treated as described above, but the primary antibody was omitted. YO-PRO-1 (Molecular Probe) or TO-PRO-3 (Molecular Probe) was used to detect the nuclei. A confocal laser scanning microscopy (FV1000; Olympus, Tokyo, Japan) was used for data acquisition. The number of $^{P}p65^{+}$ nuclei were counted in 10 microscopic fields using a 40× objective lens, and its indices were expressed as the mean number per one field. The numbers of stained cells for anti-F4/80 and anti-FSP1 were quantitatively assessed as described above.

Reverse Transcriptase (RT)-PCR

Total RNA was extracted from the homogenates of kidney tissues with TRIzol™ (Life Technologies BRL, Grand Island, NY) according to the manufacturer's instructions. All of the RNA samples were pretreated with the RNase-free DNase I (Qiagen, Basel, Switzerland). cDNA was synthesized with a kit (Ready-To-Go T-Primed First-Strand Kit; GE Healthcare, Buckinghamshire, UK). The cDNA was amplified by PCR with the use of primers for *IκBdN* (forward, 5′-CTCCAGCAGACTCCACTCCACT-3′, and reverse, 5′-ACACCAGCCACCACCTTCTGAT-3′), yielding a 712-bp fragment. Cycle programs were started with 4 minutes at 95°C, followed by 35 cycles of 1 minute at 95°C, 1.5 minutes at 60°C, and 1.5 minutes at 72°C, finishing with 5 minutes at 72°C.

Real-Time Quantitative RT-PCR (qPCR)

We performed qPCR as described previously.[41] In brief, a real-time quantitative one-step RT-PCR assay was performed to quantify mRNA levels using QuantiTect SYBR Green RT-PCR (Qiagen) and an Mx3000P QPCR system (Stratagene, La Jolla, CA). The primers used for qPCR were as follows: MCP-1 primer: forward, 5′-CTCTCTTCCTCCACCACCAT-3′, and reverse, 5′-ACTGCATCTGGCTGAGCCA-3′; FRK primer: forward, 5′-CGCGTTCTTCCATTTGTGTA-3′, and reverse, 5′-CATGATTTCGCATTTCGTCA-3′; OPN primer: forward, 5′-CCCTTTCCGTTGTTGTCCTG-3′, and reverse, 5′-CCCTCGATGTCATCCCTGTT-3′; TGF-β1 primer: forward, 5′-CAGTGGCTGAACCAAGGAGAC-3′, and reverse, 5′-ATCCCGTTGATTTCCACGTG-3′; CCN2 primer: forward, 5′-GTGGAATATTGCCGGTGCA-3′, and reverse, 5′-CCATTGAAGCATCTTGGTTCG-3′; PAI-1 primer: forward, 5′-AGGATCGAGGTAAACGAGAGC-3′, and reverse, 5′-GCGGGCTGAGATGACAAA-3′; FN-EIIIA primer: forward, 5′-ATCCGGGAGCTTTTCCCTG-3′, and reverse, 5′-TGCAAGGCAACCACACTGAC-3′; COLI primer: forward 5′-TGTAAACTCCCTCCACCCCA-3′, and reverse, 5′-TCGTCTGTTTCCAGGGTTGG-3′; and glyceraldehyde-3-phosphate dehydrogenase primer: forward, 5′-TGCAGTGGCAAAGTGGAGATT-3′, and reverse, 5′-TTGAATTTGCCGTGAGTGGA-3′. All of these oligodeoxynucleotides were designed by using Primer Express software (Perkin Elmer, Foster City, CA). Preliminary RT-PCR experiments in which these primer sets were used yielded appropriately sized, single products.

Statistical Analysis

The values are presented as the means ± SEM. The statistical differences between groups were evaluated by ANOVA, followed by a Bonferroni/Dunnett's test; significant P values were ≤ 0.05.

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DISCLOSURES

None.

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Effect of Lanthanum Carbonate Treatment on Bone in Japanese Dialysis Patients With Hyperphosphatemia

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Abstract: Lanthanum carbonate (LC), a newly developed non-calcium-containing phosphate binder, has been shown to possess high phosphate-binding capacity and safety when used for hyperphosphatemia in patients with chronic kidney disease undergoing dialysis. The effects of LC on bone metabolism in Japanese dialysis patients have not been investigated; therefore, we performed histomorphometric analysis on bone from dialysis patients with hyperphosphatemia. This was a prospective, open-label study in Japanese chronic kidney disease patients on dialysis, with a flexible daily dosage of 750–4500 mg to achieve target phosphorus levels of 3.5–5.5 mg/dL (1.10–1.78 mmol/L). Bone biopsy samples for histomorphometric analysis were obtained at baseline and after treatment with LC. The median bone lanthanum level increased during the LC treatment from 54.1 μg/kg at baseline to 4270.9 μg/kg at three years. After one year of treatment with LC, two cases with an initial classification of osteitis fibrosa improved toward normal bone turnover. The diagnosis of normal remained the same for up to three years. We also noted that two cases with a baseline classification of adynamic bone disease improved after one year, and was maintained for three years. Our data suggest that LC is effective not only for treating hyperphosphatemia, but also for improving renal osteodystrophy in Japanese dialysis patients. **Key Words:** Bone biopsy, Hyperphosphatemia, Lanthanum carbonate, Phosphate binder, Renal osteodystrophy.

