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Original Research Article

Effect of vitamin K2 on bone mineral density and serum cathepsin K in female osteoporosis patients

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Abstract

Purpose: *To investigate the influence of vitamin K_2 on bone mineral density, bone metabolism and serum tissue protease K (cathepsin K) in female patients with osteoporosis.*
Method: *A total of 210 osteoporosis patients in Affiliated Hospital of Nanjing University of Chinese Medicine who met the inclusion criteria were selected from January 2017 to January 2018. The patients were randomly divided into vitamin K_2 group, strontium renate group and blank control group (70 patients/group). Strontium ranelate group was orally given 2 g of strontium ranelate daily, while vitamin K_2 group received 15 mg of Gulikang capsule 3 times a day. Bone mineral density (BMD) and serum osteocalcin (BGP), β-collagen degradation product (β-crosslaps), type I procollagen amino terminal pro-peptide (PINP), cathepsin K (cathe K) and TRAP were measured prior to drug treatment, and six months after surgery, using standard procedures.*
Results: *Relative to the blank control, hip and lumbar spine density of vitamin K_2 and strontium ranelate groups increased to varying degrees. Strontium ranelate group had significantly higher bone mineral density (BMD) than any other groups ($p < 0.05$), and also had the lowest osteoclast activity (β-crosslaps and TRAP) and the highest osteogenic activity (BGP and PINP). On the other hand, osteoclast and osteogenic activities increased significantly ($p < 0.05$) in the vitamin K_2 group.*
Conclusion: *Appropriate vitamin K_2 treatment improves BMD in the hip and waist of women with osteoporosis by promoting osteogenic activity, and by reducing osteoclast activity and cathepsin K expression.*

Keywords: *Osteoporosis, Bone mineral density, Strontium ranelate, Vitamin K_2*

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INTRODUCTION

Post-menopausal osteoporosis (PMO) refers to changes in bone metabolism caused by decreased estrogen levels, resulting in increased loss of osteoclasts and trabecular bone. It causes bone fragility and bone fractures. At present, there are about 200 million people who suffer from osteoporosis in the world, and its incidence is now ranked 7th in the list of common diseases in the world [1]. Osteoporosis is a frequently-occurring disease, and it is marked by decreased bone mass and altered bone structure, which lead to increased bone fragility and ultimately increased risk of bone fracture [2,3].

Due to aging populations, the prevalence of osteoporosis is expected to significantly increase in the future [4,5]. Osteoporosis occurs mostly in women and elderly men [6]. Studies have found that about 25 % of trabecular bone and 3 % of cortical bone in adults undergo a dynamic balancing process of osteoclastic bone resorption and osteoblastic bone formation [7]. When the balance in bone remodeling is impaired, osteoporosis results because bone resorption becomes greater than bone formation [8]. Therefore, the aim of therapy for osteoporosis is to minimize risk of first or secondary fractures. At present, the anti-osteoporosis drugs in use are agents that inhibit bone resorption and increase bone formation. They include parathyroid hormone [9] and bisphosphonates [10]. In recent years, studies have indicated that vitamin K_2 is crucial in many aspects of bone metabolism. Vitamin K_2 ameliorates imbalance in bone tissue metabolism through regulation of bone biogenesis and inhibition of the rate of bone resorption [11].

Cathepsin K is expressed mainly in osteoclasts, and it is widely distributed in bone resorption surfaces, transcellular vesicles and lysosomes [12]. It is a key protease for bone resorption. Increased expression of cathepsin K in osteoclasts leads to increased osteoclast metabolism, accelerated bone resorption, and ultimately osteoporosis [13]. In view of this, it can be hypothesized that vitamin K_2 regulates bone metabolism in osteoporotic women by influencing cathepsin K. Therefore, the aim of this research was to study the influence of vitamin K_2 on cathepsin K, BMD and bone metabolism in patients with osteoporosis.

