

The Effects of Various Fluoride Concentrations on New Bone Formation in Intramuscular Implants of Bone Matrix in Mice

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Summary

The efficacy of fluoride therapy of osteoporosis remains a controversial issue, specially in clinical trials. The effect of fluoride on heterotopic bone formation in mice was analyzed with respect to the dose of the drug administered. Thirty days after implantation of demineralized bone matrix, the ash weight and mineral content of implants from animals receiving the highest dose of fluoride was approximately 49 % lower than that of the controls. Thirty days of fluoride administration had no effect on blood composition. On the other hand, small concentrations of fluoride ions increased the dry and ash weight of implants (36 %) and promoted bone mineralization. The results derived from this study may be directly applicable to the treatment of osteoporosis with fluoride and support our clinical trials.

Key words

Heterotopic bone formation – Fluoride concentration – Mineral content of implants

Introduction

The anabolic action of fluoride on bone is well established. Fluoride alters the composition and crystalline state of bone minerals and stimulates matrix synthesis and bone formation (Pak and Zerwekh 1995). It has been shown to cause cellular proliferation, increase alkaline phosphatase activity and enhance collagen synthesis in isolated osteoblast-like cell preparations in culture (Farley *et al.* 1983).

However, the acceptance of fluoride as effective therapy for osteoporosis has been hampered by serious potential complications and by trials showing variable results. Excessive response of bone to fluoride can cause fluoride toxicity, characterized by the formation of bone that is poorly mineralized and mechanically defective (Sogaard *et al.* 1995).

The method of inducing heterotopic bone formation described by Urist *et al.* (1983) provides the means to study new bone formation in extraskeletal sites. Upon implantation of bone matrix, undifferentiated fibroblasts migrate and differentiate into chondroblasts and osteoblasts with formation of osteoid tissue.

The aim of this investigation was to analyze the possible effects of various fluoride concentrations on new bone formation in intramuscular implants of bone matrix in mice.

Method

Our studies were performed on female mice of the H strain (Velaz, Prague). The animals were fed a standard laboratory diet (Velaz Prague) containing 23 % protein, 1.2 % calcium, 0.6 % phosphorus and water *ad libitum*. Each of the control and experimental groups comprised 8 mice. Control animals received distilled drinking water and test animals received distilled water containing 1.0, 6.0 and 8.0 mmol fluoride per litre added as sodium fluoride.

Femora were collected from growing female H strain mice (30 g) and cleansed of soft tissues. They were decalcified for 48 hours at 4 °C in 0.6 N HCl, cut into appropriate pieces and emptied of bone marrow. The metaphyseal parts were discarded and the diaphyses were washed in cold water and freeze dried. Prior to this, they were defatted with ethanol at room temperature for one hour. The mean dry weight was 6 mg (4–8 mg). Bone-inducing ability was examined by

implantation tissue fragments into the back muscles of female mice in the control and experimental groups.

After 30 days of the experiment, blood was carefully withdrawn by cardiac puncture to determine the tartrate-resistant acid phosphatase (TrACP), plasma calcium, phosphate and creatinine. Plasma and bone calcium was determined by the method of Gitelman (1967), plasma and bone phosphate by the method of Kraml (1966) and plasma creatinine by the method of Hare (1950). The activity of plasma Tr-ACP (EC 3.1.3.2) was assessed with 4-nitrophenyl phosphate as substrate in a citrate buffer at 37 °C within 10 min after sampling (Štěpán *et al.* 1983).

Observations were made on the implants 30 days after implantation into muscle to determine the changes in chemical composition after the appearance of new bone. Measurements of the dry weight, ash weight, total calcium and phosphate content were made. The ash weight was obtained by weighing the solid residue after ashing at 600 °C. After dry ashing the residue was dissolved in HCl and analyzed for total calcium and phosphate.

The results are presented as means ± S.D. Significant differences between groups were determined by analysis of variance, followed by Duncan's (1955) multiple range tests.

