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ORIGINAL ARTICLE

Impaired residual renal function predicts denosumab-induced serum calcium decrement as well as increment of bone mineral density in non-severe renal insufficiency

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Abstract

Summary Denosumab treatment of osteoporotic patients, except those with severe renal insufficiency, reduced cCa levels. Low baseline cCa, low estimated glomerular filtration rate, and high bone turnover increased the risk of lower cCa, while increasing bone mineral density. Pretreatment with antiresorptive agents was beneficial in reducing the risk of hypocalcemia.

Introduction Although denosumab-induced hypocalcemia has been frequently observed in patients with chronic kidney disease (CKD) stages 4–5D being treated with denosumab for osteoporosis, few studies have assessed the risk factors for serum-corrected calcium (cCa) reductions in patients with non-severe renal insufficiency. This study assessed the risk factors for reduced cCa concentration following denosumab administration and analyzed factors predictive of changes in bone mineral density (BMD).

Methods Seventy-seven osteoporotic patients, not including those with CKD stages 4–5D, were treated with 60 mg denosumab once every 6 months. Biochemical parameters and BMD were analyzed from prior to the initial dose until 1 month after the second dose.

Results Following the first administration of denosumab, cCa levels decreased, reaching a minimum on day 7. Multiple linear regression analyses showed that baseline cCa, estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2, tartrate-resistant acid phosphatase-5b (TRACP-5b), and bone alkaline phosphatase (BAP) or pretreatment with antiresorptive agents were significant factors independently associated with the absolute reduction in cCa from baseline to day 7 ($\Delta cCa_{0–7\ days}$). $\Delta cCa_{0–7\ days}$ after the second dose of denosumab was significantly lower than that after the first dose. After 6 months of denosumab treatment, both LS-BMD and FN-BMD significantly increased from baseline. LS-BMD and FN-BMD correlated significantly with baseline TRACP-5b or BAP and eGFR, respectively.

Conclusions Both low eGFR and high bone turnover were independent risk factors for denosumab-induced cCa decrement, and for increases in BMD. Pretreatment with antiresorptive agents may reduce the risk of hypocalcemia.

Keywords Antiresorptive agent · Bone turnover · Chronic kidney disease · Denosumab · Hypocalcemia · Osteoporosis

Introduction

Increases in the incidence of osteoporotic fractures, including hip fractures, have been associated with the aging of the population [1]. Moreover, osteoporotic fractures are a major cause of morbidity and are associated with an increased risk of mortality. Chronic kidney disease (CKD) has been associated with increased rates of morbidity, a reduced quality of life, extraskeletal calcifications, and cardiovascular mortality. CKD is very common in the general population, with its incidence increasing with age [2] as well as osteoporotic fractures [1]. Thus, osteoporotic fractures and CKD are common in the general population, and a prospective open cohort study in the United Kingdom found that CKD was a significant independent predictor of overall fracture risk in both men and women [1].

Denosumab, an inhibitor of receptor activator of nuclear factor kappa-B ligand (RANKL), is effective in the treatment of primary osteoporosis by increasing bone mineral density

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(BMD), and reducing the risk of vertebral, non-vertebral, and hip fractures [3, 4]. Although post hoc analysis of the Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months (FREEDOM) study suggested that denosumab 60 mg every 6 months was safe and likely effective at reducing fracture risk and increasing BMD in women with postmenopausal osteoporosis and CKD stages 1–4 [5], denosumab-induced hypocalcemia (DIH) has been frequently observed in patients with severe renal insufficiency (CKD stages 4–5D) [6, 7]. DIH has also been observed in patients with metastatic bone disease treated with denosumab 120 mg every month, resulting in reduced renal function [8, 9].

The aims of this study were (1) to determine the precise time course of clinical parameters during response to denosumab 60 mg, (2) to identify risk factors for reductions in serum calcium (Ca) concentration, and (3) to relate these risk factors to changes in BMD in osteoporotic patients, including those with CKD stages 1–3.

Methods

Study design and participants

This study included 77 osteoporotic patients who were started on subcutaneous injections of 60 mg denosumab once every 6 months from November 2013 to September 2016 at the Osaka City University Hospital and were followed up until 1 month after receiving the second injection of denosumab, for a total of 7 months. All participants were capable of visiting the hospital independently, and all started daily supplementation with 610 mg Ca, 400 IU cholecalciferol, and 30 mg Mg at least 1 month before the initiation of denosumab treatment. Patients were excluded if they had baseline corrected calcium (cCa) levels outside the normal range in our laboratory (8.5–10.5 mg/dL); severe renal insufficiency, as shown by an estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m^2; metabolic bone diseases, such as osteomalacia or Paget's disease of the bone; endocrine disorders, such as primary hyperparathyroidism or hyperthyroidism; poorly controlled diabetes mellitus; rheumatoid arthritis; alcohol abuse; or metastatic bone tumor.

