Alfacalcidol Increases the Therapeutic Efficacy of Ibandronate on Bone Mineral Density in Japanese Women with Primary Osteoporosis

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Bisphosphonates (BPs) increase bone mineral density (BMD) through the inhibition of osteoclast activity. Among BPs, ibandronate (IBN) is a strong inhibitor of bone resorption. However, the effects of a vitamin D analogue, alfacalcidol (ALF), on IBN treatment for osteoporosis is unknown. Fifty-three treatment-naïve post-menopausal women with primary osteoporosis were recruited and divided into IBN-treatment group (IBN group) and IBN with ALF group (IBN/ALF group). IBN (1.0 mg) was intravenously injected once a month, with or without oral ALF (1.0 μ g/day). Ultimately, 19 subjects in IBN group and 26 in IBN/ALF group were analyzed. Bone turnover markers were examined at 4, 6, 12, and 18 months, and BMD was measured at 6, 12, and 18 months. Compared with pre-treatment, bone turnover markers significantly decreased in both groups after 4 months. The levels of serum N-terminal propeptide of type-1 procollagen and tartrate-resistant acid phosphatase-5b, and urinary N-terminal telopeptide of type-I collagen were significantly lower in IBN/ALF group than those in IBN group at 12 months. Lumbar 1-4 (L)-BMD significantly increased from 6 months in IBN/ALF group and at 18 months in IBN group. L-BMD was significantly higher in IBN/ALF group (6.6% increase) than in IBN group (3.4%) at 18 months. Total hip (H)-BMD significantly increased from 6 months in IBN/ALF group and tended to improve in IBN group. H-BMD was significantly higher in IBN/ALF group (4.8%) than in IBN group (3.2%) at 18 months. In conclusion, treatment with ALF in combination with IBN improves BMD in post-menopausal women with osteoporosis.

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Introduction

Osteoporosis (OP) is a worldwide health concern, particularly in elderly women, that increases the risk of fracture leading to morbidity. Fracture prevention is therefore the primary therapeutic goal in patients with OP (Christiansen 1993). Bisphosphonates (BPs), which augment bone mineral density (BMD) through the inhibition of osteoclast activity, are the first-line drugs most commonly prescribed to prevent fracture and manage OP (Silverman and Christiansen 2012). Third-generation nitrogen-containing BPs inhibit farnesyl pyrophosphate synthetase in the mevalonate pathway in osteoclasts. This inhibition suppresses the function of osteoclasts and induces their apoptosis, thereby inhibiting osteoclastic activity (Ste-Marie et al. 2009).

In the Oral Ibandronate Osteoporosis Vertebral

Fracture Trial in North America and Europe (BONE) study (Chesnut et al. 2004), oral administration of anti-resorption osteoporotic drug, ibandronate (IBN) at 2.5 mg daily showed efficacy in preventing the incidence of new vertebral fracture. Among BPs, IBN is one of the strongest inhibitors of bone resorption.

However, one of the disadvantages of oral BP therapy is that patients are required to take the medication immediately after waking up and should not ingest food for 30 to 60 min afterwards, which reduces patient compliance (Chesnut et al. 2004). Injectable IBN formulations that allow for quarterly intravenous 3 mg bolus injections for OP treatment have since become available in the US and other countries. When administered intravenously, the bioavailability of IBN increases to as high as 100% and patients no longer need to manage food intake before and after treatment (Inderjeeth et al. 2014). In 2013 in Japan, a

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new IBN formulation enabling monthly intravenous 1 mg bolus injections was approved and marketed as Bonviva[®] (Chugai Pharmaceutical Co. Ltd., Tokyo, Japan).

IBN decreases the incidence of vertebral fracture in postmenopausal patients with OP (Miller et al. 2005; Eisman et al. 2008). Paggiosi et al. (2014) reported that IBN increased lumbar and total body BMD to a greater extent than did alendronate (ALN) or risedronate (RIS). The MOVER study (Nakamura et al. 2013) revealed that IBN was comparable to daily oral RIS in terms of reducing the incidence of vertebral fracture in Japanese patients with OP. Oral IBN at 2.5 mg daily decreased the risk of vertebral fracture by approximately 50% over a 3-year treatment period in the phase III BONE study (Inderjeeth et al. 2014). Moreover, no atypical fracture or osteonecrosis of the jaw occurred in these patients, likely due to the lower affinity of IBN for hydroxyapatite than other BPs apart from RIS (Inderjeeth et al. 2014). Thus, IBN represents a good therapeutic option in terms of fewer adverse effects than other BPs.

