Osteoclast Inhibitory Effects of Vitamin K_2 Alone or in Combination with Etidronate or Risedronate in Patients with Rheumatoid Arthritis: 2-Year Results

MINORU MORISHITA, MASAKAZU NAGASHIMA, KOICHI WAUKE, HIROSHI TAKAHASHI, and KENJI TAKENOUCHI

ABSTRACT. ***Objective.*** To investigate the effects of vitamin K_2 (Vit K_2) alone or in combination with etidronate and risedronate on bone loss, osteoclast induction, and inflammation in patients with rheumatoid arthritis (RA).

Methods. Subjects comprised 79 patients with RA who were receiving prednisolone, divided into 3 groups: Group K, Vit K_2 alone; Group KE, Vit K_2 plus etidronate; and Group KR, Vit K_2 plus risedronate. During a 24-month treatment and followup period, levels of N-terminal telopeptide of type I collagen (NTx) and bone alkaline phosphatase were measured. Bone mineral density (BMD) of the 3 groups was measured using dual-energy x-ray absorptiometry. Damage score to fingers on radiographic findings were measured according to the Larsen method. Serum levels of receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG) were measured.

Results. Falls in rate of change of BMD decreased after 18 months in groups KR and KE. Larsen damage scores indicated a significant difference between Group KE and other groups. Significant decreases in serum NTx were observed in groups KE and KR at all timepoints, but not in Group K. Levels of RANKL decreased significantly in all 3 groups.

Conclusion. Vit K_2 alone or in combination with bisphosphonates for treatment of osteoporosis in patients with RA may inhibit osteoclast induction via decreases in levels of RANKL. (First Release Feb 1 2008; J Rheumatol 2008;35:407–13)

Key Indexing Terms:
RHEUMATOID ARTHRITIS VITAMIN K_2 ETIDRONATE RISEDRONATE
RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-κB LIGAND OSTEOPROTEGERIN

Bone formation is maintained by continuous remodeling, with a balance between bone resorption by osteoclasts and osteogenesis by osteoblasts. However, loss of bone mass has frequently been observed in patients with rheumatoid arthritis (RA), and the main causes of such osteoporosis are reportedly steroid therapy, postmenopausal changes in hormone balance (postmenopausal osteoporosis), and disuse bone atrophy associated with polyarticular impairment[1,2]. Conversely, bone and cartilage damage in RA is the result of a process in which cellular and cytokine-mediated inflammation leads to an imbalance between synthesis and degradation. It is becoming clear that the increase in bone resorption in bone diseases such as osteoporosis and RA has an underlying molecular mechanism, namely, the facilitation of osteoclast differentiation and activation by the inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin 1 (IL-1)[3-5].

Osteoprotegerin (OPG) is a secreted soluble decoy receptor with homology to members of the TNF receptor family[6,7] binding to receptor activator of nuclear factor-κB ligand (RANKL) and blocking interactions with receptor activator of NF-κB[8]. OPG thus acts as an antagonist to RANKL. An imbalance in this system may play a part in the skeletal complications of RA[9]. On the other hand, several studies have reported that menatetrenone (MK4), vitamin K_2 (Vit K_2), reduces vertebral and hip fractures and improves bone quality[10], stimulating osteoblastogenesis and inhibiting osteoclastogenesis in human bone marrow cell culture[11]. Vit K_2 also inhibits bone loss induced by prednisolone (PSL) in rats[12]. Bisphosphonates (BP), which are taken up by osteoclasts and macrophages to inhibit the activity of some inflammatory cytokines, are expected to function as inhibitors of inflammation induced by these cells. Etidronate, a non-aminobisphosphonate, is reportedly effective in relieving pain in patients suffering from steroid-induced osteoporosis, postmenopausal

From the Department of Rheumatology, Tokyo Metropolitan Bokutoh Hospital; and Department of Joint Disease and Rheumatism, Nippon Medical School, Tokyo, Japan.

Supported by the Japan Osteoporosis Foundation.

M. Morishita, MD; K. Takenouchi, MD, Department of Joint Disease and Rheumatism, Nippon Medical School; K. Wauke, MD, PhD; H. Takahashi, MD, PhD, Department of Rheumatology, Tokyo Metropolitan Bokutoh Hospital; M. Nagashima, MD, PhD, Director, Department of Rheumatology, Tokyo Metropolitan Bokutoh Hospital and Department of Joint Disease and Rheumatism, Nippon Medical School.

