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ORIGINAL ARTICLE

Effects of risedronate alone or combined with vitamin K_2 on serum undercarboxylated osteocalcin and osteocalcin levels in postmenopausal osteoporosis

Yuji Kasukawa · Naohisa Miyakoshi · Toshihito Ebina · Toshiaki Aizawa · Michio Hongo · Koji Nozaka · Yoshinori Ishikawa · Hidetomo Saito · Shuichi Chida · Yoichi Shimada

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Abstract Risedronate decreases osteoporotic fracture incidence; however, its effects remain unclear in elderly osteoporotic patients. Vitamin K mediates carboxylation of osteocalcin (OC), and high undercarboxylated osteocalcin (ucOC) levels indicate vitamin K deficiency and increased osteoporotic fracture risk. We aimed to evaluate the effects of risedronate alone or combined with vitamin K_2 on serum ucOC, OC, and incidence of vertebral fractures in elderly osteoporotic patients. A total of 101 women with postmenopausal osteoporosis aged >60 years were randomly stratified into two groups—R group ($n = 51$), treated with risedronate alone; and R + K group ($n = 50$), treated with risedronate and vitamin K_2. Serum ucOC, OC and incidence of vertebral fractures were evaluated before treatment and at 6 and 12 months post-treatment. Decreased ucOC rates at 6 and 12 months were not significant between groups. However, at 6 and 12 months, decreased OC rates in the R group ($p < 0.01$ and 0.05, respectively) were significantly higher than in the R + K group, and ucOC/OC change rates in the R group ($p < 0.05$ and 0.001, respectively) were significantly lower than in the R + K group. Vertebral fracture incidence was not significantly different between the groups at 6 and 12 months. ucOC levels in patients with incident vertebral fractures were significantly higher than in patients without incident vertebral fractures in the R group at 6 months ($p < 0.05$). Although no significant difference was observed for ucOC decrease rate and incidence of vertebral fractures between treatments, ucOC levels in patients with incident vertebral fractures were significantly greater than in patients without when using risedronate alone.

Keywords Risedronate · Vitamin K_2 · Undercarboxylated osteocalcin (ucOC) · Osteocalcin · Vertebral fracture

Y. Kasukawa (✉) · N. Miyakoshi · M. Hongo · K. Nozaka · Y. Ishikawa · H. Saito · S. Chida · Y. Shimada
Department of Orthopedic Surgery, Akita University Graduate School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan
e-mail: kasukawa@doc.med.akita-u.ac.jp

T. Ebina · T. Aizawa
Department of Orthopedic Surgery, Kakunodate General Hospital, Senboku 010-0394, Japan

Introduction

Vertebral and hip fractures are serious problems in osteoporosis because of their higher dysfunction risk, resulting in increased mortality rates [1–3]. Risedronate inhibits bone resorption, leading to an increase in bone mineral density (BMD) [4, 5]. The effects of treatment for osteoporosis with drugs including risedronate prevent osteoporotic vertebral or hip fractures and therefore reduce mortality [6, 7]. However, risedronate cannot completely inhibit new fractures of the vertebra or hip, especially in elderly osteoporotic patients [8]. Many factors are considered causes of osteoporotic fractures, including low BMD [9, 10], smoking [11], prevalent fractures [12] and steroid use [13]. Nutrient deficiency, including vitamin K, vitamin D, vitamin C and calcium, can also account for increased fracture risk in elderly osteoporotic patients [14–17].

Among these vitamins, vitamin K is vital in maintaining bone strength through gamma-carboxylation of matrix glutamic acid residues in osteocalcin (OC). The fraction of imperfect gamma-carboxylation is referred to as undercarboxylated osteocalcin (ucOC), which is released from osteoblasts into the circulation during vitamin K

