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ORIGINAL ARTICLE

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Vitamin K2 inhibits glucocorticoid-induced bone loss partly by preventing the reduction of osteoprotegerin (OPG)

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Abstract We have recently demonstrated that glucocorticoid (GC) suppresses bone formation and enhances bone resorption, with resultant bone loss. This altered bone turnover is not due to the action of parathyroid hormone (PTH), but appears to be related to the suppression of osteoprotegerin (OPG). As vitamin K2 (menatetrenone) has been used for the treatment of osteoporosis, the present study was carried out to evaluate the effect of vitamin K2 on GC-induced bone loss. Twenty patients with chronic glomerulonephritis treated with GC for the first time were chosen for this study. Ten patients received GC alone (group A) and the other 10 patients each received 15 mg of vitamin K2 per day in addition to GC (group B). Markers of bone metabolism, including serum OPG, osteocalcin (OC), bone-specific alkaline phosphatase activity (BAP), PTH, tartrate-resistant acid phosphatase (TRAP), and bone mineral density (BMD), were measured before and during the treatment. OPG was significantly decreased in group A ($P < 0.001$), while no significant change was seen in group B. TRAP was markedly increased in both groups, more particularly in group A ($P < 0.01$). PTH was decreased in group A, but was increased in group B. OC was decreased at month 1 but subsequently increased until month 12 in both groups. BAP had decreased at month 3 in group A ($P < 0.05$), but not in group B. BMD of the lumbar spine was significantly reduced after 6 months ($P < 0.01$), and 12 months ($P < 0.001$) of treatment in group A, whereas there was no remarkable change in group B. The present study demonstrated that the inhibition exerted by vitamin K2 of the reduction in OPG induced by GC may, at least in part, play a role in the prevention and treatment of GC-induced bone loss.

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Introduction

An increased risk of fractures due to osteoporosis is one of the most serious problems for patients receiving long-term glucocorticoid (GC) treatment [1,2]. In general, bone loss caused by GC treatment occurs within the first 6 months of treatment. Although it is known that GC alters bone metabolism by multiple pathways [3–6], the precise mechanisms are not clear.

Osteoprotegerin (OPG), a member of the tumor necrosis factor (TNF) receptor superfamily, has recently been identified as a novel cytokine [7–8]. OPG acts as a decoy receptor, binding to RANKL (receptor activator of nuclear factor [NF]-kappa B ligand) and blocking its interaction with RANK [9], and it inhibits both the differentiation and functions of osteoclasts.

We recently showed, for the first time, that serum OPG was suppressed by GC [10]. We have also investigated the relationships between OPG and other markers of bone metabolism during GC treatment and have demonstrated that GC suppresses bone formation and enhances bone resorption, with resultant bone loss. This accelerated bone loss is not due to an increase in PTH, but appears to be related to the suppression of OPG [11].

Prevention of bone fracture is essential in patients with GC-induced osteoporosis. Vitamin K2 (menatetrenone) is approved in Japan for both prevention and treatment of osteoporosis. Recent studies have shown that vitamin K2 reduces vertebral and hip fractures and improves bone quality [12], stimulating osteoblastogenesis and inhibiting osteoclastogenesis in vitro [13]. It has also been shown that vitamin K2 mitigates the decrease in bone mineral density (BMD) induced by GC in vivo in rats [14]. However, the effect of vitamin K2 on GC-induced osteoporosis in humans has not been elucidated. The aim of the present study was to

Table 1. Characteristics of the study population

	Group A (glucocorticoid alone)	Group B (glucocorticoid plus vitamin K2)	*P*
n (men/women)	10 (6/4)	10 (6/4)	NS
Age (years)	38.5 ± 6.4	41.6 ± 7.2	NS
Body weight (kg)	68.2 ± 3.5	65.8 ± 3.2	NS
Body mass index (kg/m^2)	25.3 ± 0.9	26.3 ± 1.0	NS
Initial dose of prednisolone (mg/day)	42.0 ± 2.9	39.0 ± 2.8	NS
Final dose of prednisolone (mg/day)	10.5 ± 0.8	10.0 ± 1.1	NS
Cumulative doses of prednisolone (mg)			
1 month	1202.0 ± 85.2	1051.1 ± 66.6	NS
3 months	3058.8 ± 135.8	2907.2 ± 209.3	NS
6 months	4612.1 ± 252.1	4489.7 ± 261.1	NS
12 months	7217.3 ± 408.0	6887.5 ± 311.5	NS

Data values are means ± SEM

determine the effect of vitamin K2 on bone metabolism in GC-treated patients. To clarify whether the administration of vitamin K2 prevents GC-induced bone loss, a randomized prospective controlled study was conducted in 20 patients with chronic glomerulonephritis scheduled for treatment with GC.

