果的に家庭の機能に多少なりとも影響を及ぼし，はなはだしい場合は家庭の機能が崩壊する例も見受けられる．脊椎骨折の悲劇は患者個人の悲劇にとどまることが多いのに対し，大腿骨頸部骨折は患者個人のみならず，家庭，さらには社会的な負担増がもたらされることが問題である．この骨折の発生率は従来，わが国では少ないとされてきた．しかし折茂　肇班長による長期間の観察研究の結果，わが国における大腿骨頸部骨折の発生頻度は年々増加の一途をたどり，この増加は老年人口の増加では説明不可能と考えられている．大腿骨頸部骨折を惹起しやすい生活環境の変化が重要な要因ではないかと推定されているが，なぜわが国で大腿骨頸部骨折頻度が増加し続けるのかの本質はいまだに不明である．

Ⅲ．骨粗鬆症性骨折発生の予防可能性の拡大

以上のように骨粗鬆症性骨折の発生は脊椎，大腿骨頸部骨折においてきわめて重大な健康上の問題を提起する．しかし，これらの骨折発生の可能性は適切な治療により大幅に低下せしめうることが明らかになりつつある．すなわち近年に至り，アレンドロネート[6)7)]またはリセドロネート[8)9)]などのビスフォスフォネート製剤，もしくはラロキシフェン[10)]に代表されるSERMなどにより骨折予防効果があることが次々と明らかにされた．これら最新の治療を用いてもなお骨折発生は治療例の約半数にしか認められない．しかしそれでもなお従来の活性型ビタミンD3製剤などに比較すれば骨折予防効果はより高い[11)]．骨折が予防されるということはとりもなおさず，老年者の健康寿命の延長をもたらすことが予想される．したがって，まだ実証的データは十分ではないが，理論的に考えてもこれら治療方法を駆使することにより骨折発生予防を行うことは患者個人の福祉にとっても，家庭の機能の維持という側面からも，また医療費の節約や介護費用の節約といった面からも重要であると予想される．事実アレンドロネートの投与は医療経済的にみてペイする治療であるとの報告もみられる．

しかしこれら治療の恩恵にあずかれないまま骨折発生が起こってから治療に導入されるか，または全く放置された骨粗鬆症例のほうが治療されている，骨粗鬆症例よりもはるかに多いという問題が残されている．わが国における骨粗鬆症関連薬剤の使用量から使用されている患者の実数を逆算してみると約200万人という数字が予想されている．しかるにわが国における骨粗鬆症患者の実数は約1,000万人と予想されており，したがって約８割の骨粗鬆症例が放置されていると推定されている．骨粗鬆症治療の現場における患者捕捉率をあげ，骨折予防の実をあげることが急務と考えられる．

文　献

1) NIH Consensus development panel on osteoporosis prevention, diagnosis, and treatment. Osteoporosis prevention, diagnosis, and treatment. JAMA 285：785-795, 2001.
2) Schlaich C, Minne HW, Bruckner T, et al：Reduced pulmonary function in patients with spinal osteoporotic fractures. Osteoporosis Int 8：261-267, 1998.
3) Leech JA, Dulberg C, Kellie S, et al：Relationship of lung function to severity of osteoporosis in women. Am Rev Respir Dis 141：68-71, 1990.
4) Yamaguchi T, sugimoto T, Yamada H, et al：The presence and severity of vertebral fractures is associated with the presence of esophageal hiatal hernia in postmenopausal women. Osteoporosis Int 13：331-336, 2002.
5) Silverman SL, Minshall ME, Shen W, et al：The relationship of health-related quality of life to prevalent and incident vertebral fractures in postmenopausal women with osteoporosis. Arthritis Rheum 44：2611-2619, 2001.
6) Cummings SR, Black DM, Thompson DE, et al：Effect of alendronate on risk of fracture in women

with low bone density but without vertebral fractures : results from the fracture intervention trial. JAMA 280 : 2077-2082, 1998.

7) Black DM, Cummings SR, Karpf DB, et al : Randomized trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Lancet 348 : 1535-1541, 1996.

8) Harris ST, Watts NB, Genant HK, et al : Effects of risedronate treatment on vertebral and nonvertebral fractures in women with postmenopausal osteoporosis : A randomized controlled trial. JAMA 282 : 1344-1352, 1999.

9) McClung MR, Geusens P, Miller PD, et al : Effect of risedronate on the risk of hip fracture in elderly women. N Engl J Med 344 : 333-340, 2001.

10) Ettinger B, Black DM, Mitlak BH, et al : Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene. Results from a 3-year randomized clinical trial. JAMA 282 : 637-645, 1999.

11) Kushida K, Shiraki M, Nakamura T, et al : on behalf of the Alendronate/Alfacalcidol Fracture Intervention Study Group. The efficacy of alendronate in reducing the risk for vertebral fracture in Japanese patients with osteoporosis : A randomized, double-blind, active-controlled, double-dummy trial. Curr Ther Res Clin Exp 63 : 606-620, 2002.

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A New Active Vitamin D, ED-71, Increases Bone Mass in Osteoporotic Patients under Vitamin D Supplementation: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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Context: ED-71 has been shown to increase lumbar bone mineral density (BMD) in osteoporotic subjects. However, vitamin D insufficiency might have influenced the effect of ED-71 on BMD.

Objective: Our objective was to examine whether ED-71 can increase BMD in osteoporotic patients under vitamin D supplementation.

Design, Setting, and Patients: We conducted a randomized, double-blind, placebo-controlled clinical trial of 219 osteoporotic patients (49–87 yr of age).

Interventions: Subjects were randomly assigned to receive placebo or 0.5, 0.75, or 1.0 μg/d ED-71 for 12 months. All the subjects received 200 or 400 IU/d vitamin D_3.

Main outcome measures: We assessed changes in lumbar and hip BMD and bone turnover markers from baseline.

Results: Lumbar BMD increased with ED-71 treatment for 12 months (2.2, 2.6, and 3.1% from baseline and 2.9, 3.4, and 3.8% *vs.* placebo group in subjects receiving 0.5, 0.75, and 1.0 μg ED-71, respectively). Total hip BMD also increased with 0.75 and 1.0 μg ED-71 (−0.8, 0.6, and 0.9% from baseline and 0.1, 1.5, and 1.8% *vs.* placebo group in the 0.5, 0.75, and 1.0 μg ED-71 groups, respectively). Bone formation and resorption markers were suppressed by approximately 20% after 12 months of 0.75 and 1.0 μg ED-71 treatment. Transient hypercalcemia over 2.6 mmol/liter occurred in 7, 5, and 23% of subjects in the 0.5, 0.75, and 1.0 μg ED-71 groups, respectively, but none of them developed sustained hypercalcemia.

Conclusions: These results demonstrate that ED-71 treatment at around 0.75 μg/d can effectively and safely increase lumbar and hip BMD in osteoporotic patients with vitamin D supplementation. (***J Clin Endocrinol Metab* 90: 5031–5036, 2005**)

ED-71 [1α,25-DIHYDROXY-2β-(3-hydroxypropoxy)vitamin D_3] is an analog of 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], bearing a hydroxypropoxy residue at the 2β position. ED-71 has been demonstrated to show a pronounced effect in increasing bone mass and was able to enhance bone strength in rodents (1, 2). ED-71 binds to the vitamin D receptor (VDR) with less affinity but binds to vitamin D-binding protein with higher affinity than 1,25$(OH)_2D$, showing a long half-life in plasma (3).

An earlier open-labeled clinical trial in osteoporotic patients demonstrated that treatment with 0.25–1.0 μg/d ED-71 for 6 months increased lumbar bone mineral density (BMD) in a dose-dependent manner without causing sustained hypercalcemia or hypercalciuria (4, 5). However, because many patients had serum 25-hydroxyvitamin D [25(OH)D] levels less than 50 nmol/liter, there was a possibility that the effect of ED-71 on bone mass might have been influenced by nutritional vitamin D insufficiency of these patients and might merely reflect a correction of the vitamin D insufficiency.

The present study was undertaken to clarify whether ED-71 could increase bone mass in osteoporotic patients taking sufficient vitamin D supplementation to normalize serum 25(OH)D. The results demonstrate that ED-71 administration can effectively increase not only lumbar spine but also total hip bone mass in a dose-dependent manner under vitamin D supplementation and suggest that ED-71 can become a promising candidate for the treatment of osteoporosis.

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Abbreviations: BALP, Bone-specific alkaline phosphatase; BMD, bone mineral density; DXA, dual-energy x-ray absorptiometry; GF, glomerular filtrate; NTX, type I collagen N-telopeptide; 1,25$(OH)_2D_3$, 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3; 25(OH)D, 25-hydroxyvitamin D; VDR, vitamin D receptor.

Subjects and Methods

Subjects

We studied 219 subjects (215 females and four males, all Japanese) aged from 49–87 yr (mean, 67.3 yr) who had osteoporosis defined as low

BMD (<70% of the young adult mean, 0.708 g/cm^2 by a Hologic QDR) or osteopenia (<80% of the young adult mean, 0.809 g/cm^2 by a Hologic QDR) with at least one vertebral fracture, according to the criteria of the Japanese Society for Bone and Mineral Research (6, 7). Vertebral fractures were assessed by x-ray examination of the vertebrae, and were diagnosed according to the criteria of the Japanese Society for Bone and Mineral Research (6). Female subjects were at least 3 yr after menopause or more than 60 yr of age. Subjects were excluded if they had fractures in any of lumbar spines L2–L4 or if they had disorders such as primary hyperparathyroidism, Cushing's syndrome, gonadal insufficiency, poorly controlled diabetes mellitus (glycosylated hemoglobin over 9%), or other causes of secondary osteoporosis or a history or suspicion of active urolithiasis at any time. Subjects were also excluded if they had taken bisphosphonates until less than 12 months before entry, taken glucocorticoids, calcitonin, vitamin K, active vitamin D compounds, or hormone replacement therapy within the previous 2 months, had serum calcium levels above 10.4 mg/dl (2.6 mmol/liter) or urinary Ca excretion over 0.1 mmol/liter glomerular filtrate (GF), had serum creatinine levels above 115 μmol/liter, or had clinically significant hepatic or cardiac disorders. The subjects were enrolled at 13 centers in Japan. The protocol was approved by the internal human studies review board at each center, and informed consent was obtained from each subject.

Treatment

Subjects were randomly assigned to receive either placebo or 0.5, 0.75, or 1.0 μg ED-71 once a day for 12 months. Randomization was performed by a computerized system. The subjects were stratified into groups on the basis of serum 25(OH)D levels (<50 nmol/liter or ≥50 nmol/liter) and prevalent vertebral fractures. Upon entry, subjects were supplemented with 400 IU/d vitamin D_3 when serum 25(OH)D was less than 50 nmol/liter or with 200 IU/d vitamin D_3 when serum 25(OH)D was 50 nmol/liter or higher.

If hypercalcemia over 2.6 mmol/liter in two consecutive measurements or over 2.75 mmol/liter developed, or if hypercalciuria over 0.1 mmol/liter GF developed in two consecutive measurements, ED-71 therapy was discontinued. Compliance with the study treatment was assessed with the use of medication diaries and counts of residual medication supplies.

Assessment of BMD

The BMD of the lumbar spine in the posteroanterior projections and the total hip was measured by dual-energy x-ray absorptiometry (DXA). The 13 study centers involved in this trial were all equipped with either Hologic QDR 4500 or 2000 for BMD measurements. A central facility (Department of Nuclear Medicine, Kawasaki Medical School) performed quality assurance of the longitudinal adjustment. Adjustment for DXA machine differences was made to calibrate each machine with standardized phantoms. All the DXA measurements were analyzed at a central site by a radiologist blinded to treatment group assignment.

Assessment of chemical parameters and bone turnover

Serum and postprandial urine samples were collected at baseline and at 1, 2, 3, 4, 5, 6, 8, 10, and 12 months for routine chemical analyses, including serum and urinary Ca corrected by dLGF, hematological, hepatic, and renal functions. At baseline and at 3, 6, and 12 months, markers of bone turnover, including serum bone-specific alkaline phosphatase (BALP) (Metra-BAP; Quidel Co., San Diego, CA), osteocalcin (Bone Gla protein-radioimmunoassay Mitsubishi, Mitsubishi Kagaku Bio-Chemical, Tokyo, Japan), and urinary type I collagen N-telopeptide (NTX) (Osteomark; Ostex International, Seattle, WA), along with serum calcium-regulating hormones including intact PTH (Allegro PTH; Nichols Institute, San Juan, CA), 25(OH)D (RIA, Immunodiagnostics Systems Ltd., Tyne and Wear, UK), 1,25(OH)2D (HPLC-radioreceptor assay) and 24,25$(OH)_2$D (HPLC-competitive protein binding assay) were determined. Serum steady-state levels of ED-71 were also measured (liquid chromatography-tandem mass spectrometry, Hitachi Science Systems Ltd., Ibaraki, Japan) at 3, 6, and 12 months in all ED-71-treated patients.

