CHANGES IN THE COLLAGEN STRUCTURE OF BONE TISSUE IN EXPERIMENTAL FLUOROSIS

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SUMMARY: The changes in the regularity of collagen structure of the corticalis and spongiosa of rat femur and vertebrae, caused by daily intraperitoneal administration of 0.5 mg and 5 mg sodium fluoride, were investigated. Daily administration of 0.5 mg NaF for three months produced a slight, but significant change in the regularity of collagen fibrils; 5 mg NaF/day, a significant decrease in the regularity and disintegration of collagen fibrils. Alteration in the regularity of collagen fibrils is a part of complex disturbances of the fluorotic bone, explained by the toxic effect of fluoride.

KEY WORDS: Experimental fluorosis; Collagen structure; Disorientation of preexisting bone.

Introduction

According to experience with humans, about 10% of the entire preexisting bone tissue is reorganized in the course of one year. This perpetual process of rebuilding and remodeling bone tissue is due to the action of multi-cellular functional units (BMU, BRU or BSU), consisting of osteoclasts and osteoblasts. It is generally accepted that sodium fluoride causes enlargement of the whole bone mass.

The question arises how NaF influences bone tissue, whether enlargement of bone mass is due to increased bone formation (stimulation of osteoblasts) (1-7) and/or to decreased bone absorption (blockade of osteoclasts) (5,8-14). Authors agree that newly-formed is inferior to normal bone, the matrix is irregular (1,7,11,15), the collagen structure of newly-formed bone tissue differs from normal (11), and that mineralization is enhanced (1,3,5,7,11,12).

The aim of our experiments was to investigate the changes of collagen structure in experimental fluorosis.

Material and Methods

Forty-five female rats, each weighing 200 grams, were divided into three groups; 15 animals were given 0.5 mg, 15 received 5.0 mg NaF intraperitoneally, daily, for three months; 15 animals – the control group – received physiological saline solution in the same way.

X ray pictures were taken of the sacrificed animals (Figures 1a,1b,1c).

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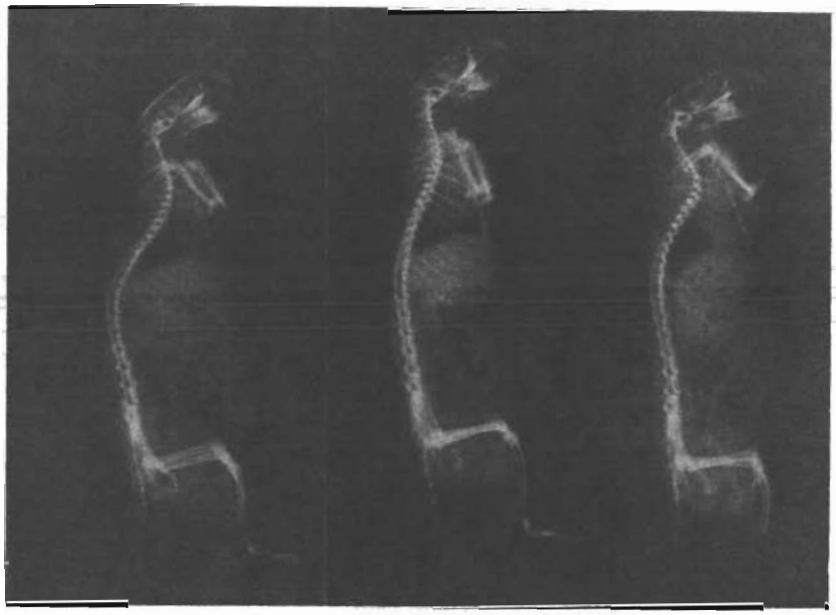


Figures 1a, 1b, 1c.

Lateral radiograph of controls and rats treated with 0.5 mg NaF. Apparent changes cannot be disclosed compared to controls. Five mg NaF daily for three months caused mainly the enlargement of the lumbar vertebrae. The thickening of corticalis of vertebrae, and formation of spicules are apparent.

Both femurs and the third, fourth and fifth lumbar vertebrae of the animals were investigated histologically, fixed in 10% formaline solution, and decalcified. The decalcifying agent consisted of 24 mL 85% formic acid, 50 mL 35% hydrochloric acid, and 125 mL distilled water. The material was embedded in paraffin, serially sectioned, and stained with picrosirius red (16).