Address reprint requests to Dr. M. Nagashima, Department of Rheumatology, Tokyo Metropolitan Bokutoh Hospital, 4-23-15 Kotobashi, Sumida-ku, Tokyo 130-8575, Japan. E-mail: m_nagashima@bokutoh-hp.metro.tokyo.jp

Accepted for publication October 15, 2007.

osteoporosis, and osteoarthritis. Similarly, the aminobisphosphonate risedronate has been reported as an effective treatment for decreased bone density and vertebral fracture in patients receiving corticosteroid therapy[13,14]. We previously examined whether intermittent cyclical etidronate inhibits bone resorption or inflammatory changes over 1.5 years and 3 years in osteoporotic patients with RA[15,16]. Etidronate also displayed inhibitory effects on the production of mediators related to inflammation, pain, and angiogenesis, including IL-6, prostaglandin E_2, substance P, and vascular endothelial growth factor in synovial cells of arthritis models[16]. However, the mechanisms of action for BP have not been completely elucidated. Few clinical reports have described the direct effects of BP on inhibition of bone resorption and bone formation. Further, very few clinical reports have examined the negative or positive effects of BP on production of TNF-α, RANKL, or OPG. Imbalances in serum type I collagen N-telopeptide (NTx) and bone-specific alkaline phosphatase (BAP), TNF-α, and RANKL and OPG may play important roles in bone loss, osteoclast induction, and inflammation.

In our review of prospectively studied patients with RA, we examined the clinical efficacy of Vit K_2 alone and in combination with etidronate or risedronate in maintaining the balance between osteogenesis and bone resorption by measuring NTX, BAP, RANKL, and OPG.

MATERIALS AND METHODS

Subjects comprised 79 patients with RA (71 women, 8 men) who fulfilled the diagnostic criteria of the American College of Rheumatology[17]. The patients were selected from the outpatient section of our department in Nippon Medical School and randomly divided into 3 groups using the double-blind envelope method: Group K, 21 patients (20 women, 1 man) administered 45 mg of Vit K_2; Group KR, 29 patients (25 women, 4 men) administered 45 mg of Vit K_2 and 2.5 mg of risedronate; and Group KE, 29 patients (26 women, 3 men) administered 45 mg of Vit K_2 and 400 mg of etidronate. In Group KE, 400 mg of etidronate was administered orally between meals for 2 weeks, and was withheld for the next 10 weeks. This 12-week period was defined as one cycle. Etidronate was administered in the designated periods for 8 cycles (96 wks, 24 mo).

Mean patient age was 61.4 ± 9.6 years in Group K, 62.3 ± 9.0 years in Group KR, and 63.4 ± 7.2 years in Group KE. Mean disease duration was 18.1 ± 14.3 years in Group K, 20.4 ± 9.8 years in Group KR, and 20.0 ± 12.0 years in Group KE. With respect to the amount of adrenal corticosteroid, 5 mg per day of PSL was administered to 17 patients in Group K, 22 patients in Group KR, and 24 patients in Group KE. Mean dose and duration of PSL were 4.3 ± 2.2 mg and 94.9 ± 79.3 months. Vitamin D_3 ± 1.0 μg/day was administered to 2 patients in Group K, 2 patients in Group KR, and 1 patient in Group KE. No calcium was administered to any of the 3 groups. Almost all patients received disease modifying antirheumatic drugs (DMARD). The most frequently used DMARD were methotrexate (MTX), followed by salazosulfapyridine and bucillamine, but no biological agents (TNF-α blockers) were prescribed. The numbers of patients who received MTX were 7, 10, and 13 patients in Groups K, KR, and KE, respectively, and mean dose of MTX was 5.3 ± 1.6 mg (range 2–8 mg). Patients continued taking medications prescribed before the study was initiated. Dosages of medications including PSL and DMARD were not increased, and no other medications were administered. Our study was approved by the Ethical Committee of Nippon Medical School. All patients provided informed consent (Table 1).

During a 24-month treatment and followup period, 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). Based on radiographic findings, damage to fingers was graded from 0 (radiographically normal) to 5 (maximum degree of joint destruction) according to the Larsen method[18]. Twenty joints, including 10 metacarpophalangeal joints, 8 proximal interphalangeal (PIP) joints, and 2 interphalangeal joints of the thumb on both hands, were evaluated using this method. Evaluation was performed by 2 rheumatologists and if evaluations differed, the lower score was adopted. Serum level of NTx was measured as a marker for bone resorption using an enzyme linked immunosorbent assay (ELISA) kit (Inverness Medical Professional Diagnostics, Princeton, NJ, USA) and serum BAP, which is known as a sensitive and specific marker of bone formation, was measured using an enzyme immunoassay (EIA) kit (Quidel, San Diego, CA, USA)[19,20].

Serum levels of RANKL measured using an ELISA kit (Biomedica, Vienna, Austria) and serum OPG also measured using an ELISA kit (Immundiagnostik AG, Bensheim, Germany) were compared between groups (Table 2).