Results

The results are summarized in Tables 1 and 2. Thirty days of fluoride administration had no effect on the growth of mice. There were no significant differences in plasma Tr-ACP, calcium, phosphate and creatinine levels between the groups of fluoride-treated mice and the group of intact mice.

The dry weight and ash content of implants were increased by 36 % in the 1 mmol/l fluoride group. However, they were slightly reduced by 22 % in the 6 mmol/l fluoride group and severely reduced by 49 % in the 8 mmol/l fluoride group in comparison with implants of the control group. The calcium and phosphate content of the implants was affected in a similar way as the dry and ash weights (Table 2).

Table 1
The body weight and blood composition in intact and fluoride-treated mice

| Fluoride (mmol/l) | Controls | 1.0 | 6.0 | 8.0 |
|-------------------|----------|-----------|-----------|----------|
| Dry weight (mg) | 14.2±1.2 | 19.4±2.1* | 11.1±1.4* | 7.2±2.1* |
| Ash weight (mg) | 8.4±1.0 | 11.2±1.1* | 7.3±1.2* | 4.8±1.1* |
| Calcium mg/ash | 3.2±0.4 | 4.3±0.6* | 2.8±0.5* | 1.8±0.6* |
| Phosphate mg/ash | 1.4±0.2 | 1.9±0.3* | 1.2±0.4* | 0.8±0.3* |

Values are means ± S.D. (n=8 in each group), * significantly different (p<0.01) from control mice

Table 2
Dry and ash weight as well as mineral content of implants from intact mice and those treated for 30 days with fluoride in different doses

| Fluoride (mmol/l) | Controls | 1.0 | 6.0 | 8.0 |
|----------------------------|----------|----------|----------|----------|
| Final body weight (g) | 34.0±1.5 | 34.1±0.8 | 33.2±1.8 | 32.8±1.6 |
| Plasma Tr-ACP (U/l) | 15.0±1.4 | 15.1±1.3 | 14.2±1.4 | 14.0±1.5 |
| Plasma calcium (mmol/l) | 2.2±0.09 | 2.2±0.07 | 2.1±0.07 | 2.1±0.08 |
| Plasma phosphate (mmol/l) | 4.2±0.12 | 4.2±0.10 | 4.1±0.12 | 4.1±0.14 |
| Plasma creatinine (μmol/l) | 78.2±7.0 | 75.8±7.5 | 76.9±6.9 | 77.8±6.2 |

Values are means ± S.D., (n=8 in each group), * significantly different (p<0.01) from control mice

Discussion

For more than two decades sodium fluoride has been a commonly used therapy for established

osteoporosis often in combination with calcium and vitamin D (Kleerekoper and Mendlovic 1993). Its ability to stimulate bone formation is well known. However, several studies have shown structural

abnormalities or mineralization defects in the bone formed during fluoride administration. The debate has arisen as to whether high doses of fluoride (70–80 mg daily) might be a toxic dose (Riggs *et al.* 1990, Fratzel *et al.* 1994). Thus the efficacy of fluoride therapy remains a controversial issue especially in clinical trials (Sogaard *et al.* 1994).

The present work demonstrates a relatively simple *in vivo* system in which completely decalcified bone induces mineral accretion. It is anticipated that the model will be useful for investigating the reactions of fluoride treatment on the formation of minerals.

In our experiments, fluoride ions in high concentrations inhibited the formation of new bone and decreased the mineralization of bone in comparison with the control group. On the other hand,

small concentrations of fluoride ions increased the formation of new bone and promoted bone mineralization. Our results are in accordance with the work of Einhorn *et al.* (1992) who reported high fluoride exposure reduced the mechanical strength of bone. Recently Lees and Hanson (1992) have shown improvement of bone quality of rabbit femurs at low fluoride dosage.

The information derived from this study concerning fluoride may be directly applicable to the treatment of osteoporosis with fluoride and support our clinical trials.

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