Patients and methods

Study design

Twenty patients (12 men and 8 women) with chronic glomerulonephritis scheduled for GC treatment were enrolled in this prospective study. The original renal diseases were minimal change nephrotic syndrome ($n = 10$), membranous nephropathy ($n = 7$), IgA nephropathy ($n = 2$), and lupus nephritis ($n = 1$). All patients were treated with GC for the first time. GC treatment was initiated at a dose of 30–50 mg/day, as prednisolone, and the dose was reduced gradually thereafter.

The patients were randomly divided into two groups before treatment. Informed consent was obtained from all subjects. Ten patients (6 men, and 2 premenopausal and 2 postmenopausal women) received GC alone (group A) and the other 10 patients (6 men and 2 premenopausal and 2 postmenopausal women) each received 15 mg of vitamin K2 (menatetrenone; Glakay; Eisai, Tokyo, Japan) three times per day in addition to GC (group B) during the study period. The profiles of the patients are shown in Table 1. Renal function was normal in all subjects (serum Cr level $<$ 1.2 mg/dl). There were no significant differences between the two groups in age, body mass index, or dose of GC (Table 1), or in various serum and urinary biochemical parameters at baseline (Table 2).

None of the patients had been prescribed drugs that affect bone metabolism, such as calcium, alfacalcidol, estrogen, calcitonin, or bisphosphonate. The study period was 12 months.

Measurements

Venous blood samples were obtained in the early morning after an overnight fast, and 24-h urine was collected in the morning. Biochemical parameters of blood and urine were measured before, and at 1, 3, 6, and 12 months after the start of GC treatment.

Bone formation was assessed by the serum intact osteocalcin (OC) and bone-specific alkaline phosphatase activity (BAP), which are known to be sensitive and specific markers of bone formation [15]. Bone resorption was assessed by the measurement of serum levels of tartrate-resistant acid phosphatase (TRAP) [16]. Serum intact OC, TRAP and BAP levels were measured by radioimmunoassay (Mitsubishi Kagaku BCL, Tokyo, Japan), enzyme-linked immunosorbent assay (ELISA; SRL, Tokyo, Japan), and enzyme immunoassay (SRL), respectively. We also measured the serum level of intact PTH by chemiluminescence immunoassay (CLIA) and serum OPG by ELISA [17]. Bone mineral density (BMD) of the lumbar spine (L3) was measured by dual-energy X-ray absorptiometry (DEXA; XR-26; Norland, Fort Atkinson, WI, USA) before and at 6 and 12 months after the start of GC treatment.

Statistics

All data values are expressed as means ± SEM. Markers of bone metabolism are expressed as percentages of initial values. All data were analyzed by analysis of variance (ANOVA) combined with Fisher's protected least significant difference (PLSD) test. The relationships between serum OPG and PTH were examined by Spearman's rank correlation analysis. Paired Student's *t*-test was used for comparisons of BMD values before and at 6 and 12 months after the start of treatment. Differences with a P value of less than 0.05 were considered to be significant.

Results

Measurement of biochemical parameters

Table 2 shows the serum and urinary biochemical parameters before treatment and the changes during treatment. There were no remarkable changes in serum creatinine, phosphorus, urea nitrogen, and creatinine clearance in either group. Serum total protein, albumin, calcium, and urinary excretion of calcium were significantly increased,

Table 2. In serum and urinary biochemical parameters before treatment and changes during treatment