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insufficiency or deficiency [18, 19]. Vitamin K_2 treatment results in a rapid decrease in serum ucOC and an increase in OC levels [20, 21]. Therefore, high ucOC serum levels are considered a marker of vitamin K deficiency or insufficiency in bone. It has been reported that the value of vitamin K deficiency for osteoporotic fractures is 266 μg/day [22] and that 4.5 ng/ml of ucOC is a cut-off value to determine vitamin K insufficiency or deficiency for osteoporotic fractures [23]. Based on these findings, it is important to evaluate vitamin K status by measuring ucOC levels in osteoporotic women during treatment for osteoporosis. With regard to ucOC levels, our previous study and other studies have shown that serum OC or ucOC levels are decreased by bisphosphonate treatment, reflecting suppression of bone turnover [24–27]. Shiraki et al. [28] reported that ucOC levels >2.6 ng/ml during treatment with amino-bisphosphonate is a risk factor for incident vertebral fractures. The ucOC cut-off value for osteoporotic vertebral fractures might be different with or without treatment for osteoporosis with bisphosphonates. Higher ucOC levels have been reported to be a risk factor for incident fractures despite the use of amino-bisphosphonates [28]. However, the effect of treatment with menatetrenone in addition to risedronate on ucOC levels or incidence of osteoporotic vertebral fractures remains unclear.

Based on prior knowledge, we hypothesized that combined treatment with risedronate and vitamin K_2 would be more effective in decreasing ucOC levels and in preventing vertebral fractures compared with risedronate therapy alone in elderly osteoporotic patients. The ucOC value is one factor to determine initiation of combined therapy. Furthermore, changes in ucOC, OC, or ucOC/OC levels during risedronate monotherapy or combined therapy with vitamin K_2 might be useful in evaluating therapeutic effects. The primary purpose of our study was to evaluate the effects of risedronate monotherapy or its combined treatment with vitamin K_2 on serum ucOC and OC levels and other bone metabolic markers prospectively. The secondary purpose was to analyze the effects of combined treatment with risedronate and vitamin K_2 on the incidence of vertebral fractures and the risk factors for incidental osteoporotic vertebral fractures during monotherapy with risedronate or combined therapy with risedronate and vitamin K_2.

Materials and methods

Patients and study design

A total of 113 osteoporotic women aged >60 years were eligible for this study. However, we could not obtain agreement from 12 patients to join this study. In all, 101 osteoporotic women were enrolled in this 1-year prospective study. Patients had no history of treatment for osteoporosis, and osteoporosis was defined with a forearm BMD < -2.5 standard deviation (SD) of the T score for Japanese women. Excluded from this study were recipients of warfarin or steroids, and patients with hyperparathyroidism, hyperthyroidism or chronic renal failure. Patients were randomly stratified into two groups—the R-group (mean age 74 years; $n = 50$) who were treated with risedronate alone at 17.5 mg/week, and the R + K-group (mean age 75 years; $n = 51$) who were treated with risedronate at 17.5 mg/week and menatetrenone at 45 mg/day. Bone metabolic markers, BMD and plain radiographs were obtained at treatment initiation and at 6 and 12 months post-treatment. Patient numbers and treatment rates at 6 and 12 months were 39 (78 %) and 29 (58 %) in the R group and 34 (67 %) and 26 (51 %) in the R + K group, respectively. This open, randomized study was conducted at the Kakunodate General Hospital. The protocol was approved by the Ethical Committee of Kakunodate General Hospital. Written consent for the study and its publication was obtained from all patients.

Vitamin K intake

Vitamin K intake was evaluated with a simple vitamin K intake questionnaire [29]. The questionnaire evaluated frequency of natto and vegetable consumption each week for the preceding month. Natto or vegetable intake frequency was categorized into four levels. Natto intake scores were—0 points = none per week; 10 points = 1−3 times weekly, 25 points = 4−5 times weekly; or 40 points = daily. Vegetable intake scores were—0 = none per week; 10 points = a little per week; 15 points = normal per week; or 25 points = greater than normal per week. A total score <40 points identified patients with inadequate vitamin K intake [29].

Bone turnover markers

Blood was collected before and after treatment in all patients. Serum levels of cross-linked N-telopeptide of type I collagen (NTX), bone-specific alkaline phosphatase (BAP), OC and ucOC were measured at the start of treatment and at 6 and 12 months after treatment initiation. NTX was measured by enzyme-linked immunosorbent assay (Osteomark; Mochida Pharmaceutical, Tokyo, Japan), and BAP was measured by enzyme immunoassay (Osteolinks-BAP; DS Pharma Biomedical, Osaka, Japan). Serum levels of OC were measured by immunoradiometric assay (BGP IRMA; Mitsubishi Chemical Medience Co., Ltd., Tokyo, Japan), and serum levels of ucOC were measured by electrochemiluminescence immunoassay (Picolumi-ucOC; Eidia Co., Ltd., Tokyo, Japan). The ucOC/OC ratio was calculated from the OC and ucOC levels.

