Low Serum Levels of Undercarboxylated Osteocalcin in Postmenopausal Osteoporotic Women Receiving an Inhibitor of Bone Resorption

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Osteocalcin, a bone-specific protein synthesized by osteoblasts, undergoes vitamin K-dependent gamma-carboxylation. Undercarboxylated osteocalcin (ucOC) represents inadequately carboxylated osteocalcin, and this fraction increases with vitamin K insufficiency. Alendronate is a bisphosphonate that inhibits bone resorption, thereby increasing bone mineral density (BMD), while also reducing bone formation closely coupled with bone resorption. The aim of this cross-sectional study was to evaluate the influence of alendronate on serum levels of ucOC, cross-linked $\eta$-telopeptide of type 1 collagen (NTx), a marker of bone resorption, and bone alkaline phosphatase (BAP), a marker of bone formation. Forty-six postmenopausal osteoporotic women were divided into three groups: patients receiving alendronate (5 mg/day or 35 mg/week) for $\geq$6 months ($n = 29$) or <6 months ($n = 7$), and patients receiving no medication related to bone metabolism ($n = 10$). Serum ucOC levels were significantly lower in patients with long-term treatment ($p < 0.0001$) or short-term treatment ($p = 0.0223$) than in untreated patients. Serum ucOC levels correlated positively with both BAP ($r = 0.695, p < 0.0001$) and NTx ($r = 0.494, p = 0.0004$) in all participants. Since low serum levels of BAP and NTx are associated with decreased levels of bone formation and bone resorption, respectively, these findings suggest that low serum ucOC levels may reflect the suppression of bone turnover. In conclusion, low serum ucOC levels reflect suppressed bone turnover and/or adequate levels of vitamin K in patients receiving an inhibitor of bone resorption.———alendronate; undercarboxylated osteocalcin (ucOC); bone alkaline phosphatase (BAP); cross-linked $\eta$-telopeptide of type 1 collagen (NTx); bone mineral density.


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Osteoporosis is a skeletal disorder resulting in reduced bone strength and subsequent increases in fracture risk. Bone strength is defined by two independent contributors: bone mineral density (BMD) and bone quality (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy 2001). BMD is expressed as grams of mineral per area or volume in bone, and indicates how compact the bone is. Bone quality includes structural strength such as microarchitecture, and intrinsic material properties such as degree of mineralization, collagen cross-linkage, and damage accumulation (Schnitzler 1993; Saito et al. 2006).

Alendronate (ALN) is a bisphosphonate that prevents osteoporotic fractures by inhibiting bone resorption, leading to subsequent increases in BMD (Black et al. 1996; Cummings et al. 1998; Cranney et al. 2002). This agent reduces biochemical markers of bone turnover for both bone formation and bone resorption, because bone formation and resorption are closely coupled. The bone formation marker osteocalcin (OC) is a bone-specific protein synthesized by osteoblasts. Serum total OC levels have been shown to decrease alongside suppression of bone turnover by ALN (Shiraki et al. 1998).

OC contains three gamma-carboxyglutamic acid residues, which facilitate the binding of OC to hydroxyapatite in bone. The fraction of OC that has undergone imperfect gamma-carboxylation is referred to as undercarboxylated OC (ucOC), and cannot be shifted from blood vessels to bone due to a decrease in the affinity for $\text{Ca}^{2+}$ and hydroxyapatite (Hauschka et al. 1989). Since vitamin K is involved in this carboxylation, inadequate levels of vitamin K lead to increased serum ucOC levels (Sokoll and Sadowski 1996; Binkley et al. 2002; Sogabe et al. 2007). Serum ucOC levels are thus regarded as a marker of vitamin K deficiency.

To date, only one study has described changes in serum ucOC levels during ALN administration (Hirao et al. 2008), and the influence of bone turnover suppression by an inhibitor of bone resorption on serum ucOC levels is not yet fully understood.
understood. We hypothesized that serum ucOC levels would be reduced through the suppression of bone turnover, as seen with decreased serum total OC levels, following administration of an inhibitor of bone resorption. The objective of this study was to evaluate serum levels of ucOC during ALN administration and to evaluate correlations between serum ucOC levels and biochemical markers of bone turnover and BMD in postmenopausal osteoporotic women.

Patients and Methods

Patients

This cross-sectional study included 46 consecutive postmenopausal women with primary osteoporosis; patients were being treated with oral ALN or were new patients who had not received pharmacotherapy for osteoporosis. The diagnosis of osteoporosis was determined with the criteria by World Health Organization (BMD T-score less than 2.5 standard deviations below the young adult mean). Participants were questioned about their medical history and were excluded if they had a history or current diagnosis of malignancy, rheumatoid arthritis, diabetes mellitus, or other disorders that could affect bone metabolism. Women with documented fresh vertebral and non-vertebral fractures within the last 6 months were also excluded.

