Original article

Vitamin K₂ and Etidronate Therapy in the Early Period after Hip Fracture

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Abstract

Objective : The purpose of the present study is to clarify the efficacy of etidronate and vitamin K₂ in sustaining bone mineral density (BMD) in patient with hip fracture by monitoring metabolic bone markers and BMD during the 36-week period after fracture.

Materials and Methods : Forty-seven hip fracture patients from 51 to 93 years old (77.2±9.6) were randomly divided into three groups : 14 patients in the intermittent cyclical etidronate-treated group (group E), 16 patients in the vitamin K₂-treated group (group K), and 17 patients in the control (group C). Drugs were administered to patients in groups E and K six weeks after their operations. Blood and urine samples were obtained just before the start of drug administration and at 12, 24, and 36 weeks thereafter. Urinary type I collagen C-terminal telopeptide (uCTX), pyridinoline (PYR), deoxypyridinoline (DPD), serum CTx (sCTX), osteocalcin (OC_{N-mid}), and undercarboxylated osteocalcin (ucOC) were measured. The contralateral proximal femur and lumbar spine BMDs were measured at baseline and at 36 weeks.

Results : Deoxypyridinoline at 12 weeks and

OC_{N-mid} at 36 weeks after treatment were lower in group E than those in group C. N-mid osteocalcin and ucOC at 24 and 36 weeks were lower in group K than those in group C. Although femoral neck BMD in groups C and E decreased compared to the baseline values at 36 weeks, femoral neck BMD in group K tended to increase. Specifically, in group K the BMD of Ward's triangle increased significantly after treatment. Bone mineral density of the lumbar spine in each group did not change significantly during the 42 weeks following hip fracture.

Conclusion : Vitamin K₂ prevented further bone loss in the contralateral proximal femur. The administration of vitamin K₂ to patients with hip fractures in the early period after fracture is potentially useful in preventing a second hip fracture on the contralateral side.

Key words : biochemical marker, bone mineral density, etidronate, hip fracture, vitamin K₂

Introduction

With an ever increasing population of elderly people in Japan and around the world, the incidence of hip fractures has been increasing year by year. The incidence of hip fractures in Asia is estimated to be approximately 1.5 million by 2025 and 3.5 million by 2050¹⁾. Those who suffer from hip fracture, even if they can walk soon after

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surgery to prevent bone atrophy from disuse, may still suffer from further fractures, such as a contralateral hip fracture or vertebral compression fractures, since bone mineral density (BMD) may decrease rapidly and not recover for a long period after the initial fracture²⁻⁶⁾. Therefore, it is important to prevent further bone loss after a hip fracture and reduce the risk of additional fragility fractures. However, drug interventions to improve osteoporosis treatment following hip fractures are not yet routine in out-patient clinics^{7,8)}. Currently, a number of drugs for improving bone metabolism and restoring BMD are available. Among these, bisphosphonate is one of the most effective drugs for increasing the BMD of the spine and the appendicular bone since it powerfully suppresses bone resorption by directly modulating the osteoclast function⁹⁾. Etidronate is a first generation bisphosphonate and is reported to reduce the rate of vertebral fractures in patients with osteoporosis after three years of treatment¹⁰⁾. On the other hand, vitamin K₂ is one of the drugs that promote bone formation. Vitamin K₂ is a cofactor of γ -carboxylase that converts undercarboxylated osteocalcin to carboxylated osteocalcin ; it also modulates the proliferation of osteoblast and the uptake of carboxylated osteocalcin into the bone¹¹⁾. In a cross-sectional study, serum concentration of vitamin K₂ was significantly lower in patients with hip fractures than in age-matched controls¹²⁾. Recently, a meta-analysis of randomized controlled trials of vitamin K₂ for osteoporotic subjects showed that supplementation with vitamin K₂ reduced bone loss, and there was a strong effect on incident fractures¹³⁾. Therefore, bone loss after hip fracture could be substantially reduced by the administration of vitamin K₂. The purpose of the present study is to clarify the efficacy of etidronate and vitamin K₂ in sustaining BMD in patients with hip fracture by monitoring metabolic bone markers and the BMD of the spine and the contralateral proximal femur during the 42-week period following the hip fracture.