BMD and incidence of vertebral fractures

BMD was measured at the distal third of the radius with dual-energy X-ray absorptiometry (Osteometer, DTX 200; Tokyo Medic, Tokyo, Japan) at the start of treatment and at 6 and 12 months after treatment. To detect vertebral fracture incidence, lateral and anteroposterior plain radiographs of the thoracic and lumbar spine were taken at identical time points. The fractures were defined semiquantitatively [30] and following quantitative assessment [31]. Prevalent fractures were identified as a grade I deformity of the vertebral body by semiquantitative assessment and following quantitative assessment, as <20 % height decrease of the center of the vertebral body compared with the anterior or posterior height of the vertebral body, or 25 % height decrease of the anterior vertebral body compared with the posterior vertebral body height. Incident vertebral fractures were defined as one grade progression of deformity of the vertebral body by semiquantitative assessment and following quantitative assessment, as a 15 % progression of the vertebral body height and a 4 mm decrease in anterior, middle, or posterior vertebral body height.

Patients were then divided into two subgroups, with or without vertebral fracture incidence at 6 or 12 months post-treatment in both the R and R + K groups. To elucidate risk factors for vertebral fracture incidence during risedronate monotherapy or combined risedronate and vitamin K_2 therapy, the average age, percentage of patients with previous vertebral fractures, bone metabolic markers and BMD at the beginning of treatment and at 6 months post-treatment were evaluated between subgroups in both the R and R + K groups.

Statistical analyses

Results are expressed as mean ± standard deviation (SD). Intergroup differences were assessed by the Mann–Whitney *U* test and differences, depending on treatment period, were assessed by the paired *t* test. A probability <0.05 was considered to indicate statistical significance. All statistical analyses were performed using Statistical Package for the Biosciences software (SPBS v9.54, Akita, Japan) [32].

Results

Baseline characteristics (Table 1) and backgrounds of the drop-out patients (Table 2)

At the beginning of treatment, patient baseline characteristics, including average age, height, body weight, body mass index (BMI) and vitamin K intake, were not significantly different between groups (Table 1). In both the R group and R + K group, the major reason for drop-out was cessation of treatment by the patients themselves ($n = 10$ in each group). Nausea due to medication was the chief complaint in four patients in the R group and in seven patients in the R + K group (Table 2).

Table 1 Patient characteristics at the beginning of treatment

	R group	R + K group	*p* value
No. of patients	50	51	
Age (years)	74.0 ± 6.9	75.4 ± 5.7	0.267
Height (cm)	147.1 ± 6.7	147.0 ± 7.0	0.945
Body weight (kg)	51.4 ± 8.8	49.8 ± 7.3	0.332
BMI (kg/m^2)	23.7 ± 3.4	23.1 ± 3.4	0.391
Vitamin K intake[a]	33.0 ± 15.5	28.1 ± 14.6	0.108

R group patients treated with risedronate alone, *R + K group* patients treated with risedronate and menatetrenone, *BMI* body mass index

[a] Evaluated with a simple vitamin K intake questionnaire (Uenishi et al. [29])

Table 2 Number of drop-out patients and the reason

	R group (*n*)	R + K group (*n*)
Drop-out by 6 months	11	17
Drop-out by 12 months	10	8
Reason for drop-out		
Cessation of treatment	10	10
Nausea	4	7
Suicide attempt/depression	3	1
Cardiac disease	1	3
Fracture of lower extremity	1	3
Death	1 (due to pneumonia)	0
Dental therapy	1	0
Brain infarction	0	1

Bone metabolic markers (Table 3)

At the beginning of treatment, bone metabolic markers, including NTX, BAP and OC were not significantly different between groups. However, ucOC in the R + K group was significantly higher than in the R group ($p < 0.05$) and ucOC/OC in the R + K group was significantly lower than in the R group ($p < 0.05$).