The 36 patients who had been treated with ALN (5 mg/day or 35 mg/week) were classified as the treated group. In addition, we further divided these patients into subgroups according to duration of ALN treatment as ≥ 6 months (n = 29; mean duration of ALN treatment, 750 days) or < 6 months (n = 7; mean duration of ALN treatment, 135 days). The remaining 10 patients, classified as the untreated group (n = 10), were new visitors to our outpatient clinic with a diagnosis of osteoporosis but no history of pharmacotherapies affecting bone metabolism. All participants provided informed consent, and the study protocol was approved by the ethics committee at Igarashi Memorial Hospital.

Assessment

Serum bone alkaline phosphatase (BAP), a marker of bone formation, was measured by enzyme immunoassay (Osteolinks-BAP; DS Pharma Biomedical, Osaka, Japan), and serum cross-linked N-telopeptide of type 1 collagen (NTx), a marker of bone resorption, was measured by enzyme-linked immunosorbet assay (Osteomark; Mochida Pharmaceutical, Tokyo, Japan). Serum ucOC was measured by electrochemiluminescence immunoassay (Picolumi-ucOC; Sanko Junyaku, Ibaragi, Japan). BMD at the first third of the distal forearm was measured with dual-energy X-ray absorptiometry (Osteometer DTX200, Toyo Medic, Tokyo, Japan). Serum markers and BMD were measured on the same day. In addition, we investigated the number of vertebral fractures on lateral radiographs according to an established method (Miyakoshi et al. 2006).

Statistical analysis

Results are expressed as mean ± standard error of the mean. Differences among the three groups were assessed by Kruskal-Wallis test and Scheffe’s method as a post-hoc test. Correlations between ucOC, BAP, NTx, age, and BMD were analyzed using Pearson’s correlation coefficients for all participants irrespective of ALN use. Values of p < 0.05 were considered significant. Statistical analyses were performed using StatView 5.03 for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Age, height, weight, number of morphometric vertebral fractures, and BMD showed no significant differences among the three groups (p = 0.403, p = 0.685, p = 0.174, p = 0.206, and p = 0.769, respectively) (Table 1). Serum levels of ucOC were significantly lower in both groups with ALN treatment for ≥ 6 months (p < 0.0001) and with ALN treatment for < 6 months (p = 0.0223) compared with the untreated group (Fig. 1A). In addition, ALN treatment for ≥ 6 months was associated with significantly lower serum ucOC levels compared with treatment for < 6 months (p = 0.0125). Serum levels of BAP and NTx were also significantly lower in patients with long-term treatment than in untreated patients (p < 0.0001 and p = 0.01, respectively) (Fig. 1B, C).

Serum ucOC levels showed a significant positive correlation with BAP (r = 0.695, p < 0.0001) and NTx (r = 0.494, p = 0.0004), and no significant correlation with age or BMD in the total population of 46 participants (Table 2).

Discussion

In the present study, serum ucOC levels were significantly lower in treated patients than untreated patients, even in patients who had received ALN for < 6 months. Conversely, BMD did not differ significantly between treated

Table 1. Background data of subjects.

<table>
<thead>
<tr>
<th>Duration of alendronate administration</th>
<th>None (n = 10)</th>
<th>&lt; 6 months (n = 7)</th>
<th>≥ 6 months (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>72.9 ± 2.6</td>
<td>78.1 ± 2.3</td>
<td>75.8 ± 1.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>147.5 ± 2.9</td>
<td>151.1 ± 6.2</td>
<td>147.1 ± 1.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>45.0 ± 3.9</td>
<td>46.3 ± 2.5</td>
<td>50.9 ± 1.5</td>
</tr>
<tr>
<td>No. of VF</td>
<td>0.8 ± 0.3</td>
<td>1.4 ± 0.4</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.278 ± 0.018</td>
<td>0.280 ± 0.025</td>
<td>0.267 ± 0.011</td>
</tr>
</tbody>
</table>

Values represent mean ± standard error of the mean. VF, vertebral fractures; BMD, bone mineral density.
Alendronate and ucOC Levels