Materials and Methods

Initially, 61 patients with hip fractures were considered for the study. All subjects suffered from hip fractures resulting from a simple fall from standing height or lower. All patients were admitted to our hospital within 24 hours after their fractures. Cemented hemiarthroplasty (Kyocera PerFix) or osteosynthesis with three 6.5 mm canulated cancellous screws (Ace Medical) for femoral neck fractures and osteosynthesis with compression hip screws (Ace Medical) or γ -nails (Howmedica) for inter-trochanteric fractures were performed within one week of admission. All subjects were allowed to walk one week after surgery. Patients were excluded if they met any of the following criteria in the two years before the fracture : 1) were admitted with a pathologic fracture, 2) had sustained a previous fracture, 3) had a thyroid disorder, 4) had suffered from alcohol abuse, 5) had taken calcium, vitamin D, bisphosphonate, vitamin K, thiazide or glucocorticoids supplements, 6) had suffered from osteomalacia after gastrectomy, 7) had had an orchidectomy, 8) were undergoing hormone replacement therapy for prostate disease, 9) were unable to walk, or 10) had pseudoarthrosis of the fracture site and osteonecrosis of the femoral head. Informed consent was obtained from each patient at the outpatient clinic. Subjects were randomly divided into three groups : the etidronate-treated group (group E), the vitamin K₂-treated group (group K), and the control group (group C). No placebo capsules were administered to the controls. Group E consisted of 22 patients given an intermittent cyclical etidronate regimen (Didronel: Sumitomo Co., 200 mg/day taken on an empty stomach for 14 days, every 3 months for a total of 4 cycles) at the start of the study. Group K consisted of 18 patients treated orally with 45 mg/day of menatetrenone (Menatetrenone, Glakay : Eisai Co. Ltd., Tokyo, Japan) for 36 weeks from the start of the study. In groups E and K, drugs were administered six weeks after the operation. Eight patients in group E and 2 patients in group

K were removed from the study due to discontinuation of drug administration. Group C consisted of 21 patients treated without drugs, but 4 patients could not be followed postoperatively because they did not return to Yayoi hospital. Consequently, a total of 47 patients (14 in group E, 16 in group K, and 17 in group C) completed this study. Blood and urine samples taken between 9 : 00 AM and 11 : 00 AM were obtained just before the start of drug administration to groups E and K. Samples were also obtained at 12, 24, and 36 weeks after the beginning of administration. In group C, blood and urine samples were collected at the same time as groups E and K (6 weeks after operation and 12, 24, and 36 weeks thereafter). Samples were stored at -30°C until analysis. Bone mineral densities of the lumbar spine and four regions (femoral neck, trochanter, Ward's triangle, and total hip) of the contralateral proximal femur were measured just before the start of treatment and at 36 weeks after beginning treatment in groups E and K. Similarly, in group C, BMD was measured at 6 weeks after operation and at the end of the study (42 weeks after operation).

Measurement of biochemical markers

Urinary type I collagen C-terminal telopeptide (uCTX) was measured by CrossLapsTM enzyme-linked immunosorbent assay (ELISA) kit according to Bonde *et al.*¹⁴⁾. The CTx is based on an immobilized synthetic peptide with an amino acid sequence specific to part of the C-telopeptide of the α I chain of type I collagen (Glu-Lys-Ala-His-Asp-Gly-Arg=CrossLaps peptide). The intra-/inter-assay coefficients of variation were less than 6% and 8%, respectively.

Urinary pyridinoline (PYR) and deoxypyridinoline (DPD) were measured by high performance liquid chromatography after acid hydrolysis using a Glison ASPEC (automated sample preparation with extraction column) in accordance with Pratt *et al.*¹⁵⁾. In our protocol, the intra-/inter-assay coefficients of variation were 6.4% and 5.9% for PYR and 6.0% and 6.0% for DPD, respectively. Values of urinary PYR, DPD,

and CTx were corrected for urinary creatinine concentration, which was measured on an autoanalyser.

The serum levels of type I collagen C-terminal telopeptide (sCTX) were measured by ELISA, using procedures discussed in Bonde *et al.*¹⁶⁾. The assay developed for measuring type I collagen degradation products in sera is based on an immobilized synthetic peptide with an isomerized amino acid sequence specific for one part of the C-terminal peptide of the α I chain of type I collagen (EKAHD- β -GGR). The intra- / inter-assay coefficients of variation were 6.7% and 9.2%, respectively.

The serum concentration of osteocalcin ($\text{OC}_{\text{N-mid}}$) was measured by ELISA (two-site N-MID Osteocalcin, Osteometer BioTech A/S, Denmark)¹⁷⁾. The intra-/inter-assay coefficients of variation were less than 4.2% and 4.0%, respectively, and the detection limit was 2.0 $\mu\text{g/l}$.