EXPERIMENTAL

Clinical profile of patients

Osteoporotic patients (210 cases) who visited our hospital from January 2017 to January 2018, and who satisfied the inclusion requirements were used as study subjects. These patients were randomly assigned to vitamin K_2 group, strontium renate group and blank control group. The selected patients were 50 - 80 years old, with a mean age of 64.07 ± 9.63 years. The average age at menopause was 48.69 ± 7.39 years, the average height was 158.82 ± 7.45 cm, and the average weight was 60.03 ± 12.39 kg. All 210 patients completed follow-up after 6 months of drug treatment. Age and menopausal time were comparable among the three groups ($p > 0.05$). The study received approval from the Ethical Committee of Affiliated Hospital of Nanjing University of Chinese Medicine (approval no. 20180726), and was carried out according to the Declaration of Helsinki promulgated in 1964 as amended in 1996 [14]. All the patients signed informed consent.

Inclusion criteria

The recommended diagnostic criterion for OP was reduction of dual-energy X-ray bone density equal or higher than 2.5 standard difference [15].

Exclusion criteria

(1) Patients with osteomalacia, calcium and vitamin D deficiency and renal tubular acidosis; (2) patients with primary and secondary hyperthyroidism; (3) patients with bone metastasis of malignant tumor; (4) multiple myeloma patients; (5) patients who had spinal hemangioma; (6) patients with suppurative myelitis; (7) patients with motor disease, chronic medical diseases and endocrine diseases caused by secondary osteoporosis, and (8) patients on estrogen, heparin, corticosteroids, bisphosphonates and other drugs related to osteoporosis.

Treatments

All patients were subjected to conditioned lifestyles, and were required to take one piece of calcium carbonate D_3 chewable tablet as a basic measure. The vitamin K_2 group was given *Gulikang* capsule (Fuji Capsule Co., Ltd. Shibakawa Plant) treatment 3 times a day (15 mg each time). Patients in the strontium ranelate group were orally administered 2 g of strontium ranelate (France Servier), daily. The 3 groups were followed up for 6 months after treatment.

Therapeutic indices

Fasting venous blood was collected before treatment, and 6 months after treatment, and serum samples were separated at room temperature. The levels of bone gla protein (BGP), beta-collagen degradation product (β-crosslaps), and PINP were determined using Roche Cobase 601 automatic electro-chemiluminescence analyzer (Royce, Switzerland). Serum cathepsin K (cathe K) and TRAP were assayed with ELISA (R & D Corporation, US).

Determination of BMD

This was carried out with dual energy x-ray absorptiometry (DXA) and dual energy x-ray bone density meter (GE-Lunar, GE) before, and 6 months after treatment. The BMD of lumbar

vertebrae L_{2-4}, femoral neck, greater trochanter, and total hip were recorded.

Statistical analysis

Data were analyzed was done with SPSS 19.0 software package. Numeric data are expressed as mean ± SD and were analyzed with Student's *t*-test. Count data was analyzed using χ^2 test. Correlation analysis was done with Spearman correlation. Statistical significance was fixed at $p < 0.05$.

RESULTS

Bone turnover index BGP, β-crosslaps, PINP, TRAP and Cathe K

As shown in Table 1, after 6 months of drug treatment, the serum levels of BGP, β-crossLaps, PINP, TRAP and cathe K were changed to different degrees, relative to their levels before treatment. Relative to the blank control group, changes in bone metabolism and cathe K were more obvious in the vitamin K2 group and the strontium ranelate group than in control ($p < 0.05$). Changes in bone metabolism in the patients given strontium ranelate group were more obvious than those in the vitamin K_2 group, while changes in cathe K were markedly higher in vitamin K_2 group than in the group that received strontium ranelate ($p < 0.05$).

