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Serum ionic fluoride concentrations are significantly decreased after treatment with alendronate in patients with osteoporosis

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ABSTRACT

Background: We determined serum ionic fluoride (SIF) concentrations before and after treatment of osteoporosis with alendronate to clarify whether SIF concentrations directly reflect a change in bone metabolism.
Methods: A total of 45 postmenopausal women with primary osteoporosis who were treated with alendronate over a 6-month period were enrolled (mean age, 64.2 years). SIF concentrations were measured by the flow injection method with an ion-selective electrode. Concentrations of bone turnover markers (serum bone alkaline phosphatase, serum osteocalcin, serum type I collagen cross-linked N-telopeptide and urinary deoxypyridinoline) and lumbar spine BMD (LsBMD) were also measured. SIF, bone turnover markers and LsBMD before and after treatment were compared.
Results: Concentrations of SIF as well as concentrations of all bone turnover markers were significantly decreased after treatment: means (standard deviations) before and after treatment were 0.62 (0.13) and 0.32 (0.09) μmol/l, respectively ($P<0.001$) and the percent change was −46.3%. LsBMD was also significantly increased by 6.7% after treatment.
Conclusions: The reduction of SIF concentrations is probably caused by inhibition of bone resorption due to the action of alendronate. The findings suggest that SIF concentrations directly reflect a change in bone metabolism.

1. Introduction

Fluoride is a trace element that is ubiquitous in the environment, and humans ingest fluoride throughout their lives. Ingested fluoride is distributed from the plasma to all tissues and organs; however, in humans, approximately 99% of the total body burden of fluoride is retained in calcified tissues such as bones and teeth, with the remainder distributed in highly vascularized soft tissues and the blood [1]. After ingestion, fluoride is rapidly absorbed from the gastrointestinal tract. Peak plasma (serum) fluoride concentrations are reached within 30–60 min, and the levels return to normal within 3–6 h. The decline in the concentration is mainly due to calcified tissue uptake and renal excretion [2]. Therefore, plasma (serum) fluoride concentrations are influenced by fluoride intake, renal function and bone metabolism [2–9].

Our previous study using 332 apparently healthy community dwelling subjects showed that serum ionic fluoride (SIF) concentrations in postmenopausal women were significantly higher than those in premenopausal women [10]. This suggests that the fluoride accumulated in bone is released into blood in large quantities due to an accelerated rate of bone resorption after menopause. Therefore, SIF concentrations may directly reflect a change in bone metabolism. To our knowledge, however, there has been only one study focusing on the relationship between SIF concentrations and change in bone metabolism in humans, and that study showed that SIF concentrations were decreased with administration of thyrocalcitonin as an antiresorptive agent for 2 days in four patients with osteoporosis [7].

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture [11]. Primary osteoporosis is caused by estrogen deficiency that results in increased bone resorption in postmenopausal women and in aging men [12,13]. If SIF concentrations directly reflect bone metabolism, it is possible that patients with primary osteoporosis have increased SIF concentrations due to increased bone resorption. Moreover, inhibition of bone resorption by antiresorptive agents may cause reduction of SIF concentrations.

Accordingly, to clarify whether SIF concentrations directly reflect a change in bone metabolism, we measured SIF concentrations, as well

Abbreviations: SIF, serum ionic fluoride; LsBMD, lumbar spine bone mineral density; FIA, flow injection analysis; BAP, bone alkaline phosphatase; NTX, type I collagen cross-linked N-telopeptide; DPD, deoxypyridinoline; OC, osteocalcin.

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as concentrations of bone turnover markers and bone mineral density, in 45 patients with primary osteoporosis before and after treatment with alendronate sodium hydrate (alendronate), an antiresorptive agent.

2. Materials and methods

2.1. Study subjects

Among the outpatients who visited the special outpatient clinic for osteoporosis in Department of Orthopaedic Surgery, Iwate Medical University Hospital from August 2003 to November 2004, 88 patients were newly diagnosed with primary osteoporosis based on diagnostic criteria according to the Japanese Society for Bone Mineral Research [14]. All of the patients were postmenopausal women, and patients with secondary osteoporosis were not enrolled in this study. Eighteen of these 88 patients did not give informed consent for participation in this study. Furthermore, 10 patients who withdrew from treatment, 4 patients who changed to other hospitals, and 11 patients who were treated with agents other than alendronate were excluded. Therefore, data for 45 (51%) of the 88 patients were used for analysis in this study.

Mean age (standard deviation (SD)) of the patients was 64.2 (6.4) years and age ranged from 49 to 82 years. All of the patients were treated daily with alendronate (5 mg) over a 6-month period. In the areas where the patients lived, public drinking water is not fluoridated and has very low fluoride concentrations (1.1 to 7.9 μmol/l) [10]. There were no patients with serum creatinine concentrations greater than 0.8 mg/dl and no patients suffered from renal dysfunction during the 6-month period. In addition, adverse events of alendronate such as pain in the muscles or joints and gastrointestinal symptoms were not observed among the patients during the study period. The study was approved by the Medical Ethics Committee of Iwate Medical University and conducted in accordance with the guidelines of the Declaration of Helsinki.