The serum concentration of ucOC was determined with a commercially available kit (Glu-OC Takara : Takara Shuzo Co, Kyoto, Japan)¹⁸⁾. The antibody used indicated that the bovine OC MoAb exhibited 100% reactivity with Glu^{17,21,23}-OC and 6% with Gla^{17,21,23}-OC. The assay methods were used according to the manufacturer's specifications. The intra-assay coefficient of variation assessed by 10 measurements was less than 7.3%. The inter-assay coefficient of variation evaluated by repeat measurements on 8 separate days over 10 weeks was less than 10%.

Measurement of bone mineral density

Bone mineral density (BMD) in grams per square centimeter was determined for the lumbar spine (L2-4 in antero-posterior projection) and four regions (femoral neck, trochanter, Ward's triangle, and total hip) in the proximal femur of the contralateral side using dual-energy X-ray absorptiometry (DXA) from an Expert 5000 instrument (Lunar, Madison, Wisconsin, U.S.A.). The precision of the integral calcium hydroxyapatite standard was less than 0.4% over time.

Statistical analysis

Data was analysed using a Statview 5.0 program on a Macintosh computer. Analysis of variance (ANOVA) followed by the Scheffe-F test was used for comparison of the baseline data including characteristics of the patients, BMDs of the lumbar spine and four regions of the contralateral proximal femur, and biochemical parameters among the three groups. To compare the biochemical parameters, percent changes from the baseline values were calculated at each time point to eliminate the effect of the baseline values on succeeding longitudinal values. Two-way analysis of variance (ANOVA), and the Scheffe-F test for two related samples were used to evaluate differences. Differences between each of the drug-treated groups (group E or group K) and the control group (group C) were analyzed using a non-parametric test, followed by a Mann-Whitney U test. Differences between baseline BMD and final BMD in each group were analyzed using a non-parametric test, followed by a Wilcoxon signed-ranks test. All values but biochemical parameters were expressed as mean±standard deviation (SD). Values of biochemical parameters were expressed as mean±standard error (SE). Statistical significance was defined as less than 0.05.

Results

The anthropometric and baseline BMD data for the three groups is shown in Table 1. There were no significant differences among the three groups in the baseline data including age, height, weight, body mass index, spine and contralateral proximal femur BMDs. Table 2 shows the baseline values of the biochemical parameters for the three groups. No significant differences among the three groups were observed in urinary and serum bone resorption markers and serum levels of vitamin K related biochemical parameters (serum OC_{N-mid} and ucOC). Figure 1 shows sequential changes in urinary CTx (upper panel) and serum CTx (lower panel) for each group. Both urinary CTx and serum CTx in each group showed a tendency to decrease over time. The values of urinary and serum CTx in groups E and C tended to be lower than those in group K at all points in time after the start of drug administration except urinary CTx in group C at 36 weeks. In groups E and C, levels of urinary CTx at 12, 24 and 36 weeks were significantly lower than the baseline values. The values of serum CTx at 36 weeks were significantly lower than the initial values in all groups. However, when comparing the values of urinary and serum CTx among the groups, no significant differences were observed at any point.

Table 1 Anthropometric and Bone Mineral Density Data of Each Group

	Group C	Group E	Group K	Differences (ANOVA)
N (male/female)	17 (3/14)	14 (3/11)	16 (2/14)	
Age (years)	77.6±10.9	74.9±7.5	78.9±10.1	n.s.
Height (cm)	146.6±10.3	151.4±11.0	148.4±8.3	n.s.
Weight (kg)	46.5±8.2	49.1±11.6	46.0±10.1	n.s.
BMI (kg/m ²)	21.6±2.6	21.3±3.1	20.8±4.0	n.s.
L ₂₋₄ BMD(g/cm ²)	0.803±0.190	0.787±0.274	0.760±0.210	n.s.
Femoral neck BMD (g/cm ²)	0.575±0.103	0.574±0.115	0.541±0.161	n.s.
Trochanter BMD (g/cm ²)	0.443±0.128	0.427±0.073	0.419±0.140	n.s.
Ward's triangle BMD (g/cm ²)	0.404±0.093	0.370±0.100	0.357±0.137	n.s.
Total hip BMD (g/cm ²)	0.581±0.130	0.576±0.094	0.545±0.149	n.s.
Trochanteric (N)/Neck fracture (N)	7/10	7/7	7/9	
Osteosynthesis (N)/Endoprosthesis (N)	11/6	10/4	9/7	

Data is shown as mean±SD.