Biochemical parameters

Serum and second-void urinary samples were collected in the morning after an overnight fast. Serum Ca, phosphate (Pi), magnesium (Mg), and creatinine (Cr) concentrations were measured by enzymatic methods using a Hitachi 7450 autoanalyzer (Hitachi Co., Tokyo, Japan). If serum albumin (Alb) concentration was below 4.0 mg/dL, cCa concentration was calculated using the following equation: cCa concentration = serum Ca concentration (mg/dL) + 4.0 − serum Alb concentration (g/dL). The incidence of laboratory hypocalcaemia by grade was classified according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. To assess renal function, eGFR was calculated using the following equation [10]: eGFR (mL/min/1.73 m^2) = 175 × serum $Cr^{-1.154}$ × $age^{-0.203}$. For women, this value was multiplied by 0.742. Serum whole PTH (wPTH) concentrations were measured by an immunoradiometric assay (Scantibodies Laboratory, Inc., Santee, CA, USA) [11], and serum 1,25$(OH)_2$D concentrations were measured by a radioimmunoassay (Immunodiagnostic Systems, Tyne & Wear, England). Serum concentrations of bone alkaline phosphatase (BAP) were measured by enzyme immunoassay (EIA) kit (Alkphase-B; Metra Biosystem, Mountain View, CA, USA) [12, 13]. The assay detected 0.7–140 U/L of BAP. The intra- and inter-assay coefficients of variation were 2.3 and 3.1%, respectively [14]. Serum concentrations of tartrate-resistant acid phosphatase-5b (TRACP-5b) were measured using a fragment-absorbed immunocapture enzymatic assay (Osteolinks TRACP-5b; DS Pharma Biomedical, Osaka, Japan) [13]. The assay detected 10–2800 mU/dL of TRACP-5b. The intra- and inter-assay coefficients of variation were 2.2 and 3.0%, respectively [15].

BMD measurements

BMD was assessed by DXA using the QDR 4500A DXA system (Hologic Inc., Marlborough, MA, USA) at baseline and after 6 months of denosumab treatment. All DXA scans were clinically performed by multiple technologists in accordance with manufacturer's recommendations. BMD measurements were recorded for the lumbar spine (LS) for L1 through L4 (L1–L4) and for the femoral neck (FN), and the analyses were reviewed by any of the investigators. Regions showing degenerative changes and/or vertebral fractures were excluded from assessments of LS-BMD. Vertebral fractures were assessed using a semiquantitative method [16]. Participants were included if they had at least two measurable lumbar spine (LSs) in the L1–4 region.

Statistical analysis

Data are expressed as the number (%), mean ± standard deviation (SD), or median (interquartile range [IQR]), as appropriate. Mean differences from baseline were compared by one-way repeated measures analysis of variance (ANOVA) followed by Dunnett's test. Because of the non-normal distribution of serum TRACP-5b and wPTH concentrations, these parameters were log-transformed before assessing correlations by linear regression analyses. The correlations between the absolute values of serum clinical parameters and their changes from baseline following the first injection of denosumab were determined using Pearson's coefficients. Linear regression analysis was performed to identify the factors associated with the

decrease in cCa concentration following denosumab administration. Multivariate linear analysis was performed to evaluate the association between the absolute decrease in cCa from baseline to day 7 ($\Delta cCa_{0-7\ days}$) and baseline characteristics (gender, age, BMI, eGFR, cCa, serum Pi, Mg, wPTH, BAP, TRACP-5b, $1,25(OH)_2D$, prior treatment for osteoporosis, and concomitant use of medications). Covariates were selected for their ability to confound the associations, as determined by univariate and stepwise models. In these analyses, gender (male, 0; female, 1), eGFR < 60 mL/min/1.73 m^2 (no, 0; yes, 1), and pretreatment with antiresorptive agents (no, 0; yes, 1) were entered as dummy variables.

Differences from baseline of the means of DXA parameters were analyzed by paired *t* tests. The values are presented as the mean ± standard error of the mean (SEM). Percent changes from baseline to 6 months were calculated as the absolute changes divided by the baseline values. Correlations between the percent changes in DXA parameters and baseline characteristics were determined by calculating Spearman's correlation coefficients.

p values < 0.05 were considered statistically significant. All statistical analyses were performed using JMP software version 11.2.0 (SAS Institute, Cary, NC, USA).

Results

Participants and treatments

Of the 77 participants enrolled, all received their first dose of denosumab and were followed for at least 6 months. Four participants did not receive the second dose of denosumab, because of relocation or side effects such as intraoral discomfort or mild liver dysfunction after the first injection of denosumab; therefore, 73 participants received the second dose of denosumab.