	Group	Baseline	1 Month	3 Months	6 Months	12 Months
TP (g/dl)	Group A	4.5 ± 0.2	5.6 ± 0.4*	6.0 ± 0.3**	6.5 ± 0.3***	6.7 ± 0.3***
	Group B	4.5 ± 0.2	5.5 ± 0.3*	6.2 ± 0.4**	6.1 ± 0.4**	6.9 ± 0.3***
Alb (g/dl)	Group A	1.9 ± 0.2	3.1 ± 0.3***	3.6 ± 0.3***	3.8 ± 0.2***	3.9 ± 0.2***
	Group B	1.8 ± 0.1	3.1 ± 0.2**	3.5 ± 0.3***	3.4 ± 0.3***	3.9 ± 0.2***
BUN (mg/dl)	Group A	20.0 ± 3.1	19.1 ± 3.5	17.5 ± 1.6	16.3 ± 2.5	15.1 ± 2.3
	Group B	14.8 ± 2.8	16.4 ± 5.0	13.9 ± 2.9	16.1 ± 3.1	14.3 ± 1.8
Cr (mg/dl)	Group A	0.95 ± 0.11	0.84 ± 0.07	0.80 ± 0.07	0.83 ± 0.09	0.84 ± 0.09
	Group B	0.79 ± 0.07	0.86 ± 0.11	0.81 ± 0.07	0.83 ± 0.09	0.90 ± 0.09
Ca (mg/dl)	Group A	7.5 ± 0.1	8.6 ± 0.2***	8.8 ± 0.2***	9.1 ± 0.2***	9.1 ± 0.2***
	Group B	7.6 ± 0.2	8.4 ± 0.2**	8.8 ± 0.3***	8.8 ± 0.3***	9.1 ± 0.2***
Co-Ca (mg/dl)	Group A	9.6 ± 0.1	9.5 ± 0.1	9.4 ± 0.2	9.4 ± 0.1	9.3 ± 0.1
	Group B	9.8 ± 0.1	9.4 ± 0.1*	9.4 ± 0.1*	9.5 ± 0.1	9.3 ± 0.2*
P (mg/dl)	Group A	3.5 ± 0.1	3.1 ± 0.2*	3.2 ± 0.1	3.4 ± 0.1	3.4 ± 0.2
	Group B	3.8 ± 0.1	3.8 ± 0.2	3.6 ± 0.3	3.6 ± 0.2	3.6 ± 0.3
CCr (ml/min)	Group A	133.7 ± 17.6	164.2 ± 23.9	137.1 ± 21.0	147.7 ± 21.0	158.3 ± 26.8
	Group B	153.5 ± 20.6	151.9 ± 19.4	150.0 ± 27.4	162.3 ± 29.8	148.3 ± 26.4
U-Ca (g/day)	Group A	0.04 ± 0.01	0.12 ± 0.02*	0.11 ± 0.02*	0.12 ± 0.03*	0.10 ± 0.02
	Group B	0.04 ± 0.02	0.10 ± 0.03	0.11 ± 0.03	0.14 ± 0.03*	0.12 ± 0.03
U-P (g/day)	Group A	0.51 ± 0.05	0.53 ± 0.08	0.52 ± 0.07	0.55 ± 0.08	0.57 ± 0.06
	Group B	0.48 ± 0.05	0.59 ± 0.06	0.50 ± 0.05	0.62 ± 0.11	0.52 ± 0.08
U-prot (g/day)	Group A	5.3 ± 1.2	2.6 ± 0.8*	1.3 ± 0.6***	1.1 ± 0.4***	0.9 ± 0.5***
	Group B	6.3 ± 1.1	1.7 ± 0.7***	1.3 ± 0.7***	1.8 ± 0.8***	0.6 ± 0.4***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs baseline value
Data values are means ± SEM
TP, total protein; Alb, albumin; BUN, blood urea nitrogen; Cr, creatinine; Ca, calcium; co-Ca, corrected calcium (= Ca + 4 − Alb); P, phosphorus; CCr, creatinine clearance; U, urinary; U-prot, urinary protein

Table 3. Baseline values of markers of bone metabolism, and BMD

	Group A	Group B	*P*
OPG (ng/ml)	1.09 ± 0.19	0.98 ± 0.16	NS
PTH (pg/ml)	43.5 ± 6.7	31.1 ± 6.3	NS
TRAP (IU/l)	3.60 ± 0.65	3.53 ± 0.73	NS
OC (ng/ml)	3.85 ± 0.80	4.89 ± 0.96	NS
BAP (mU/ml)	20.0 ± 3.1	19.8 ± 3.2	NS
BMD (g/cm^2)	0.66 ± 0.04	0.62 ± 0.06	NS

Data values are means ± SEM
OPG, osteoprotegerin; PTH, parathyroid hormone; TRAP, tartrate-resistant acid phosphatase; OC, osteocalcin; BAP, bone-specific alkaline phosphatase; BMD, bone mineral density

while urinary excretion of protein was decreased during treatment in both groups. Corrected calcium level for serum albumin was slightly decreased. These results are in agreement with our previous study [11]. However, there were no significant differences in any of these parameters between the two groups.