Assessment of adverse events

All subjects were questioned about adverse events of treatment at each visit, and all adverse events reported during one month of follow-up were analyzed regardless of the investigators' assessments of causality. The Medical Dictionary for Regulatory Activities (MedDRA, version 6) was used to categorize reported adverse events. We report all categories of adverse events for which the frequency was at least 2% in placebo or combined ED-71-treated groups and showed a higher incidence in the combined ED-71-treated group with an odds ratio higher than 3.

Statistical analysis

The predetermined primary end point was change in the posteroanterior lumbar BMD. Our prespecified analysis was to use data on the BMD measured from baseline to month 12. Analyses were performed according to the intention-to-treat principle, in which all randomized patients were included who had taken at least one dose of study drug and had both a baseline and at least one postrandomization measurement of BMD, bone markers, or other parameters.

Group means and 95% confidence intervals are given for the percent changes from baseline in lumbar and total hip BMD and are used to assess the significance of changes within each group. Medians and interquartile ranges are reported for changes in the levels of bone turnover markers. William's tests were used to determine which ED-71-treated groups in a series are significantly different from the placebo group. The comparability among the treatment groups for demographic and background information and the incidence rates of adverse events was assessed with the use of one-way ANOVA for continuous variables and χ^2 tests for dichotomous variables. The power calculation was based on the predictive percent change from baseline in lumbar BMD. A sample size of 50 patients per group was to provide more than 90% power for determining which ED-71-treated groups in a series are significantly different from the placebo group at a significance level of $\alpha = 0.025$ (one-sided), assuming a mean percent change of 0.5 in the placebo group, 1.5 in the 0.5-μg ED-71 group, 3.0 in the 0.75-μg ED-71 group, 3.0 in the 1.0-μg ED-71 group, and a pooled SD of 4.0.

Results

Baseline characteristics of the subjects

There were no statistically significant differences in baseline characteristics. As shown in Table 1, there was no significant difference in daily Ca intake among placebo and any treatment groups, and mean Ca intakes ranged from 761–840 mg/d. Mean serum 25(OH)D levels at the baseline were less than 50 nmol/liter, and 74% of the subjects showed serum 25(OH)D less than 50 nmol/liter (39 in placebo and 42, 41, and 41 nmol/liter in the 0.5-, 0.75-, and 1.0-μg ED-71-treated group, respectively). The subjects with serum 25(OH)D less than 50 nmol/liter were given 400 IU/d vitamin D_3, and those with serum 25(OH)D over 50 nmol/liter were supplemented with 200 IU/d vitamin D_3 throughout the study period. Vitamin D supplementation brought serum 25(OH)D to over 50 nmol/liter in 197 of 213 subjects (92%) at 3 months (Fig. 1).

Adherence to study treatment

Of the 25 subjects who discontinued treatment, four subjects (1.8%) were assigned to the placebo group, seven (3.2%) to the 0.5-μg ED-71 group, five (2.3%) to the 0.75-μg ED-71 group, and nine (4.1%) to the 1.0-μg ED-71 group. Among them, one in the 0.5-μg ED-71 group and two in the 1.0-μg ED-71 group withdrew from the study because of repeated hypercalcemia over 2.6 mmol/liter or moderate hypercalcemia over 2.75 mmol/liter, and one in the 1.0-μg ED-71 group

TABLE 1. Baseline characteristics of 219 enrolled subjects

Characteristic	Placebo (n = 53)	ED-71 (μg/d) 0.5 (n = 55)	0.75 (n = 55)	1.0 (n = 56)
Age (yr)	68.0 ± 7.7	67.9 ± 7.7	66.3 ± 6.6	66.8 ± 7.6
Body mass index (kg/m^2)	22.7 ± 2.9	21.8 ± 3.4	21.6 ± 3.0	22.1 ± 3.0
Lumbar BMD (g/cm^2)	0.648 ± 0.059	0.634 ± 0.052	0.653 ± 0.050	0.658 ± 0.046
Total hip BMD (g/cm^2)	0.652 ± 0.088	0.654 ± 0.091	0.652 ± 0.097	0.670 ± 0.095
BALP (U/liter)	33.3 ± 11.2	31.0 ± 9.9	32.1 ± 10.5	31.3 ± 8.8
Osteocalcin (μg/liter)	9.60 ± 3.04	8.88 ± 3.04	8.21 ± 3.03	8.67 ± 4.38
NTX (nmol BCE/mmol Cr)	66.3 ± 23.5	56.2 ± 23.9	58.0 ± 28.3	60.5 ± 25.2
Ca intake (mg/d)	761 ± 269	800 ± 310	840 ± 352	832 ± 292
25(OH)D (nmol/liter)	43.1 ± 14.2	43.0 ± 15.3	40.7 ± 12.8	44.7 ± 14.8
No. of subjects				
25(OH) < 50 nmol/liter	39	42	41	41
Existing vertebral fractures	17	17	17	17

Data are means ± SD for the indicated number of subjects in each group. BCE, Bone collagen equivalents; Cr, creatinine.

withdrew because of repeated hypercalciuria over 0.1 mmol/liter GF in two consecutive measurements. The incidence of hypercalcemia or hypercalciuria was significantly higher in the 1.0-μg ED-71 group ($P < 0.01$). Most of the other subjects who discontinued treatment did so because of reasons unrelated to adverse events.

We found no difference in adherence to treatment among the groups, and 97% of the subjects took more than 75% of the study medication.

Lumbar spine and total hip BMD

Lumbar spine BMD increased rapidly within 6 months of ED-71 treatment and gradually increased thereafter in a dose-dependent manner (Fig. 2). After 12 months of ED-71 treatment, lumbar spine BMD increased from baseline by 2.2, 2.6, and 3.1% in the 0.5-, 0.75-, and 1.0-μg ED-71 group, respectively, whereas lumbar spine BMD decreased by 0.7% in the placebo group. The change in lumbar spine BMD by the ED-71 treatment was significantly different from that in the placebo group ($P < 0.01$ in all comparisons).

Total hip BMD slightly decreased from baseline in the placebo and 0.5-μg ED-71-treated groups (−0.9 and −0.8%, respectively) but increased after 0.75 and 1.0 μg ED-71 treatment for 12 months (0.6 and 0.9%, respectively) (Fig. 2). The change in total hip BMD in the 0.75- and 1.0-μg ED-71-treated groups was significantly different from that in the placebo group ($P < 0.05$).

There was a tendency that subjects with lower serum 25(OH)D levels at the baseline (<50 nmol/liter) showed a greater increase in lumbar BMD after 12 months of 0.75 μg ED-71 treatment (2.9 and 1.6% in low and normal 25(OH)D groups, respectively). In contrast, the effect of the same amount of ED-71 on hip BMD was opposite (0.5 and 0.9% in low and normal 25(OH)D groups, respectively). The patients were also stratified at the baseline by the presence or absence of prevalent fractures, and patients with prevalent fractures tended to show a greater increase in lumbar BMD but smaller increase in hip BMD compared with those without fractures after 0.75 μg ED-71 treatment (3.6 *vs.* 2.2% in lumbar and 0.0 *vs.* 0.9% in hip BMD in patients with prevalent fracture and without fracture, respectively). However, because the number of subjects in each group was small, it was difficult to draw any conclusions from these results.

Bone turnover markers

As shown in Fig. 3, median urinary NTX as a bone resorption marker decreased by 8, 20, and 24% within 3 months of treatment by 0.5, 0.75, and 1.0 μg ED-71, respectively, and remained suppressed throughout the study period. Serum BALP and osteocalcin as bone formation markers also decreased but with more gradual time courses to reach a nadir at around 6 months of ED-71 treatment.

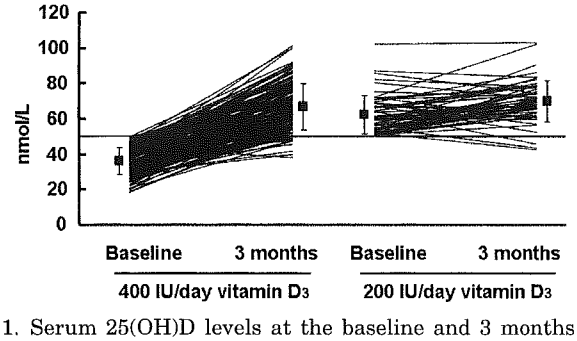

FIG. 1. Serum 25(OH)D levels at the baseline and 3 months after vitamin D_3 supplementation. Each *line* indicates data in each study subject. *Closed squares* and *I bars* indicate means ± SD.

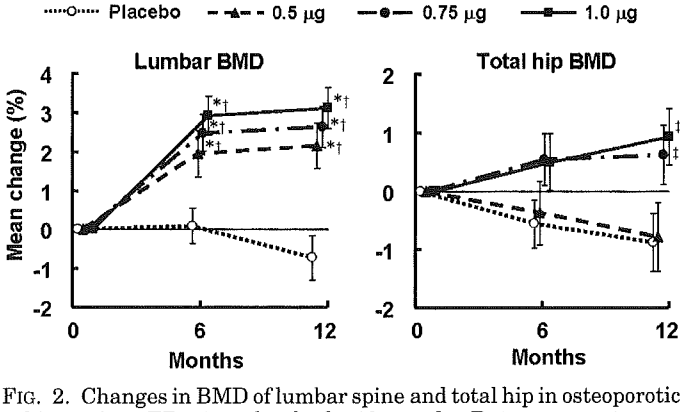

FIG. 2. Changes in BMD of lumbar spine and total hip in osteoporotic subjects given ED-71 or placebo for 12 months. Data represent mean percent changes from baseline. The *I bars* indicate SEM. *, $P < 0.01$ for the comparison with baseline; †, $P < 0.01$ for the comparison with placebo; ‡, $P < 0.05$ for the comparison with placebo.

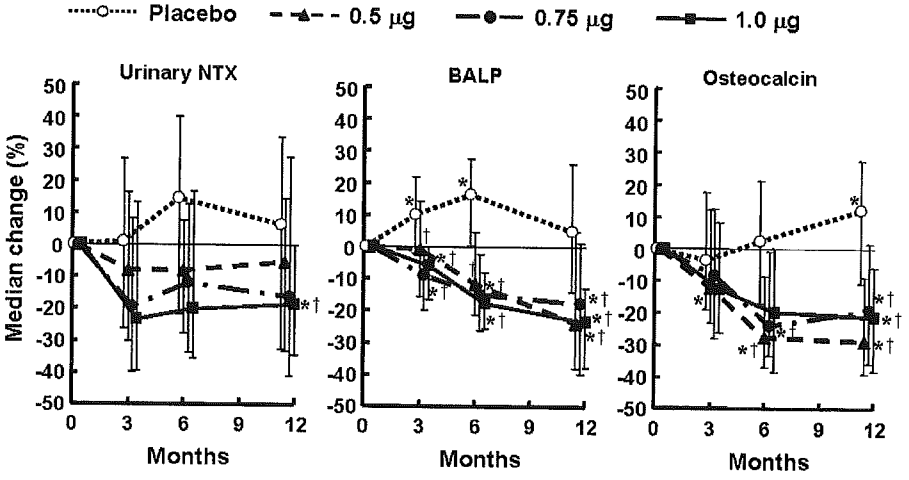

FIG. 3. Changes in urinary NTX, serum BALP, and osteocalcin in osteoporotic subjects given ED-71 or placebo for 12 months. Data represent median percent changes from the baseline. The *I bars* indicate the interquartile ranges. *, $P < 0.01$ for the comparison with baseline; †, $P < 0.01$ for the comparison with placebo.

Serum calcium-regulating hormones and ED-71 levels

Figure 4 shows changes in the levels of calcium-regulating hormones at the baseline and during ED-71 treatment. Interestingly, serum intact PTH did not change significantly despite the increase in serum Ca levels in any of the ED-71-treated groups (Fig. 5). Serum $1,25(OH)_2D$ levels were significantly suppressed by the treatment with ED-71 in a dose-dependent manner. In contrast, serum $24,25(OH)_2D$ increased not only in ED-71-treated groups but also in the placebo group. In addition, $24,25(OH)_2D$ levels were higher in ED-71-treated groups compared with those in the placebo group and increased in a dose-dependent manner with ED-71 treatment. The steady-state serum ED-71 concentration increased linearly with increasing doses of the compound.

