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Osteoporosis and Sarcopenia 2 (2016) 228–237

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Original Article

Efficacy and safety of vitamin D_3 B.O.N intramuscular injection in Korean adults with vitamin D deficiency

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Received 25 July 2016; revised 21 September 2016; accepted 29 September 2016
Available online 24 October 2016

Abstract

Objective: There has been no prospective study that examined intramuscular injection of high-dose vitamin D in Korean adults. The aim of this study was to assess the efficacy and safety of high-dose vitamin D_3 after intramuscular injection in Korean adults with vitamin D deficiency.
Method: This study was a 24-week, prospective, multicenter, randomized, double-blind, placebo-controlled trial. A total of 84 subjects ≥19 and <65 years of age were randomly allocated to either the vitamin D_3 or placebo group in a 2:1 ratio. After randomization, a single injection of plain vitamin D_3 200,000 IU or placebo was intramuscularly administered. If serum 25-hydroxyvitamin D (25[OH]D) concentrations were <30 ng/mL on week 12 or thereafter, a repeat injection was administered.
Results: After a single intramuscular injection of vitamin D_3 to adults with vitamin D deficiency, the proportion of subjects with serum 25(OH)D concentrations ≥30 ng/mL within 12 weeks was 46.4% in the vitamin D_3 group and 3.6% in the placebo group ($p < 0.0001$). The proportion of subjects with serum 25(OH)D concentrations ≥30 ng/mL within 24 weeks was 73.2% in the vitamin D_3 group and 3.6% in the placebo group ($p < 0.0001$). Mean change in serum 25(OH)D concentrations at weeks 12 and 24 after vitamin D_3 injection was 12.8 ± 8.1 and 21.5 ± 8.1 ng/mL, respectively, in the vitamin D_3 group, with no significant changes in the placebo group. Serum parathyroid hormone concentrations showed a significant decrease in the vitamin D_3 group but no change in the placebo group.
Conclusion: Intramuscular injection of vitamin D_3 200,000 IU was superior to placebo in terms of its impact on serum 25(OH)D concentrations, and is considered to be safe and effective in Korean adults with vitamin D deficiency.

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Keywords: 25-Hydroxyvitamin D; Koreans; Intramuscular injection; Cholecalciferol

1. Introduction

Vitamin D is essential in bone and mineral metabolism. Accordingly, low vitamin D status is associated with osteoporosis and fractures. Severe deficiency of vitamin D can lead to bone mineralization defects, such as rickets in children and osteomalacia in adults [1,2]. Recently, it has been suggested that vitamin D also has important roles in other tissues besides the skeletal system with its deficiency closely associated with an increased risk of several non-skeletal disorders, such as cancers, infection, autoimmune diseases, cardiovascular diseases, and diabetes mellitus [3–8]. Despite growing awareness of the multiple health benefits of vitamin D, vitamin D deficiency has become a major health concern in modern society. As more people spend a majority of their time indoors, sunlight exposure can be inadequate for cutaneous production of vitamin D. Epidemiological studies have indicated a high

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Peer review under responsibility of The Korean Society of Osteoporosis.

http://dx.doi.org/10.1016/j.afos.2016.09.004
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prevalence of vitamin D deficiency worldwide, especially in Asian countries [9–12]. When serum 25-hydroxyvitamin D (25[OH]D) level of 30 ng/mL was adopted as the cut-off value, the prevalence of vitamin D insufficiency in Korean general population was 86.8% in men and 93.3% in women, which was higher than that of the United States and Canada [13].

The increasing prevalence of limited exposure to sunlight is increasing the importance of dietary sources of vitamin D in maintaining people's vitamin D status in modern society. Fatty fish, egg yolks, and mushrooms are some of the few natural foods that contain vitamin D. In western countries, vitamin D is supplied in the diet of many people by fortifying foods. In the 1930s, a milk fortification program was implemented in the United States to combat rickets [14]. Other vitamin D fortified food products in western countries are breakfast cereals, orange juice, yogurt, and margarine. However, food fortification is uncommon in Asian countries.

A more realistic and easier way to get enough vitamin D is supplementation through either oral or intramuscular route. High-dose vitamin D administered intramuscularly has been effectively used to achieve and maintain individual's sufficient vitamin D status. However, there has been no prospective study of intramuscular injection of high-dose plain vitamin D in Korean adults. This prospective, multicenter, randomized, double-blind, placebo-controlled study was conducted to compare 200,000 IU of vitamin D_3 with placebo in terms of efficacy and safety for 24 weeks after intramuscular injection in Korean adults with vitamin D deficiency.

2. Subjects and methods

2.1. Study subjects

The study subjects were recruited at three different institutions (Yonsei University Severance Hospital, Ajou University Hospital, Dongguk University Ilsan Hospital) in South Korea. After the screening test, male or female subjects with serum 25(OH)D concentration <20 ng/mL who were between 19 and 65 years of age, were enrolled in this study. Reasons for exclusion included history of hypersensitivity reactions to cholecalciferol component; renal disorder (serum creatinine >1.25× upper limit of normal [ULN]); hypercalcemia (serum calcium >10.5 mg/dL); hypercalciuria (urine calcium >4 mg/kg/day or urine calcium [mg/dL]/creatinine [mg/dL] ratio >0.2); suspected calcium stone with clinical findings; sarcoidosis; pseudohypoparathyroidism; malignancies (a patient who was judged to have been cured as 5 years had passed since treatment was able to participate in the study); clinically significant cardiovascular and pulmonary function disorder based on the judgment of the investigator; laboratory test findings as follows: platelet <100,000/mm^3, white blood cell (WBC) < 3000/mm^3, absolute neutrophil count <1500/mm^3, albumin <3.0 g/dL, aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >1.5× ULN; treatment with phenytoin or barbiturates within 5 days prior to the investigational product injection; treatment with thiazide diuretics within 3 days prior to the investigational product injection; treatment with glucocorticoids, cholestyramine, colestipol, or cardiac glycosides within 1 day prior to the investigational product injection; requirement of vitamin D supplements during the study; use of tanning booths during the study; pregnant and lactating female; women of childbearing potential who do not use adequate contraception; and individuals deemed inappropriate for study participation by the investigator, currently participating in another clinical trial or having had the last dose of another investigational product within the past 4 weeks. A total of 84 subjects (56 in the vitamin D_3 group and 28 in the placebo group, planned to be assigned in a 2:1 ratio) were enrolled (Fig. 1). Subjects were to be stratified by serum 25(OH)D concentrations (<10 ng/mL or 10–20 ng/mL) in a 1:1 ratio. This study was carried out in accordance with principles of Korea Good Clinical Practice, International Conference on Harmonization-Good Clinical Practice, Declaration of Helsinki, and local laws and applicable regulations. This study was approved by the Institutional Review Board (IRB) at each institution (IRB No. 4-2014-0377, Yonsei University Severance Hospital; IRB NO. AJIRB-MED-CT3-13-397, Ajou University Hospital; IBR No. 2014-16, Dongguk University Ilsan Hospital). All subjects provided their written informed consent for the study after they were provided a detailed description of the experimental procedures and informed that they could withdraw from the study at any time.

