Vitamin K_2 alters bone metabolism markers in hemodialysis patients with a low serum parathyroid hormone level

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Short title: Vitamin K_2 and adynamic bone disease

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Abstract

Background: A low level of intact parathyroid hormone is an indicator of adynamic bone disease in hemodialysis patients, and is associated with a significant increase of all-cause mortality. Thus, effective treatment for adynamic bone disease is required. We previously investigated the effect of vitamin K_2 on adynamic bone disease. In this study, we assessed the efficacy of oral vitamin K_2 in a controlled trial.

Methods: Forty hemodialysis patients with low intact-PTH levels (<100 pg/ml) were randomly divided into two groups, which were a vitamin K_2 group receiving oral menatetrenone (45 mg/day) for one year and a control group without vitamin K_2. Venous blood samples were collected at baseline and during the study for measurement of bone metabolism parameters.

Results: Thirty-three patients completed follow-up. There was a significant increase of the serum intact osteocalcin level after 1 months of vitamin K_2 administration. Serum levels of intact-PTH, bone alkaline phosphatase, and cross-linked N-terminal telopeptide of type I collagen increased significantly after 12 months in vitamin K_2 group. The serum osteoprotegerin level was decreased after 12 months in the vitamin K_2 group, but the change was not significant.

Conclusion: Vitamin K_2 therapy improves bone remodeling in hemodialysis patients with a low intact-PTH level.

Key words: vitamin K_2; bone metabolism markers; intact-PTH; hemodialysis

Short summary: Oral administration of vitamin K_2 to hemodialysis patients with low intact-PTH levels increased the levels of bone formation and bone resorption markers. Accordingly, vitamin K_2 improves bone remodeling in hemodialysis patients with adynamic bone disease.

Introduction

Adynamic bone disease is an important complication of maintenance hemodialysis. In patients with adynamic bone disease, the number of osteoblasts or osteoclasts is low and bone turnover is slow, so the risk of fracture is increased(1). Adynamic bone disease is also characterized by a very low capacity of bone to buffer calcium and inability to handle a calcium load(2). Recent studies have shown that an intact parathyroid hormone (intact-PTH) level<100 pg/ml, a known indicator of adynamic bone disease, is also associated with increased arterial calcification and a higher mortality(3-4). Such reports emphasize that effective treatment for adynamic bone disease is required to improve bone metabolism and prevent vascular calcification.

Vitamin K has an important role in regulating bone mineralization and preventing vascular calcification(5). It is involved in the carboxylation of glutamate residues in vitamin K-dependent proteins to form gamma-carboxyglutamate residues (Gla-residues) that are essential for binding calcium. Vitamin K-dependent proteins with Gla domains play a role in three important physiological processes, which are blood clotting, bone metabolism, and vascular biology. Previously, recommendations about the dietary vitamin K intake have been based on hepatic requirements for the synthesis of clotting factors. However, these studies suggested that bone metabolism and vascular wall-related processes require a higher dietary intake of vitamin K(6-7). In hemodialysis patients, the vitamin K level is significantly lower than in healthy populations, and the fracture rate increases as vitamin K decreases(8).

We previously reported that oral administration of vitamin K_2 induced changes of bone metabolism markers in hemodialysis patients who had low serum intact-PTH levels without causing side effects such as A-V fistula obstruction and infarction, suggesting that vitamin K_2 therapy could safely improve bone remodeling(9). However, our previous study had no control group. Therefore, the present controlled study was performed to investigate the

effect of vitamin K_2 on bone metabolism markers and osteoprotegerin (OPG) in hemodialysis patients with a low serum intact-PTH level.