Statistical analysis. All data are expressed as mean ± standard deviation. All background data in patients with RA were analyzed using Fisher's exact test, H-test, and 1-way analysis of variance (ANOVA). Percentage changes in all measures, DEXA, NTx, and BAP at 0, 6, 12, 18, and 24 months after administration of Vit K_2, risedronate, or etidronate relative to baseline measurements were evaluated using the Wilcoxon signed-ranks test and differences in percentage changes at 0, 6, 12, 18, and 24 months among Groups K, KR, and KE were evaluated using the Mann-Whitney U-test. The relationship between any 2 items was examined using Spearman's rank correlation analysis. Differences with a value of $p < 0.05$ were considered statistically significant.

RESULTS

Measurement of BMD by DEXA. Background data in patients with RA are shown in Table 1. No significant differences in these measures were observed between the 3 groups.

Percentage changes in BMD as measured by DEXA at 6 months after the start of treatment in Groups K, KR, and KE were –2.0%, –1.2%, and 0.3%, respectively, and at 24 months were –9.0%, –5.8%, and –6.9%. All 3 groups showed significant falls until 18 months. However, reductions in percentage changes in BMD had decreased after 18 months in Group KR and appeared almost equivalent when 18 month and 24 month changes were compared. In addition, percentage changes in BMD began to increase in Group KE from 18 months. Conversely, falls continued in Group K from 18 months (Figure 1).

Percentage change in Larsen damage score increased with time in Groups K and KR, showing periarticular osteoclastic progress. On the other hand, Larsen damage scores for Group KE at baseline and at 24 months were almost equivalent, displaying a significant difference compared to Groups K and KR (Figure 2).

Measurement of serum NTx and BAP as markers of bone resorption and formation. Percentage changes in serum NTx at 6 months after start of treatment in Groups K, KR, and KE were 1.4%, –8.4%, and –16.8%, respectively, and at 24 months were 1.8%, –19.1%, and –12.1% (Figure 3). A significant decrease in serum NTx was observed at 6, 12, and 24 months in Groups KE and KR. Significant differences in NTx were observed at 6, 12, 18, and 24 months between Groups K and KE, and at 24 months between Groups K and KR. Almost

Table 1. Background data in patients with rheumatoid arthritis.

	N	Vit K_2 Group	Vit K_2 + R Group	Vit K_2 + E Group	p
Patients (n)	79	21	29	29	
Sex					
Male	8	1	4	3	0.514*
Female	71	20	25	26	
Age, yrs					
≤ 59	28	8	11	9	0.565**
60–69	34	9	13	12	
≤ 70	17	4	5	8	
Mean ± SD	62.5 ± 8.5	61.4 ± 9.6	62.3 ± 9.0	63.4 ± 7.2	0.775†
Range	42–82	42–82	44–79	52–75	
Disease duration, yrs					
≤ 9	19	9	3	7	
10–19	19	2	10	7	0.619**
20–29	28	6	12	10	
≥ 30	13	4	4	5	
Mean ± SD	19.6 ± 11.8	18.1 ± 14.3	20.4 ± 9.8	20.0 ± 12.0	0.969†
Range	2–48	3–45	4–42	2–48	
Combination drugs					
Steroid –	16	4	7	5	0.776*
+	63	17	22	24	
Vitamin D –	74	19	27	28	0.734*
+	5	2	2	1	
DMARD –	8	2	3	3	1.000*
+	71	19	26	26	

* Fisher's exact test. ** H-test, † analysis of variance. DMARD: disease modifying antirheumatic drugs; R: risedronate; E: etidronate.

Table 2. Measurement methods.

Serum Marker	Measurement
BMD*	DEXA (XR-26; Norland, Fort Atkinson, WI, USA)
NTX	ELISA (Inverness Medical Professional Diagnostica, NJ, USA)
BAP	EIA (Quidel, CA, USA)
RANKL	ELISA (Biomedica, Wien, Austria)
OPG	ELISA (Immunodiagnostik AG, Bensheim, Germany)

* BMD of the lumbar spine was measured by DEXA. BMD: bone mineral density; DEXA: dual-energy x-ray absorptiometry; NTX: N-terminal telopeptide of type I collagen; BAP: bone-specific alkaline phosphatase; EIA: enzyme immunoassay; RANKL: receptor activator of nuclear factor-κB ligand; OPG: osteoprotegerin.

no changes in NTx level were seen in Group K at all timepoints, except at 18 months. In addition to NTx, decreases in BAP were also observed, with percentage changes at 6 months after start of treatment of –2.2%, –9.6%, and –9.1%, respectively, in Groups K, KR, and KE (Figure 4). A significant decrease in serum BAP was observed from 6 months in all 3 groups, and percentage changes in BAP started to increase from 18 months.