2.2. Study design

The 24-week, prospective, multicenter, randomized, double-blind, placebo-controlled clinical trial was conducted between from November 2014 to August 2015 at the three aforementioned institutions in Korea. Following the screening visit, at which inclusion and exclusion criteria were assessed, subjects were randomly assigned to either the vitamin D_3 group or the placebo group in a 2:1 ratio. Vitamin D_3 B.O.N was manufactured by Haupt Pharma Livron (France) as a fragrance free, slightly yellow, transparent injectable solution (200,000 IU [5 mg] as cholecalciferol) contained in a colorless and transparent ampoule. All participants, investigators, pharmacists, and study personnel were blinded to treatment allocation. This study was comprised of period 1 and 2 (Fig. 2). Period 1 was the duration of time from baseline to the end of week 12. During this time, the first injection of vitamin D_3 and tests for primary efficacy assessment were carried out. Period 2 was the duration of time from week 13 to the end of week 24. During this time, subjects who had serum 25(OH)D concentrations <30 ng/mL at the time of week 12 or thereafter received a repeat single injection of the investigational product and subjects ≥30 ng/mL were followed up to week 24 without an additional injection. The enrolled subjects were scheduled to visit 9 times (at week 0, 2, 4, 6, 8, 12, 14, 18, and 24) during the trial and their clinical information and trial data were collected during individual interviews conducted by a well-trained interviewer. All subjects underwent a through medical history review and a physical examination. During the

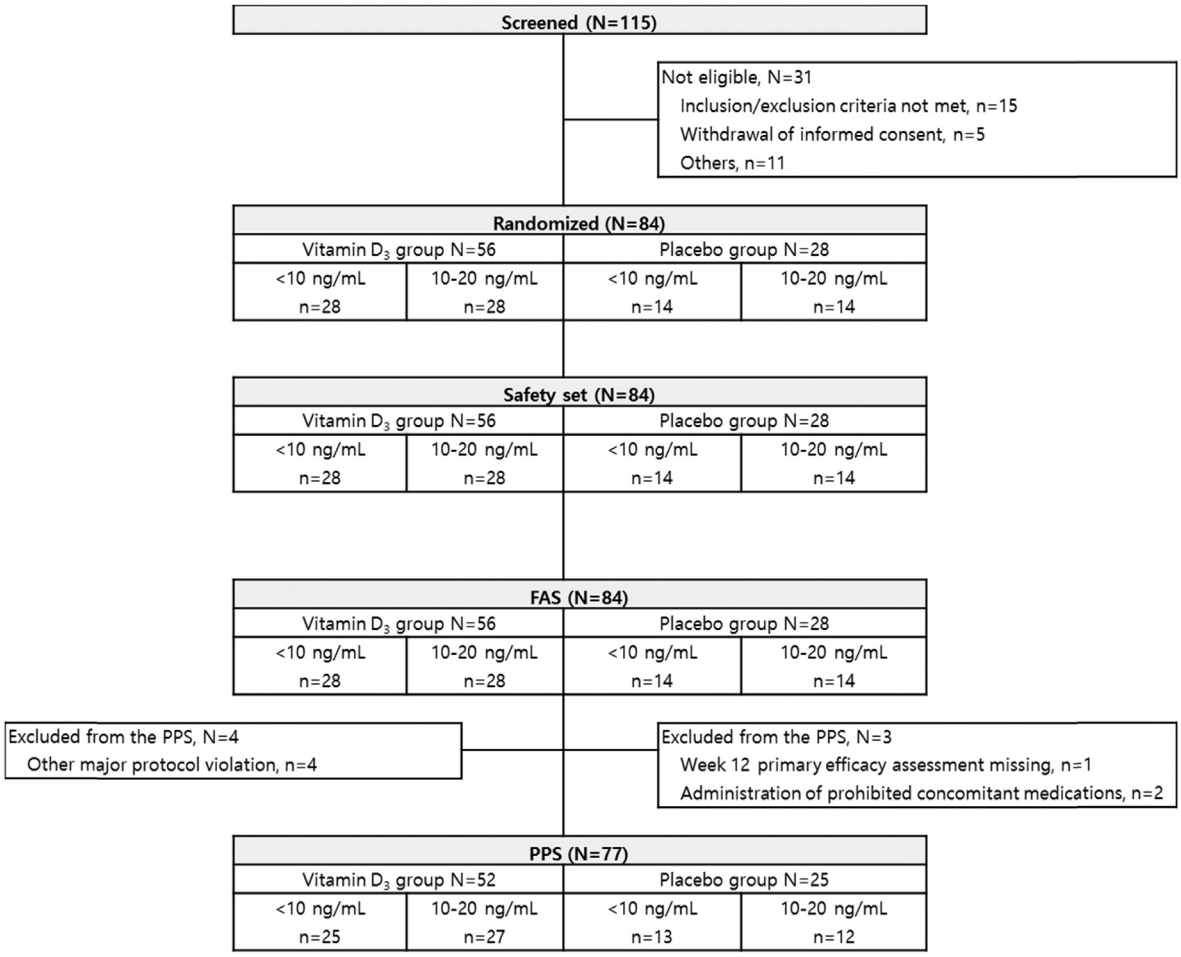

Fig. 1. Randomization scheme and subject disposition.

