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Increased Serum 25(OH)D_3 Levels in Post-Menopausal Japanese Women with Osteoporosis after 3-Year Bisphosphonate Treatment

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Osteoporosis is characterized by the systemic impairment of bone mass, strength, and microarchitecture, leading to an increased risk of fragility fracture. Bisphosphonates (BPs) are the first-line drugs for osteoporosis. Vitamin D is considered to be essential for osteoporotic treatment. However, long-term effects of BPs on the serum levels of 25-hydroxyvitamin D_3 (25(OH)D_3) are unknown. Accordingly, in this retrospective study, we collected clinical data of 41 post-menopausal Japanese women with osteoporosis treated with BP for over 3 years, without vitamin D supplementation. We measured lumbar and femoral neck bone mineral density (BMD) and serum levels of bone specific alkaline phosphatase (BAP) as a bone formation marker, and tartrate-resistant acid phosphatase (TRACP)-5b as a bone resorption marker, before and after the 3-year treatment. Serum 25(OH)D_3, 1,25$(OH)_2D_3$, and whole parathyroid hormone (PTH) were also measured. Notably, no fracture occurred during the treatment. Compared with baseline values, 25(OH)D_3 levels were significantly increased from 21.6 to 26.4 ng/mL ($P = 0.006$), despite no vitamin D supplementation. 1,25$(OH)_2D_3$ and whole PTH levels tended to be decreased from 62.6 to 57.8 pg/mL and 27.3 to 25.1 pg/mL, respectively. Both bone formation and resorption markers were significantly suppressed ($P < 0.01$). Both lumbar BMD (7.3% increase) and femoral neck BMD (4.1% increase) were significantly improved ($P < 0.0001$) after 3 years of the treatment. Thus, even without vitamin D supplementation, serum 25(OH)D_3 levels were significantly increased after 3-year BP therapy. These results suggest that vitamin D supplementation might not be required in the long-term BP therapy for osteoporosis.

Keywords: bisphosphonates; fracture; native vitamin D; osteoporosis; post-menopausal women
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Introduction

Osteoporosis (OP) is characterized by the systemic impairment of bone mass, strength, and microarchitecture leading to an increased risk of fragility fracture, disability, loss of independence, and even death. Adequate nutrition is important in achieving and maintaining optimal bone mass as well as in preventing this debilitating disease. It is widely accepted that appropriate vitamin D intake is necessary for good bone health. A deficiency in vitamin D is a prominent factor in non-vertebral and hip fractures, falls, and a loss of muscle power (Brincat et al. 2015).

Third-generation nitrogen-containing bisphosphonates (BPs) inhibit farnesyl pyrophosphate synthetase in the mevalonate pathway in osteoclasts (Ste-Marie et al. 2009). Receptor activator of nuclear factor-kB ligand (RANKL) is a cytokine that is essential for osteoclast differentiation, activation, and survival (Yasuda et al. 1998). Denosumab, a fully human monoclonal antibody against RANKL, has been shown to selectively inhibit osteoclastogenesis (Bekker et al. 2004). Both BPs and denosumab increase bone mineral density (BMD) through the inhibition of osteoclast activity (Bone et al. 2004; Suzuki et al. 2017).

Bone resorption and formation usually change in parallel due to the phenomenon of coupling (Parfitt 1982). Anti-resorption drugs, such as BPs, inhibit bone resorption as well as bone formation (Uchiyama et al. 2015). The changes in bone turnover markers are influenced by serum levels of 25-hydroxyvitamin D_3 (25(OH)D_3) in osteoporotic patients receiving alendronate (ALN), a third-generation

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nitrogen-containing BP. For therapy with ALN, additional vitamin D affected bone metabolism when $25(OH)D_3$ values were low but did not influence it when $25(OH)D_3$ was high (Olmos et al. 2012). In contrast, vitamin D insufficiency was seen among patients with low BMD, but vitamin D status did not impair a response to BPs combined with vitamin D and calcium (Ca) supplementation (Antoniucci et al. 2009). At present, vitamin D supplementation is widely included in osteoporotic treatments and is considered to be an essential component in clinical trials on OP.

Weekly ALN without vitamin D did not affect serum $25(OH)\ D_3$ levels over 3 months (Olmos et al. 2012). On the other hand, some authors reported that serum $25(OH)D_3$ levels were decreased by short-term BP therapy without vitamin D (Chung et al. 2011; Kim et al. 2014; Kamimura et al. 2017). Weekly risedronate, a third-generation nitrogen-containing BP, alone significantly decreased serum $25(OH)D_3$ levels over 16 weeks of treatment. In Korean osteoporotic patients, ALN alone significantly reduced $25(OH)D_3$ status after 16 weeks (Kim et al. 2014). Thus, short-term BP administration without vitamin D supplementation could reduce $25(OH)D_3$ level. However, there are no reports concerning the effect of long-term BP therapy on serum $25(OH)D_3$ levels.