Circulating serum 25(OH)D₃ (calcifediol) is the major and main storage form of native vitamin D (cholecalciferol) in the human body. Active vitamin D (calcitriol; 1,25-dihydroxycholecalciferol; 1,25[OH]₂D₃) regulates bone and calcium (Ca) metabolism (DeLuca 2004). Active vitamin D is synthesized as follows: cholecalciferol is hydroxylated in the liver to become 25(OH)D₃, which is then hydroxylated in the kidney to produce 1,25(OH)₂D₃ (Bouillon et al. 1995). As a vitamin D analogue, 1- α hydroxycholecalciferol (alfacalcidol; ALF; 1 α (OH)D₃), has been approved for osteoporosis in Japan (Matsumoto et al. 2011) and is frequently used in OP management. Although ALF modulates serum 1,25(OH)₂D₃ and parathyroid hormone (PTH) levels, it does not affect bone turnover markers (BTMs) (Shiraki et al. 1999).

It is generally considered that vitamin D sufficiency is important during BP treatment, and so vitamin D supplementation is often provided with BP therapy. In Japan, supplementation with vitamin D is covered by national health insurance only for combined use with denosumab. Thus, additional vitamin D in patients receiving BP therapy is not formally recognized in daily practice and vitamin D supplementation typically cannot be used in the clinical research of BPs in Japan. Combination therapy of a BP and ALF is frequently used for Japanese patients with OP since ALF has been approved as an osteoporotic drug. However, there are few reports on combined therapy using a BP and ALF, and the precise effects of ALF on BP treatment for OP is controversial (Iwamoto et al. 2003; Ringe et al. 2007; Orimo et al. 2011; Mukaiyama et al. 2015). Furthermore, little is known on the role of ALF during IBN treatment in patients with OP.

We previously examined the laboratory results of the 4-month IBN treatment either alone or with ALF in Japanese patients with OP and witnessed that IBN plus ALF improved BTMs more significantly than did IBN alone (Nakamura et al. 2016a). However, the study did not include BMD data. In the present series, we prospectively investigated lumbar 1-4 BMD (L-BMD), bilateral total hip BMD (H-BMD), BTMs, $1,25(OH)_2D_3$, and whole PTH values in treatment-naïve Japanese post-menopausal women with OP who underwent 18 months of treatment with either IBN alone or IBN plus ALF.

Patients and Methods

The inclusion criteria for this study were treatment-naïve postmenopausal Japanese women with primary OP. Exclusion criteria included the presence of obvious complications, such as chronic renal failure (eGFR < 40 mL/min/1.73 m²), bone metabolic disorders, liver dysfunction, and diabetes mellitus, all of which might affect OP, as well as fracture within 1 year prior to the study. Male patients were excluded to avoid gender-related bias. In total, 56 subjects were recruited from our institutions between April 2014 and July 2016. This study was prospectively conducted by simple randomization. Intention-to-treat (ITT) and on-treatment analyses were performed.

Of the 56 eligible patients, 3 refused to participate. Twenty-five subjects in the IBN alone group (IBN group) and 28 in the IBN and ALF group (IBN/ALF group) were analyzed by ITT analysis. During the observational period, 6 of 25 patients in the IBN group and 2 of 28 patients in the IBN/ALF group dropped out due to economic reasons, leaving 19 subjects in the IBN group (mean \pm standard deviation [SD] age: 75.3 \pm 1.9 years) and 26 subjects in the IBN/ALF group (mean \pm standard deviation [SD] age: 74.9 \pm 1.5 years) for further on-treatment analysis. The diagnosis of primary OP was made in accordance with the revised criteria established by the Japanese Society of Bone and Mineral Research (Orimo et al. 2012). All patients received 1.0 mg of IBN by monthly intravenous injection. In the IBN/ALF group, patients received 1.0 μ g of oral ALF daily after their morning meal.