Measurement of serum RANKL and OPG. RANKL levels in Groups K, KR, and KE were 25.83 pmol/l, 32.01 pmol/l, and

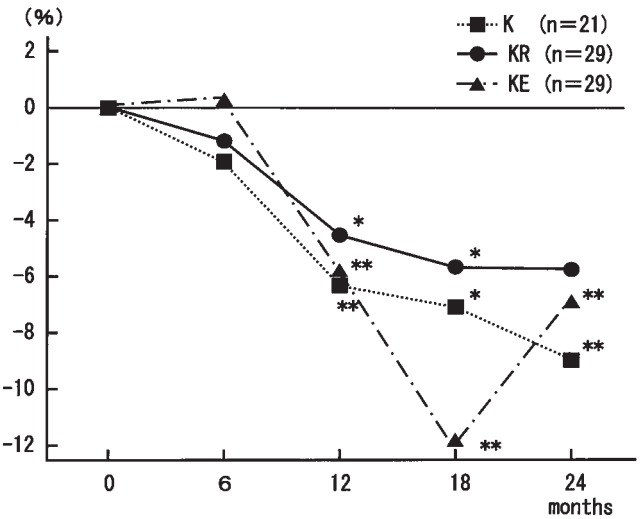

Figure 1. Percentage changes in BMD measured by DEXA at 6, 12, 18, and 24 months after start of treatment in Groups K, KR, and KE. Significant decreases in BMD, as measured by DEXA at 12, 18, and 24 months, were identified in all 3 groups. Differences between 0, 6, 12, 18, and 24 months after start of treatment were evaluated by Wilcoxon signed-rank test and differences among the 3 groups were evaluated by Mann-Whitney U-test. *p < 0.05; **p < 0.01.

33.97 pmol/l at 6 months after start of treatment and 14.15 pmol/l, 11.19 pmol/l, and 11.69 pmol/l at 24 months (Figure 5). Significant decreases in serum RANKL were observed at

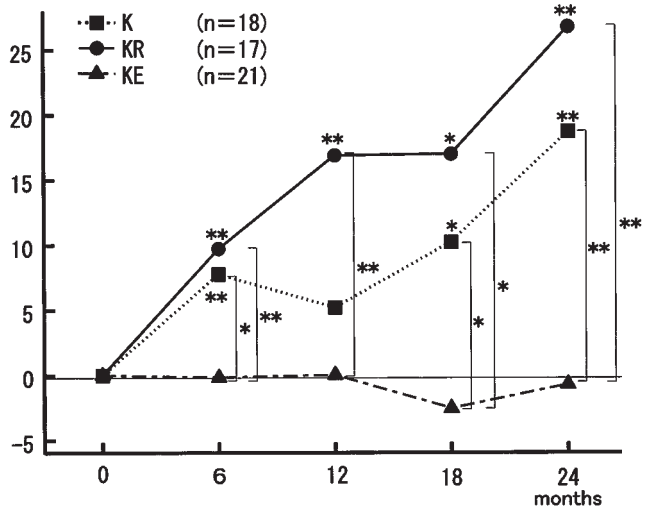

Figure 2. Percentage changes in Larsen damage score at 6, 12, 18, and 24 months after start of treatment in Groups K, KR, and KE. A significant increase in Larsen damage score was observed at 6, 12, 18, and 24 months in Groups K and KR. Evaluation was performed by 2 rheumatologists and the lower score was adopted if evaluations differed. Differences between 0, 6, 12, 18, and 24 months after start of treatment evaluated by Wilcoxon signed-rank test and differences among the 3 groups evaluated by Mann-Whitney U-test. *p < 0.05; **p < 0.01.

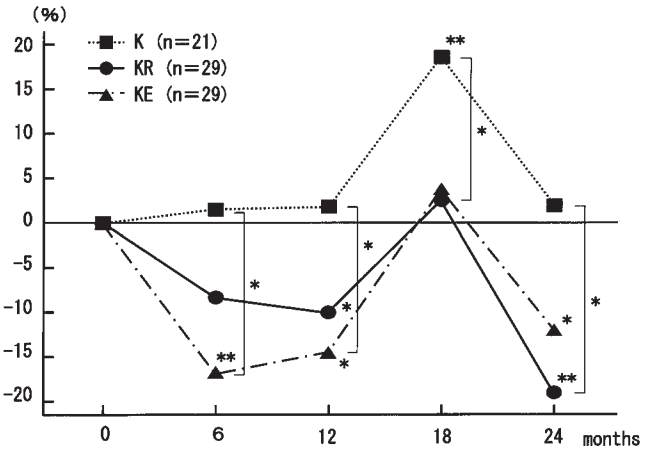

Figure 3. Percentage changes in serum NTx at 6, 12, 18, and 24 months after start of treatment in Groups K, KR, and KE. Significant decreases in serum NTx were observed at 6, 12, and 24 months in Groups KE and KR. Differences between 0, 6, 12, 18, and 24 months after start of treatment evaluated by Wilcoxon signed-rank test and differences among the 3 groups evaluated by Mann-Whitney U-test. *p < 0.05; **p < 0.01.

