SCANNING ELECTRON MICROSCOPY OF THE RAT FEMUR AFTER FLUORIDE INGESTION

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SUMMARY: Anorganic preparations of femurs from rats given 150 ppm fluoride in the drinking water for 10 weeks reveal an increase in periosteal matrix and woven bone formation with a concomitant decrease in endosteal resorptive activity. However, resorption of metaphyseal trabeculae is increased coupled with an inhibition of calcification in the epiphyseal plate.

KEY WORDS: Electron microscopy, rat femur; rats, F⁻ effect on femur

Introduction

Skeletal homeostasis, i.e., the maintenance of bone structure, is altered in fluorosis. Fluoride is known to increase crystal size and crystal perfection in biological apatites, and to reduce mineral solubility (1). As a consequence, fluoride inhibits bone resorption and promotes the stabilization of newly synthesized bone matrix (2,3). These changes may account for the reduced loss of calcium from bone with a high fluoride content (2). Thus, mineral homeostasis is altered in fluorosis as well.

It has been shown that the state of activity of bone can be diagnosed by direct examination of bone surfaces in the scanning electron microscope (4,5). Since the structure of bone is the result of the activities of cells lining bone surfaces, a disturbance of bone cell function is reflected in changes of bone surface structures (6). Therefore, the objective of this study was to examine by scanning electron microscopy the initial effects of short-term fluoride ingestion on the periosteal, endosteal and metaphyseal surfaces of the rat femur.

Materials and Methods

Young adult male Sprague-Dawley rats were divided into control and experimental groups of 12 animals each. The control group was given distilled drinking water and the experimental group was given distilled drinking water to which 150 ppm fluoride as sodium fluoride was added. All animals were given the appropriate water ad libitum for 10 weeks.

At the end of the experimental period, all rats were sacrificed by ether overdose, and the femurs were dissected free from both extremities. The proximal and distal epiphyses were removed; the diaphyses and distal epiphyses were then longitudinally sectioned. The organic matrix of the bone specimens was removed by immersing the bones in 5% sodium hypochlorite solution for 4 hours, leaving the mineral surface exposed. The an-

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Electron Microscopy of Rat Femur after F⁻ Ingestion

**Figure 1**
Periosteal Surface of Femoral Mid-Diaphysis

Woven bone formation (x 320)

**Figure 2**
Endosteal Surface of Femoral Mid-Diaphysis

Mineralization front (x 320)

**Figure 3**
Epiphyseal Plate Showing Lacunae in Zone of Mature Hypertrophied Cells

Arranged in irregular nests (x 640)

**Figure 4**
Characteristic Coarse and Rough Surface of Metaphyseal Trabeculae

(X 320)

It is of interest that in the skeleton of individuals with hyperparathyroidism, loss of synchronized collagen deposition is reflected by the loss of lamellar bone structure and by its replacement with woven bone (9-11). It appears that bone surface cells, stimulated by excess parathyroid hormone, escape the normal control mechanisms which regulate bone remodel-
organic specimens were rinsed in distilled water, air dried, and mounted on stubs. Specimens were coated with gold-palladium and examined with a Philips 500 scanning electron microscope. All surfaces of the diaphyses were examined except the areas of muscle attachment on the periosteal surfaces and the areas opposite the muscle attachment sites on the endosteal surfaces.

Results

In the fluoride-treated rats, the surface features of the periosteum of the femoral diaphyses are characteristic of woven bone formation (Fig. 1). Apposition areas appear uneven and frayed, consisting of bunches of poorly-defined needles. The uneven surface is a reflection of the underlying irregular orientation of the collagen fiber bundles. The mineralized segments vary in size with wide gaps in between, suggesting a delay in the rate of mineralization at the periosteum. Numerous osteocyte lacunae buried at various depths are evident, and the lacunar walls are irregular with mineralized segments running in all directions. These features are in contrast to the ordered arrangement of both the collagen fiber bundles and osteocytes seen in typical lamellar bone formation of the untreated animals.

On the endosteal surfaces of the diaphyses, apposition areas as well as fully mineralized areas appear similar in both groups (Fig. 2). However, well-defined resorption areas are decreased in the fluoride-treated rats. The few Howship's lacunae present on the endosteal surfaces are shallow and poorly formed.

Longitudinal sections of the distal epiphyses reveal an increase in the zone of mature hypertrophied cells of the epiphyseal plates as compared with controls. The lacunae in this zone vary considerably in size and shape, and are arranged in irregular nests rather than in parallel rows (Fig. 3). Within the cavity of the shaft, the amount of cancellous bone is reduced, particularly in the central areas of the metaphyses where the network of trabeculae is sparsely distributed. The trabeculae located in the more peripheral areas of the metaphyses are broad and heavy in appearance (Fig. 4). Areas of fully or partially mineralized bone are markedly reduced. The bone surfaces are rough, irregular and covered with numerous Howship's lacunae.