Renal osteodystrophy (ROD) is defined as an alteration of bone morphology in patients with chronic kidney disease (CKD) and one of the major complications of end-stage renal disease (ESRD) (1,2). Symptoms of ROD include pain, bone fractures, and skeletal deformities (3). Renal osteodystrophy has been classified into five major types: osteitis fibrosa (high-turnover), adynamic bone disease (low-turnover), osteomalacia (low-turnover), a mixed type with characteristics of both osteitis fibrosa and osteomalacia, and a mild type (4).

Patients on dialysis develop hyperphosphatemia because only up to 70% of the dietary load of phosphorus can be removed from circulation (5). Hyperphosphatemia in dialysis patients is important in the management of ESRD since it is a known independent factor contributing to the increased risk of cardiac death. Calcium-based phosphate binders such as calcium carbonate are widely used to reduce serum phosphate levels (6); however, prolonged administration of calcium salts and vitamin D receptor activator therapy result in over-suppression of parathyroid hormone (PTH), which leads to adynamic bone disease characterized by a low capacity for buffering calcium levels in the bone (7,8). In addition, calcium-based phosphate binders often cause hypercalcemia, and both soft-tissue and vascular calcification.

Lanthanum carbonate (LC) is a newly developed non-aluminum- and non-calcium-containing phosphate binder. Clinical studies have indicated that LC effectively reduces serum phosphorus levels and is well-tolerated for up to six years (9–12). After administration of LC, a small amount of the lanthanum

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is absorbed and distributed into body tissues and deposited in bone, although it is of low concentration (13,14). In several clinical trials conducted in the EU and USA, histomorphometric analyses suggest that lanthanum, but not calcium-based phosphate binders, exhibits significant positive effects on bone metabolism, in spite of its slight deposition to bone (15–17). However, it is not clear how much lanthanum would be distributed in bone and whether it also exhibits positive effects on bone in Asian patients.

The aim of this study was to evaluate lanthanum concentration in bone and the effect of LC on bone metabolism in Japanese dialysis patients with hyperphosphatemia who receive long-term treatment with LC.

PATIENTS AND METHODS

Study design

This study was a non-controlled, open-label, multicenter study. The protocol of this study was approved by the Institutional Review Board of each institution involved. Patients were enrolled if they consented to having two bone biopsies. After a washout period of three weeks, patients received LC for one year. Lanthanum carbonate was initiated at 750 mg/day and was subsequently titrated up to 4500 mg/day according to the serum phosphate level to achieve a target level of 3.5–5.5 mg/dL (1.10–1.78 mmol/L), if tolerated. Patients provided bone biopsy samples before and after LC treatment. All patients who completed the initial one-year study were asked to take part in the three-year follow-up assessment.

Patients

All patients were >20 years old, and either received dialysis (hemodialysis or peritoneal dialysis) or were planning to start dialysis before the initial administration of the study medication. Their predialysis serum phosphate level had to be ≥5.6 mg/dL (≥1.81 mmol/L) at three weeks after the initiation of the washout period. Exclusion criteria for the study included: severe hypocalcemia (adjusted serum calcium level <7.5 mg/dL [<1.88 mmol/L]), high risk of bleeding at bone biopsy, any significant gastrointestinal disorders, elevated serum transaminases (ALT or AST greater than three times the normal upper limit), and a history of hypersensitivity to tetracyclines.

Laboratory methods

Blood and bone biochemistry

Blood samples were taken at every study visit for routine biochemistry and hematology tests. If a patient received hemodialysis, blood samples were collected before dialysis. Osteocalcin, bone alkaline phosphatase (BAP), type I collagen cross-linked N-telopeptide (NTx) were measured according to standard methods, using appropriate assay kits. Lanthanum was measured in plasma and bone by means of inductively-coupled plasma mass spectrometry. All measurements were carried out at central laboratories.

Bone biopsy and histomorphometric analysis

Bone biopsy and histomorphometric analysis procedures were performed by traditional methods. Before each bone biopsy, patients received double tetracycline labeling. This consisted of two days of oral tetracycline hydrochloride (POLA Pharma, Sinagawa, Japan; 250 mg three times daily), followed by a 10-day tetracycline-free interval and then two days of oral tetracycline, at the previous dose. Patients temporarily stopped taking LC while tetracycline was administered.

Bone samples were taken from the ilium after double tetracycline labeling. One part of the sample (mainly cortical bone) was used to measure the bone lanthanum level; the remaining part was fixed in absolute ethanol and then embedded in a methyl methacrylate-based resin for histomorphometric analysis. Villanueva bone stain was used for staining. Sectioning was conducted by the dry section method and the thickness was set at 6 μm. All bone samples were processed and measured at the Ito Bone Histomorphometry Institute (Niigata, Japan). Classification of the various types of ROD was basically made with the cancellous histomorphometric parameters based on the criteria of Sherrard et al. (4). For some cases with limited and/or low quality samples, information from histological images was also taken into account for the classification.

Statistics

All variables were analyzed using appropriate statistical methods. Categorical variables by frequency tables and continuous variables were examined using the mean, standard deviation (SD), minimum, median, quartiles, and maximum.