6, 12, 18, and 24 months in all 3 groups. Relationships between RANKL and OPG were compared between before and 24 months after start of treatment in Groups K, KR, and KE. OPG levels in all 3 groups were independent of RANKL. No significant relationship between RANKL and OPG was observed (Figure 6).

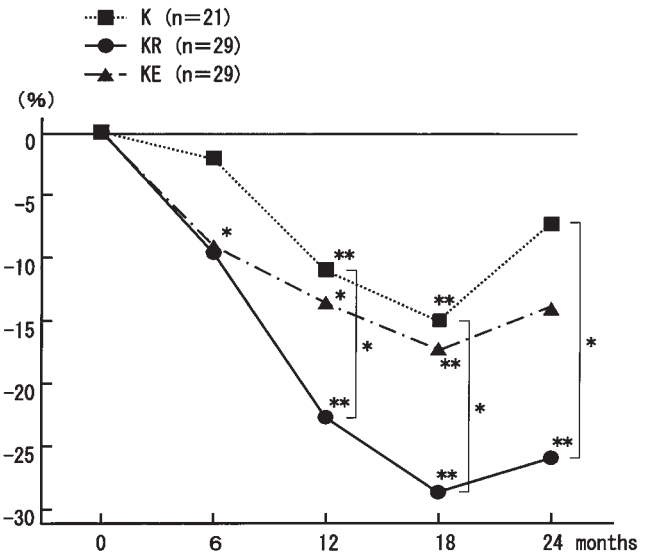

Figure 4. Percentage changes in serum BAP at 6, 12, 18, and 24 months after start of treatment in Groups K, KR, and KE. Significant decreases in serum BAP were seen at 6, 12, and 18 months in all 3 groups, but serum BAP tended to increase after 18 months in all 3 groups. Differences between 0, 6, 12, 18, and 24 months after start of treatment evaluated by Wilcoxon signed-rank test and differences among the 3 groups evaluated by Mann-Whitney U-test. *p < 0.05; **p < 0.01.

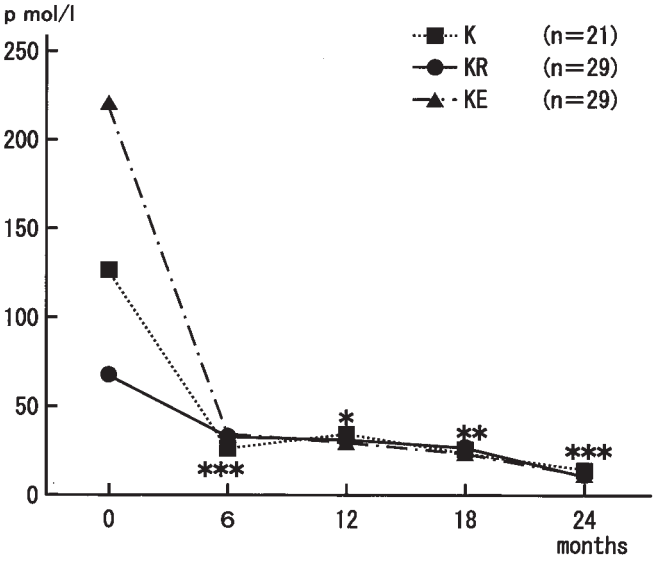

Figure 5. Percentage changes in serum RANKL levels compared to baseline at 6, 12, 18, and 24 months after start of treatment in Groups K, KR, and KE. Significant decreases in serum RANKL were observed at 6, 12, 18, and 24 months in all 3 groups. Differences between 0, 6, 12, 18, and 24 months after start of treatment evaluated by Wilcoxon signed-rank test and differences among the 3 groups evaluated by Mann-Whitney U-test. *p < 0.05; **p < 0.01; ***p < 0.001.