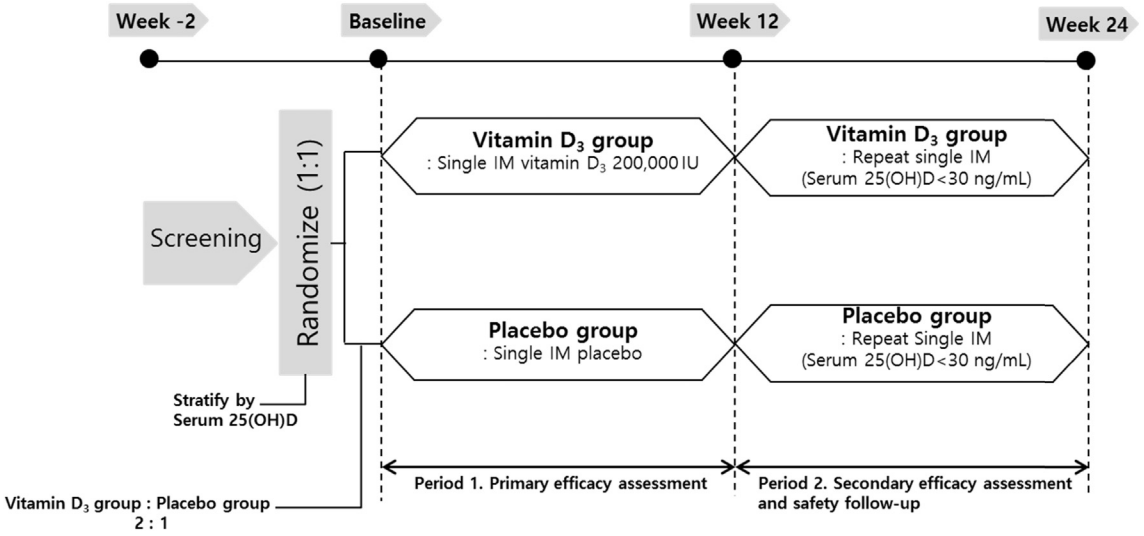

Fig. 2. Study design.

intervention period, the subjects were prohibited from any additional vitamin D supplements. Exposure to direct sunlight for 10 h or longer per week, trips to countries with more sunshine than Korea during the study, sunbath, or long hours or working under direct sunlight were also prohibited. Usual diets were to be maintained and diets containing vitamin D that could affect the efficacy of the investigational product (cod liver oil, cooked salmon and mackerel, sardine, etc.) were

restricted. The subjects had to notify drugs administered during the study to the investigators, and had to discuss in advance if he/she had to receive additional drugs or change type or dosage of drugs. If a drug administered without the judgment of the principal investigator was expected to affect efficacy and safety assessments for this study, the relevant subject was to be withdrawn.

2.3. Efficacy

The primary efficacy endpoint was proportion of subjects with improved serum 25(OH)D concentrations ≥30 ng/mL within 12 weeks after the investigational product injection. The secondary efficacy endpoints were proportion of subjects with improved serum 25(OH)D concentrations ≥30 ng/mL at week 4, 6, 8, 12, 18, and 24; proportion of subjects with improved serum 25(OH)D concentrations ≥20 ng/mL at weeks 4, 6, 8, 12, 18, and 24; and change in serum 25(OH)D concentrations at weeks 4, 6, 8, 12, 18, and 24 after the investigational product injection from baseline. Exploratory endpoints were mean change in maximum serum 25(OH)D concentration (ΔC_{max}), mean time to C_{max} (T_{max}), and area under the curve $(AUC)_{12weeks}$ for serum 25(OH)D concentrations after a single injection of the investigational product, and change in serum parathyroid hormone (PTH) concentrations at week 4, 8, 12, 18, and 24 after the investigational product injection.

2.4. Safety

Safety was assessed by adverse events (AEs) reported by subjects, physical examination, electrocardiography, and laboratory parameters. During the trial, all subjects were asked to report potential AEs. Vital signs of each subject including systolic and diastolic blood pressure and pulse rate were measured at every visit. Clinical evaluations and laboratory measurements, including serum chemistry and hematology were performed at selected visits. Proportion of subjects with increased serum 25(OH)D concentrations ≥60, ≥100, and ≥150 ng/mL after vitamin D_3 injection was determined at week 4, 6, 8, 12, 18, and 24. Serious AEs (SAEs) were immediately reported to the Kwangdong Pharmaceutical Co., Ltd within 24 h after recognition regardless of the investigator's assessment of a causal relationship to study drug.

2.5. Laboratory measurements

Serum 25(OH)D and intact PTH were analyzed at the central laboratory. Serum 25(OH)D concentrations were measured by electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics, Mannheim, Germany). The intra- and interassay coefficient of variation (CV) of 25(OH)D measurements was 2.2–6.9 and 3.4–13.1%, respectively. Intact PTH concentrations were also measured by ECLIA (Roche Diagnostics, Mannheim, Germany). The intra- and interassay CV of intact PTH measurements was 1.1–2.0 and 2.8–3.4%, respectively. Clinical chemistry test (glucose, calcium, phosphorus, blood urea nitrogen, creatinine, total bilirubin, AST, ALT, gamma glutamyl transpeptidase, alkaline phosphatase, total protein, sodium, potassium, total cholesterol, albumin, and insulin), hematologic test (hemoglobin, hematocrit, platelet count, WBC count with differential count, and hemoglobin A1c), and urinalysis (pH, protein, glucose, blood, ketones, urobilinogen, bilirubin, nitrite, leukocytes) were assessed at each site.