Baseline serum parameters of the participants ($n = 77$) are shown in Table 1. Renal function ranged widely, with eGFR ranging from 35.9 to 111.3 mL/min/1.73 m^2. Of the 77 patients, 50 (65%) were normal or had mild renal insufficiency (eGFR > 60 mL/min/1.73 m^2), whereas 27 (35.0%) had moderate renal insufficiency (eGFR 30–59 mL/min/1.73 m^2). None of these participants had severe renal insufficiency (eGFR < 30 mL/min/1.73 m^2). Serum concentrations of $1,25(OH)_2D$ were slightly higher than the upper limit of the normal range (20–60 pg/mL) and showed a significant positive correlation with eGFR ($\rho = 0.292$, $p = 0.010$). Prior treatments for osteoporosis for at least 3 months included bisphosphonates in 46 patients, selective estrogen receptor modulators (SERM) in 15, and alfacalcidol in 12. The median (interquartile range) pretreatment period for bisphosphonates, SERM, and alfacalcidol were 24.0 (22.0–48.0), 21.5 (18.0–31.5), and 12.0 (5.0–14.0) months, respectively. Also, there was no difference in renal function between alfacalcidol pretreated group and others (eGFR 67.9 ± 16.6 vs. 62.1 ± 11.7 mL/min/1.73 m^2, $p = 0.247$). In addition, 21 (27.3%) subjects were concomitantly treated with an aromatase inhibitor, and 13 (16.9%) were treated with a glucocorticoid. Fifty-five participants had four measurable LSs in their L1–4 regions, whereas 11 and 7 had 3 and 2 measurable vertebrae, respectively. The four participants lacking at least two measurable LSs in the L1–4 region were excluded from the LS-BMD analyses. No new fractures, including vertebral fractures, were observed during the study period.

Table 1 Clinical profiles of the participants

Characteristics ($n = 77$)	
Female/male, *n* (%)	68 (88.3)/9 (11.7)
Age (years)	67.9 ± 11.6
BMI (kg/m^2)	20.8 ± 3.2
Corrected calcium (mg/dL)	9.4 ± 0.3
Serum phosphate (mg/dL)	3.7 ± 0.4
Serum magnesium (mg/dL)	2.2 ± 0.2
eGFR (mL/min/1.73 m^2)	67.0 ± 16.0
wPTH (pg/mL)	22.1 (17.4–28.3)
$1,25(OH)_2D$ (pg/mL)	65.0 (52.5–89.1)
BAP (μg/L)	11.1 (8.5–14.7)
TRACP-5b (mU/dL)	356 (259–472)
LS-BMD (T-score)	− 2.92 ± 1.22
FN-BMD (T-score)	− 2.46 ± 1.10
Prior treatment for osteoporosis, *n* (%)	
Bisphosphonate	46 (59.7)
SERM	15 (19.5)
Alfacalcidol	12 (15.6)
Naïve	4 (5.2)
Concomitant use of medications, *n* (%)	
Aromatase inhibitor	21 (27.3)
Glucocorticoid	13 (16.9)

Table values are expressed as *n* (%), mean ± SD, or median (interquartile range)

BMI body mass index, eGFR estimated glomerular filtration rate, wPTH whole parathyroid hormone, $1,25(OH)_2D$ 1,25-dihydroxyvitamin D, BAP bone alkaline phosphatase, TRACP-5b tartrate-resistant acid phosphatase-5b, LS-BMD lumbar spine bone mineral density, FN-BMD femoral neck bone mineral density, SERM selective estrogen receptor modulators

Time course of denosumab-induced changes in clinical parameters

Figure 1 shows changes over time in cCa, wPTH, BAP, and TRACP-5b concentrations during the 6 months following the first denosumab injection. Concentrations of cCa decreased rapidly and significantly from day 1, with a minimum reached on day 7 (9.1 ± 0.4 mg/dL, $p < 0.001$ vs. baseline), before