DISCUSSION

RANKL, which regulates the differentiation and function of osteoclasts, and its decoy receptor (OPG) have been identified

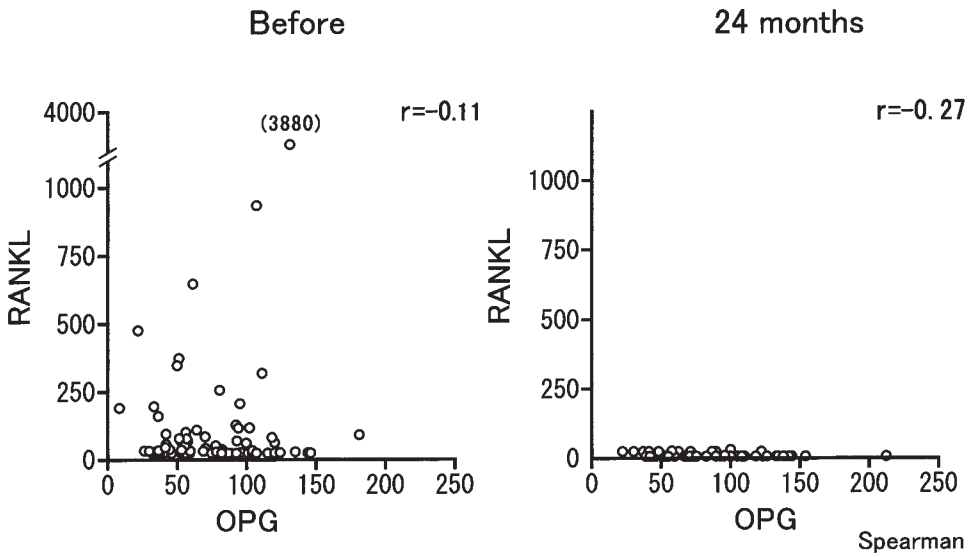

Figure 6. Relationships between RANKL and OPG were compared before and 24 months after start of treatment in Groups K, KR, and KE. RANKL decreased significantly until almost zero at 24 months after start of treatment in all 3 groups, but OPG in all 3 groups was independent of RANKL. No significant relationships between RANKL and OPG were recognized.

in recent years, and the regulatory mechanisms underlying bone resorption have gradually been clarified. Our study administered Vit K_2 alone or in combination with etidronate or risedronate to patients with RA accompanied by osteoporosis, and then investigated the effects of these drugs on bone resorption and formation based on various measures, including RANKL and OPG. First, DEXA was performed to investigate percentage changes in BMD. BMD decreased up to 18 months in all 3 groups. In Group KR, the decrease in BMD at 24 months was small, and BMD at this point was comparable to BMD at 18 months. In Group K, BMD continued to decrease at 24 months, and was significantly decreased compared to BP coadministration. Vit K_2 monotherapy reportedly reduces the risk of fractures, but does not change BMD[10] and results in significantly lower BMD when compared to etidronate alone[21]. Based on these findings, Vit K_2 should be coadministered with BP in patients with underlying diseases affecting osteoporosis, such as RA.

Many studies have found that administering BP to postmenopausal women with osteoporosis increases BMD[22,23]. In our study, the control group consisted only of patients with RA, and 79.7% took an average of 4.3 mg of prednisolone. Patients with RA experience systemic osteoporosis and produce various inflammatory cytokines that accelerate bone resorption in joints, and high concentrations of soluble RANKL have been detected from synovial fluid[24]. In RA, bone resorption is elevated[15], and when Vit K_2 and BP are combined, longterm administration for ≥ 18 months gradually decreases percentage change in BMD. Tascioglu, *et al* administered alendronate to postmenopausal women with RA accompanied by osteoporosis for at least 2 years, and reported significant increases in BMD[25].

Using Larsen damage scores, bone destruction around the metacarpophalangeal and PIP joints was assessed. In Groups K and KR, actual values and percentage change increased over time, suggesting advancing bone destruction around the joint. In Group KE, Larsen damage score remained basically unchanged from 0 to 24 months. In the past, our group found that in patients with RA, Larsen damage score was significantly lower for patients who took etidronate than for patients who did not take etidronate[16]. Also, in rats with adjuvant arthritis, the number of osteoclasts in joints around the ankle was significantly lower for etidronate than for alendronate[26]. Based on the results of past and present studies, etidronate appears to suppress bone destruction around joints in periarticular osteoporosis.

We next examined levels of NTx, a marker of bone resorption. At 6, 12, and 24 months, percentage changes in NTx remained mostly unchanged for Group K, and no significant difference was observed between 0 and 24 months. Also, in combination treatment using BP, percentage changes in NTx decreased significantly starting at 6 months for both Groups KE and KR. At 24 months, similar decreases continued, and a significant difference existed between Group K and Vit K_2 + BP groups. Several studies have documented reductions in NTx with administration of BP[23,27,28]. Also, Vit K_2 monotherapy reportedly does not bring about definite NTx changes[29], and our study obtained comparable findings. Urinary deoxypyridinoline (DPD) is a bone resorption marker like NTx, and studies have reported that Vit K_2 monotherapy does not alter urinary DPD levels[10,29,30].

