COMPRESSIVE STRENGTH OF CANCELLOUS BONE IN FLUORIDE-TREATED RATS

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SUMMARY: The effect of different fluoride concentrations in drinking water on compressive cancellous bone strength in the appendicular skeleton of young growing rats was studied. Twenty eight 6-week-old female Wistar rats were randomized into four groups. One group was given distilled water and the other three groups were exposed to fluoridated water at different concentrations (8, 30 and 60 mg F^-/L). After six weeks all rats were sacrificed and the mechanical penetration tests of the distal femoral bone were performed. The results of this study show that fluoride level at 8 mg F^-/L in drinking water increases compressive strength of cancellous bone in the appendicular skeleton in growing rats.

Keywords: Appendicular skeleton, Axial skeleton, Bone strength, Cancellous bone, Compressive strength, Fluoride exposure, Growing rats, Penetration forces, Penetration work.

INTRODUCTION

Fluoride has profound effects on the skeleton, some apparently beneficial and others definitely detrimental.[1] Although numerous studies have shown that fluoride can increase cancellous bone mass,[1-3] almost 40 years of experimental and clinical research have failed to minimize the deleterious effects of F^- on the whole skeleton.

Some studies have even reported an increase in hip fracture occurrence during and after therapeutic exposure to fluoride.[4-6]

Many studies showed a significant increase of bone mineral density (BMD) in the spine (mostly cancellous bone) of patients treated with fluoride salts.[7-9] On the other hand, some research groups found that fluoride-related increase in spinal BMD fails to decrease the number of new vertebral fractures in osteoporotic individuals.[3,10,11]

Bohatyrewicz[12] reported a significant correlation of fluoride concentration in drinking water with increased femoral bone mineral mass in growing rats exposed to fluoride at levels of 8, 30, and 60 mg F^-/L. Measurement of bending strength of the femoral shaft and failure load of the femoral neck demonstrated the detrimental effect of high fluoride intake on cortical bone quality.[12]

In order to investigate the relationship between fluoride intake and mechanical strength of cancellous bone in the appendicular skeleton of the rat, we have introduced the original osteopenetrometer which measures the force of penetration of a needle into the distal rat femur bone.

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MATERIALS AND METHODS

Animals and fluoride administration: The study comprised 28 female Wistar rats, approximately 6 weeks old at the beginning of the investigation. All rats were housed, three or four to a cage, under similar conditions at 18-24°C in a 12-hr dark-light cycle and maintained with unlimited amounts of standard laboratory pellet diet, containing: calcium 1120 mg/kg, phosphate 760 mg/kg, and fluoride 0.7 mg/kg. The four groups were differentiated by the varying concentrations of fluoride in the drinking water. The concentrations used were 0 (control), 8, 30 and 60 mg F^-/L. Drinking water was given *ad libitum*.

The rats were randomized into four groups based on their body weights, so that the mean body weight of each group was comparable.

The experiment was conducted for 6 weeks. The experimental animal procedures performed in this study were approved by the Animal Care Committee of the Pomeranian Academy of Medicine in Szczecin.

Bone specimens: At the end of experiment, the rats were anesthetized intraperitoneally with ketamine hydrochloride (50 mg/kg) and sacrificed by exsanguination. As in our earlier study,[12] the femurs were extracted and frozen in saline-soaked tissues. Prior to mechanical testing, the bones were slowly thawed overnight at 7° C and held at room temperature on the day of testing.

Mechanical testing: To perform the penetration measurement, the distal part of the femur was embedded in polymethyl methacrylate with a vertical orientation (Figure 1). The penetrometer consisted of a hydraulically powered 1.0-mm diameter needle that was advanced at constant speed (0.155 mm/s) through the surface and spongy distal part of the bone specimen. To reduce friction the needle had the end rounded. During penetration of the bone a recording was obtained of the force of penetration as a function of the depth of penetration.[13] The ultimate penetration strength was recorded; the penetration strengths from 2.0, 3.0, and 4.0 mm depth were recorded and used to calculate the mean penetration strength; and the energy absorption from 2.0 to 4.0 mm of penetration was evaluated. A typical penetration curve is shown in Figure 2.

