EXPERIMENTAL FLUOROSIS IN RATS: NaF INDUCED CHANGES OF BONE AND BONE MARROW

by

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SUMMARY: According to recent studies, excess fluoride intake can cause both osteosclerosis and osteoporosis. The effect of fluoride can be influenced by several factors. In our experiments the aim was to demonstrate the alterations in bone and bone marrow caused by sodium fluoride, and to define the relation between the bone lesion and fluoride dosage.

Of 20 white female rats, body weight 200 gr., 10 were administered 0.5 mg NaF, the other 10, 5 mg intraperitoneally for two months. NaF-induced bone changes were analyzed on ribs, vertebrae and femur. The decalcinated specimens were fixed in 8% neutral formalin, imbedded in paraffin, serially sectioned and stained with HE, or investigated by polarization optic methods. Bone and bone marrow alterations were evaluated by the morphometric method.

KEY WORDS: Fluorosis in rats; Bone changes; Rats, fluorosis in; Bone marrow

Introduction

Prolonged ingestion of excessive fluoride induces osteosclerosis, subperiosteally newly formed bone, vertebral osteophytes and calcification of paravertebral ligaments resembling the changes of ankylosing spondylitis, but the classical radiological changes in the sacroiliac joints are absent (1). The osteosclerotic effects of fluoride have been explained on the basis of secondary hyperparathyroidism (2-5). The effect of fluoride can be influenced by several factors such as vitamin D, ascorbic acid, etc.

The aim of our experiments was to study the changes in bone and bone marrow caused by sodium fluoride and to determine the degree of changes related to the dose of the fluoride compound.

Material and Methods

Ten white female rats weighing 200 grams were given 0.5 mg and 10, 5 mg of sodium fluoride intraperitoneally daily for two months. Ten rats served as controls without treatment. The ribs, vertebrae, tibia and femur were removed, fixed in 8% formaldehyde solution and decalcinated in EDTA (0.1%, pH7, 4). 5 µm thick serial sections were cut from the paraffin-embedded samples and processed for hematoxylin-eosin staining.

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Results

In treated rats, the number of osteoclasts decreased in proportion to the dose of NaF whereas the number of osteoblasts increased (only locally, not generally), or did not change. The decrease in the number of osteo-

Figure 1

Figure 2

Remodeling Zone of Tibia, HE 12.5 x 4

0.5 mg NaF

5 mg NaF

With greater dose of NaF, osteoclast number decreased, corticalis sclerosed, subperiosteal border less "lacy."

Figure 3

Figure 4

Femur Head, HE 2.5 x 4 Magnification Refers to 24 x 36 mm Negative

Extended chondral areas in epiphysis a sign of inhibited enchondral ossification caused by greater NaF dose.

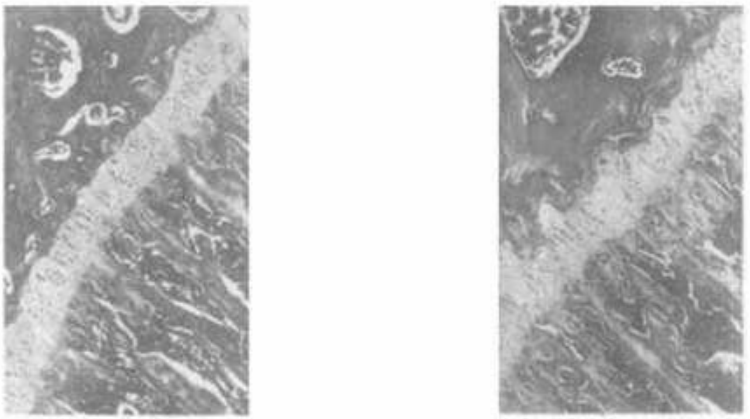
clasts was significant, particularly in the growth zone of the tibia (Figs. 1-2) and femur and in the area of enchondral ossification (Figs. 3-4). Osteoclerosis, following the treatment developed in proportion to the dose of NaF (Figs. 1-4).

Degenerative changes in proportion to the dose were noted in the epiphyseal cartilage: the chondrocytes lost their regular arrangement (Figs. 5-6).