RANKL was low for all 3 groups at 6 months after start of administration, and remained low even at 24 months. Some studies have documented that BP administration reduces RANKL[31,32]. Further, as far as Vit K_2 is concerned, MK4 sup-

presses RANKL expression[33] and geranylgeraniol (a Vit K_2 side-chain) hinders expression of RANKL to suppress formation of osteoclasts[34].

In recent years, the mechanisms of bone resorption have been roughly divided into 3 groups. The first group involves the RANK/RANKL system in osteoclast precursor cells. Second, in areas other than osseous tissue, activated T cells produce RANKL to facilitate differentiation of osteoclast precursor cells. Based on studies that found coculturing activated T cells and osteoclast precursor cells induces osteoclasts[24,35], activated T cells act directly on osteoclast precursor cells independent of the first pathway. Third, a pathway exists that does not involve RANKL. Inflammatory cytokines such as TNF-α and IL-1 accelerate the differentiation of osteoclast precursor cells via their receptors, TNF-α also accelerates differentiation of osteoclast precursor cells[36,37], and IL-1 activates mature osteoclasts[38].

In our study, RANKL was decreased by Vit K_2 monotherapy, but NTx did not decrease and remained largely unchanged. Vit K_2 monotherapy suppressed the RANK/RANKL system[33,34] and lowered RANKL, but in RA, because inflammatory cytokines such as TNF-α and IL-1 are overproduced, the third pathway, independent of RANKL, was not suppressed. This may explain why levels of NTx did not decrease.

Our findings for NTx and RANKL suggest that combining Vit K_2 and BP suppresses bone resorption. The data for BMD and BAP indicate that longterm combination therapy lasting ≥ 18 months is effective in RA patients with osteoporosis. Further, etidronate appears to suppress bone destruction around joints in periarticular osteoporosis.

REFERENCES

1. Bromley M, Woolley DE. Chondroclasts and osteoclasts at subchondral sites of erosion in the rheumatoid joint. Arthritis Rheum 1984;27:968-75.
2. Gravallese EM, Harada Y, Wang JT, Gorn AH, Thornhill TS, Goldring SR. Identification of cell types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis. Am J Pathol 1998;152:943-51.
3. Zon W, Hakim I, Tshoep K, Endres S, BarShavit Z. Tumor necrosis factor-alpha mediates RANK ligand stimulation of osteoclast differentiation by an autocrine mechanism. J Cell Biochem 2001;83:70-83.
4. Konig A, Muhlbauer RC, Fleisch H. Tumor necrosis factor alpha and interleukin-1 stimulate bone resorption in vivo as measured by urinary [3H] tetracycline excretion from prelabeled mice. J Bone Miner Res 1988;3:621-7.
5. Jimi E, Nakamura I, Ikebe T, Akiyama S, Takahashi N, Suda T. Activation of NF-kappa B is involved in the survival of osteoclasts promoted by interleukin-1. J Biol Chem 1998;273:8799-805.
6. Tsuda E, Goto M, Mochizuki S. Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. Biochem Biophys Res Commun 1997;234:137-42.
7. Simonet WS, Lacey DL, Dunstan CR. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997;89:309-19.
8. Lacey DL, Timms E, Tan HL. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998;93:165-76.
9. Takayanagi H, Lizuka H, Juji T. Involvement of receptor activator of nuclear factor-κB ligand osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. Arthritis Rheum 2000;43:259-69.
10. Shiraki M, Shiraki Y, Aoki C, Miura M. Vitamin K2 (menatetrenone) effectively prevents fractures and sustains lumbar bone mineral density in osteoporosis. J Bone Miner Res 2000;15:515-21.
11. Koshihara Y, Hoshi K, Okawara R, Ishibashi H, Yamamoto S. Vitamin K stimulates osteoblastogenesis and inhibits osteoclastogenesis in human bone marrow cell culture. J Endocrinology 2003;176:339-48.
12. Hara K, Kobayashi M, Akiyama Y. Vitamin K_2 (menatetrenone) inhibits bone loss induced by prednisolone partly through enhancement of bone formation in rats. Bone 2002;31:575-81.
13. Wallach S, Cohen S, Reid DM. Effects of risedronate treatment on bone density and vertebral fracture in patients on corticosteroid therapy. Calcif Tissue Int 2000;67:277-85.
14. Eastell R, Devogelaer J-P, Peel NFA. Prevention of bone loss with risedronate in glucocorticoid-treated rheumatoid arthritis patients. Osteoporosis Int 2000;11:331-7.
15. Hasegawa J, Nagashima M, Yamamoto M, Nishijima T, Katsumata S, Yoshino S. Bone resorption and inflammatory inhibition efficacy of intermittent cyclical etidronate therapy in rheumatoid arthritis. J Rheumatol 2003;30:474-9.
16. Yamamoto K, Yoshino S, Shue G, Nagashima M. Inhibitory effect of bone resorption and inflammation with rheumatoid arthritis for 3 years and in vitro assay in arthritis models. Rheumatol Int 2006;26:627-32.
17. Arnett FC, Edworthy SM, Bloch DA. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
18. Larsen A, Dale K, Eek M. Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. Acta Radiol Diag 1977;18:481-91.
19. Scariano JK, Glew RH, Bou-Serhal CE. Serum levels of cross-linked N-telopeptides and aminoterminal propeptides of type I collagen indicate low bone mineral density in elderly women. Bone 1998;23:471-7.
20. Garnero P, Delmas PD. New developments in biochemical markers for osteoporosis. Calcif Tissue Int 1996;59:S2-S9.
21. 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;6:487-92.
22. Dobnig H, Hofbauer LC, Viereck V, Obermayer-Pietsch B, Fahrleitner-Pammer A. Changes in the RANK ligand/osteoprotegerin system are correlated to changes in bone mineral density in bisphosphonate-treated osteoporotic patients. Osteoporos Int 2006;17:693-703.
23. Ste-Marie LG, Sod E, Johnson T, Chines A. Five years of treatment with risedronate and its effects on bone safety in women with postmenopausal osteoporosis. Calcif Tissue Int 2004;75:469-76.
24. Kotake S, Udagawa N, Hakoda M, et al. Activated human T cells directly induce osteoclastogenesis from human monocytes: possible role of T cells in bone destruction in rheumatoid arthritis patients. Arthritis Rheum 2001;44:1003-12.
25. Tascioglu F, Colak O, Armagan O, Alatas O, Oner C. The treatment of osteoporosis in patients with rheumatoid arthritis receiving glucocorticoids: a comparison of alendronate and intranasal salmon calcitonin. Rheumatol Int 2005;26:21-9.
26. Shu G, Yamamoto K, Nagashima M. Difference in osteoclast formation between proximal and distal tibial osteoporosis in rats with adjuvant arthritis: inhibitory effects of bisphosphonates on osteoclasts. Mod Rheumatol 2006;16:343-9.
27. Kushida K, Fukunaga M, Kishimoto H, et al. A comparison of