Materials and Methods

Subjects

We enrolled 40 Japanese patients who had been on hemodialysis for over 2 years at the Onomichi Clinic (Onomichi, Japan), Hakuai Clinic (Kure, Japan), Chuonaika Clinic (Kure, Japan), Clear Yakeyama Clinic (Kure, Japan), or Harada Hospital (Hiroshima, Japan). In all patients, the intact-PTH level was less than 100 pg/ml at 12 months, 6 months, and just before the present study. This inclusion criterion was selected because intact-PTH<100 pg/ml corresponds to the cut-off value for adynamic bone disease according to K/DOQI guideline 13C(10). The study began in April 2004 and was completed in January 2007. All patients who participated gave written informed consent. This study was conducted in accordance with the Declaration of Helsinki, and its protocol was approved by ethical committee of Hiroshima university faculty of medicine and other institutes. All of the subjects were clinically stable and had no acute infection. We excluded patients who had undergone parathyroidectomy and patients who had been treated with vitamin K antagonists or active vitamin D metabolites. The subjects were randomly assigned to a vitamin K_2 group that was treated with menatetrenone (45 mg/day) for 12 months (n=22) or a control group that received conventional therapy without menatetrenone (n=18). No patient received vitamin D therapy during study period, but oral administration of calcium carbonate and sevelamer hydrochloride was not regulated by the protocol. The calcium concentration of the dialysate used for hemodialysis was 3.0 mEq/l and was not changed during this study. Thirty-three patients completed the follow-up period. In the vitamin K_2 group, 9 men and 11 women completed the study. The underlying renal disease was chronic glomerulonephritis in 9 patients and diabetes mellitus in 11 patients. Withdrawal from the study occurred because of

death (n=1) and patient's will (n=1). In the control group, 9 men and 4 women completed the study. The underlying renal disease was chronic glomerulonephritis in 10 patients and diabetes mellitus in 3 patients. Withdrawal occurred because of death (n=1), transfer to another hospital (n=1), infections (n=1), and patient's will (n=2). Table 1 compares the clinical and laboratory parameters of the two groups of hemodialysis patients. There were no significant differences between the control and vitamin K_2 groups, except for the prevalence of diabetes mellitus.

Measurements

Venous blood samples were collected before hemodialysis for measurement of the serum levels of bone-specific alkaline phosphatase (B-ALP), intact osteocalcin (intact-OC), cross-linked N-terminal telopeptide of type 1 collagen (NTx), calcium, phosphate, intact-PTH, albumin, and OPG. B-ALP was measured by an immunoassay with a monoclonal anti-B-ALP antibody (Metra Biosystems, Mountain View, CA), intact-OC was measured with the Osteocalcin Test Kokusai-F kit (International Reagents Corporation, Tokyo, Japan), NTx was measured by using an Osteomark NTx serum kit (Ostex International, Seattle, WA), and intact-PTH was measured with the Nichols IRMA kit (Nichols Institute, San Juan Capistrano, CA). OPG was measured by using a human osteoprotegerin ELISA kit (BioVendor Laboratory Medicine, Brno, Czech Republic). The adjusted calcium level was calculated with Payne's formula(11). B-ALP, intact-OC, NTx, calcium, phosphate, and intact-PTH were evaluated at baseline, as well as after 1 months, 3 months, and 12 months, while OPG was only measured at baseline and after 12 months.

Statistical analysis

Results are shown as the mean ± SD. The Mann-Whitney U test was employed to assess differences of continuous variables between the two groups, while the chi-square

test was used for categorical data. The Wilcoxon signed rank test was also employed. In all analyses, $P<0.05$ was considered to indicate significance.

Results

Table 2 shows the changes of bone metabolism markers. Intact-OC increased significantly after 1 month of vitamin K_2 administration. Although there was no significant increase in the serum levels of B-ALP and NTx after 3 months of vitamin K_2 administration, a significant increase was seen after 12 months. In the control group, these bone metabolism markers were unchanged throughout the study period.

Table 3 shows the changes of adjusted calcium, phosphate, and intact-PTH in the two groups, as well as the doses of oral calcium and the phosphate binder sevelamer hydrochloride. There was a significant increase of intact-PTH after 12 months in the vitamin K_2 group, but not in the control group. In the vitamin K_2 group, the adjusted calcium level significantly increased after 3 months of treatment, while serum phosphate showed a significant decrease after 1 month. The dose of calcium carbonate was also increased significantly in vitamin K_2 group. In the control group, there were no significant changes of these parameters throughout the study period. The dose of sevelamer hydrochloride showed no significant changes throughout the study period in both groups.

Table 4 displays the levels of OPG before the study and at 12 months. Serum OPG decreased after 12 months in the vitamin K_2 group, but the change was not significant. There was no significant change of OPG in the control group.