The specimens were tested using a servo-controlled electromechanical system Instron 6022 (Instron Corp., High Wycombe, England).

Statistical analysis: Data were compared by means of nonparametric tests (Kruskal - Wallis test followed, if significant, by group comparisons with the Mann - Whitney U test). Differences were considered significant if $P < 0.05$. The results were expressed as means ± standard deviation (Mean ± SD).

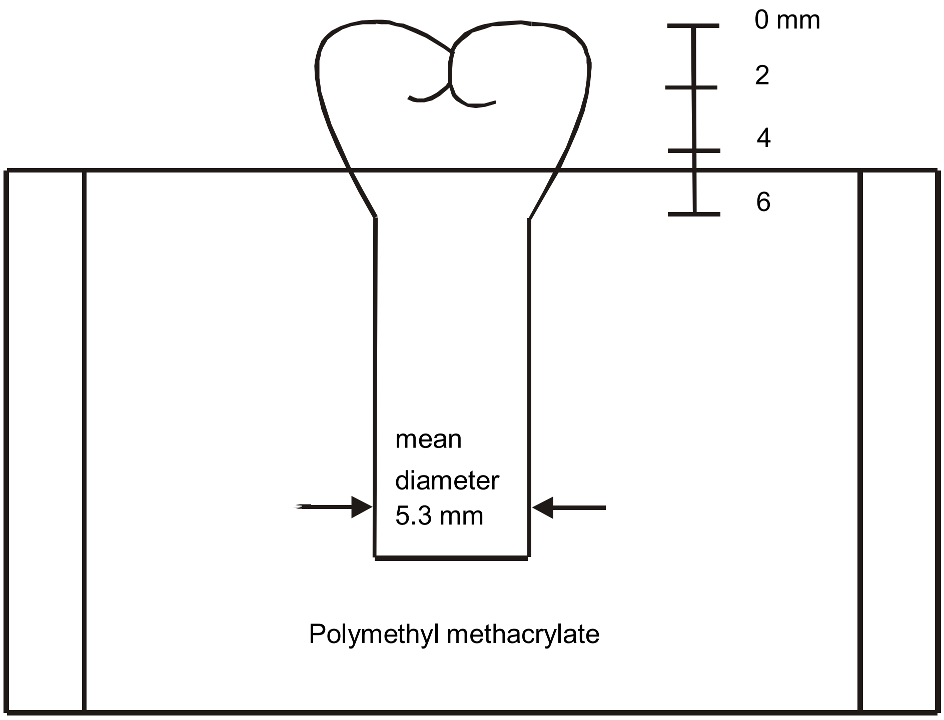

Figure 1. Rat bone prepared for penetration measurement.

