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Vitamin K_2 treatment for postmenopausal osteoporosis in Indonesia

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Abstract

Aim: To investigate the effect of vitamin K_2 (menatetrenone) treatment on bone mineral density (BMD) and a bone metabolic marker (osteocalcin) in postmenopausal women with osteoporosis living in Indonesia.

Methods: A double-blind randomized placebo-controlled study of 63 postmenopausal women with osteoporosis. The vitamin K_2 group ($n = 33$) received 45 mg menatetrenone and 1500 mg calcium carbonate per day and the control group ($n = 30$) received placebo and 1500 mg calcium carbonate per day for 48 weeks. BMD of lumbal spine (L2–L4), osteocalcin (OC) and undercarboxylated OC were measured before, 24 and 48 weeks after initiation of the treatment.

Results: After 48 weeks of treatment, the mean percentage change of lumbar BMD in the vitamin K_2 group was significantly higher ($P < 0.05$) than that of the control group. The undercarboxylated OC level decreased by 55.9% in the menatetrenone group and 9.3% in the control group compared with the baseline level. The difference between the two groups was significant ($P < 0.01$). The adverse events were three minor gastrointestinal cases, which subsided after temporary cessation of therapy.

Conclusions: Treatment with 45 mg vitamin K_2 with 1500 mg calcium per day for postmenopausal women with osteoporosis for 48 weeks resulted in a significant increase in lumbar BMD and a significant decrease in undercarboxylated OC levels.

Key words: bone mineral density, menatetrenone, osteocalcin, osteoporosis, postmenopause.

Introduction

In a woman's body, bone mass reaches its peak between 20 and 30 years of age and then gradually decreases to climacterium. If the decrease in bone mass is happening rapidly after the woman experiences menopause, it might lead to osteoporosis. Osteoporosis has taken an enormous toll among postmenopausal women. In developed countries, one in three women older than 80 years of age will sustain a hip fracture at some point.[1] Although osteoporosis is a preventable and treatable condition, many people are not diagnosed or treated, especially in developing countries. Recently, several agents such as estrogen, calcitonin and bisphosphonates have been considered in the prevention or treatment of postmenopausal osteoporosis. However, in clinical practice in Indonesia, few elderly osteoporotic patients can afford such available medications.

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Therefore, it is important to explore the use of other agents, which are affordable and yet efficacious, for long-term sustained prevention or treatment.

Since Bouckaert and Said[2] first reported the effect of vitamin K on fracture healing in 1960, studies have suggested that vitamin K_2 both increases bone formation and decreases bone resorption. Studies also demonstrated that postmenopausal bone loss and osteoporosis is associated with vitamin K deficiency.[3] Several longitudinal studies[4–7] have demonstrated that vitamin K_2 can induce significant reductions in bone loss in postmenopausal osteoporotic women. However, there are few observations during treatment of the changes in the bone metabolic marker that are affected by vitamin K_2 in postmenopausal women with osteoporosis.[7] Moreover, to our knowledge, there were few reports about vitamin K_2 treatment for postmenopausal women with osteoporosis in Indonesia. In the present study, we performed a double-blind randomized longitudinal study for 48 weeks of the effect of vitamin K_2 in postmenopausal women with osteoporosis.

Materials and Methods

Subjects consisted of healthy postmenopausal women with osteoporosis, aged between 60 and 75 years with at least 5 years menopause with no history of hysterectomy or ovarectomy, who visited the outpatient clinic at Cipto Mangunkusumo National Hospital from November 2002 to March 2004. All subjects had no previous history of any diseases known to affect bone metabolism (e.g. hypertiroidsm, renal disease, ovarian tumor) and had not previously used estrogen or any other hormonal medications. In addition, all subjects had never been treated for osteoporosis and received no medication that might have affected calcium or vitamin K_2 metabolism before the study. Postmenopausal state was defined as the absence of menstruation for at least 12 months. Osteoporosis state was defined as lumbal bone mineral density (BMD) bellow 0.880 g/cm^2 or a T score 2.5 SD below the mean peak value in young adults, as stated by World Health Organization (WHO).[8] Every patient was screened for liver and renal function, follicle simulating hormone (FSH) and serum calcium levels, and peripheral blood counts to detect any abnormalities. All of the women gave written informed consent to participate in the study, which was approved by the Ethics Committee of Indonesia University.