BMI : body mass index ; BMD : bone mineral density ; n.s. : not significant.

Table 2 Biochemical Data at the Start of Treatment in Each Group

	Group C	Group E	Group K	Differences (ANOVA)
Urinary CTx ($\mu\text{g}/\text{mmol creat.}$)	564.2 $\pm$ 80.8	523.7 $\pm$ 88.6	625.6 $\pm$ 79.7	n.s.
Serum CTx (pM)	8,403 $\pm$ 1,002	7,627 $\pm$ 1,204	8,325 $\pm$ 1,248	n.s.
Urinary PYR (nmol/mmol creat.)	115.8 $\pm$ 13.8	112.6 $\pm$ 21.9	115.9 $\pm$ 11.6	n.s.
Urinary DPD (nmol/mmol creat.)	17.8 $\pm$ 2.1	16.8 $\pm$ 3.3	17.3 $\pm$ 1.9	n.s.
Serum OC _{N-mid} (ng/ml)	15.2 $\pm$ 1.5	20.3 $\pm$ 3.7	17.9 $\pm$ 2.7	n.s.
Serum ucOC (ng/ml)	5.1 $\pm$ 0.6	7.4 $\pm$ 2.3	6.2 $\pm$ 1.3	n.s.

Data is shown as mean $\pm$ SE.

CTx : type I collagen C-terminal telopeptide ; PYR : pyridinoline, DPD : deoxypyridinoline ;

OC_{N-mid} : N-mid portion of osteocalcin ; ucOC : undercarboxylated osteocalcin ; n.s. : not significant.

Figure 1 Sequential changes in urinary (upper panel) and serum (lower panel) type I collagen C-terminal telopeptide (CTx) in the etidronate-treated group (group E), vitamin K₂-treated group (group K), and control group (group C) during the 36-week period. Data is shown as percent changes from the baseline values (mean $\pm$ SE). **p < 0.01, *p < 0.05 compared to the baseline values.

Figure 2 Sequential changes in urinary pyridinoline (PYR) (upper panel) and deoxypyridinoline (DPD) (lower panel) in the etidronate-treated group (group E), vitamin K₂-treated group (group K), and control group (group C) during the 36-week period. Data is shown as percent changes from the baseline values (mean $\pm$ SE). **p < 0.01 compared to the baseline values. At 12 weeks, the value of serum CTx in group E was significantly lower than that in group C (p < 0.05).

Figure 2 shows sequential changes in urinary PYR (upper panel) and DPD (lower panel) for each group. The values of urinary PYR and DPD at 12, 24, and 36 weeks in groups C and E and those values at 36 weeks in group K decreased significantly compared to the initial values (Figure 2). To reiterate, the values of urinary PYR and DPD in groups E and C tended to be lower than those in group K at any time point after the start

of drug administration. Comparing the values of urinary DPD among the groups, the values at 12 weeks was significantly lower in group E than in group C. Figure 3 shows sequential changes in serum OC_{N-mid} in each group. The values of OC_{N-mid} gradually increased in group C while they gradually decreased in groups E and K after 12

Figure 3 Sequential changes in serum N-mid osteocalcin (OC_{N-mid}) in the etidronate-treated group (group E), vitamin K_2 -treated group (group K), and control group (group C) during the 36-week period. Data is shown as percent changes from the baseline values (mean $\pm$ SE). Values of serum OC_{N-mid} in group K were significantly lower than those in group C at 12, 24, and 36 weeks ($p < 0.05$). The value of serum OC_{N-mid} in group E was significantly lower than the value in group C at 36 weeks ($p < 0.05$).

weeks. The values of OC_{N-mid} in group K at 12, 24, and 36 weeks and the values in group E at 36 weeks were significantly lower than those in group C. Time-dependant changes in ucOC in each group were almost the same as changes in OC_{N-mid} (Figure 4). The values of ucOC tended to be higher in group C but tended to be lower in groups E and K with time. The values of ucOC at 24 and 36 weeks in group K were significantly lower than those in group C. Figure 5 shows BMD of the lumbar spine before (pre) and after (post) treatment in each group. Although the lumbar spine BMD increased in mean value in group E compared to the initial values, no significant difference was observed. The bone mineral densities of four regions of the contralateral proximal femur are shown in Figures 6 and 7. Significant decreases in the femoral neck BMD after 36 weeks compared to the initial values were observed in groups C and group E (Figure 6, left panel). In group C and group E, bone mineral densities at the trochanter, Ward's triangle, and total hip tended to decrease after 36 weeks compared to those at the beginning (Figure 6, right panel and Figure 7). However, after 36