Measurement of serum OPG

Table 3 shows the serum levels of various markers of bone metabolism, and BMD, at the baseline. There were no significant differences in any parameters between the two groups. Percentage changes in initial values of these markers are shown in Figs. 1–5. Changes in serum OPG in the two groups are shown in Fig. 1. Serum OPG in group A had significantly ($P < 0.001$) decreased, to 76.8 ± 3.9% of the baseline value, after 1 month of GC treatment. However,

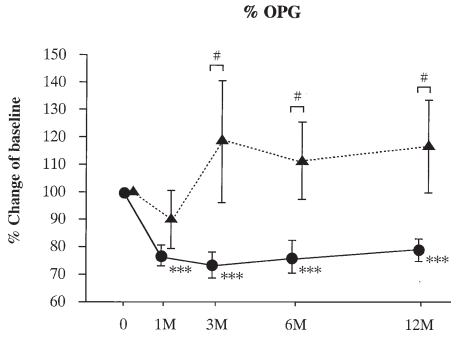

Fig. 1. Changes in serum *OPG* during glucocorticoid treatment. Data values are expressed as percentages of baseline values (means ± SEM). ***$P < 0.001$ vs baseline value; #$P < 0.05$ between groups. *Closed circles*, group A; *closed triangles*, group B; *M*, months

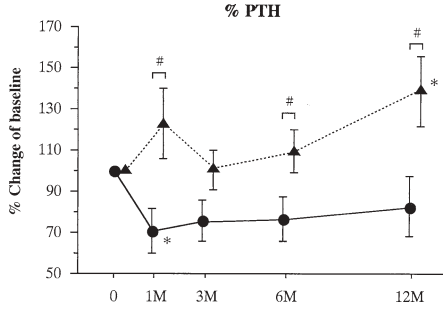

Fig. 2. Changes in serum *PTH* during glucocorticoid treatment. Data values are expressed as percentages of baseline values (means ± SEM). *$P < 0.05$ vs baseline value; #$P < 0.05$ between groups. *Closed circles*, group A; *closed triangles*, group B

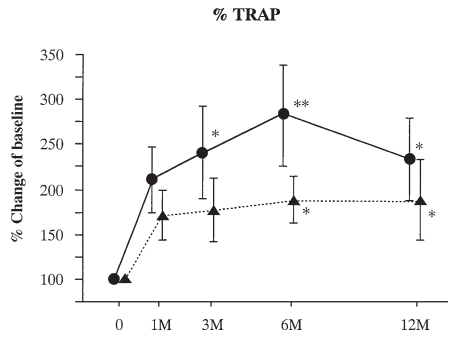

Fig. 3. Changes in serum *TRAP*, as a marker of bone resorption, during glucocorticoid treatment. Data values are expressed as percentages of baseline values (means ± SEM). $*P < 0.05$; $**P < 0.01$ vs baseline value. *Closed circles*, group A; *closed triangles*, group B

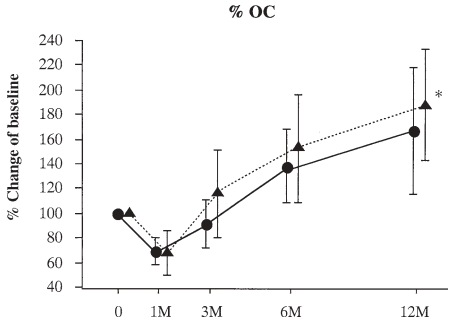

Fig. 4. Changes in serum osteocalcin (*OC*), as a marker of bone formation, during glucocorticoid treatment. Data values are expressed as percentages of baseline values (means ± SEM). $*P < 0.05$ vs baseline value. *Closed circles*, group A; *closed triangles*, group B

