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ORIGINAL ARTICLE

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Response of serum carboxylated and undercarboxylated osteocalcin to alendronate monotherapy and combined therapy with vitamin K_2 in postmenopausal women

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Abstract Alendronate decreases the risk of femoral neck fracture by suppressing bone turnover, and also decreases the serum total osteocalcin level. A low serum carboxylated osteocalcin level or high undercarboxylated osteocalcin level could be risk factors for femoral neck fracture. Vitamin K mediates the carboxylation of osteocalcin, but the effect of alendronate therapy with or without vitamin K_2 supplementation remains unknown. Forty-eight postmenopausal women were enrolled in a 1-year prospective randomized trial and assigned to alendronate monotherapy (5 mg/day) (group A, $n = 26$) or vitamin K_2 (45 mg/day) plus alendronate (5 mg/day) (group AK, $n = 22$). Bone mineral density was measured by dual-energy X-ray absorptiometry at 0 and 12 months; bone turnover parameters were measured at 0, 3, and 12 months. Four patients discontinued alendronate therapy, and we analyzed the remaining 44 patients (23 in group A and 21 in group AK) who completed 1 year of treatment. Alendronate decreased undercarboxylated osteocalcin; carboxylated osteocalcin was not affected. Addition of vitamin K_2 enhanced the decrease of undercarboxylated osteocalcin levels and led to a greater increase of femoral neck bone mineral density. Alendronate monotherapy does not decrease carboxylation of osteocalcin, and combination of vitamin K_2 and alendronate brings further benefits on both osteocalcin carboxylation and BMD of femoral neck in postmenopausal women with osteoporosis.

Key words alendronate · osteocalcin · postmenopausal woman · vitamin K_2

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Introduction

Femoral neck fracture is a major public health problem because of its high frequency, associated dysfunction, and high costs of treatment. Alendronate is one of the drugs proven to reduce the risk of femoral neck fracture by suppressing bone remodeling and increasing the bone mineral density of proximal femur [1]. It has been reported that suppression of bone remodeling by alendronate is accompanied by a decrease of serum total osteocalcin [2,3]. Several previous studies have indicated that a low serum carboxylated osteocalcin (COC) level or high undercarboxylated osteocalcin (ucOC) level could be risk factors for femoral neck fracture [4–6]. These studies have aroused interest as to whether alendronate maintains the serum COC level despite the decrease of total osteocalcin, but the changes of COC and ucOC during alendronate treatment remain unknown. Vitamin K is a cofactor of gamma-carboxylase, which converts glutamic acid residues to gamma-carboxyglutamic acid residues in osteocalcin and is essential for gamma-carboxylation of this molecule [7]. There is evidence to suggest that vitamin K_2 enhances the accumulation of osteocalcin in the extracellular matrix of osteoblasts *in vitro* [8]. In addition, it has been shown that serum ucOC decreases and COC increases rapidly during vitamin K_2 treatment [9,10]. Previous reports have indicated that vitamin K_2 has a preventive effect on vertebral fracture [11], and that combination of vitamin K_2 and etidronate is recommended to prevent the vertebral fracture in postmenopausal women with osteoporosis [12]. However, it remains unclear whether the combination of alendronate and vitamin K_2 has an additive beneficial influence on bone metabolism.

In this study, we evaluated the changes of COC and ucOC during alendronate administration and the changes of bone mineral density (BMD) and biochemical markers of bone metabolism after combined therapy with vitamin K_2 and alendronate. We performed the present study to investigate both of these issues.

Patients and methods

Patients

Forty-eight postmenopausal women were enrolled in a one-year prospective randomized trial. All of the patients had no other disease leading to secondary osteoporosis. Patients were randomized to one of two treatment groups, i.e., alendronate (5 mg/day) monotherapy (group A, n = 26) or vitamin K_2 (45 mg/day) plus alendronate (5 mg/day) (group AK, n = 22). Four patients dropped out of the study because they discontinued taking alendronate, so we analyzed the remaining 44 patients (23 patients in group A and 21 patients in group AK) who completed 12 months of treatment.