Figure 4 Sequential changes in undercarboxylated osteocalcin (ucOC) in the etidronate-treated group (group E), vitamin K_2 -treated group (group K), and control group (group C) during the 36-week period. Data is shown as percent changes from the baseline values (mean $\pm$ SE). Values of serum ucOC in group K were significantly lower than those in group C at 24 and 36 weeks ($p < 0.05$).

Figure 5 Bone mineral density of the L2-4 lumbar spine at the start of the study (pre) and 36 weeks after treatment (post) for the control group (group C), etidronate-treated group (group E), and vitamin K_2 -treated group (group K). Data is shown as the mean $\pm$ SD.

weeks of treatment the BMD in group K for all four regions in the proximal femur tended to increase compared to the initial values. Particularly noteworthy, the BMD of Ward's triangle in group K increased significantly after drug administration. None of the subjects suffered from further fractures, including second hip

Figure 6 Bone mineral densities of the contralateral femoral neck (left panel) and trochanter (right panel) at the start of the study (pre) and 36 weeks after treatment (post) for the control group (group C), etidronate-treated group (group E), and vitamin K₂-treated group (group K). Data is shown as the mean±SD. Bone mineral density of the femoral neck significantly decreased compared to the initial values in groups C and E ($p<0.05$) (left panel).

Figure 7 Bone mineral densities of the contralateral Ward's triangle (left panel) and total hip (right panel) at the start of the study (pre) and 36 weeks after treatment (post) for the control group (group C), etidronate-treated group (group E), and vitamin K₂-treated group (group K). Data is shown as the mean±SD. Bone mineral density of Ward's triangle significantly increased compared to the initial values in group K ($p<0.05$) (left panel).

fractures on the contralateral side and vertebral compression fractures, during the study. All hip fractures, including those in the etidronate-treated group, united normally by three months after the

operation. None of the subjects showed delayed union, non-union or osteonecrosis of the fractured hip or loosening of prosthesis. All subjects could walk either with or without a walking cane by the

final evaluation, and none needed a wheel chair for daily activity.

Discussion

In our results, in the control group the BMD in the contralateral femoral neck decreased significantly compared to the baseline values during the 36-week period after hip fracture. Moreover, in the control group, BMDs of the lumbar spine and other sites of the contralateral proximal femur displayed a tendency to decrease from the baseline values after 36 weeks. Several papers have reported that the BMD of the lumbar spine and the contralateral femoral neck or trochanter decreased significantly during the 48 weeks following a hip fracture without any drug intervention²⁻⁶⁾. In the current study, the BMDs in all regions, excluding the femoral neck, may not have decreased significantly compared to the baseline values in the control group since the observation period was 12 weeks shorter than in the other reports cited in this paper.

Sato *et al.* investigated bone resorption markers and calcium regulated hormones in patients with hip fractures who were treated with etidronate starting one day after surgery¹⁹⁾. Although they concluded that etidronate therapy inhibited bone resorption and improved the calcium balance in patients with hip fractures, BMD was not evaluated in their study. Iwamoto *et al.* compared the effects of etidronate and menatetrenone on forearm BMD and the incidence of new vertebral fractures in postmenopausal osteoporotic women²⁰⁾. They found a significant increase in forearm BMD and a significant reduction in the incidence of new vertebral fractures in both treated groups compared to the control group. In the present study, levels of urinary DPD in the etidronate-treated group were significantly lower than those in the control group at 12 weeks after the start of administration. In addition, levels of OC_{N-mid} in the etidronate-treated group were significantly lower than those in the control group at 36 weeks after the start of administration.

Etidronate suppressed not only bone resorption but also bone formation by decreasing activation frequency. In this study, etidronate did not sustain BMD in the contralateral proximal femur after a hip fracture although the BMD of the lumbar spine tended to increase after 36 weeks of etidronate therapy ; however, the small sample size of the etidronate-treated group may be a factor.