incidences of vertebral fracture in Japanese patients with involutional osteoporosis treated with risedronate and etidronate: a randomized, double-masked trial. J Bone Miner Metab 2004;22:469-78.
28. Eastell R, Barton I, Hannon RA, Chines A, Garnero P, Delmas PD. Relationship of early changes in bone resorption to the reduction in fracture risk with risedronate. J Bone Miner Res 2003;18:1051-6.
29. Shiraki M. A head-to-head comparative study for evaluation of effectiveness among drugs for osteoporosis [Japanese]. Nippon Ronen Igakkai Zasshi 2006;43:45-7.
30. Yasui T, Miyatani Y, Tomita J, et al. Effect of vitamin K2 treatment on carboxylation of osteocalcin in early postmenopausal women. Gynecol Endocrinol 2006;22:455-9.
31. Kobayashi A, Hirano F, Makino I. The inhibitory effect of bisphosphonates on glucocorticoid-induced RANKL expression in human cells. Scand J Rheumatol 2005;34:480-4.
32. Kananen K, Volin L, Laitinen K, Ruutu T, Valimaki MJ. Serum osteoprotegerin and receptor activator of nuclear factor-kappa B ligand (RANKL) concentrations in allogeneic stem cell transplant-recipients: a role in bone loss? Osteoporos Int 2006;17:724-30.
33. Takeuchi Y, Suzawa M, Fukumoto S, Fujita T. Vitamin K2 inhibits adipogenesis, osteoclastogenesis, and ODF/RANK ligand expression in murine bone marrow cell cultures. Bone 2000;27:769-76.
34. Hiruma Y, Nakahama K, Fujita H, Morita I. Vitamin K2 and geranylgeraniol, its side chain component, inhibited osteoclast formation in a different manner. Biochem Biophys Res Commun 2004;314:24-30.
35. Kong YY, Feige U, Sarosi I, et al. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999;402:304-9.
36. Azuma Y, Kaji K, Katogi R, Takeshita S, Kudo A. Tumor necrosis factor-alpha induces differentiation of and bone resorption by osteoclasts. J Biol Chem 2000;275:4858-64.
37. Kobayashi K, Takahashi N, Jimi E, et al. Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. J Exp Med 2000;191:275-86.
38. Jimi E, Nakamura I, Duong LT, et al. Interleukin 1 induces multinucleation and bone-resorbing activity of osteoclasts in the absence of osteoblasts/stromal cells. Exp Cell Res 1999;247:84-93.