Assessment of bone turnover markers

Blood and urine samples were obtained before the initiation of any treatment in all of the patients. Samples were collected and to measure the serum levels of bone alkaline phosphatase (BAP), carboxylated osteocalcin (COC), and undercarboxylated osteocalcin (ucOC), as well as urinary deoxypyridinoline (DPD). From the COC and ucOC levels, the ucOC/COC ratio was also calculated. After 3 and 12 months of treatment with alendronate alone or alendronate plus vitamin K_2, the tests listed above were repeated. The percent changes of these markers after 3 and 12 months of therapy were calculated and compared between groups A and AK.

Assessment of BMD

The BMD of the lumbar spine (L2–L4), left total femur, and left femoral neck were determined by dual-energy X-ray absorptiometry using a Lunar (GE, CT, USA) before the initiation of any treatment in all patients. After 12 months of treatment with alendronate alone or alendronate plus vitamin K_2, the BMD was measured again at these sites and the percent change at each site was calculated and compared between the two groups.

Statistical analysis

Results are expressed as the mean ± SE. Between-group differences were assessed by the Mann–Whitney U test, and a probability <0.05 was considered to indicate statistical significance. All statistical analyses were performed using SPSS (ver. 11.5) software (SPSS, IL, USA).

Results

Baseline characteristics

Table 1 lists the baseline characteristics and biochemical parameters of the patients. There were no significant differences of baseline age, anthropometric parameters, biochemical markers, BMD t-scores, and the number of prevalent vertebral fractures per patient (T4–L5) between groups A and AK.

Changes of BAP and DPD

As shown in Fig. 1, in both groups, there was a significant decrease of the BAP level after 3 months of treatment and the decrease was larger after 12 months. There was no significant difference in the percent change of BAP between the two groups. Both groups also showed a significant decrease of DPD after 3 months, and the decrease was greater after 12 months in group AK. There was no significant difference between groups A and AK after 3 months of treatment, but combined therapy was associated with a significantly greater decrease of DPD than alendronate monotherapy after 12 months.

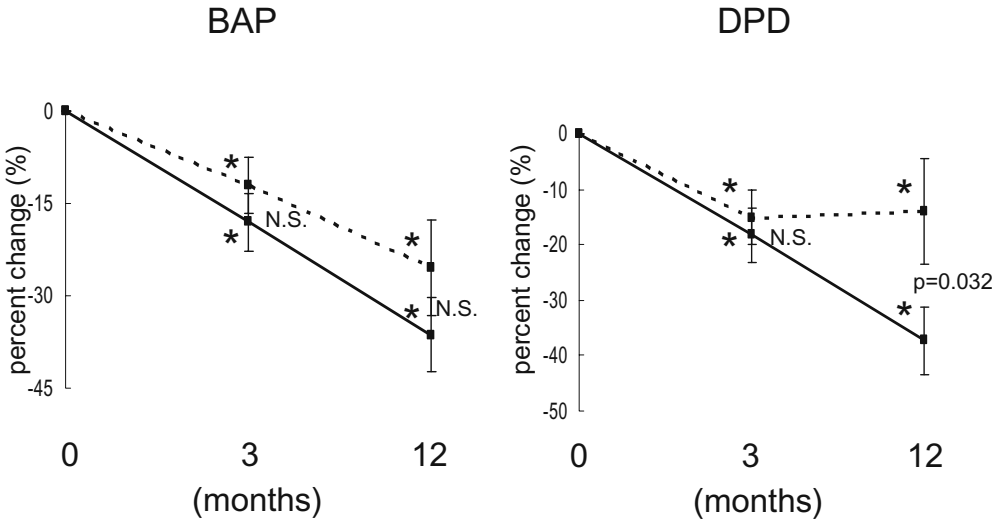
Changes of ucOC and COC

As shown in Fig. 2, alendronate monotherapy was associated with a decrease of ucOC after 3 months and this decrease persisted after 12 months, while COC was not affected. The ucOC/COC ratio was not affected by alendro-