In contrast, vitamin K₂ sustained femoral neck BMD in the contralateral proximal femur following a hip fracture. Shiraki *et al.* studied the effect of vitamin K₂ on BMD and the incidence of new fractures in 241 osteoporotic patients during a 24-month period²¹⁾. According to their results, vitamin K₂ treatment effectively prevented the occurrence of new fractures and also prevented loss of BMD in the spine. In other studies, vitamin K₂ successfully increased the BMD of disused bone in patients with hemiplegia following stroke and also prevented glucocorticoid induced reduction of BMD^{22,23)}. Vitamin K₂ is thought to act not only on bone formation but also on bone resorption. Ozuro *et al.* reported that urinary excretion of Dpyr decreased transiently but significantly after four weeks of treatment with vitamin K₂ in 34 postmenopausal women²⁴⁾. According to Sato *et al.*, pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) decreased significantly with treatment of vitamin K₂ in patients with hemiplegia following stroke compared to their control group²³⁾. Vitamin K₂ has also been reported to suppress bone resorption by inhibiting adipogenesis and osteoclastogenesis or by inhibiting prostaglandin E₂ synthesis in vitro^{25,26)}. However, all four bone resorption markers had higher values in the vitamin K₂ treated group than in the control group at almost all time points after administration. These results suggest that vitamin K₂ might not affect bone resorption soon after hip fracture.

Several papers have reported that bone formation markers increased with vitamin K₂ therapy in patients with osteoporosis or hip fractures^{23,23)}. However, reports where vitamin K₂

was administrated to patients soon after the fracture, such as in the present study, are scarce. We previously reported on sequential changes in bone resorption and formation markers after hip fracture during a 24-week period²⁷⁾. In that study, bone resorption markers (urinary PYR, DPD, CTx and serum CTx) increased soon after fracture, with the peak at 4-8 weeks, and returned to the basal levels at 24 weeks. N-mid osteocalcin increased gradually after the fracture and significantly increased at 8 and 24 weeks compared to the basal values. Therefore, according to our previous study, bone formation and bone resorption were notably accelerated at six weeks after fracture. In the current study, vitamin K₂ was administered to patients at the time when bone turnover was very high in the course of fracture healing. It is still unclear why BMDs in the contralateral femoral neck and Ward's triangle were maintained after hip fractures by vitamin K₂ administration in spite of a decrease in serum OC_{N-mid}. We have two hypotheses. First, vitamin K₂ might act in different ways depending upon the status of bone turnover before vitamin K₂ treatment. On the one hand, vitamin K₂ might accelerate bone formation in cases of low bone turnover such as with senile osteoporosis, but on the other hand, it might suppress bone formation in cases of high bone turnover such as after a fracture. The alternative hypothesis is that the demand for carboxylated OC in the bone can increase to be utilized for fracture healing or to compensate for disused bone atrophy following a fracture. However, sufficient carboxylated OC can not be incorporated into bone since the amount of vitamin K₂ available is potentially lower in patients with hip fractures¹²⁾. By administering vitamin K₂, production of OC in osteoblasts can be increased along with the conversion of ucOC to carboxylated OC so that the incorporation of carboxylated OC into bone can be accelerated, resulting in a decreased release of undercarboxylated and carboxylated OC from bone into the blood stream²⁸⁾. Therefore, in the current study circulating levels of OC_{N-mid} and ucOC decreased significantly with vitamin K₂

administration compared to group C. However, it is necessary to monitor circulating levels of vitamin K₂, intact OC and carboxylated OC to elucidate the mechanism by which vitamin K₂ prevents bone loss after a hip fracture.

Serum levels of ucOC in each group changed in almost the same manner as the changes of OC_{N-mid}. Undercarboxylated OC (ucOC) with low femoral neck BMD is thought to be a predictor of hip fracture in elderly women and also possibly reflects bone quality more than bone mineral content¹⁸⁾. As yet, no papers have reported sequential changes in serum levels of ucOC following fracture although it is believed that the role of ucOC in the process of fracture healing can be elucidated. Several studies have shown that vitamin K₂ reduced the serum levels of ucOC in the early period in patients with osteoporosis by converting ucOC to carboxylated OC^{29,30)}. In the current study, significant decreases in ucOC by vitamin K₂ therapy compared to the controls were found at 24 and 36 weeks. However, it is still unclear whether the decrease in ucOC by vitamin K₂ therapy was due to the acceleration of the conversion of ucOC to carboxylated OC.