Discussion

The surface features of the femurs from fluoride-treated rats indicate an increase in periosteal matrix and bone formation along with an inhibition of mineralization. Similar histological findings have been reported in rats treated with 100 ppm fluoride in the drinking water for 16 weeks (7). Furthermore, in the present study, the periosteal surface features are suggestive of immature, woven bone. This type of bone is characteristic found in both cortical and cancellous bone of growing animals but, during normal maturation, it is gradually replaced by lamellar bone. In the adult, however, woven bone is usually indicative of high rates of bone turnover, due to either local or systemic factors (8). Because of its organization, there is no distinct calcification front in woven bone as in lamellar bone, and mineralization occurs in a haphazard
ing. As a result, in hyperparathyroid bone disease, osteoblasts lose their ability to deposit collagen in preferential directions by the coordinated action of groups of cells. It is of further interest that direct and indirect evidence for secondary hyperparathyroidism has been reported in humans (12-15) and in animals (7, 14, 16-18) following fluoride administration. Based on these studies, it is tempting to assume that the altered periosteal bone formation seen in the present study is entirely due to increased parathyroid hormone secretion. Although there is ultrastructural evidence suggesting secondary hyperparathyroidism in rats following short-term fluoride ingestion (19), experiments in which bone organs were cultured in a medium containing fluoride have demonstrated a decrease in the rate of bone collagen synthesis (20). Therefore, it seems that fluoride, in addition to causing secondary hyperparathyroidism, may also have some direct effect on organic matrix production by osteoblasts.

In contrast to the periosteal surface, the endosteal surface in normal bone is covered by osteoblasts and osteoclasts which are engaged in modeling processes. As seen by scanning electron microscopy, fully mineralized areas and apposition areas of the endosteal surface are similar in both untreated and fluoride-treated rats. A significant difference, however, is the absence of well-defined resorption areas in the fluoride-treated rats. Studies of the long-term effects of fluoride ingestion on bone reveal an increase in endosteal resorption (7). In the present study, which deals with the short-term effects of fluoride ingestion, endosteal bone resorption was not increased, but rather decreased. This observation can be explained by the fact that the initial increase in bone resorption appears to occur in trabecular bone. A similar reduction in trabecular bone has been reported in pigs (21).

Cellular activity at the endosteal surface of cortical bone differs from that at the surface of trabecular bone in several respects. In trabecular bone, remodeling by bone surface cells is related to the adaptation of skeletal structure to load, and to the homeostasis of serum calcium. In contrast, at the endosteal surface, bone surface cells are involved in bone remodeling which mediates changes of bone shape and bone mass. Moreover, the surface to volume ratio of trabecular bone is large in comparison to dense cortical bone, and its rate of remodeling is higher. This means, all other factors being equal, that it is more reactive metabolically. For these reasons, changes in bone structure due to a variety of metabolic or local diseases are often reflected first in areas of trabecular bone (8). Furthermore, rats are highly efficient in their retention of calcium (22) so that mineral turnover occurs by remodeling of metaphyseal trabecular bone. Therefore, a pronounced increase in endosteal resorption would be expected to occur only after long-term fluoride treatment in the rat.

These findings are consistent with the hypothesis that the metabolic function of bone-resorbing cells is inhibited when the cells are exposed to high fluoride concentrations (23). This hypothesis assumes that fluoride exerts its effect by preferential accumulation in the bone mineral at osteocyte lacunar and canalicular surfaces which are adjacent to the extracellular fluid. This accumulation of fluoride is similar to other bone-seeking isotopes such as calcium, since high fluoride incorporation
occurs in bone deposited during fluoride administration, whereas bone existing prior to fluoride administration shows only a small amount of fluoride (24, 25). Any cells which resorb bone in these areas would thereby be exposed to a concentration of fluoride sufficiently high enough to potentially inhibit normal cellular processes. The inhibition of resorptive function together with the decreased level at which bone and serum calcium equilibrate after the incorporation of fluoride would lead to a fall in serum calcium and a compensatory increase in parathyroid hormone secretion. This rise in serum parathyroid hormone would stimulate the differentiation of progenitor cells into both osteoblasts and osteoclasts.

Based on the results of the present study along with a knowledge of the influence of fluoride on cellular processes and on mineral dissolution, it is possible to draw conclusions as to the initial sequence of events following fluoride ingestion in the rat. Since resorption is blocked in newly formed bone on the endosteal surfaces of cortical bone, resorption of normal non-fluoride-containing trabecular bone formed prior to fluoride ingestion increases in order to maintain the serum calcium at or near normal levels. Meanwhile, on the periosteal surfaces, the total number of osteoblasts is increased resulting in a cell-rich periosteum. However, the individual osteoblasts are less active because of the secondary hyperparathyroidism. Consequently, a less organized, more cellular woven bone is produced at the periosteal surface.

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**FLUORIDE BRIEF**

Sodium fluoride (10 or 20 mg/kg), administered to male Wistar rats, fed a standard diet, during 6 and 10 months resulted in proliferation of periosteal cells in iliac bone and fibrosis. In another group of animals treated with 10 mg/kg fluoride and calcium carbonate (CaCO₃), periosteal reactions were less pronounced.

Fatty degeneration of hepatocytes was found in the group receiving 10 and 20 mg/kg of NaF. Histological changes were observed likewise in kidneys; histochemical reactions, under the influences of NaF, were altered.

KEY WORDS: Experimental fluorosis; Bone histology, Liver and kidney changes.


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