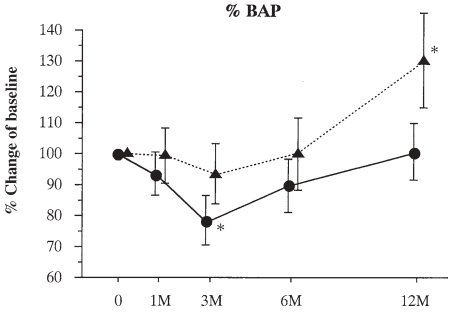

Fig. 5. Changes in serum *BAP*, as a marker of bone formation, during glucocorticoid treatment. Data values are expressed as percentages of baseline values (means ± SEM). $*P < 0.05$ vs baseline value. *Closed circles*, group A; *closed triangles*, group B

further reduction in serum OPG was not observed at 12 months after the start of treatment. These results are consistent with the results of our previous study [11]. In contrast, serum OPG was not significantly reduced in group B during vitamin K2 treatment. Percentage changes in OPG in the two groups were significantly different at 3, 6, and 12 months after the start of treatment ($P < 0.05$).

Measurement of serum PTH

As shown in Fig. 2, GC caused a significant ($P < 0.05$) reduction in serum PTH, to 70.8 ± 10.5% of the baseline value after 1 month of treatment in group A. However, no significant reduction was found during the remainder of the study period. On the other hand, serum PTH level tended to increase during the treatment period in group B, the elevation being significant ($P < 0.05$) at 12 months after the start of treatment. There were significant differences between the two groups at 1, 6 and 12 months ($P < 0.05$).

Measurement of serum TRAP as a marker of bone resorption

As shown in Fig. 3, serum TRAP increased markedly during the treatment period in both groups. The peak levels reached 282.4 ± 56.1% (group A) and 189.0 ± 25.6% (group B) of the baseline value at 6 months after the start of treatment. Notably, the vitamin K2-treated group showed lower values than those in the non-treated group, though the difference was not statistically significant.

Measurement of serum OC and BAP as markers of bone formation

As shown in Fig. 4, GC reduced serum OC level to 69.2 ± 11.1% (group A) and 67.5 ± 17.7% (group B) in the first month after the start of treatment. However, the serum OC level returned to the baseline level after 3 months, and further elevation was observed subsequently. Serum OC level in group B only showed significant elevation at 12 months after the start of treatment in comparison to the baseline value ($P < 0.05$). However, there were no significant differences between the two groups during the study period.

As shown in Fig. 5, serum BAP in group A had decreased to 78.5 ± 7.9% of the baseline value at 3 months after the start of treatment ($P < 0.05$), but further reduction in this marker was not observed during the remainder of the study period. On the other hand, there was no significant reduction in serum BAP in group B throughout the study period, and a significant elevation ($P < 0.05$) was observed at 12 months after the start of treatment.

Measurement of BMD

Changes in BMD of the lumbar spine (L3) in the two groups are shown in Fig. 6. BMD in group A was significantly ($P < 0.01$) decreased, from 0.66 ± 0.04 to 0.58 ± 0.04 g/cm^2 after 6 months ($P < 0.01$), and to 0.55 ± 0.04 after 12 months ($P < 0.001$). On the other hand, there was no significant reduction in BMD in group B. In addition, lumbar vertebral fracture was seen in one patient in group A but was not seen in any group B patients.

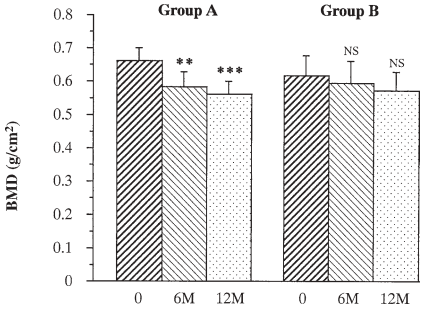

Fig. 6. Mean *BMD* of the lumbar spine (L3) before and after 6 and 12 months of glucocorticoid treatment. $^{**}P < 0.01$; $^{***}P < 0.001$ vs baseline value; *NS*, not significant

Discussion

GC-induced osteoporosis

GC is the leading cause of drug-induced osteoporosis [1,2]. Clinically, GC-induced bone loss develops rapidly, and this bone loss increases the risk of bone fractures. It has generally been thought that GC-induced osteoporosis results from suppressed bone formation [3] and enhanced bone resorption [4,5]. Possible mechanisms by which GC reduces bone density are: (1) GC-induced direct impairment of osteoblast [6] or osteoclast function and (2) secondary hyperparathyroidism, due to the increased renal excretion and decreased intestinal absorption of calcium [18]. However, the precise mechanism of GC-induced osteoporosis has not been elucidated.