Figure 5 Figure 6

Epiphyseal Cartilage from Tibia, HE 12.5 x 4

0.5 mg NaF 5 mg NaF



With greater NaF dose bone tissue sclerosed, osteocytes focally necrosed, arrangement of chondrocytes in some places irregular, chondral framework of primary spongiosa summarized in white line broadened as sign of inhibited ossification.

In some places the bone tissue became necrotic and the osteocytes lost their nuclear staining (Figs. 6-11). We have seen secondary fractures within the necrotic areas.

The hemopoietic tissue mass decreased in proportion to the dose of NaF (Figs. 7-8) and, in some places, the marrow was replaced by fibrotic connective tissue (Fig.10).

Osteophytes occurred mainly on the vertebral body, mostly in animals treated with larger doses. Only in a few cases were the osteophytes localized marginally (Fig. 9). In the majority of cases the osteophytes were present in the middle third corticals of the vertebral bodies. We have seen newly formed bone subperiosteally as well (Fig. 10).

Figure 7

Figure 8

Ribs, HE 12.5 x 4

0.5 mg NaF

5 mg NaF

Hemopoietic tissue mass decreased, bone tissue sclerosed in proportion to NaF dose.

Figure 9

Tibia - Osteophyte HE 12.5 x 4

5 mg NaF

Figure 10

Subperiosteal New Woven Bone Formation HE 12.5 x 4

5 mg NaF

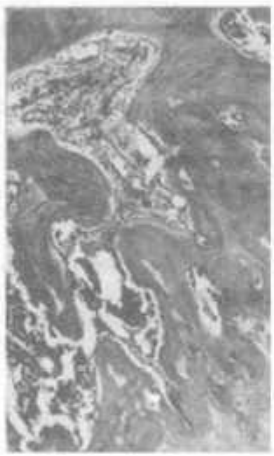
Focal calcifications were seen in paravertebral ligaments, in intervertebral discs and in the cortical Haversian canals (Fig. 12).

The articular cartillage and particularly the lower calcified zone became enlarged in proportion to the dose of NaF.

Figure 11

Femur Spongiosa, HE 12.5 x 4

5 mg NaF

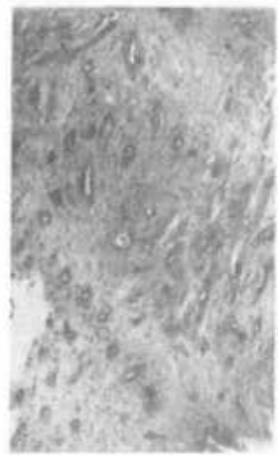


According to greater NaF dose cancellous bone sclerosed, osteocytes focally necrosed, marrow replaced by fibrotic connective tissue

Figure 12

Vertebra Corticalis, HE 12.5 x 4

5 mg NaF



Mottled calcification in Haversian canals.

Discussion

The results of our experiments suggest that increased doses of NaF cause more extensive osteosclerosis due to the decrease in number and/or activity of osteoclasts. Therefore osteosclerosis is caused primarily, not by increased bone formation but, by the inhibition of bone resorption. This view is supported by the fact that fluoride inhibits acid phosphatase activity more than alkaline phosphatase (6). The acid phosphatase activity of osteoclasts is of greater intensity than that of osteoblasts and the alkaline phosphatase activity of osteoblasts is of greater intensity than that of osteoclasts. So fluoride inhibits the osteoclasts more than the osteoblasts.

In addition, the metabolism of osteoclasts is of greater intensity

than that of osteoblasts (7,8). Therefore osteoclasts are more sensitive to fluoride poisoning, than osteoblasts.

Osteosclerosis, caused by fluoride, is really the result of a toxic effect which is reflected by the dose dependent decrease of osteoclasts and of the hemopoietic elements, the irregular arrangement of the epiphyseal cartillage chondrocytes and also by skeletal necrosis.

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PRODUCTION OF FLUOROACETATE BY CALLUS TISSUE FROM LEAVES OF Acacia georginae

by

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SUMMARY: Callus cultures of Acacia georginae were initiated from leaf discs from young leaves. Growth of callus was slow but predictable with tissue volumes up to 2.2 cm^3 being formed. Fluoride concentrations up to 80 ppm in the medium produced no adverse effect on callus growth. Reversible growth inhibition occurred at 160 ppm, whereas apparent death occurred at higher concen-

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