2.6. Statistical analyses

Efficacy was analyzed in the full analysis set (FAS) population defined as subjects who had at least one assessment of efficacy after randomization, and in the per-protocol set (PPS) defined as the FAS subjects who completed the study without major protocol deviations. Safety was analyzed in the safety set defined as randomized subjects who received at least one injection of the study drug. All statistical tests were to be performed as two-sided tests at a significance level of 0.05 in this study, in principle. Number of subjects, mean ± standard deviation (SD) or standard error (SE), median, minimum, and maximum were presented for continuous variables and frequency and percentage were presented for categorical variables. Continuous variables were tested for normality. Two-sample t-test, paired t-test, Wilcoxon rank sum test, or Wilcoxon signed rank test was performed depending on whether normality assumption was met. Categorical variables were analyzed using Pearson's chi-square test or Fisher's exact test depending on whether ≥80% had an expected value of ≥5. Calculations and statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC, USA).

3. Results

Out of 115 subjects who were screened, 84 subjects were enrolled, with 56 randomized to the vitamin D_3 group and 28 to the placebo group. Among those, 4 subjects dropped out due to withdrawal of informed consent (2 subjects in the vitamin D_3 group and 2 subjects in the placebo group) and the remaining 80 subjects (54 subjects in the vitamin D_3 group and 26 subjects in the placebo group) completed the study. All of the 84 randomized subjects were included in the safety set and the FAS. Among those, a total of 7 subjects were excluded from the PPS; they comprised 1 subject in the placebo group with missing primary efficacy assessment at week 12 visit, 2 subjects in the placebo group with administration of prohibited concomitant medications, and 4 subjects in the vitamin D_3 group with other major protocol violation. The remaining 77 subjects were included in the PPS (Fig. 1).

3.1. Demographic and other baseline characteristics of subjects

Mean age of subjects was 37.4 ± 10.4 years in the vitamin D_3 group and 36.2 ± 10.6 years in the placebo group. Mean body mass index (BMI) was 22.3 ± 2.6 kg/m^2 and 21.9 ± 2.0 kg/m^2 in the vitamin D_3 and the placebo group,

respectively. There were no statistically significant differences in demographic information and other baseline characteristics of subjects between the groups (Table 1).

3.2. Efficacy

The proportion of subjects with improved serum 25(OH)D concentrations ≥30 ng/mL within 12 weeks after the investigational product injection, the primary efficacy endpoint in this study, was 46.4% (26/56) in the vitamin D_3 group and 3.6% (1/28) in the placebo group ($p < 0.0001$), and the proportion of those ≥20 ng/mL within 12 weeks after injection was 92.9% (52/56) in the vitamin D_3 group and 10.7% (3/28) in the placebo group ($p < 0.0001$) (Fig. 3). After week 12, the investigational product repeat injection rate in the vitamin D_3 group was 76.8% (43/56), 7.1% (4/56), and 3.6% (2/56) at week 14, 18, and 20, respectively, resulting in a total repeat injection rate of 87.5% (49/56). Meanwhile, in the placebo group, all subjects except one who had been prematurely withdrawn received the repeat injection of the investigational product, resulting in a total repeat injection rate of 96.4% (27/28).

Concerning the secondary efficacy endpoints, the proportion of subjects with serum 25(OH)D concentrations ≥30 ng/mL at week 4, 6, 8, 12, 18, and 24 after the investigational product injection was 25.0% (14/56), 26.8% (15/56), 28.6% (16/56), 21.4% (12/56), 60.7% (34/56), and 62.5% (35/56), respectively, in the vitamin D_3 group. Additionally, the proportion of subjects with improved 25(OH)D concentrations ≥30 ng/mL within each time point was 28.6% (16/56), 33.9% (19/56), 44.6% (25/56), 46.4% (26/56), 71.4% (40/56), and 73.2% (41/56) within week 4, 6, 8, 12, 18, and 24, respectively (Table 2). The proportion of subjects with serum 25(OH)D concentrations ≥20 ng/mL at week 4, 6, 8, 12, 18, and 24 after the investigational product injection was 73.2% (41/56), 83.9% (47/56), 75.0% (42/56), 64.3% (36/56), 94.6% (53/56), and 96.4% (54/56), respectively, in the vitamin D_3 group. Additionally, the proportion of subjects with improved serum 25(OH)D concentrations ≥20 ng/mL within each time point was 78.6% (44/56), 91.1% (51/56), 92.9% (52/56), 92.9% (52/56), 98.2% (55/56), and 100.0% (56/56) within week 4, 6, 8, 12, 18, and 24, respectively (Table 2). The proportions of subjects with serum 25(OH)D concentrations ≥30 or ≥20 ng/mL within each time point depending on repeat injection of the investigational product were shown in Supplemental Table 1. At baseline, mean serum 25(OH)D concentration was 10.2 ± 3.9 ng/mL in the vitamin D_3 group and 10.3 ± 3.6 ng/mL in the placebo group. Mean serum 25(OH)D concentration up to week 8 following a single injection of the vitamin D_3 200,000 IU was 25.6 ng/mL, which slightly declined at week 12, and then showed an additional increase following the repeat injection, resulting in a mean serum 25(OH)D concentration of 31.7 ng/mL at week 24. In the placebo group, serum 25(OH)D concentrations at each time point showed almost no change. Changes in mean serum 25(OH)D concentrations over 24-week treatment period depending on repeat injection are shown in Fig. 4.

In an exploratory analysis, ΔC_{max} from baseline after a single injection of the vitamin D_3 200,000 IU was 21.2 ± 7.2 ng/mL and T_{max} was 55.2 ± 25.5 days. In addition, $AUC_{12weeks}$ after a single injection of the vitamin D_3 200,000 IU was 1064.7 ± 435.5 ng·day/mL (Table 3). Mean change in serum PTH concentrations at weeks 4, 8, 12, 18, and 24 after the investigational product injection in the vitamin D_3 group was −1.8 ± 13.5, −4.1 ± 10.9, −0.6 ± 24.0, −6.0 ± 9.5, and −3.3 ± 10.9 pg/mL, respectively, which indicated a statistically significant intra-group decrease ($p = 0.0009$). In the placebo group, however, the respective mean concentration change was −1.2 ± 14.0, −1.6 ± 14.8, −2.1 ± 14.6, −5.2 ± 12.3, and 1.0 ± 16.0 pg/mL, which showed no statistically significant intra-group change ($p = 0.1084$). There was a statistically significant inter-group difference at week 8, 18, and 24 (week 8: $p = 0.0045$, week 18: $p = 0.0237$, week 24: $p = 0.0319$).

