	2.36*	30.5	1.12*	37.6	3.82*
Glycosaminoglycans (5)	26.97	2.41	24.8	1.79†	18.5	2.09*	8.9	1.5*	11.52	1.12*

Data expressed as mg/24 h. Number in parentheses indicates the number of experiments carried out. * $P < 0.001$, † $P < 0.01$.

bit serum (Jha *et al.*, 1982). This may be due to diminished urinary excretion. However, it is interesting to note that unlike hydroxyproline the hydroxylysine content was reduced both in urine and serum. The reduction was significant at $P < 0.05$ (Table 2).

An earlier report on collagenolytic activity has shown increased hydroxyproline content in a variety of rabbit tissues (Susheela & Sharma, 1982). The enhanced hydroxyproline level in urinary excretion confirms the collagen breakdown due to fluoride intoxication.

Table 2. Hydroxylysine content in urine and serum

	Normal		Fluoride treated		<i>P</i> value
	mean	s.d.	mean	s.d.	
Urine	57.19*	13.20	20.64†	6.40	<0.05
Serum	75.00‡	6.25	34.06*	3.75	<0.05

Data expressed as $\mu\text{g}/100 \mu\text{l}$. * Mean of four experiments; † mean of seven experiments; ‡ mean of three experiments.

Hydroxylysine is associated with collagen cross-link formation (Bailey & Peach, 1968; Mechanic & Tanzer, 1970). Studies conducted on collagen cross-link precursors in fluoride toxicity in a variety of rabbit tissues have shown reduced saturated aldehyde content (Susheela & Sharma, 1981). The present observation on hydroxylysine content is consistent with the earlier report that due to fluoride intoxication, the collagen protein is inadequately cross-linked and the concentration of the amino acid residue in urine and serum is significantly reduced.

This report suggests the possibility of using either urinary levels of amino acid residues specific to collagen protein viz hydroxyproline and hydroxylysine or glycosaminoglycan levels as an index of fluoride intoxication.

ACKNOWLEDGMENT

This work has been financed by the grants made available by the Department of Environment, Government of India and the International Development Research Centre, Canada.

REFERENCES

- Bailey, A.J. & Peach, C.M. (1968) Isolation and structural identification of a labile intermolecular crosslink in collagen. *Biochemical and Biophysical Research Communications*, **33**, 812-819.
- Blumenkrantz, N. & Prockop, D.J. (1971) Quantitative assay for hydroxylysine in protein hydrolysates. *Analytical Biochemistry*, **39**, 59-64.
- Gold, E.W. (1979) A simple spectrophotometric method for estimating GAG. *Analytical Biochemistry*, **99**, 183-188.

- Jha Mohan & Susheela, A.K.** (1982) *In vivo* chondrogenesis and histochemical appearance of dermatan sulphate in rabbit cancellous bone. *Differentiation*, **22**, 235.
- Jha Mohan, Susheela, A.K., Neelam Krishna, Rajyalakshmi, K. & Venkiah, K.** (1982) Excessive ingestion of fluoride and the significance of the sialic acid upon glycosaminoglycans in serum of rabbit and human subjects. *Clinical Toxicology* (in press).
- Kivirikko, K.L., Laitinen, O. & Prockop, D.J.** (1967) Modification of a specific assay for hydroxyproline in urine. *Analytical Biochemistry*, **19**, 249.
- Mechanic, G. & Tanzer, M.L.** (1970) Biochemistry of collagen cross-linking isolation of a new cross-link, hydroxylysinohydroxynorleucine, and its reduced precursor, dihydroxynorleucine, from bovine tendon. *Biochemical and Biophysical Research Communications*, **41**, 1597-1604.
- Susheela, A.K. & Mukherjee, D.** (1981) Fluoride poisoning and the effect on collagen biosynthesis of osseous and non-osseous tissues of rabbit. *Toxicological European Research*, **3**, 99-104.
- Susheela, A.K. & Sharma, Y.D.** (1981) Effect of fluoride on collagen cross-link precursors of rabbit tissues. *IRCS Medical Science Biochemistry*, **9**, 862.
- Susheela, A.K. & Jha Mohan.** (1981) Effects of fluoride on glycosaminoglycans of cancellous and cortical bone of rabbits. *Experientia*, **37**, 1097-1098.
- Susheela, A.K. & Jha Mohan.** (1982) Fluoride ingestion and its influence on glycosaminoglycans in cancellous and cortical bone—a structural and biochemical study. *Fluoride*, **15**, 191.
- Susheela, A.K. & Sharma, Y.D.** (1982) On certain facets of fluoride action on collagen protein in osseous and non-osseous tissues. *Fluoride*, **15**, 177.