Adverse events

Serum Ca increased by ED-71 treatment in a dose-dependent manner, and mean serum Ca levels at 12 months of treatment were 2.38, 2.38, 2.4, and 2.43 mmol/liter in the placebo and the 0.5-, 0.75-, and 1.0-μg ED-71 groups, respectively. As shown in Fig. 5, there was a rapid increase in serum Ca during the initial 1 month of ED-71 treatment, followed by a sustained slight elevation in serum Ca levels throughout the treatment period. Postprandial urinary Ca excretion also increased with ED-71 treatment in a dose-dependent manner, and mean urinary Ca levels at 12 months were 0.045, 0.048, 0.058, and 0.06 mmol/liter GF in placebo 0.5-, 0.75-, and 1.0-μg ED-71 groups, respectively. The incidence of at least one episode of hypercalcemia over 2.6 mmol/liter was 7, 5, and 23% and that of hypercalciuria over 0.1 mmol/liter GF was 7, 9, and 25% in 0.5-, 0.75-, and 1.0-μg ED-71 groups, respectively. The incidence of serum and urinary Ca-related adverse events was significantly higher in the 1.0-μg ED-71-treated group ($P < 0.01$). Serum and urinary Ca returned to baseline levels in most of the subjects within 1 month after the end of the study period (Fig. 5).

Other adverse events that showed at least 2% in frequency in placebo or combined ED-71-treated groups with an odds ratio higher than 3 in the combined ED-71-treated group are listed in Table 2. None of them was considered by the investigators to be related to treatment. There was no difference in the incidence of serious adverse events between the placebo and combined ED-71-treated groups (7.5 and 9.6%, respectively). There was no increase in the development of hematological, hepatic, or renal abnormalities in ED-71-treated groups. Clinical fractures were captured as adverse events, and there was no significant difference in the incidence of vertebral (two in the placebo and one each in the 0.5-, 0.75-, and 1.0-μg ED-71-treated groups) or nonvertebral fractures (one in the placebo and 0.5- and 1.0-μg ED-71-treated groups and two in the 0.75-μg ED-71-treated group).

Discussion

The present study demonstrated that treatment with 0.5, 0.75, and 1.0 μg/d of a new active vitamin D, ED-71, for 12 months increased lumbar BMD in a dose-dependent manner and that 0.75 and 1.0 μg/d ED-71 significantly increased total hip BMD as well. To our knowledge, this is the first report to demonstrate the effect of a vitamin D compound in increasing BMD at the hip in osteoporotic subjects in a prospective randomized trial. Although none of the above doses of ED-71 caused sustained hypercalcemia or hypercalciuria, the incidence of Ca-related adverse events was significantly higher in the 1.0-μg ED-71-treated group. Because the lowest dose of ED-71 was unable to increase hip BMD, the therapeutic window of ED-71 in increasing lumbar and hip BMD is relatively narrow, and ED-71 at around 0.75 μg/d appears to be a safe and effective dose for the treatment of osteoporotic subjects with sufficient vitamin D supplementation.

In the present study, vitamin D_3 was supplemented to maintain serum 25(OH)D over 50 nmol/liter in 92% of the enrolled subjects, and the results demonstrated that ED-71 can increase BMD in osteoporotic subjects even with sufficient vitamin D supplementation. These results suggest that ED-71 can exert its effect on bone independently of the nutritional supplementation with native vitamin D. Supplementation with a large amount of vitamin D_3 (800 IU daily) and calcium (1200 mg daily) is associated with a reduction in the risk of nonvertebral fractures in elderly women (8). However, controversy remains whether the actions of vitamin D on the density and strength of bone is merely a result of nutritional supplementation of vitamin D. A large meta-analysis of the effects of native and hydroxylated active

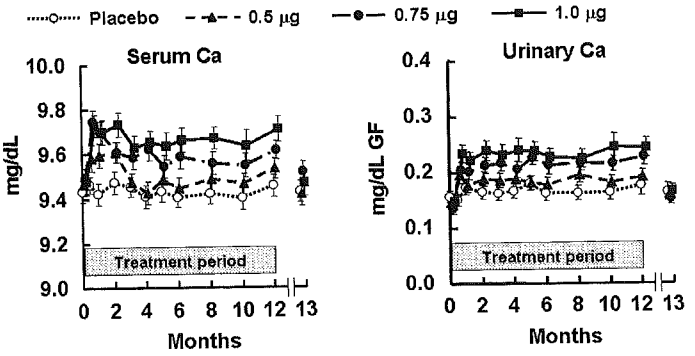

FIG. 5. Changes in serum and urinary calcium levels in osteoporotic subjects given ED-71 or placebo for 12 months. To convert serum Ca levels from mg/dl to mmol/liter, multiply by 0.25. Data are means ± SEM.

vitamin D compounds revealed that hydroxylated vitamin D exhibited a consistently larger impact on bone density than native vitamin D in postmenopausal women (9). Thus, there is a possibility that vitamin D has specific effects on bone and that some vitamin D analogs can exert stronger effects on bone. In fact, in a rat ovariectomy model of osteoporosis, ED-71 increased bone mass at the lumbar vertebra to a greater extent than alfacalcidol, while enhancing calcium absorption and suppressing serum PTH to a similar extent to alfacalcidol (2). In those animals, ED-71 lowered the biochemical and histological parameters of bone resorption more potently than alfacalcidol, with maintenance of bone formation. In addition to ED-71, some vitamin D analogs have been reported to have preferential effects on bone both *in vitro* and *in vivo*. Ro-26-9228 showed preferential gene regulation in osteoblasts over duodenum and had a bone-protective effect via induction of growth factors in osteopenic rats (10). A highly potent analog of $1,25(OH)_2D_3$, 2-methylene-19-nor-(20S)-$1,25(OH)_2D_3$, was shown to have selective

TABLE 2. Incidence of adverse events

Variable	Placebo (n = 53)	ED-71 (μg/d) 0.5 (n = 55)	0.75 (n = 55)	1.0 (n = 56)
Headache	0 (0.0)	1 (1.8)	3 (5.5)	3 (5.4)
Conjunctivitis	0 (0.0)	2 (3.6)	3 (5.5)	0 (0.0)
Stomachache NOS	0 (0.0)	4 (7.3)	0 (0.0)	1 (1.8)
Cystitis NOS	1 (1.9)	4 (7.3)	6 (10.9)	1 (1.8)
Blood calcium increased	0 (0.0)	4 (7.3)	3 (5.5)	13 (23.2)
Urine calcium increased	1 (1.9)	4 (7.3)	5 (9.1)	14 (25.0)
Any serious	4 (7.5)	6 (10.9)	7 (12.7)	3 (5.4)

Data were compiled using ICH Medical Terminology Medical Dictionary for Regulatory Activities (MedDRA version 6.0). All data are reported as number (percentage). NOS, Not otherwise specified.

effects on osteoblasts and to induce bone formation in ovariectomized rats (11). A later *in vitro* study suggested that 2-methylene-19-nor-(20S)-$1,25(OH)_2D_3$ promoted VDR binding to specific DNA elements via a formation of unique VDR conformation (12).

In our earlier clinical studies, ED-71 substantially suppressed bone resorption markers but reduced bone formation markers to a lesser extent (4, 5). In the present study, both bone formation and bone resorption markers decreased to almost a similar extent. These results are similar to those obtained by antiresorptive agents. For example, 60 mg/d raloxifene reduced osteocalcin and NTX by 27.0 and 20.9%, respectively, with a 1.5% increase in lumbar BMD after 6 months of treatment in postmenopausal Asian women (13). Nevertheless, the dose-dependent increase in BMD by ED-71 in the present study at 6 months is comparable to that observed in our earlier study in which a 2.5% increase in lumbar BMD was observed after 6 months of 0.75 μg ED-71 treatment (4). The only difference between the present study and our previous study is the supplementation of native vitamin D_3 to normalize serum 25(OH)D levels. It is not known

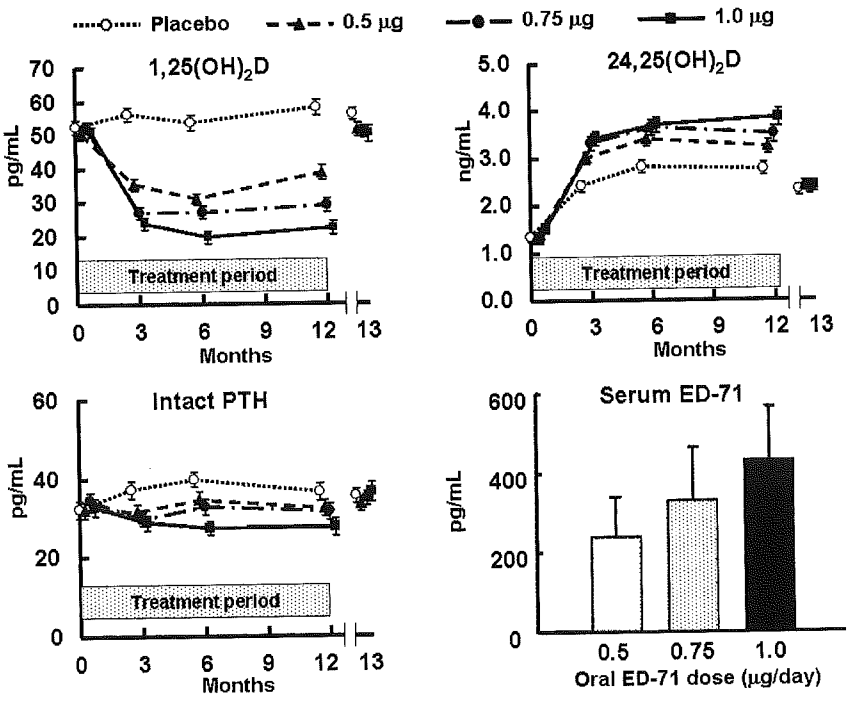

FIG. 4. Calcium-regulating hormones and steady-state serum ED-71 concentration in osteoporotic subjects given ED-71 or placebo for 12 months. Data are means ± SEM for calcium-regulating hormones and means ± SD for ED-71 concentration.

whether the difference in vitamin D status can influence responsiveness in bone turnover markers, and the reasons for the differences in the responsiveness of bone turnover markers remains unclear.

The incidence of hypercalcemia or hypercalciuria was much higher in the 1.0-μg ED-71 group compared with the 0.5- and 0.75-μg ED-71 groups. In contrast, the increase in both lumbar and total hip BMD was almost the same in the 0.75- and 1.0-μg ED-71-treated groups. When the effects of ED-71 on lumbar BMD *vs.* serum and urinary Ca were compared with those of alfacalcidol, 0.75 μg/d ED-71 increased lumbar BMD by 2.6%, serum Ca by 0.03 mmol/liter and urinary Ca by 0.023 mmol/liter GF, whereas 1.0 μg/d alfacalcidol increased BMD by 0.65%, serum Ca by 0.03 mmol/liter, and urinary Ca by 0.028 mmol/liter GF after 12 months of treatment (14). Thus, 0.75 μg ED-71 appears to be a safe, well tolerated, and effective dose in increasing both lumbar and femoral BMD and is superior to alfacalcidol in increasing BMD compared with its effects on serum and urinary Ca.

Finally, the present study has limitations. First, the effect of ED-71 was examined for only 12 months in about 50 patients in each group, which is too short a period and too small a number of patients to evaluate its effect on osteoporotic fractures. A prospective, randomized, double-blind, 3-yr study to compare the effect of ED-71 with that of alfacalcidol on fracture incidence in osteoporotic patients is currently under way. Second, the effect of ED-71 on the microstructure, mineralization, and other histological parameters is not examined. Because these parameters may influence the quality and strength of bone, additional studies are needed to clarify this issue.

In conclusion, the present observations demonstrate that ED-71 can effectively increase both lumbar and hip BMD in patients with vitamin D supplementation and suggest that ED-71 can become a promising candidate for the treatment of osteoporosis.