Changes in bone mineral density

There were no significant differences in BMDs of lumbar vertebrae (L_2- L_4), femoral neck, greater trochanter and total hip before treatment ($p > 0.05$). However, after 6 months of drug treatment, there were significant increases in BMDs of femoral neck, lumbar vertebrae, greater trochanter and total hip, when compared with their values before treatment ($p > 0.05$). Compared with the blank control group, the bone mineral densities of these tissues were significantly increased in the vitamin K_2 and strontium ranelate groups, but the increases were higher in the strontium ranelate group than in the vitamin K_2 group ($p > 0.05$). These results (Table 2) showed that vitamin K_2 enhanced the treatment of osteoporosis, but its effect was not as good as that of strontium ranelate.

DISCUSSION

In this study, 210 women with osteoporosis were selected as subjects, and they were treated with vitamin K_2 or strontium ranelate. After 6 months of treatment, the levels of indicators of changes in BMD and bone metabolism i.e. BGP, β-crossLaps, PINP, TRAP and cathe K in lumbar vertebrae (L_{2-4}) and femoral neck, greater trochanter and total hip, were measured in the three groups. The results showed that strontium ranelate and vitamin K_2 had significant therapeutic effects on osteoporosis. Both treatments significantly increased BMDs of femoral neck, lumbar vertebrae, greater trochanter and total hip. Moreover, the treatments reduced bone resorption activity (marked by β-crossLaps, TRAP, and cathe K), and promoted osteogenic activity (marked by BGP and PINP). These results indicate that strontium ranelate and vitamin K_2 are effective in the treatment of osteoporosis.

Barium ranelate is an anti-osteoporosis drug that acts by reducing bone resorption and increasing bone formation. It is used for treating osteoporosis and for reducing susceptibility to fractures, while simultaneously enhancing bone density and strength.

Table 1: Serum levels of BGP, β-crossLaps, PINP, TRAP, and Cathe K before and after 6 months of treatment (n = 70)

Group	Time	BGP (ng/mL)	β-Crosslaps (ng/mL)	PINP (ng/mL)	TRAP (U/L)	Cathe K (ng/L)
Vitamin K_2	Before treatment	18.86 ± 6.21	0.96 ± 0.08	81.66 ± 5.92	2.54 ± 0.48	34.74 ± 15.88
	After treatment	26.51±5.23$^{\#*}$	0.52 ± 0.06$^{\#*}$	93.72±8.61$^{\#*}$	2.18±0.34$^{\#*}$	18.59 ±8.86$^{\#*}$
Strontium ranelate	Before treatment	18.74 ± 5.84	0.94 ± 0.0.7	82.05 ± 7.85	2.52 ± 0.62	33.95 ± 17.26
	After treatment	33.82±7.49$^{\#*}$	0.38 ± 0.04$^{\#*}$	97.64±8.94$^{\#*}$	2.06±0.44$^{\#*}$	11.75 ±9.81$^{\#*}$
Blank control	Before treatment	18.56 ± 5.58	0.93 ±0.09	83.06 ± 8.27	2.50 ± 0.57	34.03 ± 16.88
	After treatment	19.54 ± 5.81	0.94 ± 0.07	79.28 ± 7.04	2.82 ± 0.51	35.30 ± 16.88

$^{*}P < 0.05$, relative to value before treatment in the same groups, $^{\#}p < 0.05$, relative to blank control group

Table 2: Bone mineral density (g/cm^2) before, and 6 months after treatment (mean ± SD, n = 70)

Group	Period	Lumbar vertebrae (L2-4)	Femoral Neck	Greater trochanter	Total hip
Vitamin K_2	Before treatment	0.74 ± 0.07	0.72 ± 0.09	0.54 ± 0.10	0.77 ± 0.15
	After treatment	0.87 ± 0.12* #	0.79 ± 0.10* #	0.63 ± 0.11* #	0.81 ± 0.13* #
Strontium ranelate	Before treatment	0.77 ± 0.08	0.70 ± 0.10	0.56 ± 0.12	0.75 ± 0.13
	After treatment	0.91 ± 0.10* #	0.84 ± 0.12* #	0.65 ± 0.13* #	0.84 ± 0.15* #
Blank control	Before treatment	0.78 ± 0.07	0.72 ± 0.08	0.54 ± 0.12	0.78 ± 0.13
	After treatment	0.76 ± 0.09	0.69 ± 0.09	0.52 ± 0.13	0.72 ± 0.12