Serum bone alkaline phosphatase (BAP) and N-terminal propeptide of type 1 procollagen (P1NP) were measured as bone formation markers using a chemiluminescent enzyme immunoassay with inter- and intra-assay coefficients of variation (CVs) of 3.0% and 2.5%, respectively, for BAP and 2.6% and 3.9%, respectively, for P1NP. Serum tartrate-resistant acid phosphatase (TRACP)-5b and urinary N-terminal telopeptide of type I collagen (NTX) (Osteomark, Osteox International, Seattle, WA) were assessed as markers of bone resorption. TRACP-5b was measured using an enzyme-linked immunosorbent assay (ELISA) with inter- and intra-assay CVs of 2.2% and 3.2%, respectively. Urinary NTX was measured using ELISA with inter- and intra-assay CVs of 11.5% and 12.7%, respectively. Whole PTH was measured by immunoradiometric assays with inter- and intra-assay CVs of 2.3% and 2.2%, respectively. The active form of vitamin D, 1,25(OH)₂D₃, was measured by immunoradiometric assays with inter- and intra-assay CVs of 6.0% and 9.5%, respectively (Nakamura et al. 2016b). Each marker was measured just prior to IBN administration and at 4, 6, 12, and 18 months of IBN treatment. After overnight fasting, serum and first-void urine samples were collected between 8:30 AM and 10:00 AM. Immunoassays were performed by SRL, Inc. (Tokyo, Japan). BTM scores were presented on a logarithmic scale since they were not normally distributed. Whole PTH and 1,25(OH)₂D₃ concentrations were expressed as measured values. Serum samples were stored at -80°C until assessment at the end of the study.

BMD was measured using a dual-energy X-ray absorption (DXA) fan-beam bone densitometer (Lunar Prodigy; GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA.) at the lumbar 1-4 levels of the posteroanterior spine and bilateral hips. BMD was examined before treatment administration and at 6, 12, and 18 months. Percentage changes of BTMs and BMD were determined for each time point, and comparisons were made between the groups using statistical analysis. The CVs of the BMD measurements at the lumbar spine, bilateral total hips, and bilateral femoral neck were 1.0%, 0.6%, and 1.1%, respectively (Nakamura et al. 2016b). Routine quality control was ensured using a phantom box. Fracture sites were avoided during the evaluation of BMD. H-BMD was calculated as the average BMD of the right and left hips. The physicians interpreting the BMD assessments and DXA measurements and the laboratory staff performing the bone marker assays were blinded to the treatment group identities.

Results are expressed as the mean \pm standard error of the mean. For both groups, we compared the changes in markers, L-BMD, and H-BMD at each time point using the Bonferroni correction method for multiple comparisons. Comparisons of markers, L-BMD, and H-BMD between the test groups at each measurement point were performed using Welch's *t*-test. Differences were considered statistically significant at P < 0.05.

The number of prevalent vertebral fractures in the IBN group and IBN/ALF group was 5 and 4, respectively. A history of clinical fracture was present in 3 and 7 patients in the IBN group and IBN/ ALF group, respectively.

All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The ethics committee of Showa-Inan General Hospital reviewed and approved the study protocol. This investigation was approved by Clinical Trials.gov (NCT02156999; registration date: June 1, 2014).

Results

Fig. 1 presents the patient flow throughout the trial period. Of the 53 patients who commenced the trial, 8 were lost to follow-up. Nineteen patients in the IBN group and 26 in the IBN/ALF group completed the 18-month study.

The patient characteristics prior to IBN treatment are summarized in Table 1. No significant differences in age, BTMs, 1,25(OH)₂D₃, whole PTH, or BMD were found between the groups.

Serum corrected Ca and phosphorus (P) levels

The percentage changes of serum Ca and P did not change significantly within either group at any time point compared with pretreatment values (Fig. 2a, b). There were no significant differences in the percentage changes of Ca or P concentration at any time point between the groups. Serum Ca tended to decrease in the IBN group but increase in the IBN/ALF group.

BTMs

Bone formation markers: In time point comparisons, both BAP and P1NP gradually decreased during treatment in the test groups, with significant reductions recorded from 4 to 18 months (Fig. 2c, d). Although there were no significant differences in the percentage change of BAP between the groups (Fig. 2c), a significant difference was noted for P1NP at 12 months (Fig. 2d).

Bone resorption markers: In time point comparisons, both TRACP-5b and urinary NTX were significantly decreased in the test groups from 4 to 18 months (Fig. 3a,



Fig. 1. Patient flowchart.

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Table 1. The patient characteristics before treatment.