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References

1. **Tsurukami H, Nakamura T, Suzuki K, Sato K, Higuchi Y, Nishii Y** 1994 A novel synthetic vitamin D analogue, 2β-(3-hydroxypropoxy)1α, 25-dihydroxyvitamin D_3 (ED-71), increases bone mass by stimulating the bone formation in normal and ovariectomized rats. Calcif Tissue Int 54:142–149
2. **Uchiyama Y, Higuchi Y, Takeda S, Masaki T, Shira-Ishi A, Sato K, Kubodera N, Ikeda K, Ogata E** 2002 ED-71, a vitamin D analog, is a more potent inhibitor of bone resorption than alfacalcidol in an estrogen-deficient rat model of osteoporosis. Bone 30:582–588
3. **Okano T, Tsugawa N, Masuda S, Takeuchi A, Kobayashi T, Nishii Y** 1991 A novel synthetic vitamin D_3 analogue, 2-β-(3-hydroxypropoxy)-calcitriol (ED-71): its biological activities and pharmacological effects on calcium metabolism. Contrib Nephrol 91:116–122
4. **Matsumoto T, Kubodera N** 2000 The ED-71 Study Group, 1α,25-Dihydroxy-2β-(3-hydroxypropoxy) vitamin D_3 (ED-71): a promising candidate for the treatment of osteoporosis. In: Norman A, Bouillon R, Thomasset M, eds. Vitamin D endocrine system: structural, biological, genetic and clinical aspects. Riverside, CA: Vitamin D Workshop, Inc., University of California, Riverside; 985–992
5. **Kubodera N, Tsuji N, Uchiyama Y, Endo K** 2003 A new active vitamin D analog, ED-71, causes increase in bone mass with preferential effects on bone in osteoporotic patients. J Cell Biochem 88:286–289
6. **Orimo H, Sugioka Y, Fukunaga M, Muto Y, Hotokebuchi T, Gorai I, Nakamura T, Kushida K, Tanaka H, Ikai T, Oh-Hashi Y, The Committee of the Japanese Society for Bone and Mineral Research for Development of Diagnostic Criteria of Osteoporosis** 1998 Diagnostic criteria of primary osteoporosis. J Bone Miner Metab 16:139–150
7. **Orimo H, Hayashi Y, Fukunaga M, Sone T, Fujiwara S, Shiraki M, Kushida K, Miyamoto S, Soen S, Nishimura J, Oh-Hashi Y, Hosoi T, Gorai I, Tanaka H, Igai T, Kishimoto H, Osteoporosis Diagnostic Criteria Review Committee: Japanese Society for Bone and Mineral Research** 2001 Diagnostic criteria for primary osteoporosis: revision. J Bone Miner Metab 19:331–337.
8. **Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD, Meunier PJ** 1992 Vitamin D_3 and calcium to prevent hip fractures in the elderly women. N Engl J Med 327:1637–1642
9. **Papadimitropoulos E, Wells G, Shea B, Gillespie W, Weaver B, Zytaruk N, Cranney A, Adachi J, Tugwell P, Josse R, Greenwood C, Guyatt G, Osteoporosis Methodology Group and The Osteoporosis Research Advisory Group** 2002 Meta-analyses of therapies for postmenopausal osteoporosis. VIII. Meta-analysis of the efficacy of vitamin D treatment in preventing osteoporosis in postmenopausal women. Endocr Rev 23:560–569
10. **Peleg S, Uskokovic M, Ahene A, Vickery B, Avnur Z** 2002 Cellular and molecular events associated with the bone-protecting activity of the noncalcemic vitamin D analog Ro-26-9228 in osteopenic rats. Endocrinology 143: 1625–1636
11. **Shevde NK, Plum LA, Clagett-Dame M, Yamamoto H, Pike JW, DeLuca HF** 2002 A potent analog of 1α,25-dihydroxyvitamin D_3 selectively induces bone formation. Proc Natl Acad Sci USA 99:13487–13491
12. **Yamamoto H, Shevde NK, Warrier A, Plum LA, DeLuca HF, Pike JW** 2003 2-Methylene-19-nor-(20S)-1,25-dihydroxyvitamin D_3 potently stimulates gene-specific DNA binding of the vitamin D receptor in osteoblasts. J Biol Chem 278:31756–31765
13. **Kung AW, Chao HT, Huang KE, Need AG, Taechakraichana N, Loh FH, Gonzaga F, Sriram U, Ismail NM, Farooqi A, Rachman IA, Crans GG, Wong M, Thiebaud D** 2003 Efficacy and safety of raloxifene 60 milligrams/day in postmenopausal Asian women. J Clin Endocrinol Metab 88:3130–3136
14. **Orimo H, Shiraki M, Hayashi Y, Hoshino T, Onaya T, Miyazaki S, Kurosawa H, Nakamura T, Ogawa N** 1994 Effects of 1α-hydroxyvitamin D_3 on lumbar BMD and vertebral fractures in patients with postmenopausal osteoporosis. Calcif Tissue Int 54:370–376

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ORIGINAL ARTICLE

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Therapeutic effects of alendronate 35 mg once weekly and 5 mg once daily in Japanese patients with osteoporosis: a double-blind, randomized study

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Abstract The efficacy and safety of treatment with oral alendronate (ALN) 35 mg once weekly for 52 weeks were compared with those of ALN 5 mg once daily in a double-blind, randomized, multicenter study of Japanese patients with involutional osteoporosis. The primary efficacy end point was the percent change from baseline in the lumbar spine (L1–L4) bone mineral density (BMD) after 52 weeks of treatment. In this study, 328 patients were randomized to ALN 5 mg once daily (160 patients) or ALN 35 mg once weekly (168 patients). The adjusted mean percent change from baseline in lumbar spine (L1–L4) BMD after 52 weeks of treatment was 5.8% and 6.4% in the once-daily group and the once-weekly group, respectively (both $P < 0.001$). The 95% confidence interval for the difference in spine BMD change between the two treatment groups was −0.31% to 1.48%, indicating that the two regimens were therapeutically equivalent, since the confidence interval fell entirely within the predefined equivalence criterion (±1.5%). The time course of the spine BMD increase was also similar for both regimens. Regarding total hip BMD, mean changes from baseline at 52 weeks were 2.8% and 3.0% in the once-daily group and the once-weekly group, respectively. In addition, the bone markers (urinary deoxypyridinoline, urinary type-I collagen N-telopeptides, and serum bone-specific alkaline phosphatase) were reduced to a similar level by either treatment throughout the treatment period. The tolerability and safety profiles were also similar between the treatment groups. Taken together, we conclude that the efficacy and safety of the ALN 35-mg once-weekly regimen are therapeutically equivalent to those of the ALN 5-mg once-daily regimen.

Key words Alendronate · Involutional osteoporosis · Bone markers · BMD

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Introduction

Osteoporosis is a skeletal disorder characterized by compromised bone strength predisposing a person to increased risk of fracture; bone strength reflects the integrated contributions of properties that include bone density, turnover, and microarchitecture [1,2]. The number of patients afflicted with osteoporosis is very large and increases each year in developed nations with aging populations. For example, there were approximately 11 million patients with osteoporosis in Japan in 2002 [3]. The incidence of osteoporosis-associated vertebral fracture increases with age; the estimated incidence in Japanese women is 40 per 1000 person-years for ages 70–79 and 84 per 1000 person-years for ages 80–89 [4].

Alendronate (ALN) is a nitrogen-containing bisphosphonate that is used in many countries to treat osteoporosis, and it has been extensively evaluated in clinical trials of up to 10 years duration. These trials have demonstrated that ALN has consistent efficacy in restoring bone turnover to normal levels, increasing bone mass, and reducing fracture risk [5–23]. Although daily treatment is gener-

ally well tolerated, most patients would prefer a weekly regimen, partly because oral bisphosphonates must be taken in a fasting state at least 30 min before consuming food or beverages (other than water). In the previous study (~96% Caucasian), the therapeutic equivalence of 70 mg ALN once weekly and 10 mg ALN daily was demonstrated in postmenopausal patients with osteoporosis, based on percent change in lumbar spine bone mineral density (BMD). The safety profile was comparable in the weekly and daily treatment groups in these studies [24,25].

Previous studies demonstrated that 5 mg ALN daily produced effects on bone in Japanese that were similar to those obtained using the 10-mg dose in Caucasians [19]. Consequently, the standard dose of ALN in Japan is 5 mg daily. However, the effects of 35 mg ALN once weekly have not been compared with those of 5 mg daily in Japanese. The present study was carried out in a double-blind manner in a Japanese patient population with involutional osteoporosis to compare the efficacy and safety of oral ALN 35 mg once weekly to 5 mg once daily over a 52-week period.

Patients and methods

Study design

This study was a 52-week, randomized, double-blind, multicenter, comparative trial in Japanese patients with involutional osteoporosis to compare the efficacy and safety of ALN 35 mg once weekly with those of ALN 5 mg once daily. All study staff and patients were blinded to allocation and treatment. All study medications were provided by Merck, Whitehouse Station, NJ, USA, and Banyu Pharmaceutical, Tokyo, Japan. Patients were randomized to receive either one tablet of ALN 35 mg once a week and one tablet of ALN 5 mg placebo every day (ALN 35-mg once-weekly group) or one tablet of ALN 5 mg daily and one tablet of ALN 35 mg placebo once a week (ALN 5-mg once-daily group). The study drug was allocated in a 1:1 ratio according to a randomized allocation table made by an independent organization. The patients received the study drug in the order of their enrollment at each site. All study drugs were taken orally with approximately 180 ml of water upon waking in the morning. Patients were instructed to keep their upper body upright and refrain from any food, beverage, or other drug intake for at least 30 min after dosing. One tablet of Calcichew D3 (containing 500 mg elemental calcium and 200 IU vitamin D3, Fujisawa Pharmaceutical, Osaka, Japan) was taken after the evening meal throughout the treatment period.

The study was conducted from March 2002 through October 2003, and was approved by the individual institutional review boards of the 35 participating sites (36 departments). Written informed consent was obtained from all patients enrolled, in accordance with the spirit of the Declaration of Helsinki and the Guideline for Good Clinical Practice (Ordinance of Ministry of Health, Labour and Welfare of Japan, March 27, 1997).

Subject population

At the start of the observation period, subjects were required to be ambulatory men or women aged 43 to 90 years; women were at least 2 years postmenopause. The patients were also required to have no evidence of vertebral fractures in at least three vertebrae in the L1 to L4 region on lumbar spine radiographs, and to be less than 70% of young adult mean (YAM) in lumbar spine (L2–L4) BMD, or to be less than 80% of YAM with a history of or current fragility fracture related to osteoporosis. Patients who had been treated in the past 6 months with etidronate at a dose >200 mg/day, or with other bisphosphonates (ALN, etidronate ≤200 mg/day, etc.) for ≥2 weeks were excluded.

Measurement of vertebral BMD and femoral BMD

BMD of lumbar spine (mean of L1–L4) and total hip were measured by dual-energy X-ray absorptiometry (DXA; Hologic, Bedford, MA, USA) at the start of the pretreatment observation period (2–12 weeks, depending on prior medication use), and at the start (baseline) and at weeks 12, 24, and 52 of the treatment period, or at discontinuation. The percent changes in spine and hip BMD from the start of the study drug were calculated at weeks 12, 24, and 52. After measurement, the BMD data and precision control data were forwarded, together with the thoracolumbar radiographs at baseline and lumbar radiographs at weeks 24 and 52, to the Bone Density Evaluation Committee (specifically formed for the present study, led by Prof. M. Fukunaga, Kawasaki Medical School, Okayama) to verify data quality. An auxiliary positioning instrument was used for measuring femoral BMD.

Measurement of bone markers

Biochemical markers of bone turnover, urinary deoxypyridinoline (DPD), urinary type-I collagen N-telopeptides (NTx), and serum bone-specific alkaline phosphatase (BAP), were measured by SRL Medisearch (Tokyo, Japan) at the start of the observation period and at the start and at weeks 4, 12, 24, and 52 of the treatment period, or at discontinuation. Urinary parameters were adjusted by creatinine excretion.

Safety evaluation

Safety of the study drugs was assessed using reported adverse events (AEs) and abnormal laboratory test values. The following laboratory tests were performed: hematology (red blood cell count, white blood cell count, differential white blood cells, hemoglobin, hematocrit, and platelet count), blood chemistry [albumin, total bilirubin, AST (GOT), ALT (GPT), γ-GTP, alkaline phosphatase (AL-P), LDH, CPK, BUN, serum creatinine, total cholesterol, Na, K, Ca and P], and urinalysis (protein and glucose). Fractures in the upper or lower extremities reported by investigators at each site were also included.

Statistical analyses

The primary analysis comparing the efficacy of the two regimens (ALN 35 mg once weekly and 5 mg once daily) used a per protocol set (PPS). In the PPS analysis, patients with important protocol deviations were excluded from the analyses. (A list of protocol violators was issued before unblinding the database). No missing data were imputed. Safety was evaluated in patients who received at least one dose of study drug in the treatment period. The level of statistical significance was two-sided 5% in every analysis.

The primary efficacy end point was percent change from baseline (i.e., the initiation of the treatment period) in lumbar spine (L1–L4) BMD at week 52, calculated as the least-squares (LS) mean difference between treatment groups and its 95% confidence interval (CI) from an analysis of variance (ANOVA) model that included treatment group and center as factors. The two treatments were considered therapeutically equivalent if the 95% CI of the between-group difference in spine BMD change fell entirely within the prespecified bounds of ±1.5%. Similar ANOVA models were used to calculate the least-squares mean difference of percent changes from baseline in lumbar spine BMD at weeks 12 and 24, as well as mean percent changes from baseline in total hip BMD at weeks 12, 24, and 52 and geometric mean percent changes in bone markers at weeks 4, 12, 24, and 52.

Safety and tolerability were assessed by clinical and/or statistical assessment of all relevant safety parameters, including adverse experiences, laboratory values, and vital signs. The incidence of clinical and laboratory AEs and drug-related AEs was calculated for each treatment regimen, and comparisons between the once-daily group and once-weekly group were performed using Fisher's exact test. The discontinuation rate due to drug-related adverse experiences was also calculated and compared between the two treatment groups using Fisher's exact test.