GC-induced osteoporosis should be prevented and, if present, should be treated [19]. Recent studies have shown that vitamin K2 stimulates osteoblastogenesis and inhibits osteoclastogenesis in vitro [13]. Vitamin K2 has been reported to reduce bone fracture in osteoporotic patients [12] and to prevent GC-induced bone loss [20].

Effects of vitamin K2 on serum OPG

OPG plays a key role in the physiological regulation of osteoclastic bone resorption [7,8]. Indeed, overexpression of OPG in transgenic mice resulted in osteopetrosis [8], while OPG-deficient mice developed severe osteoporosis [21]. Recent studies have suggested that GC promotes osteoclastogenesis by inhibiting OPG production in vitro [22,23], thereby enhancing bone resorption.

We recently showed, for the first time, that serum OPG was significantly suppressed by GC in vivo in humans [10]. This new finding suggests that the suppression of OPG may be associated with GC-induced osteoporosis. We have also investigated the alterations of various markers of bone metabolism in GC-induced osteoporosis and demonstrated that the increased bone resorption caused by GC may be partly mediated by the inhibition of OPG, but not by an increase in PTH [11]. A recent study has revealed that vitamin K2 enhanced the expression of OPG and inhibited osteoclastic bone resorption in stromal cells [24]. In clinical studies, it has been found that vitamin K2 effectively prevents fractures and sustains lumbar BMD in patients with osteoporosis [12] and also prevents GC-induced bone loss [20].

In the present study, while the inhibitory effect of GC on OPG remained during the treatment period (Fig. 1, group A), no significant reduction in serum OPG was observed with vitamin K2 treatment (Fig. 1, group B). These results imply that vitamin K2 administration inhibited the GC-induced reduction of OPG. Therefore, it is assumed that the prevention of GC-induced osteoporosis by vitamin K2 was achieved partly through this mechanism.

Effects of vitamin K2 on serum PTH

The present study and our previous study [11] confirmed that serum PTH was decreased during GC treatment. Thus, secondary hyperparathyroidism could not explain the GC-induced osteoporosis. This result is in agreement with previously reported results [25,26]. In the present study also, serum PTH did not significantly change until 6 months after the start of treatment, and was elevated at 12 months in the vitamin K2-treated group (group B). Though there have been very few reports on the relationship between PTH and vitamin K2, this result suggested that vitamin K2 may have direct effects on PTH secretion in vivo, as there were no significant differences in calcium or phosphorus metabolism between the two groups (Table 2). Of note, a recent study showed that vitamin K2 inhibited PTH-induced osteoclastic bone resorption in vitro [27].

Among various markers of bone metabolism, only serum PTH was positively correlated with serum OPG ($r = 0.42$; $P < 0.0001$). It appears reasonable, for protection against bone loss, that PTH is suppressed in compensatory response to the reduction of OPG caused by GC. These results are not consistent with the results of a previous study showing that PTH inhibited OPG mRNA expression in bone in vitro [28], which would result in the stimulation of osteoclast formation. Though the relationship between OPG and PTH is not clear, it is likely that PTH regulates bone resorption, at least in part via its effect on OPG expression.

Effects of vitamin K2 on a marker of bone resorption

Vitamin K2 has been reported to inhibit osteoclastic bone resorption [29], due to suppression of RANKL expression, and to stimulate osteoblastic bone formation in a murine bone marrow cell culture [30]. It was suggested that vitamin K2 may alter bone metabolism to spare bone mass, not only as a nutrient factor but also as a substance that modulates cellular differentiation and function in bone marrow.

TRAP is known to reflect the activity of osteoclasts [16]. A previous in vitro study demonstrated that treatment with GC increased the level of TRAP activity, resulting in the promotion of osteoclastic bone resorption [23]. The increase in the level of TRAP activity was remarkably inhibited by vitamin K2 administration in an in vitro study [27].

It is therefore thought that vitamin K2 has a suppressive effect on osteoclastogenesis. The present study also showed that GC significantly increased serum TRAP during the treatment period (Fig. 3, group A) and that the increase tended to be inhibited by vitamin K2 administration (Fig. 3, group B). These findings suggest that vitamin K2 may suppress GC-induced bone resorption partly through an elevation of OPG level, independent of PTH.