RESULTS

A total of 15 patients at two centers were enrolled in this study. One patient was excluded during the washout period for efficacy and safety reasons because it was difficult to perform a bone biopsy on this patient due to severe obesity. Nine out of the 14 patients completed the one year treatment. Five

TABLE 1. *Demographic and baseline information*

Gender	Male	11 (79%)
	Female	3 (21%)
Age (years)	Mean ± SD	59.2 ± 14.9
	Range	24–76
Height (cm)	Mean ± SD	164.6 ± 9.4
	Range	150–180
Weight (kg)	Mean ± SD	69.6 ± 16.6
	Range	42.5–103.4
Dialysis duration (years)	Mean ± SD	8.0 ± 6.3
	Range	2.4–27.8
Type of dialysis	Hemodialysis	12 (86%)
	Peritoneal dialysis	2 (14%)
Primary cause of ESRD	CGN	6 (43%)
	Diabetic nephropathy	7 (50%)
	Alport syndrome	1 (7%)
Prior medications	Calcium carbonate	9 (64%)
	Sevelamer hydrochloride	8 (57%)
	Others	2 (14%)

$N = 14$. CGN, chronic glomerulonephritis; ESRD, end-stage renal disease.

patients terminated the study prematurely during the one year observation period due to: an adverse event ($N = 2$), investigator decision ($N = 2$), and changing hospital ($N = 1$). Six out of the nine patients consented to enter an extension period (>1 year), and four patients completed three years of treatment. Two patients discontinued the treatment during one- and three-year observation periods because of investigator decision ($N = 1$) and clinical site closure ($N = 1$). At baseline, one year, and three years, bone biopsies were taken from 12, 8, and 4 patients, and ROD classification was carried out on 7, 4, and 4 patients, respectively. The first patient was enrolled in June 2005 and the final patient completed their last clinic visit in October 2008. Demographic data and other characteristics are presented in Table 1.

The treatment was started with a daily dose of 750 mg and titrated to higher doses (up to 4500 mg). The median (minimum–maximum) value of the LC dosage at the end of study was 1875 (750–4500) mg/day.

Among these 14 patients, 7 were on intravenous (i.v.) and 1 was on oral (p.o.) vitamin D receptor activator therapy at baseline. At one year, six patients (4 on i.v. and 2 on p.o. therapy) of the nine remaining patients were on vitamin D receptor activator therapy, and at three years this had changed to three patients (2 on i.v. and 1 on p.o. therapy) of the remaining four. None of the patients were treated with cinacalcet during the LC treatment phase.

Biochemistry

Serum phosphate levels decreased from 9.28 mg/dL (3.00 mmol/L) at baseline to 5.89 mg/dL (1.90 mmol/L) at one year, and then to 5.82 mg/dL (1.88 mmol/L) at three years. The mean change in serum phosphate levels from baseline to the end of study was −3.51 mg/dL (−1.13 mmol/L). Corrected serum calcium levels slightly increased, but the change was not significant (Table 2).

The median (Q1, Q3) of the serum intact PTH level decreased from 425 (277, 603) pg/mL (45.1 pmol/L) at baseline ($N = 14$) to 329 (206, 476) pg/mL (34.9 pmol/L) at one year ($N = 13$), then to 276 (150, 566) pg/mL (29.3 pmol/L) at three years ($N = 6$). The median (Q1, Q3) of the changes from baseline to the end of study were −10.0 (−298.0, 178.0) pg/mL (−1.1 pmol/L).

TABLE 2. *Summary of serum phosphate, calcium, Ca × P product levels (Mean + SD)*

	N	Inorganic phosphorus (mg/dL)	Adjusted calcium (mg/dL)	Ca × P product (mg^2/dL^2)
Baseline	14	9.28 ± 1.47	9.53 ± 0.59	85.34 ± 13.21
1 year	13	5.89 ± 1.06	9.88 ± 1.06	56.70 ± 10.68
2 years	5	5.32 ± 0.88	10.46 ± 0.79	53.81 ± 8.26
3 years	6	5.82 ± 1.03	10.37 ± 1.40	57.10 ± 9.42
Change from Baseline	14	−3.51 ± 1.85	0.41 ± 1.01	−30.36 ± 16.87
95% CI		(−4.57, −2.44)	(−0.17, 0.99)	(−40.10, −20.62)

The patients who had treated 1-year or 3-years treatment of LC were included in this table.