Risedronate monotherapy and combined risedronate and vitamin K_2 therapy significantly decreased NTX ($p < 0.001$ and 0.01, respectively) and BAP ($p < 0.001$) levels at 6 and 12 months after treatment. Risedronate monotherapy significantly decreased ucOC levels at 6 ($p < 0.01$) and 12 months ($p < 0.001$) and OC levels at

Table 3 Serum levels of bone metabolic markers

	Beginning		6 months		12 months	
	R group ($n = 50$)	R + K group ($n = 51$)	R group ($n = 39$)	R + K group ($n = 34$)	R group ($n = 29$)	R + K group ($n = 26$)
NTX (nmol BCE/L)	20.1 ± 6.4	19.7 ± 6.3	16.0 ± 5.3**	15.5 ± 4.8*	14.7 ± 3.7**	14.3 ± 5.9*
BAP (U/L)	21.2 ± 9.7	20.7 ± 11.0	13.8 ± 5.8**	12.0 ± 4.1**	13.5 ± 8.4**	11.3 ± 3.5**
ucOC (ng/ml)	7.7 ± 4.0	5.7 ± 4.2$^{\#}$	4.8 ± 3.1*	2.6 ± 1.0**^{,B}	4.0 ± 2.8**	1.9 ± 0.8**^{,C}
OC (ng/ml)	7.5 ± 2.3	6.9 ± 2.4	6.4 ± 3.6	6.3 ± 1.7	4.8 ± 1.2**	5.4 ± 1.6
ucOC/OC	1.0 ± 0.4	0.8 ± 0.3$^{\#}$	0.8 ± 0.4	0.4 ± 0.1**^{,C}	0.8 ± 0.4	0.4 ± 0.1**^{,C}
% change						
NTX (%)			−20.7 ± 19.5	−16.1 ± 17.8	−27.7 ± 16.6	−22.4 ± 27.2
BAP (%)			−31.6 ± 20.4	−33.7 ± 22.7	−38.3 ± 23.7	−36.2 ± 16.7
ucOC (%)			−41.5 ± 26.9	−35.2 ± 36.0	−49.1 ± 38.3	−55.7 ± 23.5
OC (%)			−23.5 ± 26.2	−1.79 ± 31.2^{B}	−34.2 ± 29.6	−16.3 ± 29.4^{A}
ucOC/OC (%)			−19.8 ± 29.6	−35.8 ± 20.8^{A}	−22.8 ± 36.3	−48.6 ± 15.6^{C}

Mean ± SD

R group patients treated with risedronate alone, *R + K group* patients treated with risedronate and menatetrenone, *NTX* cross-linked N-telopeptide of type I collagen, *BAP* bone alkaline phosphatase, *ucOC* undercarboxylated osteocalcin, *OC* osteocalcin, *ucOC/OC* ratio of ucOC to OC, *% change* percentage change in maker compared with the value at the beginning of treatment, *BCE* bone collagen equivalent

$^{\#}$ $p < 0.05$ versus R group, * $p < 0.01$, ** $p < 0.001$ versus each marker compared with the value at the beginning of treatment. ^{A} $p < 0.05$, ^{B} $p < 0.01$, ^{C} $p < 0.001$ versus R group at each time point

12 months only ($p < 0.001$). However, ucOC/OC was not significantly decreased in the R group at both time points. Combined risedronate and vitamin K_2 treatment significantly decreased ucOC and ucOC/OC levels at 6 ($p < 0.001$) and 12 months ($p < 0.001$) after treatment. OC levels were not significantly decreased in the R + K group at both time points.

After 6 and 12 months, ucOC ($p < 0.01$ and 0.001, respectively) and ucOC/OC ($p < 0.001$ and 0.001, respectively) in the R + K group were significantly lower than in the R group. However, other markers, including NTX, BAP and OC, were not significantly different between groups at both time points. The magnitude of OC level decrease in the R + K group was significantly lower than in the R group at 6 ($p < 0.01$) and 12 months ($p < 0.05$). Furthermore, the percentage change of ucOC/OC in the R + K group was significantly larger than in the R group at 6 ($p < 0.05$) and 12 months ($p < 0.001$). The percentage change of other markers, including NTX, BAP and ucOC, were not significantly different between groups at both time points.

Bone mineral density (Table 4)

BMD and *T* score were not significantly different between groups at the beginning of treatment. BMD, *T* score and percentage change in these parameters in both the R and R + K groups were also not significantly different at 6 and 12 months after treatment.