After all the inclusion criteria were fulfilled, subjects were randomized by a third party (who had no conflict of interest in the study) using a random table generated by SPSS version 10.5 (SPSS, Chicago, IL, USA). Treatment allocations were blinded to both the investigator and the patient until the study was finished. The vitamin K_2 group received 45 mg/day menatetrenone and 1500 mg/day calcium carbonate; and the control group received placebo and 1500 mg/day calcium carbonate. Both groups were given three divided doses per day for 48 weeks. The placebo capsule's dimension and appearance was matched with menatetrenone capsules and contained only sucrose. Diets which were overabundant in vitamin K-rich food (e.g. green vegetables and fermented foods) were likewise not permitted.

BMD, osteocalcin (OC) and undercarboxylated osteocalcin were measured before, 24 and 48 weeks after the initiation of the treatment. BMD in the lumbar spine was determined by dual energy X-ray absorptiometry (DXA: Lunar DPX-L 1994, Hologic, Bedford, MA, USA). Scans were made along three lumbar vertebrae (L2–L4) and BMD was measured in g/cm^2. Rates of change in BMD were calculated. The correlations of BMD with age and initial BMD were investigated. Blood samples for OC and undercarboxylated OC analysis were collected in the morning before meals. Serum was separated with centrifugation at 3000 rpm for 15 min and kept at minus 80°C for analysis by the osteocalcin EIA kit (Biomedical Technologies, Stoughton, MA, USA).

Data were expressed as the mean and standard error of the mean (SEM) where indicated. Data were analyzed statistically by the one-way analysis of variance (ANOVA) or the Wilcoxon signed-rank test. Statistical analysis was carried out using SPSS version 10.5. and a $P < 0.05$ was considered statistically significant.

Results

Out of a total of 212 postmenopausal women who were screened for possible entry into the study, only 69 were eligible. Six patients declined to participate in the study. Table 1 shows the baseline characteristics of the subjects. There was no significant difference in age, number of births, age of menopause, body mass index, bone metabolic markers, blood chemistries or BMD of the lumbar spine between the two groups at baseline.

Figure 1 presents the rates of lumbal BMD changes in both groups during the treatment study. At week 24 of treatment, the mean percentage of BMD change was $1.36 \pm 0.22\%$ and $-0.06 \pm 0.05\%$ in the vitamin K_2 and control group, respectively. At week 48, the mean

Table 1 Baseline characteristics of the control and vitamin K_2 groups (mean ± SD)

	Control ($n = 30$)	Vitamin K_2 ($n = 33$)	P-value
Age (years)	60.6 ± 5.7	60.9 ± 4.9	NS
Age of menopause	48.6 ± 3.7	49.6 ± 3.8	NS
Number of births	4.1 ± 2.4	4.5 ± 2.6	NS†
Body mass index	22.6 ± 3.9	23.8 ± 3.9	NS
FSH (pg/mL)	85.6 ± 28.9	77.0 ± 26.2	NS†
Osteocalcin (ng/mL)	18.9 ± 6.5	15.3 ± 8.5	NS†
Undercarboxylated OC (ng/mL)	5.4 ± 2.19	6.3 ± 4.13	NS†
Serum calcium (mg/dL)	9.3 ± 4.5	9.2 ± 0.5	NS
Lumbar BMD (g/cm^2)	0.766 ± 0.063	0.792 ± 0.06	NS

BMD, bone mineral density; FSH, follicle stimulating hormone; NS, not significant. Independent t-test, except † which used the Mann–Whitney U-test.

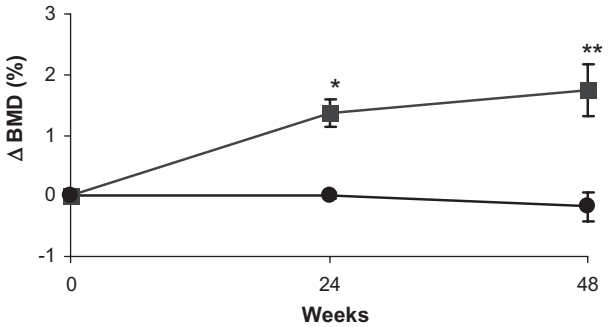

Figure 1 Changes (%) in lumbal bone mineral density (BMD) in both groups at weeks 0, 24 and 48 of treatment. (■) Vitamin K_2; (●) control. *Not significant versus control. **$P < 0.05$ versus control (each value in the mean ± SEM)