Table 1. Characteristics of the patients at baseline

	A	AK	P value
Age (years)	69.8 ± 1.7	67.0 ± 1.4	N.S.
BMI	20.9 ± 0.64	21.0 ± 0.57	N.S.
Lumbar (L2–L4) BMD (t-score)	–2.9 ± 0.18	–3.0 ± 0.17	N.S.
Total femur BMD (t-score)	–2.2 ± 0.16	–2.1 ± 0.17	N.S.
Femoral neck BMD (t-score)	–2.0 ± 0.20	–2.1 ± 0.16	N.S.
Serum BAP	28.4 ± 1.7	29.1 ± 2.2	N.S.
Urine DPD	6.0 ± 0.39	6.4 ± 0.48	N.S.
Serum ucOD	3.1 ± 0.44	2.4 ± 0.34	N.S.
Serum COC	5.6 ± 0.64	6.4 ± 0.65	N.S.
ucOC/COC	0.65 ± 0.10	0.64 ± 0.26	N.S.
Number of prevalent vertebral fractures per patient (T4–L5)	1.09 ± 0.44	1.00 ± 0.39	N.S.

A, alendronate monotherapy group; AK, vitamin K_2 plus alendronate group; BMI, body mass index; BMD, bone mineral density; BAP, bone alkaline phosphatase; DPD, deoxypyridinoline; ucCOD, undercarboxylated osteocalcin; COC, carboxylated osteocalcin



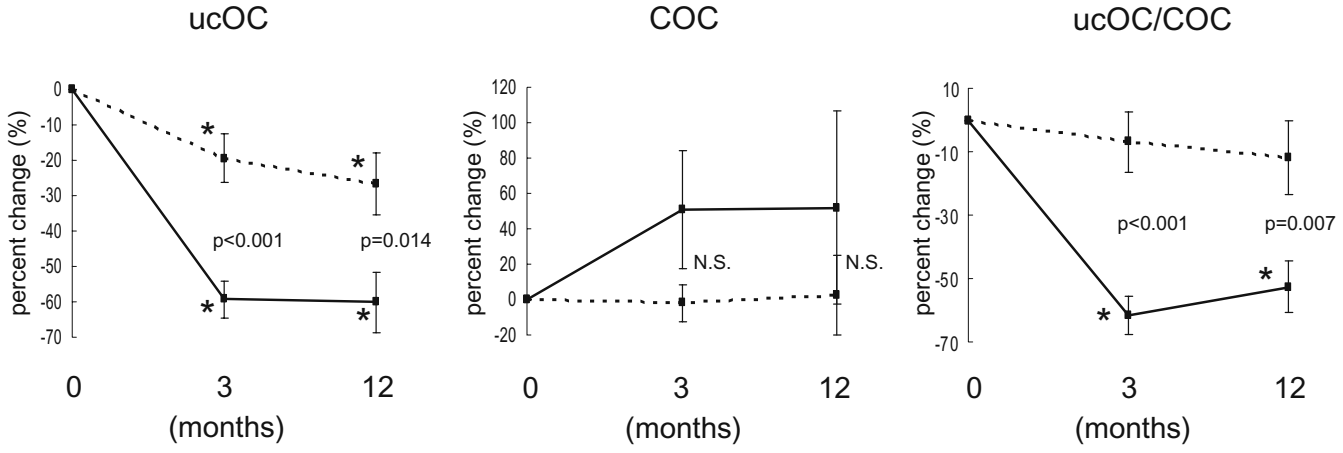


Fig. 1. Percent changes of serum bone alkaline phosphatase (*BAP*) and urinary deoxypyridinoline (*DPD*) after 3 and 12 months of alendronate therapy with or without vitamin K_2. *Dotted lines*, group A; *unbroken lines*, group AK. *Significant difference compared with before treatment; N.S., no significant difference between groups A and AK; p, *P* value for the difference between groups A and AK