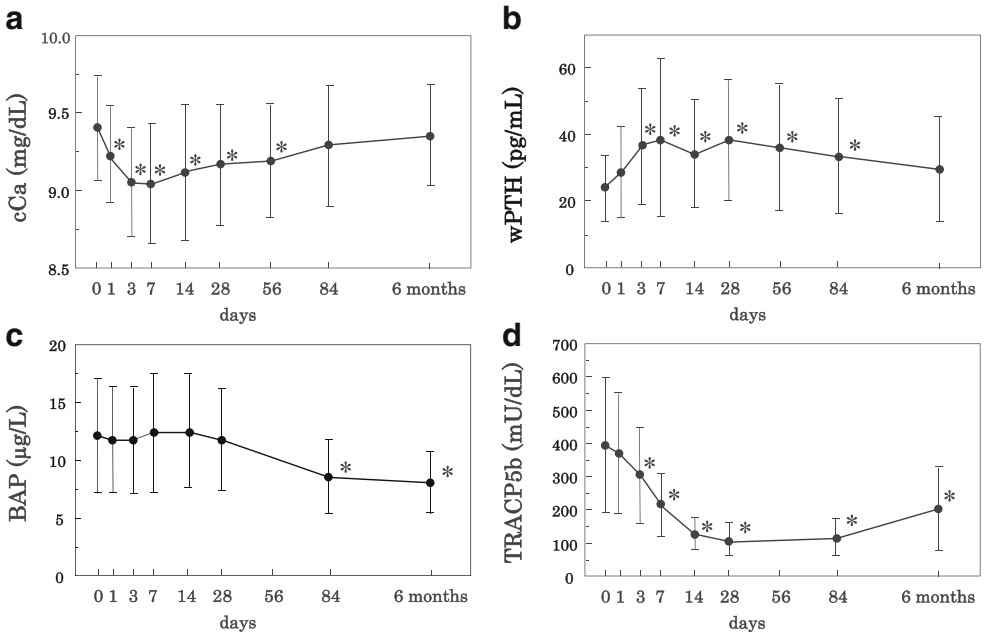

Fig. 1 Time course of changes in clinical parameters following the first administration of denosumab. Changes in serum concentrations of **a** cCa, **b** wPTH, **c** BAP, and **d** TRACP-5b for 6 months after injection of denosumab. Data are presented as mean ± SD, *$p < 0.05$ from 0 month

returning to baseline levels at 84 days (9.3 ± 0.4 mg/dL, $p = 0.330$). Although none of these subjects exhibited symptomatic hypocalcemia during the study, grade 1 hypocalcemia, defined as a corrected calcium level less than 8.5 mg/dL, which is the lower limit of the standard at our facility, was observed in eight patients (10.4%), all of which occurred during the initial course of denosumab treatment. Serum wPTH concentrations were significantly higher than baseline on day 3, peaked on day 7 (38.8 ± 22.9 pg/mL, $p < 0.001$), and returned to baseline at 6 months (30.0 ± 15.2 pg/mL, $p = 0.138$). Mean serum $1,25(OH)_2D$ concentration, which was 72.3 ± 31.7 pg/mL at baseline, was significantly higher on day 7 (95.8 ± 35.2 pg/mL, $p = 0.001$), and remained stable through 6 months (88.5 ± 35.9 pg/mL) (data not shown). BAP concentration, which was 12.1 ± 4.9 μg/L at baseline, significantly decreased from 3 months (8.7 ± 3.3 μg/L, $p < 0.001$), stabilizing until 6 months (8.2 ± 2.7 μg/L). TRACP-5b concentration, which was 391 ± 200 mU/dL at baseline, decreased significantly on day 3, reached a nadir at day 28 (116 ± 45.7 mU/dL, $p < 0.001$), and began to increase through 6 months (204 ± 123 mU/dL), although the concentration remained significantly lower at 6 months than at baseline. Changes in log wPTH from 0 to 7 days ($r = -0.662$, $p < 0.001$) and in log TRACP-5b from 0 to 28 days ($r = 0.360$, $p = 0.002$) were also significantly correlated with $\Delta cCa_{0–7\ days}$ (data not shown).

Linear regression analysis between $\Delta cCa_{0–7\ days}$ and baseline characteristics

The 7-day reduction in cCa after the first denosumab injection ($\Delta cCa_{0–7\ days}$) showed a significant positive correlation with baseline eGFR ($r = 0.310$, $p = 0.006$) and a significant negative correlation with both log baseline TRACP-5b ($r = -0.292$, $p = 0.009$) and log baseline BAP ($r = -0.282$, $p = 0.013$) (Fig. 2a–c). Table 2 shows the results of multiple linear regression analyses of baseline characteristics independently associated with $\Delta cCa_{0–7\ days}$. In model 1, which included gender, age, BMI, cCa, log wPTH, eGFR, and log TRACP-5b as independent variables, cCa_{7day} ($\beta = -0.392$, $p = 0.001$), eGFR ($\beta = 0.225$, $p = 0.044$), and log TRACP-5b ($\beta = -0.235$, $p = 0.0023$) were significantly and independently associated with $\Delta cCa_{0–7\ days}$. Replacement of eGFR by a categorical variable, such as whether eGFR was or was not < 60 mL/min/1.73 m^2 (model 2), showed a more significant correlation ($\beta = -0.265$, $p = 0.018$). Replacement of log TRACP-5b by log BAP (model 3) showed that this variable was also independently associated with $\Delta cCa_{0–7\ days}$ ($\beta = 0.200$, $p = 0.047$). When both log TRACP-5b and log BAP were added (model 4), both factors failed to emerge as an independent variables significantly associated with $\Delta cCa_{0–7\ days}$. Replacement of log TRACP-5b by a categorical variable, such as pretreatment with an antiresorptive agent such as bisphosphonates or SERM (model 5), showed that this variable was also independently associated with $\Delta cCa_{0–7\ days}$ ($\beta = 0.205$, $p = 0.047$). The addition of log TRACP-5b and pretreatment with antiresorptive agents to model 5 eliminated the latter as an independent variable (model 6). Similarly, the addition of log BAP and pretreatment with antiresorptive agents to model 5 eliminated the latter as an independent variable (model 7).