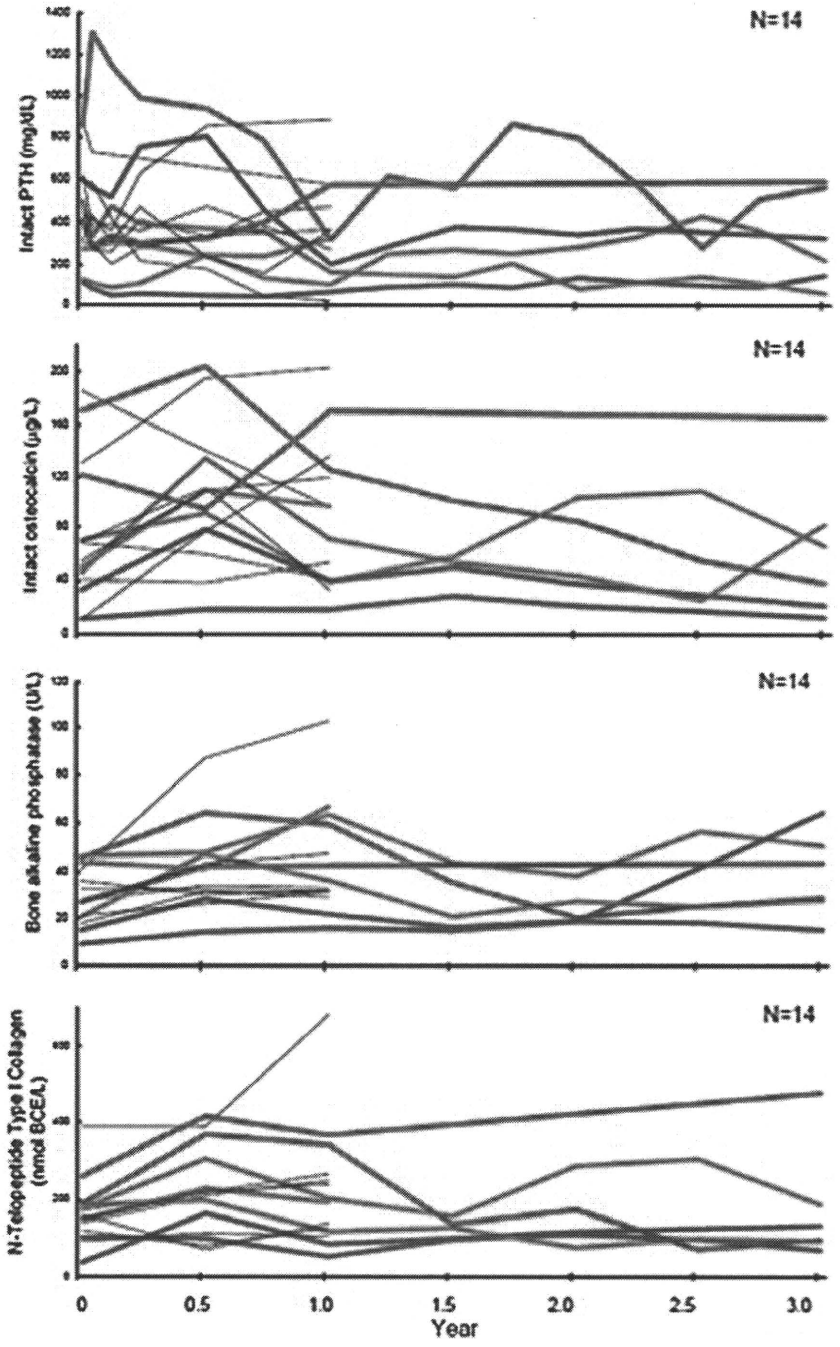

FIG. 1. Time-dependent changes in individual intact parathyroid hormone (PTH) and plasma bone metabolism markers. Each line represents an individual patient. The colored lines indicate patients with baseline renal osteodystrophy classifications as follows: red, osteitis fibrosa-type disorder at baseline; blue, adynamic bone disease-type disorder at baseline; and green, mild-type disorder at baseline.

Changes in individual intact PTH and plasma bone metabolism markers are shown in Figure 1. No clear trends for time-dependent change in these four parameters were observed.

The plasma lanthanum level at the baseline was below the limit of quantification (<0.0312 μg/L). At one year, two years, and three years after administration of LC, plasma lanthanum levels (geometric mean (geometric SD)) were 0.329 (2.06), 0.464 (2.06), and 0.487 (2.08) μg/L, respectively. The bone lanthanum levels (level per wet weight) were 54.1 μg/kg (median) at baseline, 983.8 μg/kg at one year, and 4270.9 μg/kg at three years (Table 3). All patients showed the same trend.

Histomorphometric analysis

Figures 2 and 3 show two representative cases (cases 01-03 and 01-06). Table 4 summarizes the parameters for histomorphometric analysis in these two patients. According to the criteria of Sherrard

TABLE 3. *Bone lanthanum levels (μg/kg)*

	N	Geometric mean	Geometric SD	Median	Minimum	Maximum
Baseline	12	49.0	1.8	54.1	20.8	129.1
1 year	8	1055.7	2.0	983.8	323.6	2985.3
3 year	4	3512.0	2.0	4270.9	1408.4	6452.0

Data provided based on the wet weight. Not all patients had bone lanthanum determinations done due to technical reasons.