The regularity of collagen fibrils of the preexisting bone tissue was measured by a polarized optic method according to Brace-Kohler in 550 nm monochromatic light using an Opton Standard microscope. Measurements were performed on the corticalis and spongiosa of both femurs and vertebrae using five visual fields in each case; 10 measurements were made in all fields. Average retardation values, characterizing the regularity of collagen fibrils, were calculated. Analysis of significance was performed between the retardation values obtained according to T and Welch (modified T) tests (Figures 1a,1b,1c).

Results

Retardation values, measured in the spongiosa and corticallis of the femurs and vertebrae, are presented in Table 1.

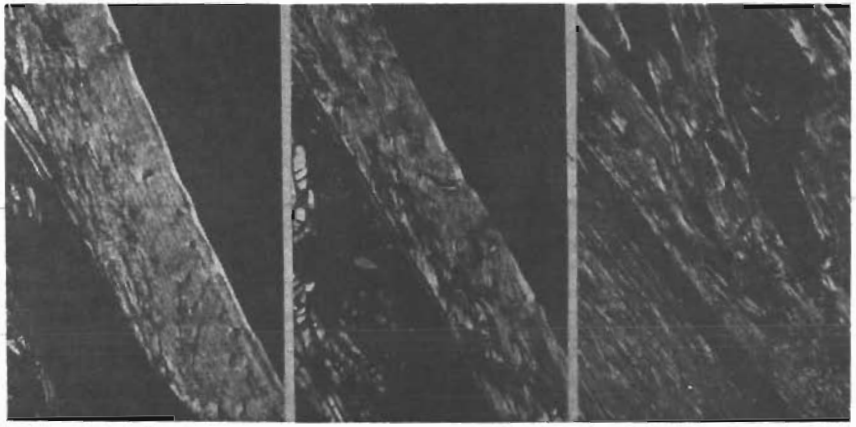
Table 1

Retardation of Collagen Fibrils in the Corticalis and Spongiosa of Femurs and Vertebrae

	FEMUR		VERTEBRA	
	CORTICALIS	SPONGIOSA	CORTICALIS	SPONGIOSA
Control	0.7083 ±0.0240	0.6780 ±0.0304	0.6901 ±0.0261	0.6878 ±0.0393
0.5 mg NaF	0.6078 ±0.0281	0.5754 ±0.0531	0.6153 ±0.0298	0.5950 ±0.0230
5 mg NaF	0.4390 ±0.0408	0.4284 ±0.0444	0.4212 ±0.0328	0.4275 ±0.0385

Control Σ 0.6910 ±0.0332; 0.5 mg NaF Σ 0.5991 ±0.0376; 5 mg NaF Σ 0.4275 ±0.0385

The regularity of collagen fibrils in the corticalis and spongiosa of femurs and vertebrae decreased compared to normal (Figure 2a) in rats which received 0.5 mg NaF daily. The observed difference is significant (Figure 2b). On rats which received 5 mg NaF daily, for three months, the regularity of collagen fibrils decreased significantly compared to normal in the corticalis and spongiosa on both femur and vertebrae (Figure 2c).



Figures 2a, 2b, 2c.

Histologic picture of the diaphysis of femur of a control (a) rat treated daily with 0.5 mg NaF (b), and 5.0 mg NaF (c).
2a: Well differentiated, lamellar bone.
2b: Effect of 0.5 mg NaF collagen structure of preexisting bone tissue slightly disoriented, disintegrated.
2c: Significantly disoriented, disintegrated; effect of 5.0 mg NaF.

The intercellular matrix of bone tissue consists of a collagen structure, embedded in proteoglycan aggregates. The process of formation and mineralization of the intercellular matrix are closely related. Isolated injury of any of these components is inconceivable: injury to one of the components is always associated with injury to the others. During the recent investigation irregularity of collagen structure of preexisting bone tissue could be disclosed by a specific topooptic method.

Conclusion

The investigations disclosed that fluoride causes the regularity of the collagen structure of preexisting, differentiated, lamellar bone to decrease; fluoride exerts its effect not only on the newly generated (newly formed woven) bone tissue, but it also changes the collagen structure of preexisting bone. In our opinion these changes can be considered part of the toxic effect of fluoride exerted on osteocytes. Changes in collagen structure are followed by damage to the matrix (proteoglycan aggregate). We are planning in future to direct our attention to this field of investigation.

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