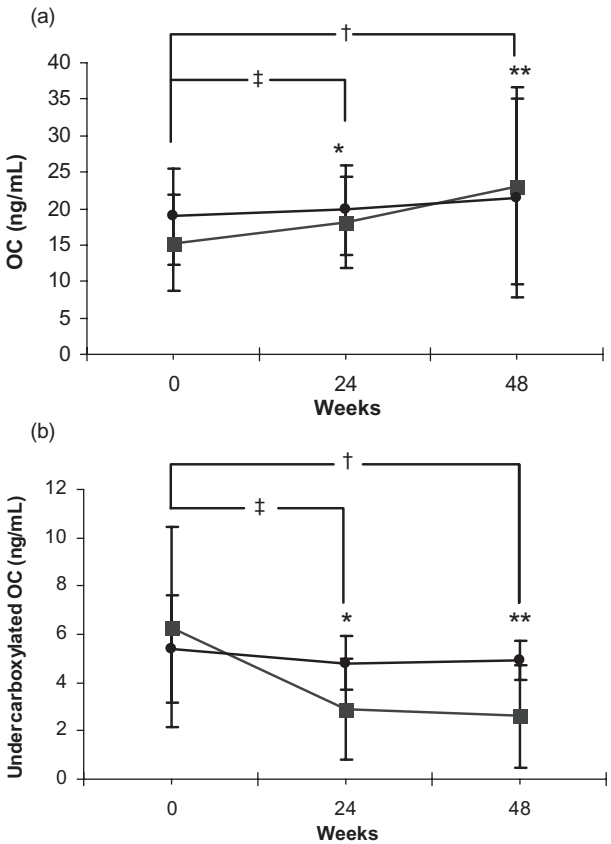

Figure 2 Concentration of (a) osteocalcin (OC) and (b) undercarboxylated OC at weeks 0, 24 and 48 of treatment in both groups. (■) Vitamin K_2; (●) control. †$P < 0.05$ and ‡not significant (NS) in vitamin K_2 group, † and ‡NS in control group, * and **NS. K_2 versus control (value in the mean ± SD).

percentage BMD was 1.74 ± 0.43% and –0.18 ± 0.24% in the vitamin K_2 and control group, respectively. There was a significant difference in BMD between the two groups at week 48 ($P < 0.05$).

Figure 2 presents the change of OC and undercarboxilated OC concentration in both groups during the treatment study. At week 24 and 48 of treatment, the mean (±SD) of OC concentrations in the vitamin K_2 group was 18.1 ± 6.2 and 23.1 ± 13.6 ng/mL, respectively, while in the control group it was 19.8 ± 7.7 and 21.5 ± 12.4 ng/mL, respectively. In all observations of OC concentrations, there were no significant differences between both groups. At week 48, the OC concentration in the vitamin K_2 group was significantly different compared with baseline. At week 24 and 48 of treatment, the mean (±SD) of undercarboxylated OC concentrations in vitamin K_2 group were 2.9 ± 2.1 and 2.6 ± 2.15 ng/mL, respectively, while in control group these were 4.8 ± 1.1 and 4.9 ± 0.8 ng/mL, respectively. The OC undercarboxylated concentration in the vitamin K_2 group was significantly different compared with baseline and showed significant differences compared with control at week 24 and 48 of treatment. In the control group, there was no significant change compared to baseline in both OC and undercarboxylated OC concentration.

The adverse events were two minor gastrointestinal symptoms, which subsided after temporary cessation of therapy. There was no report of allergic reactions in the treated group.

Discussion

It has been established that osteoporosis treatment with estrogen, calcitonin and bisphosphonates are reducing bone turnover and preventing osteoporotic vertebral fracture in postmenopausal women with osteoporosis. Although many clinical trials have proven its efficacy, in clinical practice some elderly osteoporotic patients have responded little or could not bear the increased risk of thromboembolism from these treatments. Moreover, to provide such medication for sustained long-term prevention or treatment, the cost would be relatively high for elderly patient in developing countries. One of the alternative agents is vitamin K_2, because of its action on bone metabolism and its relatively lower cost compared with other agents. In Indonesia, vitamin K has been relatively affordable and widely used by midwives to reduce the risk of bleeding in newborns.

Most studies showed a slight increase or decline in BMD after 1 year of vitamin K_2 treatment for osteoporosis in postmenopausal women. In a controlled clinical trial of vitamin K_2 use in 172 osteoporotic/osteopenic women for 24 months, vitamin K_2 resulted in only a $0.135 \pm 5.44\%$ increase in BMD; higher, but not significantly higher, than baseline values.[6] A slight increase ($0.23 \pm 0.47\%$) was also reported by Iwamoto *et al*.[4] in a longitudinal study of 17 postmenopausal women. Ishida *et al*.[9] also found that vitamin K reduced the risk of vertebral fractures without substantial improvement in BMD.