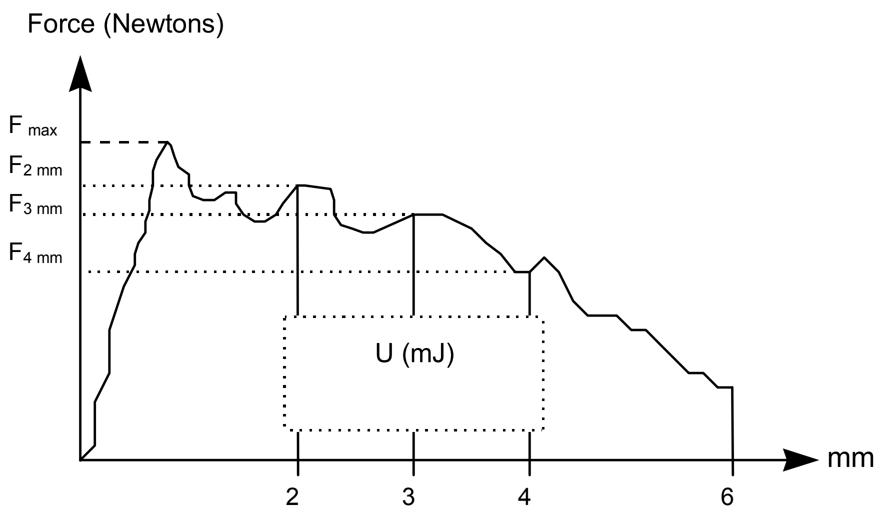

Figure 2. The typical load penetration curve - F_{max} - ultimate penetration strength (N), F_{mean} - mean penetration strength by 2, 3 and 4 millimeters (N), U - energy absorption (mJ).

RESULTS

After six weeks the highest values of all measurements such as maximal penetration force, mean penetration strength, and energy absorption were found in the group exposed to fluoride at a concentration of 8 mg F^{-}/L (Table). The maximal penetration force of the group treated with 8 mg F^{-}/L was significantly higher than in the control group and in the 60 mg F^{-}/L group. The mean penetration strength was significantly higher in the 8 mg F^{-}/L group than in the control group and the 60 mg F^{-}/L group. The penetration work of the group exposed to 8 mg F^{-}/L was also significantly higher than in the control group.

TABLE. Mechanical properties of the trabecular bone

Group	Ultimate penetration strength (Newtons)	Mean penetration strength (Newtons)	Energy absorbed (mJ)
Control	95.8 ± 9.46	62.4 ± 7.71	297.2 ± 45.9
8 mg F^{-}/L	104.2 ± 8.02	72.8 ± 7.97	362.6 ± 43.8
30 mg F^{-}/L	97.5 ± 8.75	70.3 ± 9.88	343.0 ± 45.2
60 mg F^{-}/L	94.8 ± 6.58	64.2 ± 7.23	329.4 ± 43.5

Values are mean ± standard deviation.
Values in following groups are significantly different: ultimate penetration strength: 8 mg F^{-}/L *vs* controls: $P < 0.05$; 60 mg F^{-}/L *vs* 8 mg F^{-}/L: $P < 0.01$; mean penetration strength: 8 mg F^{-}/L *vs* controls: $P < 0.01$; 60 mg F^{-}/L *vs* 8 mg F^{-}/L: $P < 0.05$; energy absorption: 8 mg F^{-}/L *vs* controls: $P < 0.001$.

DISCUSSION

This study has shown that exposure to fluoride in drinking water increases the compressive strength of cancellous bone in growing rats only at a concentration 8 mg F^{-}/L. Higher concentrations decrease the cancellous bone strength when compared to 8 mg F^{-}/L. This observation corresponds to that of Turner *et al*[14] and agrees with an earlier study by Bohatyrewicz[12] that bone strength follows a biphasic relationship with bone fluoride content.

The anabolic action of fluoride demonstrated in the form of increased bone mineral mass of whole femoral bones reported by Bohatyrewicz et al.[15] was not paralleled here by an increase of the mechanical strength of cancellous bone of the distal femur in present study. These experimental observations are similar to those of controlled clinical trials demonstrating a rapid and significant increase in spinal BMD after treatment with fluoride salts without an expected reduction in vertebral fracture rate.[10,11]

Fluorotic bone is more resistant to compressive forces, but more easily fractured by bending and torsional forces.[14,16,17] Femoral neck and shaft fractures representing appendicular skeleton fractures mostly occur from a combination of torsion and bending, whereas spinal fractures representing axial skeleton fractures generally occur from compression and tension forces. This fact might possibly explain why the experimental intensive exposure to fluoride fails to show the detrimental effect on the axial skeleton to the same extent as on the appendicular skeleton.

We conclude that when fluoride is administered in lower concentrations (8 mg F^-/L), it increases the compressive strength of cancellous bone in the appendicular skeleton of growing rats. However, higher fluoride concentrations (30 and 60 mg F^-/L) decrease the mechanical strength of cancellous bone compared to 8 mg F^-/L.

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