Discussion

In the present study, both bone formation markers (Intact-OC and B-ALP) and a bone resorption marker (NTx) were increased significantly by vitamin K_2 therapy. These changes of bone metabolism markers suggested that vitamin K_2 can improve bone remodeling in

hemodialysis patients with low serum PTH levels.

Intact-OC began to increase after 1 month of vitamin K_2 administration, and B-ALP also showed a significant increase after 12 months. Vitamin K_2 is an essential cofactor for site-specific carboxylation of osteocalcin and fully carboxylated osteocalcin (Gla-OC) binds to the calcium ions of hydroxyapatite(12). It is widely known that Gla-OC is increased by vitamin K_2 therapy. In our previous study, the Gla-OC level was increased by at least 1.5-fold after 1 month of vitamin K_2 administration. Moreover, Shiraki et al.(13) have reported that 6 months of vitamin K_2 therapy increases intact-OC as well as Gla-OC in postmenopausal osteoporosis patients. In this study, intact-OC began to increase from one month and continued to increase for 12 months. These data indicate that vitamin K_2 enhanced the secretion of intact-OC in hemodialysis patients with low intact-PTH levels, and also stimulated bone formation.

In our patients, NTx also increased significantly after 12 months of vitamin K_2 therapy. NTx showed a decrease after 1 month of vitamin K_2 administration, but this was not significant. In the control group, the serum level of NTx was unchanged throughout the study period. NTx has been reported to be the most sensitive marker of bone resorption(14), with serum or urinary NTx levels being decreased by effective antiresortive therapy(15). According to previous reports about osteoporosis patients and healthy athletes, administration of vitamin K_2 reduces bone resorption(16-17). Conversely, Shiraki et al.(13) reported that NTx excretion was increased in osteoporosis patients after 6 months of vitamin K_2 treatment. In our previous study, NTx also showed a significant increase with vitamin K_2 administration(9). Vitamin K_2 has opposing effects that can promote or inhibit osteoclast activity. Vitamin K_2 inhibits osteoclastic bone resorption via its side chain and it also causes osteoclasts to undergo apoptosis(18-19). Conversely, osteocalcin production is stimulated by vitamin K_2 and it increases the secretion of osteopontin and fibronectin, thus promoting the activation of osteoclasts(20). In the present study, osteocalcin showed a rapid increase

after the start of vitamin K_2 administration, while the increase of NTx was seen after 12 months. This suggests that the stimulatory effect of vitamin K_2 on osteoclasts via osteocalcin became predominant over the direct inhibitory effect of vitamin K_2 itself at 12 months.

Adynamic bone disease is the most frequent type of renal osteodystrophy in hemodialysis patients. According to K/DOQI guideline 13C, adynamic bone disease in hemodialysis patients (as determined from either bone biopsy or an intact-PTH level<100 pg/ml) should be treated by allowing the serum intact-PTH level to rise in order to increase bone turnover(10). There was a significant increase of intact-PTH after 12 months in vitamin K_2 group. Oral administration of calcium carbonate was not regulated by our protocol, and the dose of oral calcium was increased significantly after 1 month in vitamin K_2 group. High-dose calcium supplementation led to an increase of serum calcium, but the serum intact-PTH level was not suppressed even though the serum calcium level rose significantly. It has been considered that vitamin K_2 has no direct effect on parathyroid gland, but it is possible that the intact PTH level increased because bone metabolism was improved by vitamin K_2 therapy. If oral calcium intake had been limited, the effect of vitamin K_2 therapy might have been stronger.

An intact-PTH level<100 pg/ml is not only an indicator of adynamic bone disease, but is also a risk factor for higher all-cause mortality(4). Previously, secondary hyperparathyroidism has been suggested to play a key role in the high prevalence of vascular calcification among hemodialysis patients. However, recent studies have indicated that low levels of PTH could also be associated with a higher risk of death in hemodialysis patients(21-22).

The relationship between bone mineralization markers and OPG has attracted attention recently, because OPG is an important physiological regulator of osteoclastogenesis and elevated OPG levels are associated with increased vascular calcification(4). In this study, the OPG level showed a decrease after 12 months of vitamin K_2 therapy, but the change did

not reach significance. There was also no significant change in the control group. We previously found that the serum OPG level showed a negative correlation with bone metabolism markers in hemodialysis patients(23). Though we could not detect any strong effect of vitamin K_2 on serum OPG levels in this study, the decrease of OPG might have been involved in the changes of bone metabolism markers in the vitamin K_2 group.