Characteristic	IBN (n=25)	IBN with ALF (n=28)	P value
Age (years)	73.8 ± 1.9	72.3 ±1.7	0.57
BMI (kg/m ²)	21.6 ± 0.6	21.3 ± 0.6	0.70
Serum corrected Ca (mg/dl)	9.2 ± 0.1	9.3 ± 0.1	0.70
Serum phosphorus (mg/dl)	3.6 ± 0.1	3.6 ± 0.1	0.52
Serum BAP ($\mu g/l$)	19.4 ± 2.2	21.4 ± 2.0	0.51
Serum P1NP (ng/mL)	63.6 ± 7.0	65.4 ± 7.6	0.86
Serum TRACP-5b (mU/dl)	541.1 ± 36.1	547.6 ± 37.3	0.90
Urinary NTX (nmol BCE/mmol CRE)	66.5 ± 9.6	67.0 ± 11.6	0.97
1,25(OH) ₂ D ₃ (pg/ml)	59.9 ± 3.1	58.1 ± 2.7	0.66
Serum whole PTH (pg/ml)	33.7 ± 2.6	31.2 ± 2.7	0.50
Lumbar1-4 BMD (g/cm ²)	0.837 ± 0.03	0.822 ± 0.03	0.74
Total hip BMD (g/cm ²)	0.669 ± 0.02	0.685 ± 0.02	0.62
Prevalent vertebral fractures, n	5	4	
History of clinical fracture, n	3	7	
	Clavicle fracture: 1	Proximal femoral fracture: 3	
	Trochanteric fracture: 1	Distal radial fracture: 2	
	Sacral fracture: 1	Rib fracture: 1	
		Proximal humeral fracture: 1	





The percentage changes of (a) serum Ca and (b) serum P did not differ significantly between the IBN and IBN/ALF group. There were no significant differences in either parameter at any time point compared with pretreatment values. (c) Serum BAP was significantly lower at every time point in both groups compared with pretreatment values. There were no significant differences in BAP between the groups during the observation period. (d) Serum P1NP was significantly lower at every time point in both groups compared with pretreatment values. There is a significant difference in P1NP at 12 months between the groups.

Closed circles indicate the IBN group and closed triangles indicate the IBN/ALF group. Double asterisks indicate a significant difference of P < 0.01. The hash mark indicates a significant difference of P < 0.05.



Fig. 3. Percentage changes of TRACP-5b, urinary NTX, whole PTH, and 1,25(OH)₂D₃.

(a) Serum TRACP-5b was significantly lower at every time point in both groups compared with pretreatment values. There was a significant difference in TRACP-5b at 12 months between the groups. (b) Urinary NTX was significantly lower at every time point in both groups compared with pretreatment values. There was a significant difference in urinary NTX at 12 months between the groups. (c) Serum whole PTH increased slightly during the observation period in the IBN group and decreased slightly in the IBN/ALF group. There were no significant differences in whole PTH during the observation period between the groups. (d) Serum $1,25(OH)_2D_3$ in both groups increased slightly during the observation period compared with pretreatment values. There were no significant differences in $1,25(OH)_2D_3$ during the observational period between the groups.

Closed circles indicate the IBN group and closed triangles indicate the IBN/ALF group. Double asterisks indicate a significant difference of P < 0.01. The hash mark indicates a significant difference of P < 0.05. The double hash marks indicate a significant difference of P < 0.01.

b). The percentage changes of TRACP-5b and urinary NTX were significantly different between the groups at 12 months (Fig. 3a, b).

Serum whole PTH and $1,25(OH)_2D_3$: There were no significant differences in whole PTH or $1,25(OH)_2D_3$ at any time point compared with pretreatment values in either group (Fig. 3c, d), nor were there any significant differences between the groups (Fig. 3c, d). PTH tended to increase in the IBN group but decrease in the IBN/ALF group (Fig. 3c).

L-BMD and H-BMD: L-BMD increased steadily for 18 months in the IBN group $(3.4 \pm 1.0\% \text{ at } 18 \text{ months})$ and in the IBN/ALF group $(6.6 \pm 1.0\% \text{ at } 18 \text{ months})$. In the IBN/ALF group, we recorded significant differences in L-BMD at every time point compared with baseline values. In the IBN group, there was a significant difference in

L-BMD at 18 months compared with pretreatment levels. At 18 months, there was a significant difference in the percentage change of L-BMD between the groups (P < 0.05) (Fig. 4a).

H-BMD increased steadily for 18 months in the IBN group $(3.2 \pm 0.7\% \text{ at } 18 \text{ months})$ and in the IBN/ALF group $(4.8 \pm 0.7\% \text{ at } 18 \text{ months})$. The percentage change of H-BMD was significantly increased at every time point in the IBN/ALF group but never in the IBN group (Fig. 3b). The percentage change of H-BMD was significantly higher in the IBN/ALF group (4.8%) than in the IBN group (3.2%) at 18 months according to ITT analysis (P < 0.05) (Fig. 4b).

No obvious adverse effects were recorded during the study period, nor did any spinal fracture occur, as confirmed by plain radiographs.



Fig. 4. Percentage changes of BMD at the lumbar spine and hips.