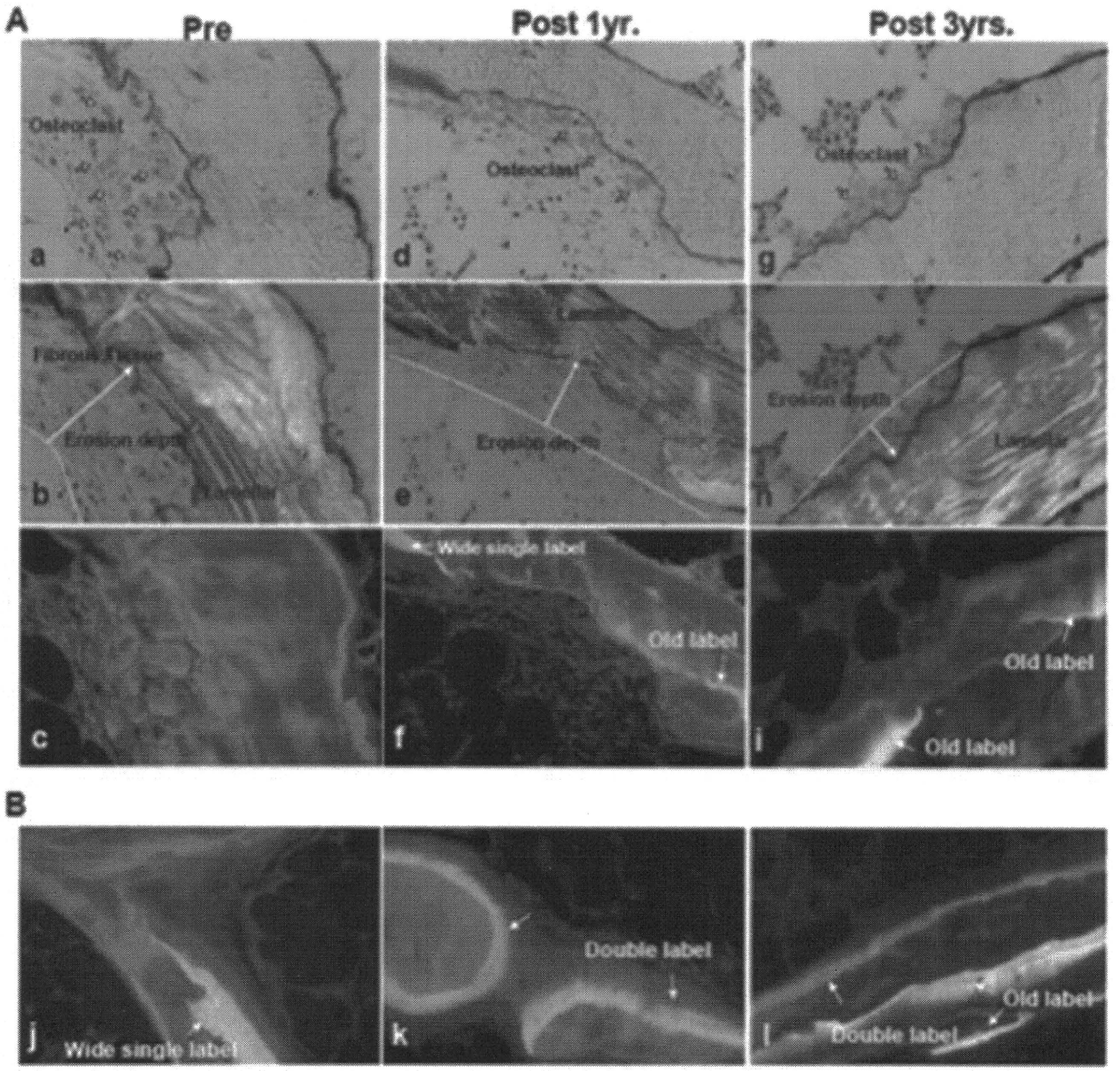

FIG. 2. Microscopic images of pre- and post treatment cancellous bone from a patient with a fibrous osteitis-type disorder (Case: 01-03). Light (a, d, g), polarizing (b, e, h), and fluorescent (c, f, i, j, k, l) microscopy at a magnification of ×400. Panel A shows microscopic images from one field of each specimen from pre-treatment and post-treatment bone. Panel B shows fluorescent microscopic images from a different field of the specimen.