Prevalence or incidence of vertebral fractures (Table 5)

At the beginning of treatment, 18 patients (36 %) in the R group and 25 patients (49 %) in the R + K group had prevalent vertebral fractures. These included 30 and 45 vertebral fractures in the R and R + K groups, respectively, with the most common site being in the T12 or L1 vertebrae. The number of patients with vertebral fracture incidence was three (7.7 %) in the R group and four (11.8 %) in the R + K group at 6 months after treatment. At 12 months after treatment, vertebral fracture incidence was 10.3 % in the R group and 15.4 % in the R + K group. Four vertebral fractures occurred 6 months after treatment in both the R and R + K groups. At 12 months after treatment, six and four vertebral fractures were observed in the R and R + K groups, respectively. There was no statistically significant difference in the incidence of vertebral fractures between groups.

Bone metabolic markers in patients with and without incident vertebral fractures (Table 6)

The average age of patients with incident vertebral fractures in the R group only, was significantly greater ($p < 0.05$) than that of patients without incident vertebral fractures. Six patients (100 %) in the R group and 7 patients (87.5 %) in the R + K group with incident vertebral fractures had prevalent vertebral fractures at the beginning of treatment. NTX and BAP levels in patients

Table 4 Bone mineral density and *T* score

	Beginning		6 months		12 months	
	R group ($n = 50$)	R + K group ($n = 51$)	R group ($n = 39$)	R + K group ($n = 34$)	R group ($n = 29$)	R + K group ($n = 26$)
BMD (g/cm^2)	0.305 ± 0.061	0.286 ± 0.062	0.313 ± 0.064	0.295 ± 0.060	0.305 ± 0.065	0.288 ± 0.067
T score	−4.9 ± 1.7	−5.4 ± 1.4	−4.7 ± 1.8	−5.2 ± 1.7	−4.8 ± 1.8	−5.3 ± 1.9
% change						
BMD (%)			1.3 ± 6.0	0.74 ± 7.5	1.2 ± 8.1	0.5 ± 6.1
T score (%)			0.3 ± 16.3	1.5 ± 12.9	2.1 ± 13.7	0.2 ± 14.2

Mean ± SD

R group patients treated with risedronate alone, *R + K group* patients treated with risedronate and menatetrenone, *BMD* bone mineral density, *% change* percentage change in each parameter compared with the value at the beginning of treatment

Table 5 Number of patients with prevalent and incident vertebral fractures

	Beginning		6 months		12 months	
	R group ($n = 50$)	R + K group ($n = 51$)	R group ($n = 39$)	R + K group ($n = 34$)	R group ($n = 29$)	R + K group ($n = 26$)
No. of patients with prevalent fracture (%)	18 (36)	25 (49)	–	–	–	–
Total no. of prevalent vertebral fractures	30	45	–	–	–	–
No. of patients with incident vertebral fractures	–	–	3	4	3	4
Incidence of new vertebral fractures (%)	–	–	7.7	11.8	10.3	15.4
Total no. of incident vertebral fractures	–	–	4	4	6	4

R group patients treated with risedronate alone, *R + K group* patients treated with risedronate and menatetrenone

with incident vertebral fractures in the R group, only at the beginning of treatment, were significantly higher than those without incident vertebral fractures ($p < 0.05$ and 0.01, respectively). Six months after treatment, NTX and ucOC levels in patients with incident vertebral fractures in the R group only were significantly higher than those without incident vertebral fractures ($p < 0.0001$ and 0.05, respectively). BMD and *T* score in patients with incident vertebral fractures in the R group only were significantly lower than those in patients without incident vertebral fractures ($p < 0.05$).

Discussion

In this prospective study, serum ucOC levels decreased after risedronate monotherapy and combined risedronate with vitamin K_2 therapy in similar magnitude after 1 year of treatment. A decrease in OC level was observed after 1 year of treatment with risedronate monotherapy only. Hirao et al. [27] revealed that alendronate monotherapy decreased ucOC, while carboxylated OC (COC) and ucOC/COC were unaffected. Conversely, combined therapy with alendronate and vitamin K_2 decreased the ucOC level and ucOC/COC ratio in post-menopausal osteoporotic patients. OC is measured as a total of ucOC and COC. In the present study, the magnitude of ucOC level decrease was similar between risedronate monotherapy and combined therapy; however, the magnitude of ucOC/OC ratio decrease was significantly lower in combined therapy compared with monotherapy. Vitamin K_2, menatetrenone, stimulates the post-translational gamma-carboxylation of glutamic acid residues in pro-osteocalcin and mediates the conversion of ucOC to COC as a cofactor of gamma-carboxylase [33]. Monotherapy with risedronate decreased the serum levels of ucOC and OC via suppression of bone turnover, and resulted in no significant difference in the ucOC/OC ratio after 1 year of treatment. On the other hand, menatetrenone stimulated carboxylation of OC and resulted in decreased serum ucOC levels and increased serum COC levels during combined therapy with risedronate. The effects of menatetrenone caused no significant changes in serum OC levels and significant decreases in the ucOC/OC ratio 1 year later with combined therapy with risedronate and menatetrenone. In corticosteroid-induced osteoporosis, there was no added benefit of combined risedronate and vitamin K_2 therapy on bone metabolic markers, including OC and ucOC, compared with risedronate monotherapy [34]. Therefore, evaluation of not only the ucOC level,