Alendronate reportedly decreases risk of osteoporotic fractures as early as 6 months after starting treatment, with only modest early effects on BMD (Liberman et al. 1995; Black et al. 2000). Several studies have confirmed that bisphosphonates improve bone quality such as trabecular architecture, mean degree of mineralization of bone and bone material properties of collagen (Boivin et al. 2000; Recker et al. 2005; Borah et al. 2006; Durchschlag et al. 2006). These findings imply that improvements in bone quality contribute to a reduction in fracture risk before any significant increase in BMD occurs during ALN treatment. Furthermore, serum ucOC levels have been shown to represent a marker of bone quality as well as a marker of vitamin K deficiency in postmenopausal women, as previous studies have demonstrated high ucOC levels as a risk factor for hip fracture independent of BMD (Szulc et al. 1996; Vergnaud et al. 1997). The lower serum ucOC levels seen in the present study may thus reflect early improvements in bone quality. We speculate that serum ucOC levels offer a possible candidate for evaluating bone quality, independent of BMD, in all postmenopausal women irrespective of administration of inhibitors of bone resorption.

As shown in previous studies, serum BAP and NTx levels were significantly decreased through suppressed bone turnover in osteoporotic postmenopausal women treated with ALN (Greenspan et al. 1998; Braga de Castro Machado et al. 1999). In addition to the effects of ALN on bone markers, the present study indicates significant positive cor-

Table 2. Correlations between age, BMD, BAP, NTx, and ucOC.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>BMD</th>
<th>BAP</th>
<th>NTx</th>
<th>ucOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.000</td>
<td>-0.574*</td>
<td>0.220</td>
<td>0.044</td>
<td>0.027</td>
</tr>
<tr>
<td>BMD</td>
<td>1.000</td>
<td>-0.360**</td>
<td>-0.361***</td>
<td>-0.221</td>
<td></td>
</tr>
<tr>
<td>BAP</td>
<td>1.000</td>
<td>0.716*</td>
<td></td>
<td>0.695*</td>
<td></td>
</tr>
<tr>
<td>NTx</td>
<td>1.000</td>
<td>0.494****</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ucOC</td>
<td>1.000</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

BMD, bone mineral density; BAP, bone alkaline phosphatase; NTx, N-telopeptide of type 1 collagen; ucOC, undercarboxylated osteocalcin.

*p < 0.0001, **p = 0.0135, ***p = 0.0130, ****p = 0.0004
relations between serum ucOC levels and serum BAP/NTx levels. Our results are consistent with the findings of Hirao et al. (2008), who demonstrated that serum ucOC levels were reduced 20% after 1 year of ALN therapy. However, correlations between ucOC and BAP/NTx were not evaluated in that study (Hirao et al. 2008). The present study is the first report to demonstrate correlations between serum ucOC levels and serum BAP/NTx levels in postmenopausal osteoporotic women according to ALN treatment status. No correlation was detected between ucOC and age in the present study, although previous studies have shown that serum ucOC levels increased in an age-dependent manner (Liu and Peacock 1998; Tsugawa et al. 2006). ALN may modify age-related increases in serum ucOC levels. These results suggest that serum ucOC levels are affected by suppressed bone turnover.

The present study shows several limitations. First, the number of patients enrolled in this study was small and a cross-sectional analysis was applied. We therefore could not confirm any reasons for lower serum ucOC levels in patients receiving ALN, instead only showing that levels were lower in patients receiving ALN than in untreated patients. Second, we did not evaluate vitamin K intake. A fermented soybean product called “natto”, which contains a large amount of vitamin K, is a common food in Japan. Kaneki et al. (2001) reported that natto intake affected the gamma-carboxylation of OC. Third, we only measured serum ucOC levels and did not consider serum total OC levels. Shiraki et al. (1998) showed that ALN decreased serum total OC levels accompanied by suppression of bone turnover. As both ucOC and carboxylated OC are released from osteoblasts into circulation, bone turnover may convert a proportion of ucOC. The ratio of ucOC to total OC may thus be more appropriate in seeking to understand the association between ucOC and bone turnover. Finally, evaluation of BMD was performed using the forearm. BMD evaluation at multiple sites, including the lumbar spine and femoral neck, is a preferable method. Further prospective studies are required to evaluate the effects of ALN on ucOC levels and relationships to bone quality and turnover.

In conclusion, this is the first study to report a relationship between ucOC levels and BAP/NTx in women treated with ALN. Serum ucOC levels were lower in osteoporotic patients treated with ALN than in untreated patients. Significant positive correlations were seen between serum ucOC levels and serum BAP or NTx levels in postmenopausal osteoporotic women according to ALN treatment status. These results suggest that serum ucOC levels represent a marker of both bone turnover and vitamin K status in bone. Further studies are needed to confirm these findings.

References


Alendronate and ucOC Levels