Effects of osteoporosis pretreatments or denosumab on $\Delta cCa_{0–7\ days}$

Analyses also showed that $\Delta cCa_{0–7\ days}$ after the first injection of denosumab was significantly lower in patients pretreated

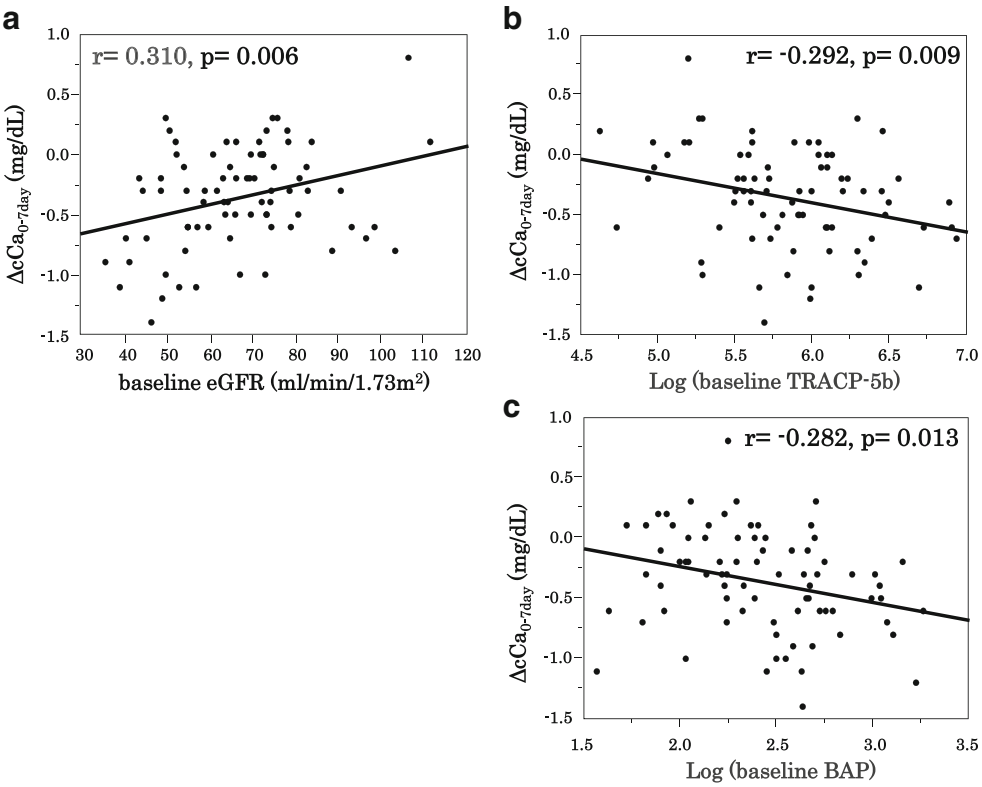

Fig. 2 Correlations between the absolute reduction in cCa concentration from baseline to day 7 ($\Delta cCa_{0-7\ days}$) and clinical parameters following denosumab administration. Baseline eGFR (**a**) showed a significant positive correlation with $\Delta cCa_{0-7\ days}$. Both baseline TRACP-5b (**b**) and baseline BAP (**c**) showed a significant negative correlation with $\Delta cCa_{0-7\ days}$. Statistical analyses were performed by calculating Pearson's correlation coefficients

with bisphosphonates (− 0.30 ± 0.4 mg/dL, $p = 0.018$) and SERM (− 0.27 ± 0.3 mg/dL, $p = 0.043$) than in patients not receiving non-antiresorptive therapy (− 0.61 ± 0.5 mg/dL; Fig. 3a). There were no associations between the change in cCa and each pretreatment duration of bisphosphonates ($\rho = 0.121$, $p = 0.430$) and SERM ($\rho = -0.060$, $p = 0.845$) (data

Table 2 Multiple linear regression analysis of baseline characteristics significantly associated with $\Delta cCa_{0-7\ days}$