We earlier described that after 3 years of BP treatment, bilateral total hip BMD was increased, but the surrounding skeletal mass was not (Uchiyama et al. 2015). The present study examines if 3-year BP therapy without additional vitamin D significantly change the serum $25(OH)D_3$ level.

Patients and Methods

We retrospectively reviewed the clinical data of 41 post-menopausal women with OP who had also been recruited for the study of long-term BP treatment on skeletal muscle and BMD. In the current study, a total of 32 patients were investigated and the results reported elsewhere (Uchiyama et al. 2015). Another 9 patients were added for this series.

The background clinical data of the cohort is summarized in Table 1. The average age at the time of BP initiation was 73.1 years (range 43-88 years). All patients were diagnosed as having primary OP (Soen et al. 2013) and received BPs without vitamin D supplementation. No patient had any of the following conditions: systemic glucocorticoids, grade 3 or more chronic kidney disease, hyperparathyroidism, abnormalities in serum calcium or phosphorus, or diabetes. ALN was prescribed to 35 patients, risedronate was given to 5 patients, and minodronate, which is a third-generation nitrogen-containing BP was administered to 1 patient. The mean (standard deviation) height and weight were 150.5 (7.6) cm and 47.1 (7.5) kg, respectively. Prior fragility fracture was reported by 4 patients: 3 of the distal radius and 1 spinal compression fracture. Measured variables included BMD, bone specific alkaline phosphatase (BAP), tartrate-resistant acid phosphatase (TRACP)-5b, $1,25(OH)_2D_3$, $25(OH)D_3$, and whole parathyroid hormone (PTH).

Table 1. Background patient data.

Parameter	Value (n = 41)
Body height (cm)	150.5 ± 7.6 (130-167)
Body weight (kg)	47.1 ± 7.5 (32.6-69.0)
Age (years)	73.1 ± 9.2 (43-88)
History of fracture prior to BP treatment (+ : −)	4 : 37
BP treatment	Alendronate 35 cases
	Risedronate 5 cases
	Minodronate 1 case

Values are presented as mean ± standard deviation (range) where applicable.

BMD assessment

BMD values of the L2-4 lumbar spine and left femoral neck were determined by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy; GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA). Calibration of the instrument using the manufacturer's internal standard was performed before each measurement. The coefficients of variation for the lumbar spine and femoral neck were 0.7% and 1.1%, respectively (Uchiyama et al. 2015).

Biochemical markers and hormones associated with bone metabolism

BAP was determined by the chemiluminescent enzyme immunoassay, TRACP-5b was measured by the enzyme immunoassay, whole PTH was detected by immunoradiometric assay (IRMA), and $1,25(OH)_2D_3$ and $25(OH)D_3$ were assessed by radioimmunoassays. All measurements were performed by SRL Inc. (Tokyo, Japan). Blood samples were collected between mid-morning and early afternoon.

Statistical analysis

Measurements of each variable were compared immediately before and at 3 years after initiation of BPs using paired *t*-tests. The Bonferoni correction was used for multiple comparisons. A P-value of less than 0.05 was considered to be statistically significant. All statistical analyses were performed using JMP ver. 7 software (Tokyo, Japan).

This study was approved by the institutional ethics review board (Protocol No. 2796) of Shinshu University School of Medicine prior to its start and was conducted in accordance with the ethical tenets outlined in the revised 2014 Declaration of Helsinki for research involving human subjects. Written informed consent was obtained from all patients.

Results

The results for BMD and biochemical markers of bone metabolism are presented in Table 2 and described below. No fracture or other adverse effects occurred during the observational period for 3 years.

BMD values

BMD of the lumbar spine and femoral neck were significantly increased at the study end point ($P < 0.001$). The percent changes of lumbar and femoral neck BMD were 7.3% and 4.1%, respectively (Table 2).

Bone turnover markers

Bone formation and resorption markers were both significantly decreased at 3 years of BP treatment. BAP was significantly decreased from 15.6 ± 7.3 to 9.5 ± 2.7 μg/L ($P < 0.001$). TRACP-5b was significantly reduced from 424 ± 202 to 270 ± 137 mU/dL ($P < 0.001$). The percent decreases of BAP and TRACP-5b were 39.1% and 36.3%, respectively (Table 2).