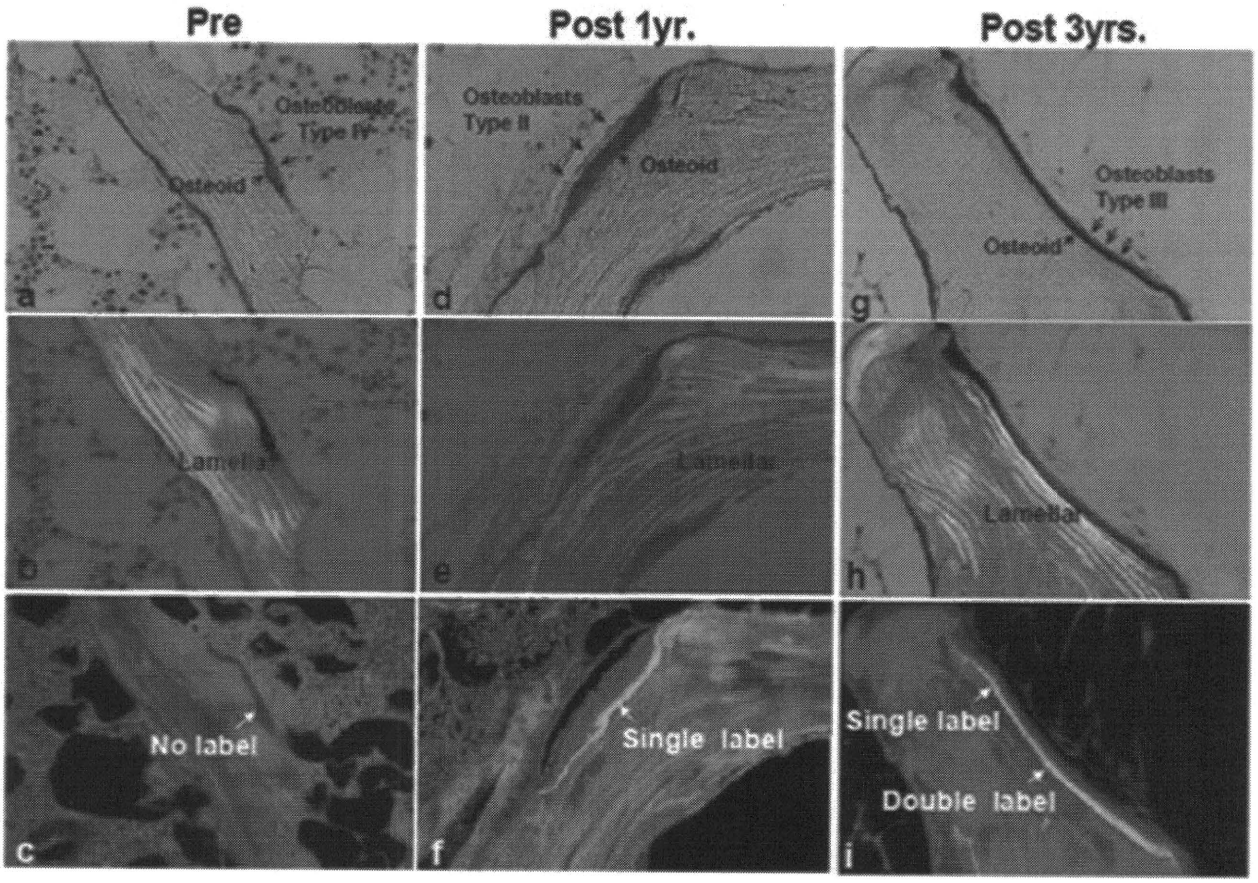

FIG. 3. Microscopic images of pre- and post treatment cancellous bone from a patient with an adynamic bone-type disorder (Case: 01-06). Light (a, d, g), polarizing (b, e, h), and fluorescent (c, f, i) microscopy at a magnification of ×400.

TABLE 4. *Parameters for histomorphometric analysis of cancellous bone*

Parameters	Case 01-03			Case 01-06		
	Baseline	1 year	3 years	Baseline	1 year	3 years
Bone volume (%)	17.3	17.1	18.5	9.0	NC	18.0
Formation						
Osteoid volume (OV/BV) (%)	1.99	1.88	0.79	1.60	3.58†	4.18
Osteoid thickness (μm)	10.0	13.6	8.8	5.9	9.8†	9.8
Resorption						
Eroded surface (%)	7.0	15.0	12.0	1.4	2.2†	5.7
Fibrous volume (Fb.V/TV) (%)	0.79	0.26	0.00	0.00	0.00	0.00
Mineralization						
Mineral apposition rate (μm/day)	0.54	0.43	0.68	0.34	NC	0.29
Bone formation rate (BFR/BV) (%/year)	22.8	40.1	32.6	0.1	NC	11.3
Bone formation rate (mm^3/mm^2/year)	0.014	0.024	0.021	0.0003	NC	0.007
Activation frequency (*N*/year)	NC	NC	0.62	0.01	NC	0.18

†Reference data. Case 01-03 was diagnosed as an osteitis fibrosa-type disorder based on OV/BV < 15%, Fb.V/TV > 0.5% at baseline. After one year of treatment, the diagnosis had improved to a milder type of disorder based on OV/BV < 15%, Fb.V/TV < 0.5%, and BFR/BV > 3.9%/year. After three years of treatment, the diagnosis of a milder type was maintained. Case 01-06 was diagnosed as an adynamic bone disease-type disorder based on OV/BV < 15%, Fb.V/TV < 0.5%, and BFR/BV < 3.9%/year at baseline. After three years of treatment, the diagnosis had improved to a milder type disorder based on OV/BV < 15%, Fb.V/TV < 0.5%, and BFR/BV > 3.9%/year. BS, bone surface; NC, not calculated; TV, tissue volume.

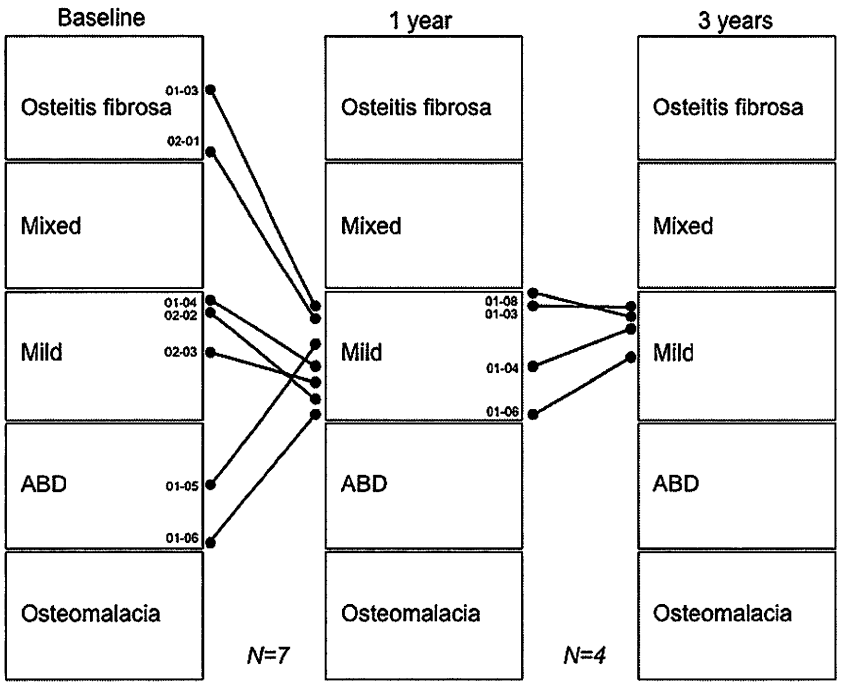

FIG. 4. Summary of the changes to the classification of renal osteodystrophy occurring in each case over the three-year follow-up period. Each line represents an individual patient. Not all biopsies were suitable for histomorphometric analysis. Pre-treatment diagnosis was a fibrous osteitis-type disorder in two patients, a milder type disorder in three patients, and an adynamic bone disease (ABD)-type disorder in two patients. After one year of treatment the diagnosis changed to a milder type disorder in all patients. After three years of treatment a bone biopsy was conducted in a total of four patients. The diagnosis of a milder type disorder was maintained in all four patients.

et al. (4), the baseline classifications of ROD for cases 01-03 and 01-06 were defined as a fibrous osteitis-type disorder and an adynamic bone-type disorder, respectively.