Fig. 2. Percent changes of serum undercarboxylated osteocalcin (ucOC), carboxylated osteocalcin (COC), and the ucOC/COC ratio after 3 and 12 months of alendronate therapy with or without vitamin K_2. *Dotted lines*, group A; *unbroken lines*, group AK. *Significant difference compared with before treatment; N.S., no significant difference between groups A and AK; p, *P* value for the difference between groups A and AK

nate monotherapy. Combined therapy with vitamin K_2 and alendronate also led to a decrease of ucOC after 3 months, and the decrease was significantly greater than with monotherapy. COC showed an increase, but it was not significant, and there were no significant differences of COC levels between monotherapy and combined therapy. However, the ucOC/COC ratio was significantly decreased by combined therapy and the change was significantly greater than with monotherapy.

Changes of BMD

As shown in Fig. 3, the BMD of the lumbar spine (L2–L4) was significantly increased in both groups and there was no significant difference between the two groups. Total femoral BMD only showed an increase in group AK, but there was no significant difference between the two groups. At the femoral neck, however the BMD showed almost no change in group A, while it was significantly increased in group AK and the latter group showed a significant increase of femoral neck BMD compared with the former group.

Discussion

Because femoral neck fracture impairs the survival of elderly patients, prevention of this type of fracture is important. Alendronate is one of the most potent therapeutic agents to prevent the femoral neck fracture. Recently, combination of alendronate plus raloxifene [13], alendronate plus vitamin D_2 [14] and hormone replacement plus alendronate [15] are shown to have additive effects on BMD and biochemical markers of bone. Combination of vitamin K_2 and etidronate is also recommended, because it prevents new vertebral fractures in postmenopausal women with osteoporosis [12]. However, combination of vitamin K_2 and alendronate is not focused previously. In this study, the response of bone metabolism marker and BMD after

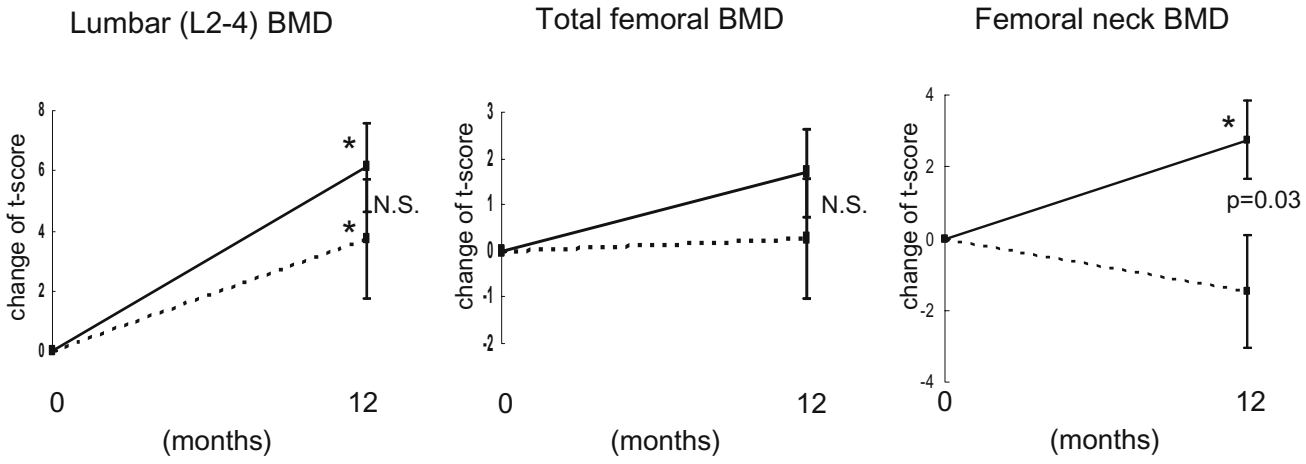


Fig. 3. Percent changes of the t-score for lumbar (L2–L4) bone mineral density (*BMD*), total femoral BMD, and femoral neck BMD after 12 months of alendronate therapy with or without vitamin K_2. *Dotted lines*, group A; *unbroken lines*, group AK. *Significant difference compared with before treatment; N.S., no significant difference between groups A and AK; p, *P* value of the difference between groups A and AK