Independent variables	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Gender (female = 1, male = 0)	0.015	0.888	− 0.010	0.924	0.034	0.749	− 0.014	0.892	− 0.042	0.690	− 0.015	0.884	− 0.034	0.745
Age (years)	0.161	0.130	0.157	0.133	0.125	0.227	0.150	0.157	0.122	0.239	0.150	0.146	0.125	0.221
BMI (kg/m^2)	− 0.013	0.903	0.008	0.941	0.037	0.722	0.016	0.878	0.022	0.831	0.009	0.930	0.033	0.746
cCa (mg/dL)	− 0.392	0.001	− 0.388	0.001	− 0.387	0.001	− 0.383	0.001	− 0.408	0.001	− 0.284	0.001	− 0.380	0.001
Log [wPTH (pg/mL)]	0.036	0.733	0.030	0.774	0.051	0.628	0.037	0.729	− 0.003	0.976	0.001	0.989	0.014	0.896
eGFR (mL/min/1.73 m^2)	0.225	0.044	–	–	–	–	–	–	–	–	–	–	–	–
eGFR < 60 mL/min/1.73 m^2 (yes = 1, no = 0)	–	–	− 0.265	0.018	− 0.271	0.016	− 0.265	0.019	− 0.250	0.028	− 0.243	0.030	− 0.242	0.031
Log [TRACP-5b (mU/dL)]	− 0.235	0.023	− 0.231	0.024	–	–	− 0.177	0.240	–	–	− 0.188	0.075	–	–
Log [BAP (μg/L)]	–	–	–	–	− 0.200	0.047	− 0.072	0.622	–	–	–	–	− 0.174	0.081
Pretreatment with antiresorptive agents[a] (yes = 1, no = 0)	–	–	–	–	–	–	–	–	0.205	0.047	0.151	0.154	0.179	0.082
R^2	0.288		0.304		0.292		0.296		0.292		0.314		0.313	
p	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	

Values are the standard regression coefficients (β). R^2 = multiple coefficient of determination

[a] Including bisphosphonates (46 patients) and SERM (15 patients)

$\Delta cCa_{0-7\ days}$ absolute decrease from baseline to day 7 in corrected calcium concentration, BMI body mass index, wPTH whole parathyroid hormone, eGFR estimated glomerular filtration rate, TRACP-5b tartrate-resistant acid phosphatase-5b, BAP bone alkaline phosphatase, SERM selective estrogen receptor modulator

not shown). The $\Delta cCa_{0-7\ days}$ after the first injection of denosumab was significantly lower in patients with eGFR > 60 mL/min/1.73 m^2 than in those with eGFR < 60 mL/min/1.73 m^2 (−0.25 ± 0.4 vs. −0.55 ± 0.5 mg/dL, $p = 0.002$). In addition, $\Delta cCa_{0-7\ days}$ was significantly lower after the second than after the first injection of denosumab (−0.09 ± 0.3 vs. −0.36 ± 0.4 mg/dL, $p < 0.001$) (Fig. 3b), and $\Delta cCa_{0-7\ days}$ after the second injection of denosumab showed a significant correlation with baseline eGFR ($r = 0.287$, $p = 0.017$), but not with any other independent variable, including log TRACP-5b (data not shown).

Correlations between changes in BMD and risk factors for denosumab-induced reductions in cCa

Six months after the first injection of denosumab, the percent changes in LS-BMD (2.77% ± 0.5%, $p < 0.001$) and FN-BMD (1.84% ± 0.8%, $p = 0.034$) were significantly higher than at baseline. As shown in Table 3, the percent change in LS-BMD was significantly correlated with both baseline TRACP-5b ($\rho = 0.452$, $p < 0.001$) and baseline BAP ($\rho = 0.338$, $p = 0.005$), and the percent change in FN-BMD was significantly correlated with eGFR ($\rho = -0.310$, $p = 0.006$). Moreover, both of these factors were identified as risk factors for denosumab-induced reduction in cCa (Fig. 2a–c).

Table 3 Correlations between the percent changes in DXA parameters after 6 months of denosumab therapy and risk factors for denosumab-induced reductions in cCa

	%LS-BMD		%FN-BMD	
	ρ	p	ρ	p
Baseline eGFR	−0.210	0.084	−0.310	0.006
Baseline TRACP-5b	0.452	< 0.001	0.133	0.253
Baseline BAP	0.338	0.005	0.033	0.780

ρ Spearman's correlation coefficient, eGFR estimated glomerular filtration rate, TRACP-5b tartrate-resistant acid phosphatase-5b, BAP bone alkaline phosphatase, LS-BMD lumbar spine bone mineral density, FN-BMD femoral neck bone mineral density

Discussion

This study, involving osteoporotic patients without severe renal insufficiency, showed that renal function and high bone turnover were independently associated with denosumab-induced reductions in cCa, with most of these changes observed 7 days after denosumab administration. Pretreatment with antiresorptive agents was beneficial in preventing denosumab-induced reductions in cCa. The second dose of denosumab was less likely to reduce cCa than the first dose.