3.3. Safety

During the study, the incidence rate of treatment emergent AEs was 57.1% in the vitamin D_3 group and 67.9% in the placebo group (Supplemental Table 2A). Of the total 62 AEs in the vitamin D_3 group, 59 events (95.2%) were mild and 3 events (4.8%) were moderate. Of the total 32 AEs in the placebo group, 30 events (93.8%) were mild and 2 events (6.3%) were moderate. Neither group had severe AEs. Among the mild AEs, 9 events (14.5%) in the vitamin D_3 group and 3 events (9.4%) in the placebo group were adverse drug reactions (Supplemental Table 2B). The incidence rate of SAEs was 1.8% in the vitamin D_3 group and 3.6% in the placebo group (Supplemental Table 2C). All of the 3 SAEs (2 instances of appendicitis and 1 instance of cholecystitis) were assessed as not related in terms of causal relationship to the investigational product. In the analysis of laboratory tests, vital signs, physical examination, and ECG, several items showed statistically significant differences between baseline and week 24 results, all of which, were changes within clinically normal ranges and were not clinically significant. In addition, no

Table 1
Demographic and other baseline demographic characteristics of the study participants.

Variable	Vitamin D_3 group (N = 56)	Placebo group (N = 28)	p
Age (years)	37.4 ± 10.4	36.2 ± 10.6	0.6079[b]
Gender (M/F)	9/47	3/25	0.7426[c]
Height (cm)	163.2 ± 6.3	163.3 ± 6.2	0.7609[b]
Body weight (kg)	59.5 ± 8.0	58.5 ± 7.5	0.6135[a]
BMI (kg/m^2)	22.3 ± 2.6	21.9 ± 2.0	0.4662[a]
Serum 25(OH)D (ng/mL)	10.2 ± 3.9	10.3 ± 3.6	0.8736[a]
10–20 at baseline	13.4 ± 2.3	13.1 ± 2.7	0.6694[b]
<10 at baseline	7.0 ± 2.1	7.5 ± 1.5	0.3527[a]
Serum calcium (mg/dL)	9.2 ± 0.3	9.2 ± 0.3	0.6401[a]
Serum phosphorus (mg/dL)	3.4 ± 0.5	3.6 ± 0.4	0.2082[a]
Hemoglobin A1C (%)	5.4 ± 0.3	5.3 ± 0.3	0.2927[b]

Data are mean ± SD, except gender.
BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D.
[a] Two-sample *t*-test.
[b] Wilcoxon rank sum test.
[c] Fisher's exact test.

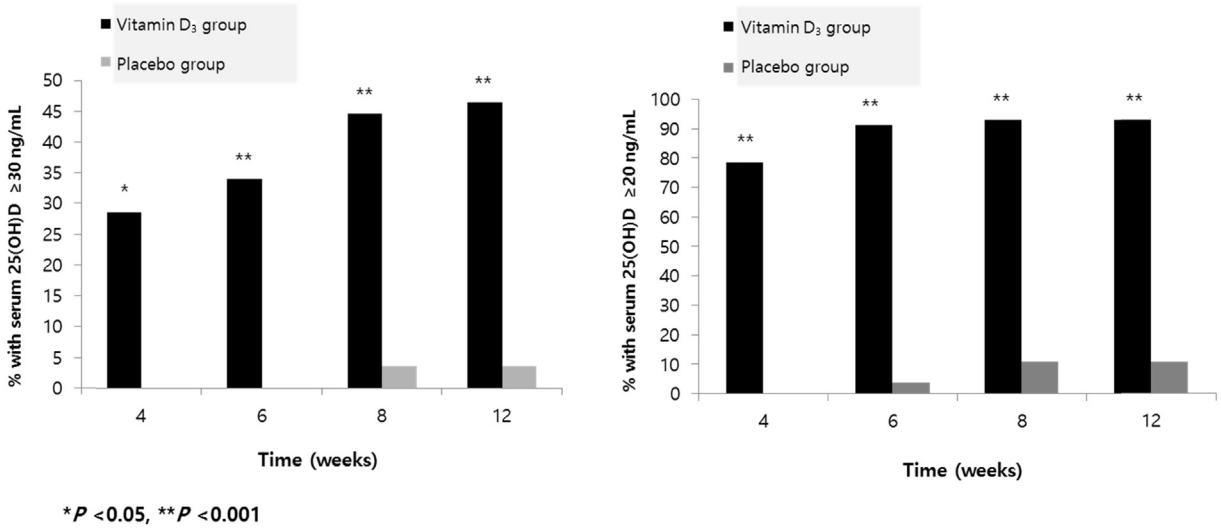

Fig. 3. Proportion of subjects whose 25(OH)D levels increase to 20 or 30 ng/mL or higher during the 12-week treatment period. 25(OH)D, 25-hydroxyvitamin D.

Table 2
Proportion of subjects whose 25(OH)D concentrations increased ≥30 or ≥20 ng/mL during the 24-week treatment period.

	Serum 25(OH)D concentrations ≥30 ng/mL			Serum 25(OH)D concentrations ≥20 ng/mL		
	Vitamin D_3 group (N = 56)	Placebo group (N = 28)	p	Vitamin D_3 group (N = 56)	Placebo group (N = 28)	p
	n (%)	n (%)		n (%)	n (%)	
Within week 4	16 (28.6)	0 (0.0)	0.0017[a]	44 (78.6)	0 (0.0)	<0.0001[a]
Within week 6	19 (33.9)	0 (0.0)	0.0005[a]	51 (91.1)	1 (3.6)	<0.0001[a]
Within week 8	25 (44.6)	1 (3.6)	0.0001[a]	52 (92.9)	3 (10.7)	<0.0001[a]
Within week 12	26 (46.4)	1 (3.6)	<0.0001[a]	52 (92.9)	3 (10.7)	<0.0001[a]
Within week 18	40 (71.4)	1 (3.6)	<0.0001[a]	55 (98.2)	3 (10.7)	<0.0001[a]
Within week 24	41 (73.2)	1 (3.6)	<0.0001[a]	56 (100.0)	4 (14.3)	<0.0001[a]

25(OH)D, 25-hydroxyvitamin D.
[a] Pearson's chi-square test.