In Case 01-03, the classification of ROD at one year and three years after treatment was of the milder-type. Polarizing images showed that the fibrous tissues (stained brown) were present at pre-treatment, most of which disappeared after one year of treatment, and were no longer observed after three years of treatment (Fig. 2b,e,h).

The fluorescent image of pre-treatment bone shows a wide single label indicative of a mineralization defect (Fig. 2j), whereas the post-one-year bone image shows separated double labels, indicative of improvement in the mineralization defect (Fig. 2k). The post-three-years fluorescent image reveals narrow double labels, indicating that the bone condition has further improved toward normal mineralization (Fig. 2l).

In Case 01-06, the classification of ROD at one year and three years after treatment was also of the milder-type. The pre-treatment fluorescent image showed no labels, indicative of severe mineralization defect with complete loss of mineralization (adynamic bone disease) (Fig. 3c). The post-one-year fluorescent image showed slightly wide single labels (Fig. 3f); the post-three-years image showed double labels, indicating that mineralization had further improved and was partially normalized (Fig. 3i). These findings suggest that the mineralization defect was gradually relieved over time from the pre-treatment phase through to one and three years post-treatment.

In a similar way, other cases are evaluated and summarized in Figure 4. In a total of seven patients, the pre-treatment diagnosis was a fibrous osteitis-type disorder in two patients, a milder type disorder in three patients, and an adynamic bone disease-type disorder in two patients. After one year of treatment, the diagnosis changed to a milder type disorder in all seven patients. After three years of treatment, a bone biopsy was conducted for a total of four patients. The diagnosis of a milder type disorder was maintained in all four patients.

Safety

Drug-related adverse events were reported nine out of 14 patients. The most frequently reported drug-related adverse events were mainly gastrointestinal disorders (nine cases): nausea (five cases), vomiting (four cases), and stomach discomfort (two cases). A drug-related serious adverse event was reported in one case (ventricular extrasystole). There were no deaths in this study, and were no clinically meaningful changes in laboratory parameters or vital signs. Two patients discontinued the study treatment because of adverse events (gastric discomfort [drug-related] and cerebral infarction [non-drug-related]). There was no bone-related adverse event.

DISCUSSION

In this study, we evaluated lanthanum concentration in bone and the effect of LC on bone metabolism in Japanese dialysis patients with hyperphosphatemia who received long-term treatment with LC.

The median of the bone lanthanum level was 54 μg/kg at baseline, and it increased to 984 μg/kg after one year of treatment, which was within the range of previous reports (770 and 1770 μg/kg [median]) (17,18). After continuous treatment for three years, a further increase in the bone lanthanum level was observed. In a previous report from Western countries, the bone lanthanum level was approximately 5000 μg/kg (median) after 4.5 years of treatment (19). In our study with Japanese patients, the bone lanthanum level was 4271 μg/kg after three years of treatment. Although the direct comparison is impossible due to differences in the dose range and the duration of the treatment, there seem to be no major differences in both lanthanum levels. Therefore, ethnic differences in the bone lanthanum level between Caucasian and Japanese patients seem to be minor, while there are large variations within each patient. The similar levels of lanthanum distribution in the bone of Caucasians and Japanese may be due to similar plasma lanthanum levels at one-year treatment (0.329 μg/L in this study and 0.51 to 1.08 μg/L in studies in Western countries) (15,20). There was no significant correlation between bone lanthanum concentration and cumulative dosage of LC, perhaps due to the small sample size (Pearson's method, data not shown). Further evaluation would be necessary.

The bone deposition of lanthanum showed generalized distribution and no association with pathological features, unlike aluminum. Aluminum is concentrated at mineralizing fronts and is toxic to osteoblasts (21). Consistent with these findings, clinical evidence published to date has suggested no evidence of an adverse effect of LC on bone (22).

The results of bone histomorphometric analysis of cancellous bone showed that treatment with LC improved the osteoid condition toward normalization in both cases of excessive and insufficient osteoid, and that the treatment also improved the resorption condition toward normalization in cases with both enhanced and reduced resorption. After one year of treatment: "osteitis fibrosa", noted in the two patients at baseline, changed to "mild" after LC treatment; "mild" noted in the three patients at baseline remained unchanged; and "adynamic bone disease" noted in the two patients at baseline changed to "mild". After three years of treatment, in a total of four patients, the diagnosis remained the same, but with further histomorphometric improvement in all four patients. These findings were consistent with previous studies, in which the bone turnovers were improved from either low or high at baseline toward normal after treatment with LC at follow-up in the majority of patients (15–17). In addition, they concluded that there is no evidence of adverse effects of LC on bone mineralization (15–17).

Malluche et al. reported correlations between osteocalsin and BAP with histomorphometrically assessed bone formation rate/bone surface (BFR/BS) (16). Therefore, in order to examine a possibility of bone metabolism markers to predict ROD classification, we measured intact PTH and plasma bone metabolism markers (Fig. 1). The correlation between BAP and BFR/BS was confirmed and the tendency of the correlation between osteocalsin and BFR/BS was observed in our study (data not shown). However, there seemed to be no correlations between bone metabolism markers and ROD classifications.