Although persistently reduced serum Ca levels have been observed in patients with moderate to severe CKD [6, 17], the earliest changes in serum Ca were observed after 7 days in patients with osteoporosis [18] and CKD stage 5D [19], after 7–14 days in osteoporotic patients [7, 20], and after 14 days in subjects aged ≥ 70 years with fragility fractures admitted to hospital [21] and patients with CKD stage 5D [6]. A pharmacokinetic study of denosumab in participants with various grades of kidney function showed precise time-course changes in serum Ca [17]; however, these changes were not considered reliable because of the small number of participants at each CKD stage. By contrast, the present study evaluated precise time-course changes in cCa in a sizable number of the participants. Measuring serum Ca 7 days after denosumab injection may be important in daily clinical practice. In our

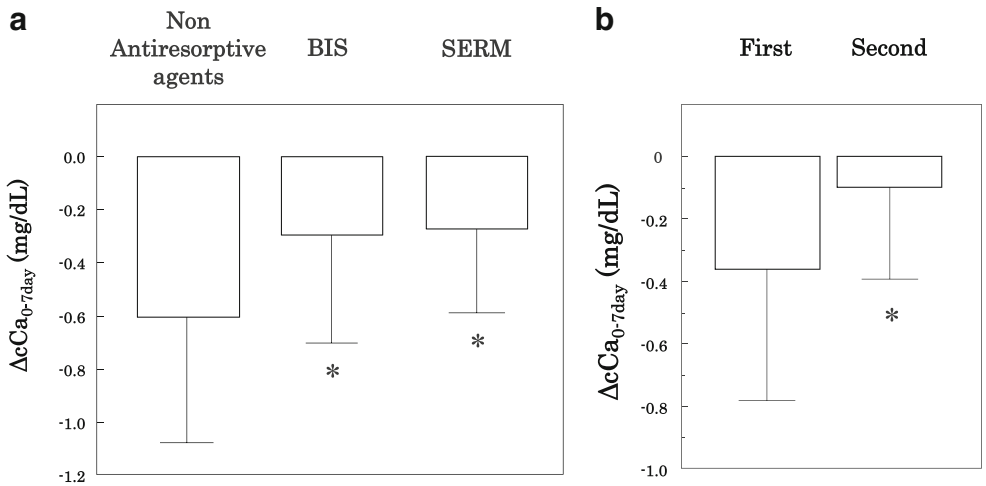

Fig. 3 Effects of previous treatment, including denosumab, on $\Delta cCa_{0-7\ days}$. Patients treated with non-antiresorptive agents were naïve to treatment for osteoporosis and the administration of alfacalcidol. $\Delta cCa_{0-7\ days}$ after the first injection of denosumab was significantly lower in patients pretreated with either bisphosphonates or SERM than in those pretreated with non-antiresorptive therapy (**a**). $\Delta cCa_{0-7\ days}$ was significantly lower after the second than after the first injection with denosumab (**b**). Mean ± SD, *significant differences ($p < 0.05$)

study, grade 1 hypocalcemia was observed in eight patients (10.4%). Although the grade of laboratory hypocalcaemia was less severe than those in previous report in patients with metastatic bone disease receiving 120 mg denosumab (grade 1 3.0%, grade 2 4.1%, grade 3 1.8%, grade 4 0.7%, total 9.6%) [22], the identified risk factors associated with decrements in serum Ca concentration in our study were similar to this previous report. We confirmed that the potential risk factors for denosumab-induced hypocalcemia are the same despite the dose of denosumab.

The pharmacokinetics and pharmacodynamics of denosumab were shown to be stable in subjects with renal function ranging from normal to CKD stage 5D [17]. These findings indicated that accumulation of denosumab caused by renal insufficiency had no effect on reductions in serum Ca. The major reason why renal impairment is more likely to develop denosumab-induced decrement in cCa possibly related to the resistance of bone and kidney to PTH and the decline in renal production of $1,25(OH)_2D$ seen in CKD. Although PTH rapidly regulates serum Ca by increasing Ca efflux from bone and renal Ca reabsorption, denosumab suppressed Ca efflux from bone. Uremic toxins such as indoxyl sulfate suppress both bone formation and bone resorption [23], in which bone is resistant to PTH. Even in patients with mild CKD, hypocalcemia was likely caused by reduced renal tubular reabsorption of Ca [24], with higher PTH required for normal renal tubular reabsorption of Ca [25]. PTH also increases serum Ca by promoting the formation of $1,25(OH)_2D$ in the kidney, thereby facilitating Ca absorption by the small intestine [26]. Decreased intestinal Ca absorption has been reported in patients with early stage CKD [27]. Supplementation with cholecalciferol did not improve intestinal Ca absorption in patients with CKD [28], suggesting that this impairment may be associated with a uremia-specific factor (e.g., metabolic acidosis) [29]. Although all participants in the present study received adequate cholecalciferol supplementation at least 1 month before the first injection of denosumab, intestinal Ca absorption may have been reduced in subjects with CKD. Because it is reported that renal function impairment did not appear to be a risk factor for DIH in patients with postmenopausal osteoporosis receiving denosumab with eldecalcitol, an analogue of the active form of vitamin D [20], patients with eGFR below 60 mL/min/1.73m^2 may be recommended the denosumab plus active vitamin D analog (alfacalcidol or eldecalcitol) combination therapy.