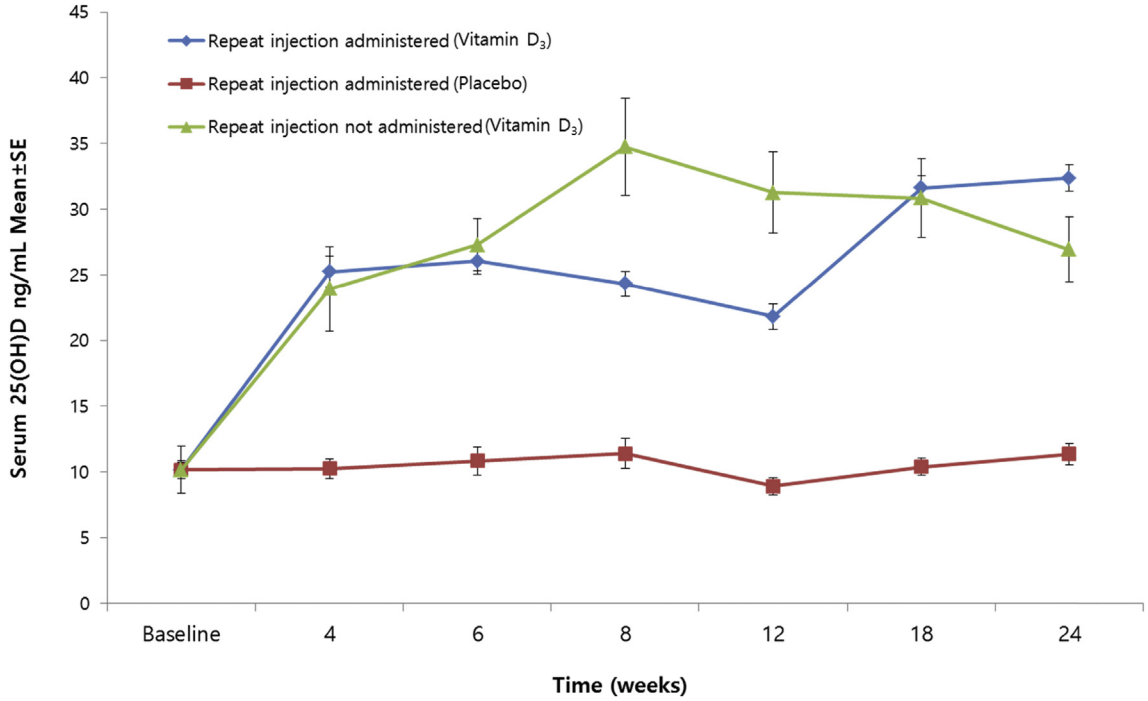

Fig. 4. Mean serum 25(OH)D levels over the 24-week treatment period. 25(OH)D, 25-hydroxyvitamin D.

Table 3
Pharmacokinetic parameters including ΔC_{max}, T_{max}, and $AUC_{12weeks}$ after a single injection of the vitamin D_3 200,000 IU.

Pharmacokinetic parameters (n = 56)	Mean ± SD	Median	Minimum, maximum
ΔC_{max} (ng/mL)	21.2 ± 7.2	20.4	7.9, 41.3
T_{max} (day)	55.2 ± 25.5	55.0	13.0, 102.0
$AUC_{12weeks}$ (ng·day/mL)	1064.7 ± 435.5	1096.7	197.5, 2136.0

subject showed an increase in serum 25(OH)D concentrations to ≥60 ng/mL at any time point up to week 24 after the investigational product injection.

4. Discussion

The present study shows that a single injection of vitamin D_3 200,000 IU was able to increase serum 25(OH)D concentrations to ≥30 ng/mL in almost half of subjects, and ≥20 ng/mL in over 90% of the subjects within 12 weeks. A repeat injection of vitamin D_3 200,000 IU was able to increase serum 25(OH)D concentrations to ≥30 ng/mL in more than 70% of subjects and to ≥20 ng/mL in all subjects within 24 weeks. These results indicate that intramuscular injection of vitamin D_3 200,000 IU is an effective way to increase serum 25(OH) concentrations and achieve an adequate or sufficient vitamin D status in Korean adults with vitamin D deficiency defined as serum 25(OH)D concentration <20 ng/mL. However, some subjects (28.57%) still could not achieve serum 25(OH)D concentrations ≥30 ng/mL by week 24 after a repeat injection, which means that multiple injections (≥2 times) of high-dose vitamin D_3 for more than 24 weeks are needed to achieve sufficient vitamin D level in many patients with vitamin D deficiency. Nonetheless, all subjects achieved their serum 25(OH)D concentrations above 20 ng/mL within week 24. Although serum 25(OH)D concentration ≥30 ng/mL is generally accepted as the optimal level for skeletal health, especially in those with established osteoporosis [15], a number of researchers and organizations support a serum 25(OH)D concentration of 20 ng/mL as an enough level for general population [14,16]. Likewise, both the Korean Society of Osteoporosis and the Korean Society for Bone and Mineral Research also recommend a serum 25(OH)D concentration ≥20 ng/mL as an appropriate level for prevention of osteoporosis in general population of Korea [17].