Table 6 Patient parameters with and without incident vertebral fractures at the beginning and at 6 months after treatment

	Beginning				6 months			
	R group		R + K group		R group		R + K group	
	$F-$ ($n = 33$)	$F+$ ($n = 6$)	$F-$ ($n = 26$)	$F+$ ($n = 8$)	$F-$ ($n = 33$)	$F+$ ($n = 6$)	$F-$ ($n = 26$)	$F+$ ($n = 8$)
Age (years)	73.0 ± 7.5	79.7 ± 5.7*	75.6 ± 5.5	75.6 ± 6.7				
% of patients with prevalent fractures (%)	30.3	100	46.2	87.5				
NTX (nmol BCE/L)	19.3 ± 5.4	28.3 ± 9.0**	18.7 ± 5.1	19.0 ± 4.4	14.7 ± 3.8	23.3 ± 7.0****	15.4 ± 4.6	15.8 ± 5.7
BAP (U/L)	19.9 ± 7.7	31.9 ± 19.0*	18.3 ± 5.0	19.6 ± 6.2	13.1 ± 5.6	17.7 ± 6.0	11.5 ± 3.5	13.3 ± 5.9
ucOC (ng/ml)	7.9 ± 5.2	10.3 ± 9.0	4.6 ± 1.8	4.4 ± 2.6	4.2 ± 2.9	7.9 ± 7.0***	2.6 ± 1.0	2.5 ± 0.9
OC (ng/ml)	7.4 ± 2.5	8.6 ± 1.9	6.6 ± 1.6	5.9 ± 1.7	5.5 ± 2.9	8.1 ± 5.4	6.0 ± 1.7	6.2 ± 1.9
ucOC/OC	1.0 ± 0.4	1.0 ± 0.6	0.7 ± 0.2	0.8 ± 0.5	0.8 ± 0.4	0.9 ± 0.6	0.4 ± 0.1	0.4 ± 0.1
BMD (mg/cm^2)	0.317 ± 0.006	0.269 ± 0.005	0.299 ± 0.053	0.272 ± 0.066	0.322 ± 0.062	0.263 ± 0.053***	0.297 ± 0.059	0.288 ± 0.067
T score	−4.5 ± 1.8	−5.9 ± 1.3	−5.0 ± 1.5	−5.8 ± 1.8	−4.4 ± 1.7	−6.0 ± 1.5***	−5.1 ± 1.6	−5.4 ± 1.8

Mean ± SD

R group patients treated with risedronate alone, *R + K group* patients treated with risedronate and menatetrenone, *F −* patients without incident vertebral fractures 12 months after treatment, *F +* patients with incident vertebral fractures 12 months after treatment, *NTX* cross-linked N-telopeptide of type I collagen, *BAP* bone alkaline phosphatase, *ucOC* undercarboxylated osteocalcin, *OC* osteocalcin, *ucOC/OC* ratio of ucOC to OC, *BCE* bone collagen equivalent

* $p < 0.05$, ** $p < 0.01$ versus $F-$ of the R group at the beginning of treatment, *** $p < 0.05$, **** $p < 0.0001$ versus $F-$ of the R group 6 months after treatment

but also the OC level and the ucOC/OC ratio, would be useful for analyzing the effects of several medicines on bone metabolism in osteoporosis.