This study has several limitations. In our previous study, we found a significant difference in sequential changes of OC_{N-mid} according to the method of operation, osteosynthesis or endoprosthesis, for the hip fractures²⁷⁾. Serum levels of OC_{N-mid} increased significantly after a hip fracture was treated by osteosynthesis, while they did not change in hip fractures treated by endoprosthesis. Therefore, subjects should have been divided according to the operative method, but the sample size did not lend itself to further division into subgroups. A second possible limitation is the lower values of BMD data for group K compared to those in groups C and E at extractions of the samples, although there were no significant differences in the lumbar and the proximal femur BMDs at the start of the study.

In this study, we used vitamin K₂ or etidronate alone to treat patients with hip fracture. Iwamoto *et al.* recommended a combined treatment of

vitamin K₂ and etidronate to prevent new vertebral fractures rather than a single treatment of vitamin K₂ or etidronate for postmenopausal osteoporosis³¹). Combined therapy with vitamin K₂ and etidronate for patients with hip fractures may be efficacious and it is well worth consideration.

In summary, vitamin K₂ prevented further bone loss of the femoral neck and Ward's triangle in the contralateral side in patients with hip fractures, although the mechanism for how vitamin K₂ maintains BMD following a fracture is still unclear. Etidronate suppressed both bone resorption and formation in a high turnover state after a hip fracture ; however, it did not sustain BMD in the contralateral proximal femur. This latter result may be partly due to the relatively small number of subjects in the etidronate-treated group. Administration of vitamin K₂ to patients with hip fractures in the early phase after fracture is potentially useful in preventing a second hip fracture of the contralateral side.

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Reference

1. Cooper C, Campion G, Melton LJ 3rd. Hip fractures in the elderly : a world-wide projection. *Osteoporos Int* 1992 ; 2 : 285–289.
2. Dirschl DR, Henderson RC, Oakley WC. Accelerated bone mineral loss following a hip fracture : a prospective longitudinal study. *Bone* 1997 ; 20 : 79–82.
3. Hedstrom M, Sjoberg K, Svensson J, *et al.* Changes in biochemical markers of bone metabolism and BMD during the first year after a hip fracture. *Acta Orthop Scand* 2001 ; 72 : 248–251.
4. Karachalios T, Lyritis GP, Kaloudis J, *et al.* The effects of calcitonin on acute bone loss after peritrochanteric fractures. A prospective, randomized trial. *J Bone Joint Surg [Br]* 2004 ; 86-B : 350–358.
5. Wehren LE, Hawkes WG, Hebel JR, *et al.* Bone mineral density, soft tissue body composition, strength, and functioning after hip fracture. *J Gerontol* 2005 ; 60A : 80–84.
6. Yu-Yahiro JA, Michael RH, Dubin ND, *et al.* Serum and urine markers of bone metabolism during the year after hip fracture. *J Am Geriatr Soc* 2001 ; 49 : 877–883.
7. Carnevale V, Nieddu L, Romagnoli E, *et al.* Osteoporosis intervention in ambulatory patients with previous hip fracture : a multicentric, nationwide Italian survey. *Osteoporos Int* 2006 ; 17 : 478–483.
8. Gardner MJ, Brophy RH, Demetrakopoulos D, *et al.* Interventions to improve osteoporosis treatment following hip fracture. *J Bone Joint Surg [Am]* 2005 ; 87 : 3–7.
9. Murakami H, Takahashi N, Sasaki T, *et al.* A possible mechanism of the specific action of bisphosphonates on osteoclasts : tiludronate preferentially affects polarized osteoclasts having ruffled borders. *Bone* 1995 ; 17 : 137–144.
10. Storm T, Thamsborg G, Steiniche T, *et al.* Effect of intermittent cyclical etidronate therapy on bone mass and fracture rate in women with postmenopausal osteoporosis. *N Engl J Med* 1990 ; 322 : 1265–1271.
11. Akedo Y, Hosoi T, Inoue S, *et al.* Vitamin K₂ modulates proliferation and function of osteoblastic cells in vitro. *Biochem Biophys Res Commun* 1992 ; 187 : 814–820.
12. Hodges SJ, Akesson K, Vergnaud P, *et al.* Circulating levels of vitamins K1 and K2 decreased in elderly women with hip fracture. *J Bone Miner Res* 1993 ; 8 : 1241–1245.
13. Cockayne S, Adamson J, Lanham-New S, *et al.* Vitamin K and the prevention of fractures. *Arch Intern Med* 2006 ; 166 : 1256–1261.
14. Bonde M, Qvist P, Fledelius C, *et al.* Immunoassay for quantifying type I collagen degradation products in urine evaluated. *Clin Chem* 1994 ; 40 : 2022–2025.