There has been no randomized controlled trial (RCT) that investigated the efficacy of vitamin D replacement in terms of fracture or fall in Korean population. Only a few prospective RCTs including current one have assessed the efficacy of vitamin D replacement in Korean population in terms of change in serum 25(OH)D concentration. Two previous RCTs investigated the efficacies of orally administered vitamin D combined with bisphosphonates [18,19]. One RCT compared the efficacy of once weekly alendronate 70 mg plus vitamin D_3 5600 IU with alendronate 70 mg without additional vitamin D in Korean osteoporotic women whose mean serum 25(OH)D concentration was 18.71 ± 6.50 ng/mL at baseline [18]. Once weekly alendronate plus vitamin D_3 5600 IU was able to increase the serum 25(OH)D levels to 15 ng/mL or greater in over 98% of subjects after 16-week treatment. The mean serum 25(OH)D levels after 8 and 16 weeks were 28.35 ± 5.03 and 30.08 ± 5.87 ng/mL, respectively, in the once weekly alendronate plus vitamin D_3 5600 IU group compared with 17.99 ± 5.47 and 17.14 ± 6.04 ng/mL in the alendronate alone group, which makes the mean differences in serum 25(OH)D levels between two groups as 10.41 and 12.94 ng/mL at week 8 and 16, respectively. Another RCT also compared the efficacy of a once monthly ibandronate 150 mg plus vitamin D_3 24,000 IU with a once monthly ibandronate 150 mg without additional vitamin D in postmenopausal women whose mean serum 25(OH)D concentration was about 21 ng/mL at baseline [19]. This study showed that the proportion of patients with a 25(OH)D level >20 ng/mL increased from 46.9% to 63.5% in the ibandronate plus vitamin D_3 24,000 IU group but decreased from 40.0% to 26.3% in the ibandronate alone group after 16-week treatment. The mean serum 25(OH)D levels after 8 and 16 weeks were 24.8 ± 9.0 and 25.3 ± 9.9 ng/mL, respectively, in the once monthly ibandronate plus vitamin D_3 24,000 IU group compared with 18.3 ± 7.4 and 17.4 ± 9.2 ng/mL in the ibandronate alone group, which made the mean differences in serum 25(OH)D levels between two groups as 6.5 and 7.9 ng/mL at week 8 and 16, respectively. Therefore, although it is difficult to directly compare the efficacy of vitamin D replacement between these previous RCTs and our study, intramuscular injection of vitamin D_3 200,000 IU seems to be a better strategy to improve serum 25(OH)D concentration considering that the mean differences in serum 25(OH)D levels between vitamin D_3 and placebo group were 14.1, 13.9, 21.0, and 20.3 ng/mL at week 8, 12, 18, and 24, respectively, in the present study.

As an exploratory endpoint in the present study, mean serum PTH concentrations showed a significant decrease over 24 weeks after investigational product injection in the vitamin D_3 group, but not in the placebo group, which made statistically significant inter-group difference at week 8, 18, and 24. Because high PTH may affect bone metabolism negatively by increasing bone turnover, it is expected that decreased PTH level after vitamin D_3 injection can be beneficial for skeletal health. Results from the subgroup analyses in the present study showed that mean change in serum 25(OH)D concentrations at week 4, 6, 8, and 12 after the vitamin D_3 injection from baseline was an increase of 16.6 ng/mL, 17.2 ng/mL, 17.5 ng/mL, and 14.8 ng/mL, respectively, among the subjects with serum 25(OH)D concentrations <10 ng/mL at screening and an increase of 13.2 ng/mL, 14.9 ng/mL, 13.4 ng/mL, and 10.9 ng/mL, respectively, among the subjects with serum 25(OH)D concentrations 10–20 ng/mL at screening. These results indicate a greater increase of serum 25(OH)D concentrations in the subgroup with serum 25(OH)D concentrations <10 ng/mL prior to administration of vitamin D_3 than in the 10–20 ng/mL subgroup, which suggests that vitamin D_3 injection may increase serum 25(OH)D concentrations more effectively in those with more severe vitamin D deficiency.

With regards to the safety, replacement of high-dose vitamin D, especially with calcium can cause toxicity

including hypercalcemia and hypercalciuria [17]. The Women's Health Initiative study also showed an increased incidence of urinary tract stones in those who were taking vitamin D and calcium supplements [20], although it is unlikely that treating vitamin D deficiency with vitamin D alone would increase the risk of urinary tract stones. Recently, some observational cohort studies have shown a reverse-J-shaped curve for the relationship between serum 25(OH)D concentration and all-cause or cardiovascular mortality with increased risk at both lower and higher serum levels of 25(OH)D [21,22]. In the present study, hypercalcemia occurred in only 1.8% (1/56 subjects, 1 event) in the vitamin D_3 group, which was found to be mild. Hypercalciuria occurred in 5.4% (3/56 subjects, 3 events) in the vitamin D_3 group and 7.1% (2/28 subjects, 3 events) in the placebo group without significant difference. In addition, no subject showed an increase in serum 25(OH)D concentrations to ≥60 ng/mL at any time point up to week 24 after the investigational product injection. Therefore, there is little chance of excessive increase of vitamin D level after intramuscular injection of vitamin D_3 200,000 IU.

In conclusion, intramuscular injection of vitamin D_3 200,000 IU in adults with vitamin D deficiency was demonstrated to be superior to placebo in terms of the proportions of subjects with achieved serum 25(OH)D concentrations ≥30 or ≥20 ng/mL. In addition, change in serum 25(OH)D concentrations over 24 weeks after vitamin D_3 injection also indicated a statistically significant increase in the vitamin D_3 group compared with the placebo group, and change in serum PTH concentrations showed a statistically significant decrease both within the vitamin D_3 group and between the vitamin D_3 and placebo groups. Therefore, mega-dose intramuscular injection of vitamin D_3 200,000 IU is considered to be an effective and safe strategy to improve vitamin D level in Korean adults at the age of ≥19 and < 65 with vitamin D deficiency.

Conflict of interest

YSC (Yoon-Sok Chung) has received funding and/or honoraria from Pfizer, Takeda, MSD, Sanofi, Yuyu, Hanlim, Kwangdong, and GSK, and is a member of the advisory boards of MSD and Yuyu. Other authors have no conflict of interest.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2015R1C1A1A01054333), and also sponsored by Kwangdong Pharmaceutical Co., Ltd.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.afos.2016.09.004.

Appendix

Appendix Table 1
Proportion of subjects whose 25(OH)D concentrations increased ≥30 or ≥20 ng/mL during the 24-week treatment period depending on repeat injection (subgroup analyses).