In the present study, at the end of treatment (week 48) the vitamin K_2 group had a $1.74 \pm 0.43\%$ increase in bone mass of the lumbal spine, compared with a $0.18 \pm 0.24\%$ decrease in the control group. At week 48, the differences between the two groups were significant. In most studies of postmenopausal women with osteoporosis, calcium treatment is not included in the vitamin K_2 or control group. Therefore, in the present study, the higher and significant increase in BMD might be caused by calcium supplementation in the vitamin K_2 group. The 1500 mg calcium treatment alone, as given in the control group, did not demonstrate increased BMD. In the present study, calcium 1500 mg might not be sufficient to reduce bone loss for long in postmenopausal women with osteoporosis. It might also be speculated that calcium supplementation in the vitamin K_2 group had synergistic effects on increasing the bone mass. However, as bone metabolism is multifactorial – bone mass is influenced by physical activity, ethnicity, nutrition and genetic factors – other causes for the difference might be uncertain. All these factors might have influenced the subjects' response to vitamin K_2 treatment. Bunyaratavej *et al*.,[7] in a study of Thai subjects (who might have similar bone anthropometry to Indonesian subjects), included 800 mg calcium in the vitamin K_2 group and showed an insignificant increase in lumbal BMD (+0.6%) compared to the control group.

The effect of vitamin K_2 on bone metabolism is not yet established. It has been reported that the mechanisms by which menatetrenone (vitamin K_2) inhibits osteoclast is through the inhibition of cyclooxygenase-2 (COX-2) and prostaglandin E2 production.[10] Conversely, vitamin K_2 is essential for the γ-carboxylation of glutamic acid residues of osteocalcin, a bone matrix collagen protein,[11] which allows binding of hydroxyapatite to occur and its accumulation in the bone matrix. It is thought that a deficiency in vitamin K_2 causes insufficient γ-carboxylation of osteocalcin, leading to an increase in non-carboxylated or undercarboxylated forms of OC. Undercarboxylated OC has been reported to be increased in osteoporotic patiets,[12] and to be correlated with the risk of hip fracture.[13]

In the present study, we could not determine the mechanisms behind the improved lumbal BMD in vitamin K_2 group. However, there was a significant increase in OC concentration compared with baseline in the vitamin K_2 group and insignificant differences between these two groups. This slight increase and insignificant difference compared to the control has been demonstrated previously in other observations.[7,14] In the vitamin K_2 group, the undercarboxylated OC decreased significantly compared to baseline and control. These findings might indicate that the vitamin K_2 treatment improved γ-carboxylation of OC and improved OC concentration in the vitamin K_2 group compared to baseline, but insignificantly compared to the control group. This significant increase in the vitamin K_2 group might subsequently contribute to the significant increase in lumbal BMD.

In this study, the undercarboxylated OC concentration at week 48 decreased by 55.9% in the menatetrenone group and 9.3% in the control group compared with the baseline value. The difference between the two groups was significant ($P < 0.001$). Although the reduction in undercarboxilated OC levels was smaller

compared with Bunyaratavej *et al*.'s[7] study (87.27%), the baseline undercarboxylated OC of the menatetrenone group in our study was lower (6.2 ng/dL) compared with Bunyaratavej *et al*.[7] (10.47 ng/dL). This could lead to a higher percentage drop in the Bunyaratavej *et al*.[7] study compared with this study. It is also worth noting that treatment appeared to have a steeper curve of BMD and bone metabolic marker in the first 24 weeks, but thereafter the mean rate of increase declined. This decline might indicate that the bone metabolic profile was almost stabilized by treatment at week 24 and was then affected by persistent bone loss, as previously demonstrated.[7]

This study has several limitations. Like other clinical trials, the major drawback is that we could not make the same controlled condition in all patients (e.g. restricted diets, same levels of serum menatetrenone before treatment, work or exercise) that may affect the response of subjects to treatment. Also, the duration of the treatment was limited to 48 weeks. This implies that a longer treatment period and a more controlled environment would be of benefit in future studies. Moreover, as BMD and fracture rates are commonly used as the end-point of efficacy in osteoporosis drug trials, we did not address the risk of fractures in this study.

In summary, this is the first study of menatetrenone use in Indonesia. This study indicated that vitamin K_2 might be an alternative in the treatment of postmenopausal osteoporosis.

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