Bone regulatory metabolic hormones

The serum levels of 25(OH)D_3, 1,25(OH)$_2D_3$ and whole PTH are presented in Fig. 1 and Table 1. Serum 25(OH)D_3 levels were significantly increased at 3 years of BP treatment, from 21.6 ± 8.2 to 26.4 ± 12.3 ng/mL ($P = 0.006$). The percent increase of 25(OH)D_3 was 22.2% (Fig. 1). Serum 1,25(OH)$_2D_3$ and whole PTH levels were decreased from 62.6 ± 18.0 to 57.8 ± 21.0 pg/mL and 27.3 ± 13.8 to 25.1 ± 8.8 pg/mL, respectively, albeit not significantly. The percent decreases of 1,25(OH)$_2D_3$ and whole PTH were 7.7% and 8.1%, respectively (Fig. 1, Table 2).

Table 2. BMD and biochemical markers of bone metabolism before and at 3 years of BP monotherapy.

	Before treatment (n = 41)	3 years (n = 41)	P-value	Percent change
Lumbar spine BMD (g/cm^2)	0.844 (0.169)	0.906 (0.197)*	< 0.0001	7.3 %
Femoral neck BMD (g/cm^2)	0.585 (0.100)	0.609 (0.101)*	< 0.0001	4.1 %
BAP (μg/L)	15.6 (7.3)	9.5 (2.7)*	< 0.0001	−39.1 %
Reference range: 3.8-22.6				
TRACP-5b (mU/dL)	424 (202)	270 (137)*	< 0.0001	−36.3 %
Reference range: 120-420				
1,25(OH)$_2$D (pg/mL)	62.6 (18.0)	57.8 (21.0)	0.208	−7.7 %
Reference range: 20-60				
25(OH)D_3 (ng/mL)	21.6 (8.2)	26.4 (12.3)*	0.006	22.2 %
deficiency: 20 >				
PTH (pg/mL)	27.3 (13.8)	25.1 (8.8)	0.313	−8.1 %
Reference range: 9-39				

Values are presented as mean (standard deviation).
BMD, bone mineral density; BAP, bone specific alkaline phosphatase; TRACP-5b, tartrate-resistant acid phosphatase 5b; PTH, whole parathyroid hormone.
*$P < 0.05$ between baseline and 3 years of treatment.

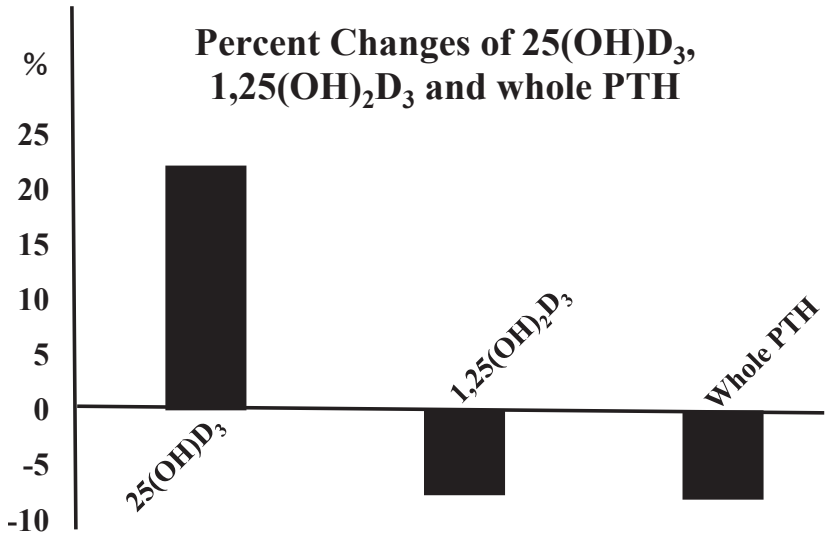

Fig. 1. Percent changes of $25(OH)D_3$, $1,25(OH)_2D_3$, and whole PTH after 3-year BP therapy, compared with the levels before treatment.

Discussion

This study revealed that serum $25(OH)D_3$ levels had become significantly increased from 21.6 to 26.4 ng/mL (22.2%) by 3 years of BP treatment without vitamin D supplementation ($P = 0.006$), whereas the levels of whole PTH and $1,25(OH)_2D_3$ were slightly decreased at the study end point.