Results

Patient allocation

A total of 328 patients were randomized. After excluding four patients because they did not take the study drug or no efficacy/safety data were available, 324 patients were included in the safety analysis; 156 in the once-daily group and 168 in the once-weekly group. Of the 328 patients randomized, 277 (84.5%) completed the study, while 51 patients (15.5%) were discontinued; 24 in the once-daily group and 27 in the once-weekly group. Major reasons for discontinuation were manifestation of adverse experiences (18 in the once-daily group and 19 in the once-weekly group) or failure to meet the inclusion/exclusion criteria (two in the once-daily group and two in the once-weekly group). For the primary efficacy assessment, the PPS analysis included 297 patients; 150 in the once-daily group and 147 in the once-weekly group. Table 1 shows the baseline characteristics of the 324 patients who were included in the safety analysis, with no significant difference between the two treatment groups.

Lumbar spine and total hip BMD

After 52 weeks of treatment, the LS means of percent change from baseline in spine BMD in the ALN 5-mg once-daily and the 35-mg once-weekly treatment groups were 5.76% and 6.35%, respectively (Table 2). These represent statistically significant increases compared with baseline ($P < 0.001$ for both groups). The LS mean difference (95% CI) of the percent change from baseline in the lumbar spine BMD between the two groups was 0.58% (95% CI; −0.31, 1.48), thereby meeting the prespecified criterion for equivalence, since the 95% CI fell entirely within the bounds of ±1.5% (Table 2). Moreover, the two dose regimens were not significantly different, since the 95% CI of the mean difference between groups included zero. The LS means of the percent change from baseline in the total hip BMD after 52 weeks in the once-daily and once-weekly groups were 2.81% and 2.96%, respectively (Table 2), representing statistically significant increases compared with

Table 1. Baseline characteristics of the patients ($n = 324$)

Characteristics	Dosing regimen of ALN	
	5 mg once daily ($n = 156$)	35 mg once weekly ($n = 168$)
Sex, no. (%)		
Female	149 (96)	163 (97)
Male	7 (4)	5 (3)
Age, years	67.4 (6.9)	66.0 (6.9)
Height, cm	149.6 (6.8)	150.3 (5.5)
Body weight, kg	48.6 (6.3)	49.5 (6.9)
BMI, kg/m^2	21.7 (2.6)	21.9 (2.9)
Spine BMD (L1–L4), g/cm^2	0.62 (0.06)	0.62 (0.06)
Total Hip BMD, g/cm^2	0.65 (0.08)	0.66 (0.09)

All values are presented as mean (SD), except sex
BMI, body mass index; BMD, bone mineral density; ALN, alendronate

Table 2. Percent change from baseline in lumbar spine and total hip BMD at week 52

Site	Dosing regimen of ALN		Intergroup difference
	5 mg once daily	35 mg once weekly	
Spine	5.76 (4.98, 6.55)	6.35 (5.58, 7.11)	0.58 (−0.31, 1.48)
Total hip	2.81 (2.10, 3.51)	2.96 (2.27, 3.65)	0.15 (−0.65, 0.95)

Values are presented as mean (95% confidence intervals). Analyses based on the per protocol set

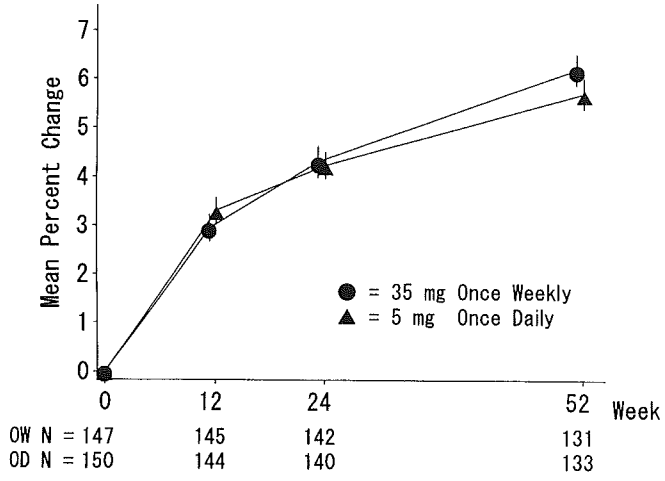

Fig. 1. Mean percent change from baseline in the lumbar spine (L1–L4) bone mineral density (BMD) (mean ± SE). *OW*, once weekly; *OD*, once daily

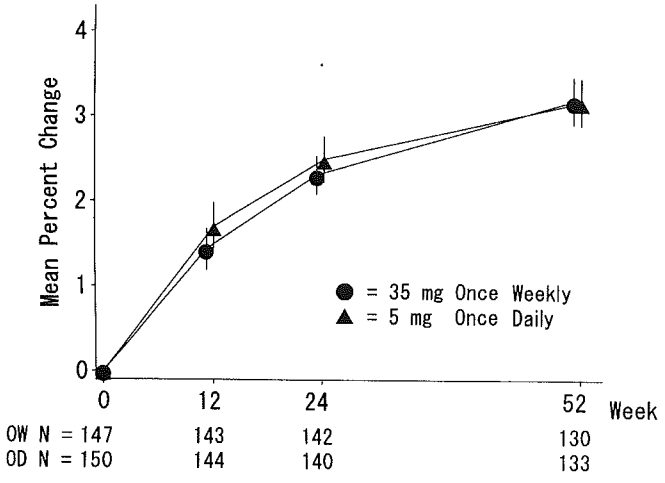

Fig. 2. Mean percent change from baseline in total hip BMD (mean ± SE)

baseline ($P < 0.001$ for both groups). Figures 1 and 2 show the time course of percent BMD change in the lumbar spine and total hip for the two ALN treatment groups. BMD increased significantly ($P < 0.001$) at each time point of measurement in both the spine and total hip, with a similar profile of BMD changes over time for both treatments.

Bone markers (DPD, NTx, and BAP)

The time course of geometric mean percent change from baseline in bone markers (urinary DPD, urinary NTx, and serum BAP) is shown in Fig. 3. In both ALN treatment groups, all three markers were decreased significantly during the period from 4 weeks to 52 weeks ($P < 0.001$ at each measurement point for DPD and NTx; $P \leq 0.017$ for BAP). The geometric mean percent change at week 52 in the once-daily and once-weekly groups, respectively, were –42.0% and –44.9% for DPD (Fig. 3a), –49.2% and –51.5% for NTx (Fig. 3b), and –50.3% and –52.1% for BAP (Fig. 3c). The bone markers (DPD, NTx, and BAP) were thus reduced to a similar level by either treatment throughout the treatment period.

Safety

The incidence of clinical AEs was 89.7% (140/156 patients) in the once-daily group and 85.1% (143/168 patients) in the once-weekly group, with no significant difference between the treatment groups (Table 3). The incidence of drug-related clinical AEs was 17.9% (28/156 patients) in the once-daily group and 13.1% (22/168 patients) in the once-weekly group. Regarding upper gastrointestinal AEs, which have been the major issue with respect to the safety of ALN (and other related drugs), the incidence of drug-related upper gastrointestinal AEs was 9.0% (14/156 patients) and 10.7% (18/168 patients), respectively, in the once-daily and once-weekly groups, with no statistical difference between the treatment groups. The discontinuation rates due to clinical drug-related AEs in the once-daily and once-weekly groups were 8.3% (13/156 patients) and 5.4% (9/168 patients), respectively, with no statistical difference between the treatment groups. Serious AEs were reported in 7 patients in the once-daily group and 11 patients in the once-weekly group. Among those, two patients in the once-daily group had serious AEs that were judged to be drug-related (one case was reflux esophagitis, and the other was aggravation of allergic bowel syndrome). No drug-related laboratory AE occurred at an incidence of more than 3%. None of the laboratory AEs were serious or resulted in discontinuation. The number of patients with new fractures in the upper and lower extremities was two in the once-daily group and three in the once-weekly group.

No remarkable differences were observed between the two groups with regard to any of the safety parameters.

Discussion

This study demonstrated that the effects of daily 5 mg and weekly 35 mg ALN were similar with regard to changes in BMD of the lumbar spine and hip and biochemical markers of bone turnover during 1 year of followup; safety and tolerability were also similar for both dosing regimens. Based upon the prespecified statistical criterion, the 5-mg-daily and 35-mg-weekly dosing regimens were declared therapeutically equivalent. Supporting evidence comes from a recent pharmacokinetic study in Japanese, which reported that the urinary excretion rate of oral ALN 35 mg was similar to that of ALN 5 mg, as was the safety and tolerability profile [26]. The similar urinary excretion rate of ALN 35 mg and 5 mg indicates that overall the same amount of ALN is obtained from each regimen.

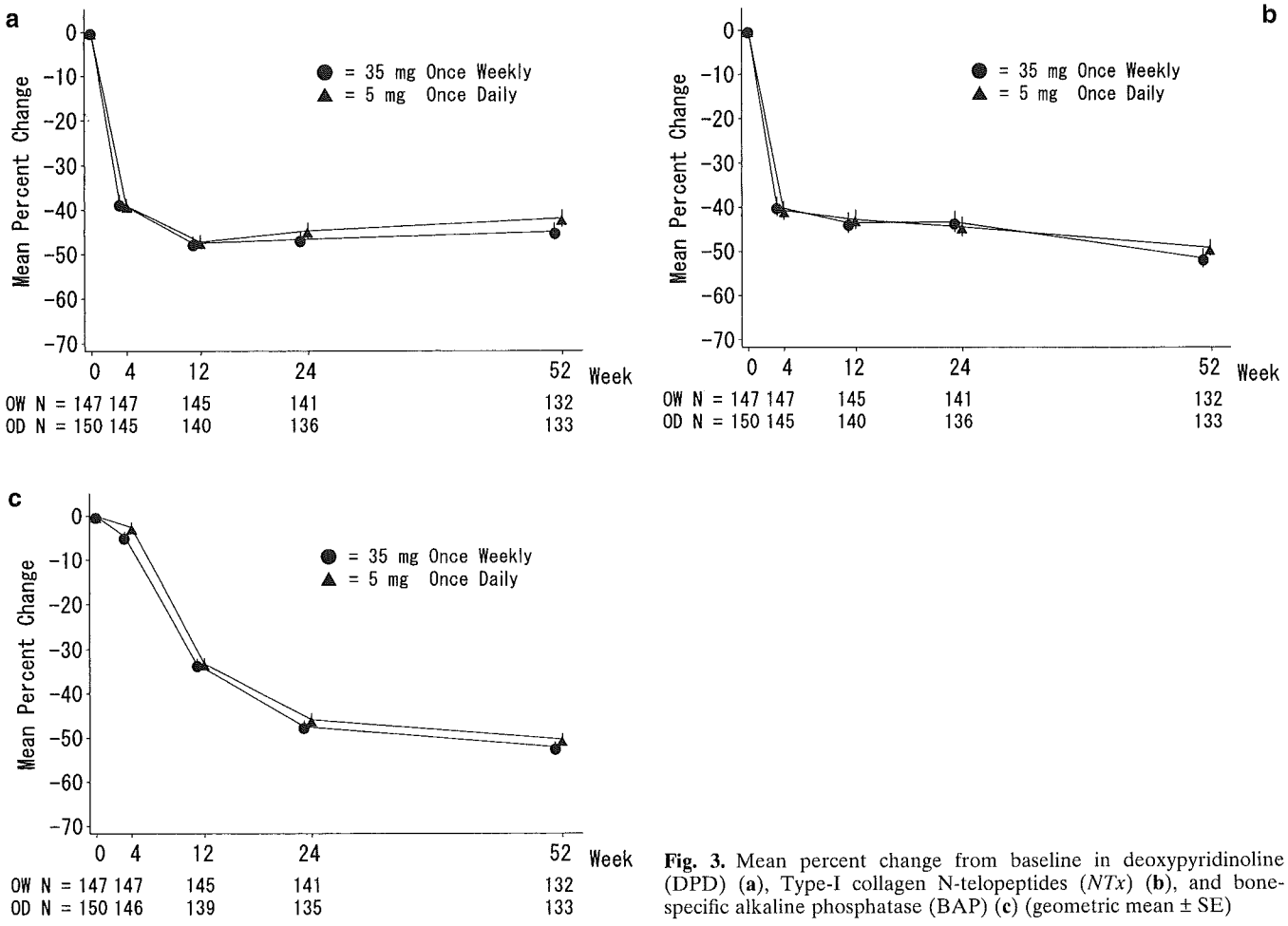

Fig. 3. Mean percent change from baseline in deoxypyridinoline (DPD) (**a**), Type-I collagen N-telopeptides (*NTx*) (**b**), and bone-specific alkaline phosphatase (BAP) (**c**) (geometric mean ± SE)

Table 3. Summary of clinical adverse events (AEs)

	Total *n* (%)	Dosing regimen of ALN	
		5 mg once daily *n* (%)	35 mg once weekly *n* (%)
No. of patients	324	156	168
Any AE	283 (87.3)	140 (89.7)	143 (85.1)
Drug-related AE	50 (15.4)	28 (17.9)	22 (13.1)
Serious AE	18 (5.6)	7 (4.5)	11 (6.5)
Serious drug-related AE	2 (0.6)	2 (1.3)	0 (0.0)
Discontinued due to AE	37 (11.4)	18 (11.5)	19 (11.3)
Discontinued due to drug-related AE	22 (6.8)	13 (8.3)	9 (5.4)
Discontinued due to serious AE	7 (2.2)	4 (2.6)	3 (1.8)
Discontinued due to serious drug-related AE	2 (0.6)	2 (1.3)	0 (0.0)

Less frequent dosing with any medication may improve compliance and thereby improve efficacy [24,27]. The mechanism of action, together with animal studies, predicts that weekly dosing with ALN should provide efficacy that is equivalent to comparable cumulative daily dosing, without compromising safety and tolerability. This result is because bisphosphonates bind to active bone remodeling sites and remain there for extended periods; at sufficient concentrations, this effectively inhibits osteoclastic bone resorption throughout the typical 2–3 week resorption phase of bone remodeling [24,28]. Less frequent (such as weekly) bisphosphonate dosing might also reduce the risk of upper gastrointestinal irritation [24,28]. ALN has demonstrated unsurpassed efficacy with regard to restoring bone turnover

to premenopausal levels, increasing bone density, and reducing the risk of all types of fractures including hip fractures. Furthermore, extensive experience with ALN in clinical trials of up to 10 years' duration has demonstrated continued efficacy and a good safety and tolerability profile [16].