*P< 0.05, relative to pre-treatment value; #p < 0.05, relative to control

It overcomes the drawbacks of one-way treatments with other drugs [16]. Many animal experiments have shown that strontium ranelate accelerates the formation of new bone while slowing down the resorption of old bone. Strontium ranelate not only inhibits bone resorption in ovariectomized rats, it also increases bone mass of normal rats, thereby increasing the strength of bone [17]. It increases bone quality by inhibiting bone breakage and stimulating osteogenesis, thereby accelerating bone formation in healthy mice, and improving bone quality of vertebrae [18]. These results clearly showed the therapeutic impact of strontium ranelate on menopausal osteoporosis. The significant increase in bone mineral density of femoral neck, lumbar spine, greater trochanter and total hip are the most direct effects. At the same time, the increases in BGP and PINP, and the decreases in β-crossLaps, TRAP and cathe K further indicate that strontium ranelate promotes osteogenesis and inhibits osteolysis.

Vitamin K_2 mitigates imbalance in bone tissue metabolism, and has a two-way regulatory effect by promoting the rate at which bones are formed, while inhibiting the rate at which bones are resorbed. Vitamin K_2 promotes bone formation mainly through two routes [19]. One pathway involves the vitamin K_2-mediated carboxylation of glutamate residues in osteocalcin to form carboxylated osteocalcin (CO), and promotion of calcium salt deposition, thereby improving bone mineralization. The second pathway is involved in steroid and heterogene receptor (SXR)-mediated transcriptional regulation, up-regulation of osteogenesis and extracellular matrix-related gene expressions, and increased collagen aggregation.

Vitamin K_2 inhibits the expression of COX-2 and the formation of PEG2; it also inhibits the activation of interleukin-1 (IL-1) and nuclear factor B (NF-B), and induces osteoclast apoptosis and other pathways that inhibit osteoclast activity [20]. A number of clinical studies in China and elsewhere have demonstrated that vitamin K_2 increases bone mineral density and reduces incidence of fractures. A study has shown that vitamin K_2 significantly improved hip strength and resistance to fracture in women, and decreased incidents of fractures by 53 % [20]. The results obtained in this study clearly showed that vitamin K_2 significantly increased bone mineral density of femoral neck, lumbar vertebrae, greater trochanter and total hip, and also enhanced balance in bone metabolism by increasing osteogenic activity and reducing osteoclast activity.

Study limitations

This study has some limitations. Firstly, the number of subjects used was small, and the follow-up time (6 months) was too short for any meaningful comparison of the effects of the two treatments. Secondly, the dose of the drugs used may not have been appropriate, and this could have influenced the outcome of the study. These limitations should be addressed in subsequent studies.

CONCLUSION

The results obtained in this study clearly show that vitamin K_2 increases bone density, promotes osteogenesis and inhibits bone fracture. It is more effective in treating osteoporosis than strontium ranelate, and it can be taken for a long time. It inhibits the expressions of β-crossLaps and TRAP, promotes the expressions of BGP and PINP, and inhibits the activity of osteoclasts by inhibiting the synthesis of cathe K. Thus, vitamin K_2 is a safe, effective and suitable drug for protection from, and treatment of osteoporosis in the elderly.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

This work was done by the authors named in this article and the authors accept all liability resulting from claims which relate to this article and its contents. The study was conceived and designed by Mao Jun; Gu Yuanyang, Xing Runlin, Xu Bo, Fan Donghua, Mao Jun collected and analysed the data; Gu Yuanyang and Xing Runlin wrote the text and all authors have read and approved the text prior to publication. Gu Yuanyang and Xing Runlin contributed equally to this work and should be considered as co-first authors.

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