However, serum TRAP increased above the baseline during GC treatment in spite of the unchanged OPG level in the vitamin-K2 treated group (group B). Thus, the changes in TRAP cannot be explained simply by changes in OPG, suggesting that bone resorption is modulated not only by changes in OPG but also by other factors related to osteoclastogenesis.

Effects of vitamin K2 on markers of bone formation

It is well established that GC-induced bone loss is caused by the inhibition of bone formation, which includes the suppression of osteoblastic proliferation [4]. Koshihara and Hoshi [13] reported that vitamin K2 enhanced the mineralization of osteoblasts in vitro. With the addition of vitamin K2, accumulation of OC in the extracelluar matrix of osteoblasts was enhanced. Koshihara and colleagues [24] also demonstrated that vitamin K2 both stimulated osteoblastogenesis and inhibited osteoclastogenesis in human bone marrow cell culture. An in vivo study has shown that vitamin K2 prevents the bone loss induced by GC partly through the enhancement of bone formation [14].

In the present study, serum OC and BAP levels initially declined. The reductions in OC and BAP were most apparent at 1 month (OC) and 3 months (BAP; $P < 0.05$) after the start of treatment in group A (Figs. 4, 5). However, serum OC and BAP levels in group A returned to baseline levels at 3 and 6 months after the start of treatment. It is assumed that the decrease in serum OC and BAP levels that occurred during the first few months was due to the high dose of GC, and that the subsequent recovery in OC and BAP levels may have been related to the reduction in the dose of GC. However, it seems strange that serum OC had increased above the baseline during GC treatment even in the non-vitamin K2-treated group (group A). The increase in serum OC may be associated with the improved general status of the nephrotic patients, because body edema was markedly reduced and serum albumin was increased with the decrease of urinary protein excretion in response to GC treatment. Conversely, both OC and BAP levels in group B had significantly increased at 12 months after the start of treatment ($P < 0.05$). These results suggest that vitamin K2 administration prevents GC-induced bone loss partly through the enhancement of bone formation.

It has been previously shown that the level of undercarboxylated OC (ucOC) is elevated in osteoporotic patients and correlates with the risk of hip fracture [31]. Vitamin K2 is essential for the carboxylation of OC and has been reported to decrease the serum level of ucOC [32]. In the present study, we measured ucOC by ELISA. Serum ucOC in the vitamin K2-treated group (group B) showed a lower level than that in the other group (group A), and the difference was statistically significant at 6 and 12 months after the start of treatment (data not shown). These results show that the effect of vitamin K2 may be related to bone quality, by its promotion of the carboxylation of OC. Further studies are needed to clarify the role of the carboxylation of OC in bone, and these studies will provide an another insight into the mechanism of vitamin K2 treatment in osteoporosis.

Effects of vitamin K2 on BMD

It has been reported that the circulating level of vitamin K is lower in osteoporotic patients with bone fractures than healthy age-matched women as controls [33]. A recent study has shown that the decrease in BMD induced by GC was inhibited by vitamin K2 treatment in rats [14]. It has also been reported that vitamin K2 increased lumbar BMD, probably through the carboxylation of OC, which may be related to bone quality [34], in long-term GC-treated children [35]. In postmenopausal women with osteoporosis, vitamin K2 administration also improved BMD [36]. Therefore, it is well established that vitamin K2 prevents bone loss and promotes bone formation.

In the present study, GC treatment resulted in decreased BMD of the lumbar spine in group A (Fig. 6), but the BMD in group B was not significantly reduced by vitamin K2 administration. Thus, GC–induced reduction of BMD was prevented by vitamin K2 administration. As no adverse effect related to the use of vitamin K2 was observed in the present study, this drug should be tolerated well by most patients. Therefore, vitamin K2 is recommended for the prevention and treatment of GC-induced osteoporosis.

Conclusion

The present study demonstrated that GC treatment of patients with chronic glomerulonephritis resulted in bone loss, which was associated with the sustained enhancement of bone resorption rather than the rapid suppression of bone formation. Notably, this bone loss was suppressed by vitamin K2 administration, partly due to the prevention of the GC-induced reduction of OPG. The results of the present study have provided a new insight into the role of vitamin K2 in GC-induced bone loss, but further studies are needed to clarify these mechanisms.

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