In this study, the ucOC value in patients with incident vertebral fractures (7.9 ± 7.0 ng/ml) was higher than in patients without incident vertebral fractures (4.2 ± 2.9 ng/ml) 6 months after risedronate monotherapy. In a retrospective study by Shiraki et al. [28], ucOC serum levels in patients with incident vertebral fractures (2.75 ± 0.19 ng/ml) was higher than in patients without incident vertebral fractures (2.28 ± 0.13 ng/ml) with amino-bisphosphonate treatment. In the study, the ucOC cut-off value for osteoporotic fracture incidence was found to be 2.6 ng/ml and patients without incident vertebral fractures were found to have a greater ucOC value of 4.2 ng/ml [28]. In this prospective study, the aim was to evaluate vertebral fracture incidence; however, fracture occurrence at other sites was not reported in participants. Results from both Shiraki et al. [28] and our study have shown that a higher ucOC level in patients treated with bisphosphonates correlates with a higher risk of osteoporotic fracture incidence. Thus, combined bisphosphonate and vitamin K_2 therapy should be considered for patients showing higher ucOC levels during bisphosphonate therapy.

Previous studies have shown the effects of combined bisphosphonate and menatetrenone therapy on the incidence of vertebral fractures. Iwamoto et al. [35] reported that combined etidronate and vitamin K_2 therapy reduced the incidence of vertebral fractures after treatment for 24 months compared with etidronate, vitamin K_2, or calcium monotherapies. In our study, combined risedronate and vitamin K_2 therapy did not show significant effects on prevention of vertebral fracture incidence compared with risedronate monotherapy. This may be due to the difference in type of bisphosphonate (etidronate or risedronate) and patient population, including average age, which was approximately 10 years greater in this study compared with the study by Iwamoto et al. [35]. Furthermore, the ucOC values in combined risedronate and vitamin K_2 therapy with or without incident vertebral fractures, were not significantly different and lower than the reported cut-off value (2.6 ng/ml) for osteoporotic fracture during treatment with alendronate or risedronate in the study by Shiraki et al. [28]. In our study, the number of patients at 1 year after treatment was insufficient to conclude the effects of combined therapy with risedronate and menatetrenone on the prevention of incidental osteoporotic vertebral fractures in elderly osteoporotic patients with prevalent vertebral fractures. Many factors including spinal alignment, muscle weakness, or increased fall risk in elderly patients might influence the incidence of vertebral fractures [36]. Therefore, it could be important to consider not only combined risedronate and menatetrenone therapy, but improvement

of spinal alignment, muscle strength, or decreased fall risk as well.

Our study has several limitations. First, the baseline ucOC level was different between the R and R + K groups at the beginning of treatment, even after patient randomization. Also, the vitamin K intake score using a simple questionnaire, and the OC and other metabolic marker values, were not significantly different at the beginning of treatment. Horiuchi et al. [37] have demonstrated that serum ucOC levels were higher in osteoporotic than in non-osteoporotic elderly patients with type II diabetes mellitus (DM). Although we did not exclude the presence of type II DM in our subjects, coexisting type II DM might have influenced the difference in serum ucOC levels between the two groups at the baseline of this study. Second, the follow-up rates of both groups were not high, at 78 and 67 % at 6 months and 58 and 51 % at 12 months in each group, respectively. Lower continuous rates of weekly bisphosphonate treatment, which have been reported at 50–60 % at 12 months in previous studies [38], are a current clinical issue. However, the number of patients at the final follow-up was insufficient to conclude the effects of combined therapy with risedronate and vitamin K_2 on preventing incidental osteoporotic vertebral fractures. The power of analysis, with a 1-β error, was 0.44 between the groups at the final follow-up to evaluate the significant effects of combined therapy to prevent osteoporotic fractures. Therefore, further studies are required to evaluate the effects of this combined therapy on bone metabolic markers and vertebral fracture incidence in aged osteoporotic patients.

In conclusion, risedronate monotherapy significantly decreased ucOC and OC levels, but not the ucOC/OC ratio after 1 year of treatment. Conversely, combined risedronate and vitamin K_2 therapy significantly decreased the ucOC level and the ucOC/OC ratio, but not OC levels 1 year after treatment. The magnitude of decrease in the serum ucOC level was similar between the monotherapy and combined therapy groups. The ucOC level in patients with incident vertebral fractures was higher than in the patients without incident vertebral fractures during risedronate monotherapy. However, combined treatment could not prevent incident vertebral fractures in aged osteoporotic patients with prevalent vertebral fractures.

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Conflict of interest All authors have no conflicts of interest.

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