A single criterion for evaluating the therapeutic equivalence of treatment groups based on changes in BMD has not been well established. In a previous study of Caucasians, equivalence was declared when the 90% CI of the difference in spine BMD change between the two groups (daily 10 mg and weekly 70 mg ALN) fell within the range of ±1.5% [24]. In the current study, we used a somewhat stricter criterion, requiring the 95% CI of the difference between the two groups in spine BMD change to fall entirely within a range of ±1.5% to establish equivalence. When the results of this study were compared with the results from the Caucasian study [24], the percent changes from baseline in the lumbar spine (L1–L4) BMD were similar.

Regarding the safety of ALN in the present study, the incidence of AEs overall and gastrointestinal AEs (including upper gastrointestinal AEs and drug-related AEs) did not differ significantly between the treatment groups. These findings are consistent with those observed in Caucasian patients with osteoporosis [24]. Furthermore, a placebo-controlled clinical trial and an endoscopy study both reported that oral administration of ALN 70 mg once weekly had a safety and tolerability profile similar to that of a placebo [29,30]. The lack of differences in upper gastrointestinal AEs between daily 5 mg and weekly 35 mg in our study suggests that the weekly 35 mg dose may not increase the risk of upper gastrointestinal events in Japanese patients with osteoporosis, if administered appropriately [31].

A trial in the United States that surveyed patients who had received treatment with both once-daily and once-weekly ALN in a cross-over design showed that the large majority of patients preferred the 70-mg once-weekly regimen compared with the 10-mg once-daily regimen [32]. Similarly, in a separate, multinational study, which included 406 postmenopausal women with osteoporosis from 19 countries, 84% of patients preferred the once-weekly over the once-daily regimen [33]. In actual medical practice, the once-weekly formulation is now in extensive use, accounting for about 90% of new prescriptions for ALN. Although some patients may wish to take the once-daily formulation for various reasons, the existence of the once-weekly formulation will provide patients with a broader range of treatment options. As noted earlier, oral drug compliance is increased if the frequency of drug administration is reduced [27]. Accordingly, it can be surmised that increasing the convenience of ALN by reducing the frequency of administration to once weekly will increase patient acceptance of treatment of osteoporosis, compliance, and continuation of therapy, leading to better long-term benefits.

In conclusion, our data confirmed that the effects of a weekly 35-mg dose of ALN were similar to those of a daily 5-mg dose in Japanese patients with osteoporosis. The safety and tolerability profiles of both regimens were also similar to each other. These data, together with previous findings, suggest that weekly dosing with ALN provides an alternative that is more convenient and preferred by many patients, which may result in better patient acceptance and continuation of therapy.

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References

1. Consensus Development Conference (1993) Diagnosis, prophylaxis, and treatment of osteoporosis. Am J Med 94:646–650
2. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy (2001) Osteoporosis prevention, diagnosis, and therapy. JAMA 285:785–795
3. Japan Osteoporosis Society Working Group (2002) Guideline to Clinical Evaluation Methods for Agents Used in Treatment of Osteoporosis, 2002 Revised Edition (in Japanese). Osteoporos Jpn 10:7–79
4. Fujiwara S, Kasagi F, Masunari N, Naito K, Suzuki G, Fukunaga M (2003) Fracture prediction from bone mineral density in Japanese men and women. J Bone Miner Res 18:1547–1553
5. Black DM, Cummings SR, Karpf DB, Cauley JA, Thompson DE, Nevitt MC, Bauer DC, Genant HK, Haskell WL, Marcus R, Ott SM, Torner JC, Quandt SA, Reiss TF, Ensrud KE (1996) Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Lancet 348:1535–1541
6. Cummings SR, Black DM, Thompson DE, Applegate WB, Barrett-Connor E, Musliner TA, Palermo L, Prineas R, Rubin SM, Scott JC, Vogt T, Wallace R, Yates AJ, LaCroix AZ (1998) Effect of alendronate on risk of fracture in women with low bone density but without vertebral fractures: results from the fracture intervention trial. JAMA 280:2077–2082
7. Liberman UA, Weiss SR, Broll J, Minne HW, Quan H, Bell NH, Rodriguez-Portales J, Downs RW Jr, Dequeker J, Favus M, Seeman E, Recker RR, Capizzi T, Santora AC II, Lombardi A, Shah RV, Hirsch LJ, Karpf DB (1995) Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. N Engl J Med 333:1437–1443
8. Tucci JR, Tonino RP, Emkey RD, Peverly CA, Kher U, Santora II AC (1996) Effect of three years of oral alendronate treatment in

postmenopausal women with osteoporosis. Am J Med 101:488–501
9. Devogelaer JP, Broll H, Correa-Rotter R, Cumming DC, Nagant de Deuxchaisnes C, et al. (1996) Oral alendronate induces progressive increases in bone mass of the spine, hip, and total body over 3 years in postmenopausal women with osteoporosis. Bone 18:141–150
10. Karpf DB, Shapiro DR, Seeman E, Ensrud KE, Johnston CC Jr, Adami S, Harris ST, Santora AC II, Hirsch LJ, Oppenheimer L, Thompson D (1997) Prevention of nonvertebral fractures by alendronate. A meta-analysis. JAMA 277:1159–1164
11. Sharpe M, Noble S, Spencer CM (2001) Alendronate: an update of its use in osteoporosis. Drugs 61:999–1039
12. Cranney A, Wells G, Willan A, Griffith L, Zytaruk N, Robinson V, Black D, Adachi J, Shea B, Tugwell P, Guyatt G (2002) II. Meta-analysis of alendronate for the treatment of postmenopausal women. Endocr Rev 23:508–516
13. Tonino RP, Meunier PJ, Emkey R, Rodriguez-Portales JA, Menkes CJ, Wasnich RD, Bone HG, Santora AC, Wu M, Desai R, Ross PD (2000) Skeletal benefits of alendronate: 7-year treatment of postmenopausal osteoporotic women. Phase III Osteoporosis Treatment Study Group. J Clin Endocrinol Metab 85:3109–3115
14. Pols HA, Felsenberg D, Hanley DA, Stepán J, Muñoz-Torres M, Wilkin TJ, Qin-sheng G, Galich AM, Vandormael K, Yates AJ, Stych B (1999) Multinational, placebo-controlled, randomized trial of the effects of alendronate on bone density and fracture risk in postmenopausal women with low bone mass: results of the FOSIT study. Osteoporos Int 9:461–468
15. Black DM, Thompson DE, Bauer DC, Ensrud K, Musliner T, Hochberg MC, Nevitt MC, Suryawanshi S, Cummings SR (2000) Fracture risk reduction with alendronate in women with osteoporosis: the fracture intervention trial. J Clin Endocrinol Metab 85:4118–4124
16. Bone HG, Hosking D, Devogelaer JP, Tucci JR, Emkey RD, Tonino RP, Rodriguez-Portales JA, Downs RW, Gupta J, Santora AC, Liberman UA (2004) Ten years' experience with alendronate for osteoporosis in postmenopausal women. N Engl J Med 350:1189–1199
17. Wehren LE, Hosking D, Hochberg MC (2004) Putting evidence-based medicine into clinical practice: comparing anti-resorptive agents for the treatment of osteoporosis. Curr Med Res Opin 20:525–531
18. Shiraki M, Kushida K, Fukunaga M, Kishimoto H, Kaneda K, Minaguchi H, Inoue T, Tomita A, Nagata Y, Nakashima M, Orimo H (1998) A placebo-controlled, single-blind study to determine the appropriate alendronate dosage in postmenopausal Japanese patients with osteoporosis. Endocr J 45:191–201
19. Nakamura T, Kushida K, Shiraki M, Fukunaga M, Taga M, Kishimoto H, Tomita A, Inoue T, Minaguchi H, Kaneda K, Nagata Y, Nakashima M, Orimo H (1998) A double-blind, dose-ranging study of alendronate in patients with involutional osteoporosis, osteoporotic osteopenia or artificial menopause (in Japanese). Med Consult New Remedy 35:3–17
20. Kishimoto H, Shiraki M, Fukunaga M, Kushida K, Kaneda K, Inoue T, Tomita A, Yamamoto K, Orimo H (1998) A long-term study of alendronate in patients with involutional osteoporosis (in Japanese). Med Consult New Remedy 35:19–41
21. Shiraki M, Kushida K, Fukunaga M, Kishimoto H, Taga M, Nakamura T, Kaneda K, Minaguchi H, Inoue T, Morii H, Tomita A, Yamamoto K, Nagata Y, Nakashima M, Orimo H (1999) A double-masked multicenter comparative study between alendronate and alfacalcidol in Japanese patients with osteoporosis. Osteoporos Int 10:183–192
22. Kushida K, Shiraki M, Nakamura T, Kishimoto H, Morii H, Yamamoto K, Kaneda K, Fukunaga M, Inoue T, Nakashima M, Orimo H (2002) The efficacy of alendronate in reducing the risk for vertebral fracture in Japanese patients with osteoporosis: a randomized, double-blind, active-controlled, double-dummy trial. Curr Ther Res 63:606–620
23. Kushida K, Shiraki M, Nakamura T, Kishimoto H, Morii H, Yamamoto K, Kaneda K, Fukunaga M, Inoue T, Nakashima M, Orimo H (2004) Alendronate reduced vertebral fracture risk in postmenopausal Japanese women with osteoporosis: a 3-year follow-up study. J Bone Miner Metab 22:462–468
24. Schnitzer T, Bone HG, Crepaldi G, Adami S, McClung M, et al. (2000) Therapeutic equivalence of alendronate 70 mg once-weekly and alendronate 10 mg daily in the treatment of osteoporosis. Aging Clin Exp Res 12:1–12
25. Rizzoli R, Greenspan SL, Bone H III, Schnitzer TJ, Watts NB, Adami S, Foldes AJ, Roux C, Levine MA, Uebelhart B, Santora AC II, Kaur A, Peverly CA, Orloff JJ (2002) Two-year results of once-weekly administration of alendronate 70 mg for the treatment of postmenopausal osteoporosis. J Bone Miner Res 17:1988–1996
26. Naruse S, Kato Y, Tani K, Hisaka A, Watanabe H, Arizono H, Taniguchi T (2004) Pharmacokinetic study of alendronate in postmenopausal Japanese women – Evaluation of urinary excretion and safety profile of alendronate after single oral administration of 35 mg and 5 mg tablet formulations (in Japanese). Rinsyo Iyaku 20:1–8
27. Greenberg RN (1984) Overview of patient compliance with medication dosing: a literature review. Clin Ther 6:592–599
28. Bone HG, Adami S, Rizzoli R, Favus M, Ross PD, Santora A, Prahalada S, Daifotis A, Orloff J, Yates J (2000) Weekly administration of alendronate: rationale and plan for clinical assessment. Clin Ther 22:15–28
29. Lanza F, Sahba B, Schwartz H, Winograd S, Torosis J, Quan H, Reyes R, Musliner T, Daifotis A, Leung A (2002) The upper GI safety and tolerability of oral alendronate at a dose of 70 milligrams once weekly: a placebo-controlled endoscopy study. Am J Gastroenterol 97:58–64
30. Greenspan S, Field-Munves E, Tonino R, Smith M, Petruschke R, Wang L, Yates J, DePapp AE, Palmisano J (2002) Tolerability of once-weekly alendronate in patients with osteoporosis: a randomized, double-blind, placebo-controlled study. Mayo Clin Proc 77: 1044–1052
31. Cryer B, Bauer DC (2002) Oral bisphosphonates and upper gastrointestinal tract problems: what is the evidence? Mayo Clin Proc 77:1031–1043
32. Simon JA, Lewiecki EM, Smith ME, Petruschke RA, Wang L, Palmisano JJ (2002) Patient preference for once-weekly alendronate 70 mg versus once-daily alendronate 10 mg: a multicenter, randomized, open-label, crossover study. Clin Ther 24:1871–1886
33. Kendler D, Kung AWC, Fuleihan GEH, Gonzalez JGG, Gaines KA, Verbruggen N, Melton ME (2004) Patients with osteoporosis prefer once weekly to once daily dosing with alendronate. Eur Menopause J 48:243–251