A. Serum 25(OH)D concentrations ≥30 ng/mL.

	Repeat injection administered					Repeat injection not administered[c]				
	Vitamin D_3 group (N=49)		Placebo group (N=27)		p	Vitamin D_3 group (N=7)		Placebo group (N=1)		p
	n	(%)	n	(%)		n	(%)	n	(%)	
Within week 4	13	(26.5)	0	(0.0)	0.0028[b]	3	(42.9)	0	(0.0)	1.0000[b]
Within week 6	16	(32.7)	0	(0.0)	0.0008[a]	3	(42.9)	0	(0.0)	1.0000[b]
Within week 8	20	(40.8)	1	(3.7)	0.0005[a]	5	(71.4)	0	(0.0)	0.3750[b]
Within week 12	20	(40.8)	1	(3.7)	0.0005[a]	6	(85.7)	0	(0.0)	0.2500[b]
Within week 18	34	(69.4)	1	(3.7)	<0.0001[a]	6	(85.7)	0	(0.0)	0.2500[b]
Within week 24	35	(71.4)	1	(3.7)	<0.0001[a]	6	(85.7)	0	(0.0)	0.2500[b]

B. Serum 25(OH)D concentrations ≥20 ng/mL.

	Repeat injection administered					Repeat injection not administered				
	Vitamin D_3 group (N=49)		Placebo group (N=27)		p	Vitamin D_3 group (N=7)		Placebo group (N=1)		p
	n	(%)	n	(%)		n	(%)	n	(%)	
Within week 4	39	(79.6)	0	(0.0)	<0.0001[a]	5	(71.4)	0	(0.0)	0.3750[b]
Within week 6	44	(89.8)	1	(3.7)	<0.0001[a]	7	(100.0)	0	(0.0)	0.1250[b]
Within week 8	45	(91.8)	3	(11.1)	<0.0001[a]	7	(100.0)	0	(0.0)	0.1250[b]
Within week 12	45	(91.8)	3	(11.1)	<0.0001[a]	7	(100.0)	0	(0.0)	0.1250[b]
Within week 18	48	(98.0)	3	(11.1)	<0.0001[a]	7	(100.0)	0	(0.0)	0.1250[b]
Within week 24	49	(100.0)	4	(14.8)	<0.0001[a]	7	(100.0)	0	(0.0)	0.1250[b]

25(OH)D, 25-hydroxyvitamin D.
[a] Pearson's chi-square test.
[b] Fisher's exact test.
[c] Repeat injection was not administered in 1 subject in vitamin D_3 group and 1 subject in placebo group due to dropout.

Appendix Table 2
Safety assessment.

A. Adverse events.

Category	Test group (N=56)			Comparator group (N=28)		
	n	(%)	[event]	n	(%)	[event]
Infections and infestations	18	(32.1)	[23]	10	(35.7)	[11]
Gastrointestinal disorders	6	(10.7)	[7]	4	(14.3)	[5]
Nervous system disorders	4	(7.1)	[4]	3	(10.7)	[3]
Renal and urinary disorders	4	(7.1)	[5]	2	(7.1)	[3]
General disorders and administration site conditions	5	(8.9)	[7]	0	(0.0)	[0]
Injury, poisoning and procedural complications	2	(3.6)	[2]	3	(10.7)	[3]
Investigations	2	(3.6)	[3]	2	(7.1)	[2]
Musculoskeletal and connective tissue disorders	2	(3.6)	[2]	0	(0.0)	[0]
Reproductive system and breast disorders	1	(1.8)	[1]	1	(3.6)	[1]
Respiratory, thoracic and mediastinal disorders	1	(1.8)	[1]	1	(3.6)	[1]
Skin and subcutaneous tissue disorders	1	(1.8)	[1]	1	(3.6)	[1]
Vascular disorders	2	(3.6)	[2]	0	(0.0)	[0]
Blood and lymphatic system disorders	1	(1.8)	[1]	0	(0.0)	[0]
Cardiac disorders	0	(0.0)	[0]	1	(3.6)	[1]
Ear and labyrinth disorders	0	(0.0)	[0]	1	(3.6)	[1]
Eye disorders	1	(1.8)	[1]	0	(0.0)	[0]
Hepatobiliary disorders	1	(1.8)	[1]	0	(0.0)	[0]
Metabolism and nutrition disorders	1	(1.8)	[1]	0	(0.0)	[0]
Total	32	(57.1)	[62]	19	(67.9)	[32]

B. Adverse drug reactions.

Category	Test group (N=56)			Comparator group (N=28)		
	n	(%)	[event]	n	(%)	[event]
Renal and urinary disorders	3	(5.4)	[3]	2	(7.1)	[3]
Hypercalciuria	3	(5.4)	[3]	2	(7.1)	[3]
General disorders and administration site conditions	3	(5.4)	[4]	0	(0.0)	[0]
Injection site granuloma	1	(1.8)	[1]	0	(0.0)	[0]
Injection site nodule	1	(1.8)	[1]	0	(0.0)	[0]
Injection site reaction	1	(1.8)	[1]	0	(0.0)	[0]
Injection site urticaria	1	(1.8)	[1]	0	(0.0)	[0]
Infections and infestations	1	(1.8)	[1]	0	(0.0)	[0]
Injection site infection	1	(1.8)	[1]	0	(0.0)	[0]
Metabolism and nutrition disorders	1	(1.8)	[1]	0	(0.0)	[0]
Hypercalcemia	1	(1.8)	[1]	0	(0.0)	[0]
Total	6	(10.7)	[9]	2	(7.1)	[3]

C. Serious adverse events.

Category	Test group (N=56)			Comparator group (N=28)		
	n	(%)	[event]	n	(%)	[event]
Infections and infestations	1	(1.8)	[1]	1	(3.6)	[1]
Appendicitis	1	(1.8)	[1]	1	(3.6)	[1]
Hepatobiliary disorders	1	(1.8)	[1]	0	(0.00)	[0]
Cholecystitis	1	(1.8)	[1]	0	(0.00)	[0]

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