Some limitations of the present study should be noted. First, we account with a relatively small size and this data needs confirmation in larger materials. Second, instead of random selection, the numbers of patients are different between vitamin K group and control group.

In conclusion, vitamin K_2 therapy improved bone remodeling in our patients. Vitamin K_2 is one of the candidate to treat the hemodialysis patients with an intact-PTH level<100 pg/ml. Due to the small sample size and short observation period, our findings require confirmation in a larger patient population with longer follow-up.

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Legends for tables

Table 1. Clinical and laboratory parameters of the two groups

	Control group (n=13)	Vitamin K_2 group (n=20)
Age (years)	65.3±12.6	64.7±10.2
Gender (male,%)	69.2%	45.0%
Duration (years)	8.9±6.4	9.5±8.1
Diabetes (%)	23.1%	55.0%*
intact-PTH (pg/ml)	46.7±29.6	50.3±28.0
B-ALP (U/l)	19.3±7.6	18.3±5.5
intact-Osteocalcin (ng/ml)	22.5±15.8	19.1±14.1
NTX (nmol BCE/l)	51.3±52.0	47.2±31.8
Adjusted calcium (mg/dl)	9.2±0.5	9.1±0.5
Phosphate (mg/dl)	5.2±1.2	5.4±1.2

Result are shown as the mean±SD. $P^*<0.05$ vs. control group by the Mann-Whitney U test or chi-square test

Table 2. Changes of bone metabolism markers

	Group	Baseline	1 month	3months	12months
B-ALP(U/l)	Vitamin K_2	18.3±5.5	17.9±6.0	22.1±10.9	25.9±14.1*
	Control	19.3±7.6	20.5±10.3	20.4±8.8	20.6±8.6
intact-Osteocalcin(ng/ml)	Vitamin K_2	19.1±14.1	23.6±14.2*	25.5±13.2*	27.2±15.3*
	Control	22.5±15.8	21.3±15.5	22.6±15.1	22.1±11.8
NTX(nmol BCE/l)	Vitamin K_2	47.2±31.8	42.8±27.6	52.1±32.7	55.2±31.5*
	Control	51.3±52.0	47.5±43.9	54.8±43.9	51.1±25.7

Results are shown as the mean±SD. $P^*<0.05$ vs. baseline by the Wilcoxon signed rank test.

Table 3. Changes of intact-PTH,adjusted calcium, and phosphate,as well as the dose of oral calcium and phosphate binders

	Group	Baseline	1 month	3 months	12 months
Adjusted calcium (mg/dl)	Vitamin K_2	9.1±0.5	9.3±0.4	9.5±0.5*	9.4±0.8
	Control	9.2±0.5	9.3±0.4	9.2±0.8	9.3±0.8
Phosphate (mg/dl)	Vitamin K_2	5.4±1.2	4.7±1.0*	4.9±0.9	4.8±1.1
	Control	5.2±1.2	5.2±1.3	5.1±1.3	5.0±1.4
intact-PTH (pg/ml)	Vitamin K_2	50.3±28.0	48.7± 30.4	56.9± 56.0	91.1± 64.8*
	Control	46.7±29.6	44.5± 27.6	43.8± 24.1	47.4± 28.4
Dose of calcium carbonate (g/day)	Vitamin K_2	1.6±1.5	2.0±1.7*	2.0±1.7*	2.0±1.8
	Control	1.7±1.7	1.7±1.7	1.8±1.8	1.8±1.8
Dose of sevelamer hydrochloride (g/day)	Vitamin K_2	1.5±2.2	1.5±2.2	1.4±2.3	1.4±2.3
	Control	1.6±1.9	1.6±1.9	1.7±2.1	1.7±2.2

Results are shown as the mean±SD. $P^*<0.05$ vs. baseline by the Wilcoxon signed rank test.

Table 4. Changes of osteoprotegerin

	Group	Baseline	After 12 months	P value
Osteoprotegerin (pmol/l)	Vitamin K_2	6.82±2.34	6.16±1.70	0.080
	Control	5.97±2.11	6.10±2.82	0.721

Results are shown as the mean±SD. P: vs. baseline by the Wilcoxon signed rank test.