2.2. Collection of blood and urine samples

Overnight fasting blood samples were obtained in the morning before and 6 months after treatment. Samples of spot urine were obtained at the same time as collection of blood samples. For measurements of bone turnover marker concentrations, the blood and urine samples were stored immediately after collection in ice and were transported to a laboratory (BML General Laboratory, Kawagoe, Japan). For measurement of SIF concentrations, blood samples were collected in polypropylene tubes. The samples were transported to our laboratory and were allowed to clot at room temperature and then centrifuged at 1500×*g* for 10 min. After separation, the serum samples were stored at −80 °C until measurements.

2.3. Measurement of serum ionic fluoride concentrations

SIF concentrations were determined by flow injection analysis (FIA) with a fluoride ion-selective electrode as a detector in our laboratory [10,15]. The FIA system consisted of 2 double-plunger reciprocal pumps (Uniflose, Tokyo, Japan), a sample injector with a sample loop (0.4 ml), and a flow cell. An Orion 94-09 fluoride electrode (Orion Research, Cambridge, MA) and reference electrode (Model 4400, DKK, Tokyo, Japan) were set in the flow cell. Purified water was used for the carrier solution and the flow rate was set to 1.0 ml/min. The flow rate of the buffer solution was set to 1.0 ml/min. The flow rates of the 2 pumps were kept within ±0.3% of the desired values.

Each serum sample (0.2 ml) was mixed with 1.2 ml of a diluting solution (0.05 mol/l sodium acetate solution, pH 5.0) and pH was adjusted to 5.4±0.2 by 0.1 or 0.5 mol/l of hydrogen chloride or sodium hydroxide within 10 μL. The sample was centrifuged at 10,000 rpm for 3 min and the supernatant was prepared for determinations. Standard or sample solutions were injected into the FIA system with a 1-ml syringe. Measurement for every standard and sample solution was performed twice. The working curve of the peak heights of potential differences to the fluoride concentrations was calculated using a curve fitness program.

2.4. Other measurements

Concentrations of serum bone alkaline phosphatase (BAP; Beckman Coulter Inc. CA, USA), serum type I collagen cross-linked N-telopeptide (NTX; Inverness Medical Innovations Inc. MA) and urinary deoxypyrridinoline (DPD; Quidel Corp. San DiegoCA) were measured by an enzyme immunoassay. Concentrations of serum osteocalcin (OC; Mitsubishi Medience Inc, Tokyo, Japan) were measured by an immunoradiometric assay. Lumbar spine bone mineral density (LsBMD) was measured as the mean BMD of the first through fourth lumbar vertebrae by dual-energy X-ray absorptiometry using QDR4100A (Hologic Inc. MA) before and 6 months after treatment.

2.5. Statistical analysis

Distributions of SIF concentrations before and 6 months after treatment with alendronate in patients are presented by histograms. We calculated mean values of SIF concentrations, bone turnover marker concentrations and LsBMD before and after treatment. Comparisons of the mean values before and after treatment were performed using the paired *t*-test except for LsBMD. Data for LsBMD before and after treatment were compared by Wilcoxon's rank test because the distribution was not normal. Percent change in each variable was also calculated [16]. Correlations between percent changes in SIF, bone turnover markers and LsBMD were estimated using Pearson's correlation coefficients. A 2-sided $P<0.05$ was considered to be statistically significant. All analyses were performed by using the Statistical Package for Social Science version 11J (SPSS Japan Inc, Tokyo, Japan).

3. Results

Fig. 1 shows two histograms of SIF concentrations before and 6 months after treatment. Before treatment, the range of SIF concentrations was from 0.36 to 0.90 μmol/l (median, 0.62 μmol/l). The distribution before treatment seemed to be a normal distribution and the peak of the distribution was in the range of 0.53 to 0.63 μmol/l (Fig. 1 (A)). After treatment, the range of SIF concentrations was from 0.17 to 0.54 μmol/l (median, 0.30 μmol/l). The distribution after treatment moved to the left, and the peak of the distribution was in the range of 0.21 to 0.32 μmol/l. (Fig. 1 (B)).

Table 1 shows concentrations and percent changes of SIF concentrations, bone turnover marker concentrations and LsBMD before and 6 months after treatment. SIF concentrations were significantly decreased after treatment: means (SDs) before and after treatment were 0.62 (0.13) and 0.32 (0.09) μmol/l, respectively ($P<0.001$), and the mean percent change (SD) was −46.3 (16.9)%. Concentrations of bone turnover markers were also significantly decreased after treatment: means (SDs) before and after treatment were 34.7 (9.8) and 20.5 (6.4) U/l for BAP, 8.4 (2.2) and 4.9 (1.4) ng/mL for OC, 23.0 (7.1) and 13.9 (2.8) nmolBCE/l for NTX, and 8.7 (2.9) and 4.5 (1.4) nmolCr for DPD, respectively (all $P<0.001$). Mean percent changes (SD) of BAP, OC, NTX and DPD concentrations were −39.6 (15.7)%, −37.5 (27.4)%, −34.5 (21.8)% and −46.0 (18.3)%, respectively. The percent changes of BAP, NTX and DPD exceeded the minimum significant changes of each bone turnover marker [16]. In addition, LsBMD was significantly increased after treatment: means (SDs) before and after treatment were 0.61 (0.06) and 0.65 (0.06) g/cm^2, respectively ($P<0.001$), and the mean percent change (SD) was 6.7 (4.0)%.

Table 2 shows correlations between percent changes in SIF, bone turnover markers and LsBMD. Percent change in OC was inversely