The present study also found that elevated TRACP-5b and BAP was a significant independent risk factor for denosumab-induced reduction in cCa, even after adjusting for baseline cCa and eGFR. Similarly, elevated total P1NP, TRACP-5b, and urinary NTX were found to be risk factors for postmenopausal osteoporosis [20], whereas NTX [22] and alkaline phosphatase (ALP) [22, 30] were elevated in patients with metastatic bone disease. Our results may explain the concept of why patients dependent on high bone turnover to maintain normal serum Ca are more susceptible to bone turnover suppression by denosumab. Specifically, denosumab strongly prevented bone resorption by inhibiting osteoclast activity, thereby reducing Ca mobilization as a consequence of bone turnover. The presence of large populations of osteoblasts, similar to the hungry bone syndrome after parathyroidectomy, may shift Ca into bone [31, 32]. In the present study, pretreatment with antiresorptive agents, such as bisphosphonates or SERM, significantly reduced the change in cCa when compared with non-antiresorptive therapy. These results suggested that the decrease in bone turnover induced by antiresorptive agents was beneficial in reducing the risk of reductions in cCa [8, 9]. Because it is reported that serum TRACP-5b decreased significantly at first 3 months of both bisphosphonate [33] and SERM [34] treatment, patients with high baseline bone turnover, as evidenced by elevated TRACP-5b and BAP, may be recommended to pretreat with antiresorptive drugs at least 3 months before the initiation of denosumab treatment. Also, the second injection of denosumab resulted in a significantly smaller reduction in cCa than the first injection. This phenomenon is clinically important; in that, patients who did not become hypocalcemic after the first injection of denosumab were less likely to develop hypocalcemia after the second and subsequent injections.

After 6 months, denosumab treatment increased LS-BMD by 2.77% and FN-BMD by 1.84% in these patients, magnitudes consistent with those previously reported [35]. We also found that these 6-month changes in LS-BMD and FN-BMD correlated significantly with baseline TRACP-5b or BAP and eGFR, respectively, and that changes in both LS-BMD and FN-BMD were identified as risk factors for denosumab-induced reductions in cCa. Baseline and early changes in serum biochemical markers have been reported to predict BMD alterations in patients treated with denosumab. A substudy of the FREEDOM trial, involving 160 postmenopausal osteoporotic women treated with denosumab, showed significant correlations between reduced CTX and increased LS- or total hip (TH)-BMD [36]. A subanalysis of 27 denosumab-treated women in the DATA study found that higher baseline concentrations of CTX and P1NP were associated with greater increases in BMD at the LS and TH [37]. Furthermore, lower eGFR was associated with a greater improvement in FN-BMD in patients with CKD who received denosumab [38].

The major limitations of this study included its retrospective design and its lack of appropriate untreated control subjects. This study included not only patients with postmenopausal osteoporosis but also those with osteoporosis induced by drugs such as glucocorticoids and aromatase inhibitors. Although these baseline characteristics were considered in statistical analysis, we did not identify any concomitant medications as risk factors associated with denosumab-induced decrement in serum Ca. Also, the independent risk factors

for denosumab-induced decrement in serum Ca were not influenced after adjusted for any concomitant medications. In addition, similar to results in postmenopausal women with osteoporosis, several clinical studies showed that treatment with denosumab significantly increased LS- and FN-BMD in patients with osteoporosis associated with long-term glucocorticoid treatment [39], in women with breast cancer receiving aromatase inhibitors [40, 41], and in patients previously treated with bisphosphonates [42, 43], suggesting that the denosumab-induced change in BMD in this study was clinically significant. Another limitation was that CKD was diagnosed based on eGFR calculated from serum Cr levels, age, and gender, without assessment of microalbuminuria or other factors.

In summary, this study is the first to show that high bone turnover and renal insufficiency were independent risk factors for denosumab-induced reductions in cCa among osteoporotic patients, including those with CKD stages 1–3. Other risk factors for denosumab-induced reductions in cCa included high TRACP-5b, high BAP, and low eGFR, which also predicted BMD gains at the LS and FN, respectively. Monitoring of serum Ca, approximately 1 week after the first dose of denosumab for these identified higher-risk patients, is strongly recommended. Pretreatment with antiresorptive agents or a second dose of denosumab within 6 months may be beneficial at reducing the risk of cCa reduction.

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Compliance with ethical standards

Conflicts of interest MI received grant support and lecture fees from Daiichi Sankyo Co., Limited. YI, KM, EM, and MI received lecture fees from Daiichi Sankyo Co., Limited. DM, MO, NH, YN, and SY have no conflicts of interest to report.

Human and animal rights and informed consent All participants provided written informed consent. The study protocol was approved by the institutional ethics committee of Osaka City University Graduate School of Medicine. This study was conducted in accordance with the principles of the Declaration of Helsinki.

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