(a) L-BMD increased steadily over 18 months in the IBN group $(3.4\% \pm 1.0\%$ at 18 months) and in the IBN/ALF group $(6.6 \pm 1.0\%$ at 18 months). There were significant differences in L-BMD at every time point in the IBN/ALF group and at 18 months in the IBN group compared with pretreatment values. There was a significant difference in L-BMD between the groups at 18 months. (b) H-BMD increased steadily over 18 months in the IBN group $(3.2 \pm 0.7\%$ at 18 months) and in the IBN/ALF group $(4.8 \pm 0.7\%$ at 18 months). There were significant differences in H-BMD at every time point in the IBN/ALF group. The percentage change of H-BMD was significantly higher in the IBN/ALF group (4.8%) than in the IBN group (3.2%) at 18 months by ITT analysis.

Closed circles indicate the IBN group and closed triangles indicate the IBN/ALF group. The asterisk indicates a significant difference of P < 0.05. Double asterisks indicate a significant difference of P < 0.01. The hash mark indicates a significant difference at P < 0.05. The dagger indicates a significant difference at P < 0.05 by ITT analysis.

Discussion

This study evaluated the results of 18 months of IBN alone or IBN plus ALF for the treatment of OP in postmenopausal Japanese women. We observed that IBN plus ALF improved BMD and BTMs more substantially than did IBN alone, thus representing an effective osteoporotic drug combination.

In a large clinical trial in Japan, combination therapy with ALN and ALF reduced the risk of fracture compared with ALN alone, but only in the early phase (Orimo et al. 2011). A significant difference in fracture risk was noted between the groups based on sub-analyses of severe osteoporotic cases (Orimo et al. 2011). Ringe et al. (2013) evaluated the results of treatment with ALN alone versus a combination of ALN plus native vitamin D or ALF. The BMD increase in the BP alone group was minimal and was increased significantly higher in the ALN plus ALF group than in the ALN plus native vitamin D group (Ringe et al. 2013). In the present study, BMD was significantly more improved in the IBN/ALF group than in the IBN group. Based on these findings, combination therapy with a BP and ALF may be more effective in osteoporotic treatment.

Shiraki et al. (1999) compared the treatment effects of ALF alone versus ALN alone and witnessed that serum Ca decreased significantly in the ALN group but tended to increase in the ALF group. $1,25(OH)_2D_3$ was significantly improved in both groups. Serum PTH was significantly decreased in the ALF group but significantly increased in the ALF group but significantly increased in the ALF group (Shiraki et al. 1999). We speculate that BP

therapy decreases serum Ca by inhibiting bone metabolism to thereby increase the concentrations of serum PTH and $1,25(OH)_2D_3$. ALF may be converted to $1,25(OH)_2D_3$, which raises serum Ca level and decreases serum PTH (Nakamura et al. 2015). In this study, serum Ca level was lowered in the IBN group and raised in the IBN/ALF group, neither significantly, while serum PTH tended to increase in the IBN group and decrease in the IBN/ALF group. We hypothesize that the changes in PTH were caused by serum Ca values.

PTH receptor signaling in osteoblasts and osteocytes can increase the RANKL/osteoprotegerin (a decoy receptor of RANKL) ratio, thereby enhancing osteoclast recruitment and activity and stimulating bone resorption (Wong et al. 2016). Thus, the inhibitory effects of the ALF-induced increase in PTH might have reduced bone turnover to a greater extent in the IBN/ALF group. We recently reported that in patients being treated with denosumab, less inhibition of bone metabolism and diminished gains in BMD were achieved without vitamin D supplementation. Moreover, the percentage changes of whole PTH in the non-vitamin D group were significantly higher than those in the vitamin D addition group (Nakamura et al. 2016a). We presume that the greater BMD increase in the IBN/ALF group is facilitated by PTH regulation, which has strong bone-inhibitory effects, via the addition of ALF.

In the present study, bone metabolism and BMD in the IBN/ALF group showed remarkably greater improvements than in the IBN group. Based on these findings, a BP plus ALF is recommended for combination therapy for OP.

The main limitations of this study are a small sample size and the inability to generalize results beyond Japanese women with primary OP.

In conclusion, this is the first study examining both BMD and bone metabolism in Japanese women with OP undergoing treatment with either IBN alone or IBN combined with ALF. Combination therapy more significantly improved BMD and bone metabolism than did IBN alone, thus representing an effective option for OP treatment.

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Author Contributions

Y.N. directed this study. Y.N., T.S., and M.K. wrote the main manuscript. S.I., S.U., and H.K. gave suggestions on this study. All authors reviewed and approved the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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