15. Pratt DA, Daniloff Y, Duncan A, *et al.* Automated analysis of the pyridinium crosslinks of collagen in tissue and urine using solid-phase extraction and reversed-phase high-performance liquid chromatography. *Ann Biochem* 1992 ; 207 : 163–175.
16. Bonde M, Garnero P, Fledelius C, *et al.* Measurement of bone degradation products in serum using antibodies reactive with an isomerized form of an 8 amino acid sequence of the C-telopeptide of type I collagen. *J Bone Miner Res* 1997 ; 12 : 1028–1034.
17. Rosenquist C, Qvist P, Bjarnason N, *et al.* Measurement of a more stable region of osteocalcin in serum by ELISA with two monoclonal antibodies. *Clin Chem* 1995 ; 41 : 1439–1445.
18. Vergnaud P, Garnero P, Meunier PJ, *et al.* Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women : the EPIDOS study. *J Endocrinol Metab* 1997 ; 82 : 719–724.
19. Sato Y, Kanoko T, Yasuda H, *et al.* Beneficial effect of etidronate therapy in immobilized hip fracture patients. *Am J Phys Med Rehabil* 2004 ; 83 : 298–303.
20. Iwamoto J, Takeda T, Ichimura S. Effect of menatetrenone on bone mineral density and incidence of vertebral fractures in postmenopausal women with osteoporosis: a comparison with the effect of etidronate. *J Orthop Sci* 2001 ; 6 : 487–492.
21. Shiraki M, Shiraki Y, Aoki C, *et al.* Vitamin K₂ effectively prevents fractures and sustains lumbar bone mineral density in osteoporosis. *J Bone Miner Res* 2000 ; 15 : 515–521.
22. Sasaki N, Kusano E, Takahashi H, *et al.* Vitamin K₂ inhibits glucocorticoid-induced bone loss partly by preventing the reduction of osteoprogenin (OPG). *J Bone Miner Metab* 2005 ; 23 : 41–47.
23. Sato Y, Honda Y, Kuno H, *et al.* Menatetrenone ameliorates osteopenia in disuse-affected limbs of vitamin D- and K-deficient stroke patients. *Bone* 1998 ; 23 : 291–296.
24. Ozuro R, Sugimoto T, Yamaguchi T, *et al.* Time-dependent effects of vitamin K₂ (Menatetrenone) on bone metabolism in postmenopausal women. *Endocrine Journal* 2002 ; 49 : 363–370.
25. Hara K, Akiyama Y, Tajima T, *et al.* Menatetrenone inhibits bone resorption partially through inhibition of PGE₂ synthesis in vitro. *J Bone Miner Res* 1993 ; 8 : 535–542.
26. Takeuchi Y, Suzawa M, Fukumoto S, *et al.* Vitamin K₂ inhibits adipogenesis, osteoclastogenesis, and ODF/RANK ligand expression in murine bone marrow cell cultures. *Bone* 2000 ; 27 : 769–776.
27. Ohishi T, Takahashi M, Kushida K, *et al.* Changes of biochemical markers during fracture healing. *Arch Orthop Traum Surg* 1998 ; 118 : 126–130.
28. Koshihara Y, Hoshi K. Vitamin K₂ enhances osteocalcin accumulation in the extracellular matrix of human osteoblasts in vitro. *J Bone Miner Res* 1997 ; 12 : 431–438.
29. Miki T, Nakatsuka K, Naka H, *et al.* Vitamin K₂ (menaquinone 4) reduces serum undercarboxylated osteocalcin level as early as 2 weeks in elderly women with established osteoporosis. *J Bone Miner Metab* 2003 ; 21 : 161–165.
30. Takahashi M, Naitou K, Ohishi T, *et al.* Effect of vitamin K and/or D on undercarboxylated and intact osteocalcin in osteoporotic patients with vertebral or hip fractures. *Clin Endocrinol* 2001 ; 54 : 219–224.
31. Iwamoto J, Takeda T, Ichimura S. Combined treatment with vitamin K₂ and bisphosphonate in postmenopausal women with osteoporosis. *Yonsei Med J* 2003 ; 44 : 751–756.