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SPECIAL REPORT

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Randomized trial comparing low-dose hormone replacement therapy and HRT plus 1α-OH-vitamin D_3 (alfacalcidol) for treatment of postmenopausal bone loss

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Abstract We conducted a prospective, randomized, multicenter, open-label 2-year trial with 76 postmenopausal women aged ≥60 years with low (T-score less than −1) lumbar bone mineral density (BMD). The hormone replacement therapy (HRT) group received a low dose of conjugated estrogen (CEE) at a dose of 0.31 mg/day ± medroxyprogesterone acetate (MPA) 2.5 mg/day. Group HRT/D received the same dose of HRT together with alfacalcidol in a daily dose of 1.0 μg/day. Changes in lumbar BMD measured by dual energy X-ray absorptiometry (DXA) were followed every 6 months for 2 years. The lumbar BMD of group HRT increased 3.37% [95% confidence interval (CI) 1.6%–5.2%], 4.00% (95%CI 1.6%–6.4%), and 2.32% (95%CI −0.7% to 5.3%) at 12, 18, and 24 months, respectively, when the baseline value was taken as 0%. Lumbar BMD of group HRT/D showed a significant increase beyond 6 months. The percent increases for this group at 6, 12, 18, and 24 months were 6.18 (95%CI 1.3%–6.6%), 6.18% (95%CI 3.9%–8.5%), 7.17% (95%CI 4.3%–10.0%), and 8.75% (95%CI 6.0%–11.5%), respectively. In addition, there was a significant difference in the changes of the lumbar BMD between the two groups at 24 months, suggesting that the combination of HRT and alfacalcidol is more effective than HRT alone in terms of the BMD effect. This study is the first prospective trial demonstrating an additive effect of alfacalcidol on lumbar BMD in postmenopausal women receiving low-dose HRT. It suggests that the combination therapy can be considered to be a promising mode of treatment for bone loss after menopause.

Key words bone loss · low-dose HRT · osteoporosis · 1α-OH-vitamin D_3

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Introduction

The use of lower doses of estrogens for hormone replacement therapy (HRT) has been proposed as an effective modality for preventing and treating menopause-related disorders [1–3], including postmenopausal bone loss [4–7]. Lindsay et al. [7] demonstrated that 0.31 mg of conjugated equine estrogen (CEE) with or without progesterone significantly increased BMD at both spine and femur. The advantages of using lower doses of estrogens are primarily to reduce side effects [1,8] such as unexpected genital bleeding, breast tenderness, and most importantly to reduce the incidence of breast cancer, stroke, and deep vein thrombosis which are now shown to be associated with long-term use of HRT [9,10]. Disadvantages of lower doses of estrogen therapy, on the other hand, are less efficacy of the estrogen action than the regular dose because of its dose-dependence. Currently, a daily dose of 0.625 mg is recommended as a standard dose of CEE particularly for preventing postmenopausal bone loss, but continuous use of this dose of CEE with 2.5 mg of medroxyprogesterone acetate (MPA) has been extremely restricted after the Women's Health Initiative (WHI) [9] and the Million Women Study [10]. Therefore, it is a matter of urgent concern to establish a new modality of HRT with efficacy but without reducing the optimal effects.

Osteoporosis is a disease caused by multiple factors. Decreased calcium absorption in aged women is also considered an important cause for bone loss. Therefore, there is a rationale for testing combination therapy with HRT and alfacalcidol. Gorai et al. [11] showed a synergistic effect of alfacalcidol and HRT on early postmenopausal bone loss. Gallagher et al. [12] could not find significant differences in BMD between HRT alone and HRT plus calcitriol therapy, but they found that the decrease in BMD after discontinuing the therapy was significantly low in subjects treated with HRT plus calcitriol [13]. Both studies clarified the protective effect of CEE at a dose of 0.625 mg but failed to confirm whether the addition of vitamin D_3 to HRT is promising. Ettinger et al. [14] and MacLenann et al. [15], on the other hand, have shown that a daily dose of 0.31 mg of CEE administered with calcium was effective in preventing postmenopausal bone loss as much as that obtained by HRT at a standard dose, suggesting that the efficacy of combination therapy of HRT and alfacalcidol is augmented if the calcium balance is improper. Based on this hypothesis, the present study was conducted to determine the effect of combination therapy of HRT using a lower dose of estrogen with alfacalcidol on spinal BMD of postmenopausal Japanese women.

Materials and methods

Subjects

Postmenopausal women aged ≥60 years old were recruited for this study. The principal entry criterion was a spinal BMD of −1.0 SD and below the young adult mean (YAM) by dual energy X-ray absorptiometry (DXA). Exclusion criteria were treatment with estrogen during the past 6 months and any treatment with bisphosphonates or other drugs intended to treat osteoporosis. Women who had any of following were also excluded from this study: a history of breast cancer, uterine bleeding with unknown origin, hypercalcemia and a history of vertebral fractures, which may affect BMD measurement by DXA. Endometrial screening, breast examination, and biochemical analysis were performed before the study, and all women were confirmed to be free of endometrial cancer, or hyperplasia, breast cancer, and hepatic, renal, or other diseases with a known influence on bone metabolism. During the study, breast and endometrial screening was performed depending on each institutional method and criteria. All subjects were placed on a general diet without calcium supplementation. The protocol was reviewed by the board of each institution, and written informed consent was obtained by each subject.

Study protocol

Subjects were randomly assigned to one of two groups. One group received a daily dose of 0.31 mg CEE and 2.5 mg of MPA (HRT group), and the other received an identical regimen of HRT plus alfacalcidol 1 μg/day (HRT/D group).

Measurements

The primary endpoint of the present study was the percentage changes in BMD of the L2-4 vertebral bodies from baseline to the time of the final evaluation. The lumbar (L)2-4 BMD was measured by DXA before initiation of the study and at 6, 12, 18, and 24 months thereafter. Because the BMD measurement was done at each institution using different types of DXA (DPX series from Lunar, QDR series from Hologic, and XR-26 from Norland), the values obtained from different machines were calibrated relative to the QDR-1000 equivalent values according to the equations previously described [15]. The BMD of each subject was measured by the identical machine throughout the study period. Changes in BMD were expressed in terms of percentage change from the baseline value.

Blood and urine samples were collected for biochemical assessment every 6 months and transferred to the center for measurement of biochemical markers. In serum we measured calcium (Ca), phosphorus, alkaline phosphatase (Alp), parathyroid hormone (PTH-intact, immunoradiometric assay; Allegro intact PTH, Sumitomo Seiyaku Biomedical, Osaka, Japan), and N-terminal osteocalcin (radiommunoassay; CIS International, Tokyo, Japan). In urine we measured deoxypyridinoline (DPD, high-performance liquid chromatography) and pyridinoline (Pry). All measurements were carried out by SRL (Tokyo). Urinary parameters were normalized by the urinary concentration of creatinine.

Statistical analysis

Results were expressed as the mean ± SD. Statistical differences between baseline and treatment values were analyzed by the two-sided paired *t*-test and the difference between groups by the Wilcoxon rank-sum analysis. $P < 0.05$ was considered significant.

Results

A total of 83 subjects were registered for this study: 42 for CEE with or without MPA (group HRT) and 41 for the combination HRT and alfacalcidol (group HRT/D). Among them, a total of seven subjects were excluded because of violation of inclusion criteria. As a result, 76 were enrolled for the full analysis set (FAS). The baseline data of the two groups are shown in Table 1. There were no significant differences in any mean between the two groups except age.

The effects of each regimen on the lumbar BMD are shown in Fig. 1. Lumbar BMD of the HRT group showed a significant increase at 12 months and 18 months, with increases of 3.37% (95% confidence interval (CI) 1.6%–5.2%) and 4.00% (95%CI 1.6%–6.4%), respectively. The mean percent increase at 24 months for this group was 2.32%, but it was not statistically significant when compared with the baseline level. The lumbar BMD of the HRT/D group showed a significant increase after 6 months. The increases of this group at 6, 12, 18, and 24 months were 3.96% (95%CI 1.3%–6.6%), 6.18% (95%CI 3.9%–8.5%), 7.17% (95%CI 4.3%–10.0%), and 8.75% (95%CI 6.0%–11.5%), respectively. In addition, there was a significant difference in the change in lumbar BMD between the two groups at 24 months, suggesting that HRT/D is more effective than HRT alone.

Changes in serum OC, PTH, and Ca levels and urinary D-Pry and Pry levels are shown in Table 2. Serum OC showed a significant decrease in both groups, but the magnitude of the decrease was larger and it was more rapid in the HRT/D group than in the HRT group. Serum PTH in the HRT/D group at 12 months decreased significantly compared to the baseline value, and it was significantly lower than the corresponding value of the HRT group. Serum Ca levels at 6 months in the HRT group decreased significantly from the baseline level, and the value was significantly lower than the corresponding value of the HRT/D group. Urinary D-Pry and Pry both showed a significant decrease from baseline in both groups without any significant difference between the two groups.

Dropping out of the study because of an adverse event, such as uterine bleeding or breast pain, occurred in 12 (15.9%) of the subjects. Seven subjects discontinued because of uterine bleeding and three because of breast pain. The symptoms disappeared after discontinuing the medication. Breast and endometrial examinations were performed according to each institutional method and criteria, and no abnormal findings were reported.

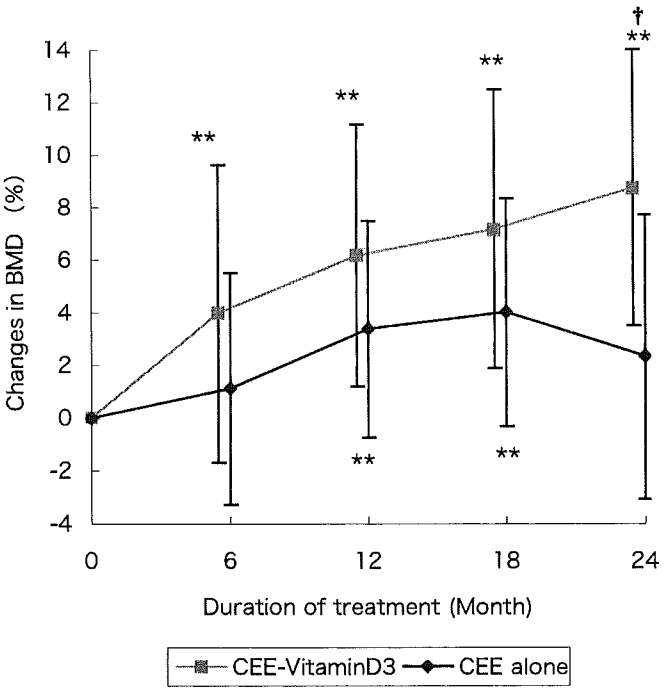

Fig. 1. Changes in lumbar spine bone mineral density (*BMD*). Values are the mean ± SD. $*P < 0.05$ and $**P < 0.01$ versus the baseline value, respectively; $^{\dagger}P < 0.05$ versus CEE alone. *CEE*, conjugated estrogen

Table 1. Baseline characteristics

Characteristic	CEE alone ($n = 39$)	CEE-vitamin D_3 ($n = 37$)	*P*
Age (years)	64.3 ± 5.8	67.3 ± 5.7	0.0234
Lumbar spine BMD (g/cm^2)	0.7608 ± 0.0892	0.7432 ± 0.0887	0.3933
Serum osteocalcin (ng/ml)	11.29 ± 4.63	12.72 ± 4.02	0.1657
Urinary deoxypyridinoline (pM/μM Cr)	7.06 ± 3.10	7.24 ± 3.10	0.9267
Urinary pyridinoline (pM/μM Cr)	30.19 ± 9.72	33.55 ± 14.68	0.6413
Serum PTH (pg/ml)	34.5 ± 14.9	41.1 ± 44.1	0.7250
Serum Ca (mg/dl)	9.27 ± 0.38	9.36 ± 0.37	0.3487
Ca/Cr (mg/mg)	0.2249 ± 0.1079	0.1917 ± 0.0974	0.1977

Values are unadjusted means ± SD
BMD, bone mineral density; PTH, parathyroid hormone