Serum levels of Ca, $1,25(OH)_2D_3$, and PTH are tightly regulated in the human body. Circulating $25(OH)D_3$ is the major and main storage form of vitamin D. It is generally accepted that $25(OH)D_3$ levels will remain constant if nutritional conditions do not change. However, several reports have shown that short-term BP therapy reduced serum $25(OH)D_3$ (Chung et al. 2011; Kim et al. 2014). We previously reported that serum 25(OH)D levels were significantly decreased from 21.6 ± 1.4 ng/m to 18.3 ± 1.4 ng/ml (15.7%) with minodronate (1mg/day), and from 21.9 ± 1.5 ng/ml to 18.6 ± 1.5 ng/ml (13.9%) with risedronate (17.5mg/week) at 4 months (Kamimura et al. 2017). It has been unknown why serum $25(OH)D_3$ levels are decreased by short-term BP therapy. Shiraki et al. (1999) and Nakamura et al. (2015) showed that $1,25(OH)_2D_3$ levels were significantly increased soon after administration of BPs and denosumab. As the half-life of $1,25(OH)_2D_3$ is comparatively short, we hypothesize the following events: 1) BP treatment decreases serum Ca, which increases $1,25(OH)_2D_3$ levels, 2), conversion from $25(OH)D_3$ to $1,25(OH)_2D$ increases, and consequently 3) serum $25(OH)D_3$ level may be decreased (Kamimura et al. 2017).

The present study revealed that both $1,25(OH)_2D_3$ and PTH levels were slightly decreased at 3 years of BP treatment. During ALN therapy, serum $1,25(OH)_2D_3$ gradually increased and peaked at 24 weeks before returning to baseline levels (Shiraki et al. 1999). After daily 2.5 mg risedronate treatment, $1,25(OH)_2D_3$ levels were slightly increased and then became decreased at 36 weeks as compared with pretreatment values. In the long term, increased $1,25(OH)_2D_3$ levels returned to baseline levels or became decreased by BP treatment (Shiraki et al. 2003). Meanwhile, BP therapy transiently increased serum PTH and $1,25(OH)_2D_3$ levels, the levels of which were returned to baseline values within a year. Thus, long-term BP treatment slightly decreased those values, compared with the values prior to the treatment. Another important question was why serum $25(OH)D_3$ levels were increased by long-term BP therapy. We have speculated the following events: 1) a return to normal Ca level, 2) decreased serum $1,25(OH)_2D_3$, 3) a decrease in the requirement of $1,25(OH)_2D_3$ by bone metabolism inhibition, and thereby 4) increased $25(OH)D_3$ level.

Low serum levels of $25(OH)D_3$ are generally considered to be a risk factor for fracture (Gerdhem et al. 2005; Ikegami et al. 2011; Tanaka et al. 2014). The maintenance of serum $25(OH)D_3$ above 20 ng/mL was appropriate for the prevention of OP. On the other hand, elevated $25(OH)D_3$ is also a risk factor for fracture: $25(OH)D_3$ and fracture are highly associated with each other, like a U-shape (Sanders et al. 2010).

In a Korean report, serum $25(OH)D_3$ increased by combined therapy with RIS and cholecalciferol (Chung et al. 2011). Serum $25(OH)D_3$ rose 4-fold after only 3 months of ALN plus cholecalciferol treatment (Olmos et al. 2012) Furthermore, daily oral administration of high-dose cholecalciferol resulted in an increased risk of falls and fractures (Sanders et al. 2010). Alfacalcidol (ALF), 1α (OH) vitamin D3 is a vitamin D analogue that is frequently combined with BPs and other anti-resorptive drugs for the treatment of OP in Japan (Mukaiyama et al. 2015). ALF undergoes 25-hydroxylation in the liver (Orimo and Schacht 2005). And, ALF alone significantly increased $1,25(OH)_2D_3$ values (Shiraki et al. 1999). We speculate that ALF addition

increases 1,25$(OH)_2D_3$ values, which increases 25(OH)D_3 values during BP therapy.

The combination therapy of ALN and ALF showed no remarkable overall reduction in vertebral fracture following a significant decrease after the first half year (Orimo et al. 2011), implying that combined therapy may have rather increased fracture risk after 6 months. Odds ratio and 95% confidence interval for the vertebral fracture risk during 0 to 180 days and 0 to 360 by ALN therapy or by ALN plus ALF were 0.53 and 0.28 - 0.99 (P = 0.07), and 0.89 and 0.59-1.34 (P = 0.57), respectively. We speculated that in ALN alone group, decreased 25(OH)D_3 level by ALN alone therapy might raise fracture risk in short-term, while in long-term the excessive increased 25(OH)D_3 by the combined therapy of ALN and ALF also might increase fracture risk.

This study showed that 25(OH)D_3 values were significantly increased by long-term BP treatment without additional vitamin D. Based on these results of OP treatment using anti-resorptive drugs, administration of vitamin D may be essential only in the short term.

The limitations of this study were its retrospective design and relatively small number of patients.

In conclusion, the serum 25(OH)D_3 levels were significantly increased, while those of 1,25$(OH)_2D$ were slightly decreased by 3 years of BP treatment without vitamin D supplementation. From the point of views of 25(OH)D_3 level, in long-term BP therapy, vitaminD administration might not be required.

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Author Contributions

Y.N. directed this study. S.I. performed statistical analyses. S.U., M.K., M.K., and H.K. conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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