administration of vitamin K_2 plus alendronate was assessed. Maintenance of BMD and suppression of accelerated bone turnover are thought to be one of the factors associated with a reduced risk of fracture, although these factors explain the part of the anti-fracture effects. Suppression of bone resorptive activity induced by alendronate is accompanied with the reduction of osteoblast activity, and this condition is so called the suppression of bone turnover. This condition is accompanied by decrease of the serum total osteocalcin level [2,3]. In addition, a decrease of COC or an increase of ucOC, and same individual changes against the total osteocalcin increase the risk of femoral neck fracture [4–6]. Therefore, it would be interesting to know how these molecules change when alendronate therapy suppresses the total osteocalcin level, but there have been no previous investigations of ucOC and COC during alendronate treatment. In this study, alendronate monotherapy caused ucOC to decrease significantly, while COC was not affected. The reduction of the ucOC level without decrease of COC by alendronate may contribute to reduce the risk of femoral neck fracture.

Next, we investigated the influence of vitamin K, because synthesis of osteocalcin depends on vitamin D and K, and vitamin K is also required for the post-translational gamma-carboxylation of glutamic acid residues in pro-osteocalcin [16]. Vitamin K is a cofactor of gamma-carboxylase that mediates the conversion of ucOC to COC. Before starting this study, we hypothesized that vitamin K may help to resist the up-regulation of ucOC or down-regulation of COC levels by alendronate, so that combined therapy with vitamin K_2 and alendronate could be a useful regimen. Indeed, vitamin K rapidly decreases the ucOC level and increases the COC level [9,10], but the changes of ucOC and COC after combined vitamin K_2 and alendronate therapy have been unclear until this study was performed. The combined therapy group showed a significant decrease of ucOC levels after 3 months and 12 months of treatment, while COC levels showed no significant changes, as was the case during alendronate monotherapy. However, the ucOC/COC ratio was also significantly decreased by addition of vitamin K_2. From these observations, combined therapy with vitamin K_2 and alendronate contributes to a further decrease of ucOC compared with alendronate monotherapy, so this combined therapy might be desirable in the aspect of the change of biochemical marker. Furthermore, femoral neck BMD was significantly increased by combined therapy compared with alendronate monotherapy although lumbar (L2–4) and total femoral BMD showed no significant differences between the two groups, These phenomena suggest that vitamin K and alendronate combined therapy seems to have beneficial and selective effect on cortical bone at femoral neck on which the mechanical stress caused by walking or weight-bearing is focused. It is possible that vitamin K may modulate the osteocytes residing in cortical bone, since osteocyte would regulate bone matrix mineralization and volume as the primary mechanosensory cells in bone [17–19]. In this study, combined therapy led to a more significant decrease of DPD than monotherapy after 12 months (Fig. 1). This finding is reasonable because it was previously reported that vitamin K_2 inhibits osteoclast activity [20,21] and that vitamin K_2 supplementation may induce a decrease of bone resorption markers [22,23].

This study has too small in number of subject and too short in observation period to assess the incidence of fracture. This is the limitation of this study and the further studies were needed to clear whether the combination of vitamin K and alendronate have additive beneficial effect on the prevention of femoral neck fracture. However, this study could show the additive beneficial effect of combined therapy on the changes in biochemical markers of bone and femoral neck BMD.

In summary, alendronate monotherapy caused a decrease of ucOC, while COC and the ucOC/COC ratio were not affected. Addition of vitamin K_2 brought a significant further

decrease of ucOC, the ucOC/COC ratio, and DPD, and also brought a significant further increase of femoral neck BMD than alendronate monotherapy.

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