Table 2. Percent changes in serum OC, PTH, urinary D-Pry, and Pry

Measurement	Baseline	6 Months	12 Months	18 Months	24 Months
CEE alone					
OC	0	−8.7 ± 51.2	−7.5 ± 32.8	−36.7 ± 12.5**	−32.6 ± 20.4**
PTH	0	2.9 ± 34.9	10.3 ± 38.8	2.6 ± 33.9	2.9 ± 38.3
Ca (mg/dl)	9.3 ± 0.4	9.0 ± 0.4*†	9.2 ± 0.3	9.3 ± 0.4	9.2 ± 0.5
D-Pry	0	−17.4 ± 45.5	−8.9 ± 80.0	−31.9 ± 28.4*	−27.6 ± 51.1
Pry	0	−2.5 ± 38.3	14.3 ± 108.8	−16.2 ± 27.7	−16.2 ± 44.5
CEE-vitamin					
OC	0	−24.4 ± 29.7**	−33.2 ± 24.9*††	−43.6 ± 17.8**	−55.1 ± 16.4*†
PTH	0	−15.0 ± 44.6	−17.3 ± 32.2*†	−8.8 ± 64.8	−4.8 ± 53.2
Ca (mg/dl)	9.4 ± 0.4	9.3 ± 0.5	9.2 ± 0.4	9.2 ± 0.3	9.2 ± 0.3
D-Pry	0	−28.3 ± 31.7**	−29.6 ± 35.4**	−34.2 ± 14.2**	−45.1 ± 18.2**
Pry	0	−15.9 ± 27.4*	−14.7 ± 30.7	−15.3 ± 12.4**	−30.2 ± 23.0**

* $P < 0.05$ vs. baseline value; ** $P < 0.01$ vs. baseline value; † $P < 0.05$ vs. that of CEE alone; †† $P < 0.01$ vs. that of CEE alone
Values are unadjusted means ± SD
OC, osteocalcin; D-Pry, deoxypyridinoline (DPD); Pry, pyridinoline

Discussion

Although the daily dose of 0.625 mg of CEE has been recommended as standard for preventing postmenopausal bone loss, recent studies [4–7], including this one, have shown that CEE at a dose of 0.31 mg/day is still effective in protecting bones. Because the effect of estrogen is dose-dependent and the estrogen requirement may differ among individuals, the efficacy of HRT using a low dose of estrogens should be evaluated on an individual basis. Ettinger et al. [14] demonstrated first that combining 0.3 mg CEE with 1500 mg calcium was as effective as 0.6 mg CEE alone. In addition, Recker et al. [17] showed that continuous therapy with a low dose of HRT significantly increased BMD either by supplementation of calcium (maintaining calcium intake above 1000 mg/day) or by oral 25-hydroxyvitamin D (maintaining the serum 25-hydroxyvitamin D level at a minimum of 75 nmol/l), respectively. Those studies indicated that the improved calcium metabolism using calcium or vitamin D supplementation are expected to have additional benefit when the patients are being treated with a low dose of estrogen. The results of the present study demonstrated that low-dose HRT was effective in increasing BMD of postmenopausal women, and that the addition of alfacalcidol significantly enhanced the effect of low dose HRT.

Gorai et al. [11], on the other hand, could find only a marginal effect of alfacalcidol on postmenopaual bone loss in patients treated with a daily estrogen dose of 0.625 mg in combination with alfacalcidol. In addition, neither Gallagher et al. [12] nor Komulainen et al. [18] could find any further benefit in the combined treatment with CEE plus vitamin D cholecalciferol compared to the effect of CEE alone. The discrepancies between these and the present study may be accounted for by differences in the estrogen dose. The antiresorption activity of daily administration of CEE at a dose of 0.625 mg is potent in terms of reducing bone turnover, and it is similar to that seen after administration of alendronate, one of the most potent antiresorption drugs [19]. Recent studies of alendronate and PTH combined treatment indicated that the PTH effect on bone was abolished by the concurrent use of alendronate, possibly through the reduction of bone turnover by alendronate [20]. Therefore, it is likely that the effect of concomitant administration of vitamin D_3 may be masked in the conditions when bone resorption is maximally suppressed. On the other hand, it is suggested that the effect of vitamin D is manifested in subjects receiving a low dose of estrogen and probably in patients who are under low calcium intake.

Because serum 25-hydroxyvitamin D_3 levels decline with the advancement of age, mainly owing to decreased cutaneous production, it is postulated that vitamin D_3 deficiency is common in the elderly and that deficiency in vitamin D_3 causes secondary hyperparathroidism, high bone turnover, bone loss, mineralization defects, and fractures [21]. Furthermore, calcium nutrition is known to be deficient in elderly people because of low intake of calcium from food and impaired intestinal calcium absorption. Therefore, administrating any active vitamin D is beneficial for correcting any calcium imbalance in elderly subjects [21]. Alfacalcidol has been reported to increase lumbar BMD in osteoporotic women and can prevent fractures in those with senile osteoporosis [22]. It is shown that long-term administration of antiresorption drugs such as estrogen reduces serum calcium levels as a result of inhibiting bone resorption; it also causes secondary hyperparathyroidism, which in turn is postulated to lessen the effect of estrogen. Therefore, correction and maintenance of proper calcium balance is a rational measure to take to decrease PTH during HRT and to enhance the effect of HRT. Indeed, it is shown that calcium administration increased BMD of subjects whose BMD remained stable during long-term HRT [23]. As shown in Table 2, administration of alfacalcidol significantly increased the serum calcium level at 6 months and decreased PTH, supporting the hypothesis.

Among several management modalities for osteoporosis, HRT can reduce menopausal symptoms and prevent bone loss simultaneously. In fact, despite of the Women's Health Initiatives' report [9], which emphasized the disadvantages of HRT and proposed restricted use of HRT for

postmenopausal health care, HRT still plays a large part of the management of postmenopausal health care. It has been shown that HRT doses that are lower than the current standard doses can decrease the incidence of side effects without sacrificing efficacy and safety [1–3,24,25]. Therefore, it will not take long for low-dose HRT to replace standard HRT for management of menopausal symptoms. Although it has been shown that the effect of estrogen on bone is dose-dependent, the increase in BMD observed in the HRT/D group of this study was compatible with that previously reported elsewhere in Japanese women by using the standard dose of HRT [5,11], indicating that the combination of low-dose HRT and alfacalcidol is potent and can be a therapeutic option for preventing osteoporosis in elderly postmenopausal women. The limitations of the present study were that the population of the study was small and our study did not have an arm receiving HRT with 0.625 mg of CEE. Whether this therapy can prevent osteoporotic fracture is another matter of concern. Further investigations are required on this matter.

References

1. Utian WH, Shoupe D, Bachmann G, Pinkerton JV, Pickar JH (2001) Relief of vasomotor symptoms and vaginal atrophy with lower doses of conjugated equine estrogens and medroxyprogesterone acetate. Fertil Steril 75:1065–1079
2. Pickar JH, Yeh I, Wheeler JE, Cunnane MF, Speroff L (2001) Endometrial effects of lower doses of conjugated equine estrogens and medroxyprogesterone acetate. Fertil Steril 76:25–31
3. Lobo RA, Bush T, Carr BR, Pickar JH (2001) Effects of lower doses of conjugated equine estrogens and medroxyprogesterone acetate on plasma lipids and lipoproteins, coagulation factors, and carbohydrate metabolism. Fertil Steril 76:13–24
4. Gambacciani M, Monteleone P, Genazzani AR (2002) Low-dose hormone replacement therapy: effects on bone. Climacteric 5:135–139
5. Mizunuma H, Okano H, Soda M, Kagami I, Miyamoto S, Tokizawa T, Honjo S, Ibuki Y (1997) Prevention of postmenopausal bone loss with minimal uterine bleeding using low dose continuous estrogen/progestin therapy: a 2-year prospective study. Maturitas 27:69–76
6. Gambacciani M, Ciaponi M, Cappagli B, Monteleone P, Benussi C, Bevilacqua G, Genazzani AR (2003) Postmenopausal femur bone loss: effects of a low dose hormone replacement therapy. Maturitas 45:175–183
7. Lindsay R, Gallagher JC, Kleerekoper M, Pickar JH (2002) Effect of lower doses of conjugated equine estrogens with and without medroxyprogesterone acetate on bone in early postmenopausal women. JAMA 287:2668–2676
8. Gambacciani M, Ciaponi M, Cappagli B, Genazzani AR (2001) Effects of low-dose continuous combined conjugated estrogens and medroxyprogesterone acetate on menopausal symptoms, body weight, bone density, and metabolism in postmenopausal women. Am J Obstet Gynecol 185:1180–1185
9. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J, Writing Group for the Women's Health Initiative Investigators (2002) Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA 288:321–333
10. Beral V, Million Women Study Collaborators (2003) Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet 362:419–427
11. Gorai I, Chaki O, Taguchi Y, Nakayama M, Osada H, Suzuki N, Katagiri N, Misu Y, Minaguchi H (1999) Early postmenopausal bone loss is prevented by estrogen and partially by 1α-OH-vitamin D_3: therapeutic effects of estrogen and/or 1α-OH-vitamin D_3. Calcif Tissue Int 65:16–22
12. Gallagher JC, Fowler SE, Detter JR, Sherman SS (2001) Combination treatment with estrogen and calcitriol in the prevention of age-related bone loss. J Clin Endocrinol Metab 86:3618–3628
13. Gallagher JC, Rapuri PB, Haynatzki G, Detter JR (2002) Effect of discontinuation of estrogen, calcitriol, and the combination of both on bone density and bone markers. J Clin Endocrinol Metab 87:4914–4923
14. Ettinger B, Genant HK, Cann CE (1987) Postmenopausal bone loss is prevented by treatment with low-dosage estrogen with calcium. Ann Intern Med 106:40–45
15. MacLennan AH, MacLennan A, Wenzel S, Chambers HM, Eckert K (1993) Continuous low-dose oestrogen and progestogen hormone replacement therapy: a randomised trial. Med J Aust 159:102–106
16. Shiraki M, Kushida K, Fukunaga M, Kishimoto H, Taga M, Nakamura T, Kaneda K, Minaguchi H, Inoue T, Morii H, Tomita A, Yamamoto K, Nagata Y, Nakashima M, Orimo H (1999) A double-masked multicenter comparative study between alendronate and alfacalcidol in Japanese patients with osteoporosis: the Alendronate Phase III Osteoporosis Treatment Research Group. Osteoporos Int 10:183–192
17. Recker RR, Davies KM, Dowd RM, Heaney RP (1999) The effect of low-dose continuous estrogen and progesterone therapy with calcium and vitamin D on bone in elderly women: a randomized, controlled trial. Ann Intern Med 130:897–904
18. Komulainen M, Kroger H, Tuppurainen MT, Heikkinen AM, Alhava E, Honkanen R, Jurvelin J, Saarikoski S (1999) Prevention of femoral and lumbar bone loss with hormone replacement therapy and vitamin D_3 in early postmenopausal women: a population-based 5-year randomized trial. J Clin Endocrinol Metab 84:546–552
19. Cranney A, Wells G, Willan A, Griffith L, Zytaruk N, Robinson V, Black D, Adachi J, Shea B, Tugwell P, Guyatt G, Osteoporosis Methodology Group and The Osteoporosis Research Advisory Group (2002) Meta-analyses of therapies for postmenopausal osteoporosis. II. Meta-analysis of alendronate for the treatment of postmenopausal women. Endocr Rev 23:508–516
20. Black DM, Greenspan SL, Ensrud KE, Palermo L, McGowan JA, Lang TF, Garnero P, Bouxsein ML, Bilezikian JP, Rosen CJ, PaTH Study Investigators (2003) The effects of parathyroid hormone and alendronate alone or in combination in postmenopausal osteoporosis. N Engl J Med 349:1207–1215
21. Lips P (2001) Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev 22:477–501
22. Shiraki M, Kushida K, Yamazaki K, Nagai T, Inoue T, Orimo H (1996) Effects of 2 years' treatment of osteoporosis with 1 alpha-hydroxy vitamin D_3 on bone mineral density and incidence of fracture: a placebo-controlled, double-blind prospective study. Endocr J 43:211–220
23. Mizunuma H, Okano H, Soda M, Tokizawa S, Kagami I, Miyamoto S, Honjo S, Ibuki Y (1996) Calcium supplements increase bone mineral density in women with low serum calcium levels during long-term estrogen therapy. Endocr J 43:411–415
24. Li C, Samsioe G, Borgfeldt C, Bendahl PO, Wilawan K, Aberg A (2003) Low-dose hormone therapy and carbohydrate metabolism. Fertil Steril 79:550–555
25. Goudev A, Georgiev D, Koycheva N, Manasiev N, Kyurkchiev S (2002) Effects of low dose hormone replacement therapy on markers of inflammation in postmenopausal women. Maturitas 43:49–53