In our study, there was no adverse effect concerning bone metabolism due to treatment with LC. In rats, mineralization defects were caused by very high doses of LC (1000 mg/kg/day) (23); however, this has been conclusively demonstrated to be due to phosphate depletion rather than a direct effect of lanthanum on bone (24). The bone kinetics of lanthanum in humans have recently been reported and modeled in detail (25). Bronner et al. estimated that lanthanum was cleared from bone at approximately 13% per year after treatment cessation. According to this estimation, bone lanthanum concentrations would approach pretreatment concentrations in about eight years; they concluded that this clearance rate would be high compared with the bone turnover rate in a healthy individual, generally considered to be 3.6% per year (25).

CONCLUSION

In spite of the increase of bone lanthanum levels after long term treatment with LC, beneficial effects rather than effects concerning bone metabolism were observed in Japanese dialysis patients. Lanthanum carbonate is therefore expected to improve the symptoms of renal osteodystrophy.

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ORIGINAL ARTICLE

Three-year extension study of lanthanum carbonate therapy in Japanese hemodialysis patients

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Abstract
Background Lanthanum carbonate is a non-aluminum, non-calcium phosphate binder. Its efficacy and its safety profile up to 1 year have been reported in Japanese hemodialysis patients.
Methods The present study was an extension of the earlier study. One hundred and forty-five patients were enrolled in the original 1 year observational Phase III study. After 1 year of treatment, 63 patients continued with further lanthanum treatment. Lanthanum carbonate was administered at 750–4,500 mg/day for up to 156 weeks (3 years). The reduction in serum phosphate was used to evaluate efficacy, and laboratory markers of bone turnover were monitored.
Results The serum phosphate level was maintained at a significantly lower level ($P < 0.05$) than the baseline level during the 3-year study period. Most of the drug-related adverse events were mild and were mainly gastrointestinal disorders. The safety profile of lanthanum during 3 years of treatment was similar to that seen in the previous study. There were no clinically relevant changes in vital signs or the electrocardiogram. Bone turnover markers, such as osteocalcin, bone-specific alkaline phosphatase, and crosslinked *N*-telopeptide of type I collagen, showed no clinically relevant changes.
Conclusion Lanthanum therapy was able to reduce and maintain the serum phosphate level within the K/DOQI and JSDT guideline ranges in Japanese dialysis patients for 3 years.

Keywords Hyperphosphatemia · Lanthanum carbonate · Phosphate binder · Hemodialysis

Introduction

Hyperphosphatemia is a common complication of renal failure in patients with chronic kidney disease (CKD), and is associated with increased cardiovascular mortality of dialysis patients [1–3]. Dialysis and restricted dietary phosphate intake are effective at reducing the serum phosphate level to some extent, but are usually not sufficient to achieve the target level. Accordingly, the majority of CKD patients will require treatment with phosphate binders to trap dietary phosphate and reduce its absorption [4].

Lanthanum carbonate (LaC) is a non-aluminum, non-calcium phosphate binder that has been available in the US and Europe and was also approved in Japan in 2009. Preclinical studies have shown that lanthanum binds phosphate throughout the gastrointestinal tract regardless of the pH and is poorly absorbed from the gut [5, 6].

Both short-term and long-term clinical trials have demonstrated the safety and efficacy of LaC in dialysis patients, but mainly in Western countries [7–18]. The longest duration of LaC treatment in these trials is 6 years [19]. In Japan, a randomized, placebo-controlled Phase IIb dose-finding study, a double-blind comparative Phase III study, a long-term (12-month) Phase III study in hemodialysis patients, and a small open-label single-arm Phase III study in patients on continuous ambulatory peritoneal dialysis have been conducted, and these studies have

The co-investigators in the Lanthanum Carbonate Research Group are listed in the Appendix.

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demonstrated the efficacy and safety of LaC in Japanese dialysis patients [20–22]. Since the control of phosphate is required in the majority of CKD patients on dialysis, and the life expectancy of Japanese patients is longer than that of patients in Western countries [23], long-term safety data on LaC in a Japanese dialysis population is needed. In order to obtain such long-term data, we extended the previous 1-year Phase III study to a total of 3 years.

Materials and methods

Study design

This extension of the long-term Phase III study was an uncontrolled, open-label, observational multicenter trial conducted in Japan to obtain safety and efficacy data of LaC for up to 3 years.

The long-term Phase III study was originally designed to run for 1 year, but it was extended to a 2-year study and then to a 3-year study. The design of the original 1-year Phase III study has been reported previously [22]. In brief, patients treated with LaC during the Phase II dose-finding study ($n = 107$) continued to receive LaC therapy in the 1-year Phase III study. In addition, patients who had received placebo in the previous Phase II dose-finding study ($n = 27$) and newly recruited patients ($n = 11$) initiated LaC treatment for the 1-year study (Fig. 1). The Institutional Review Boards approved extensions of the study on both occasions. Informed consent for further participation in the study was obtained from the patients upon the completion of 1 and 2 years of treatment. The study was conducted in accordance with the Good Clinical Practice Guidelines and the Declaration of Helsinki.

Subjects

Patients who had completed the 1-year study and were judged to be eligible to continue by the investigators were eligible to enter the extension study if they agreed to continue their participation by providing written informed consent.

Based on the serum phosphate level (target 3.5–5.5 mg/dL) and the tolerability of treatment, the dose of LaC was adjusted within the range of 750–4,500 mg/day in increments of 750 mg/day on a weekly basis. Patients who continued from the Phase II dose-finding study received LaC at a starting dose of 750 mg/day, as described

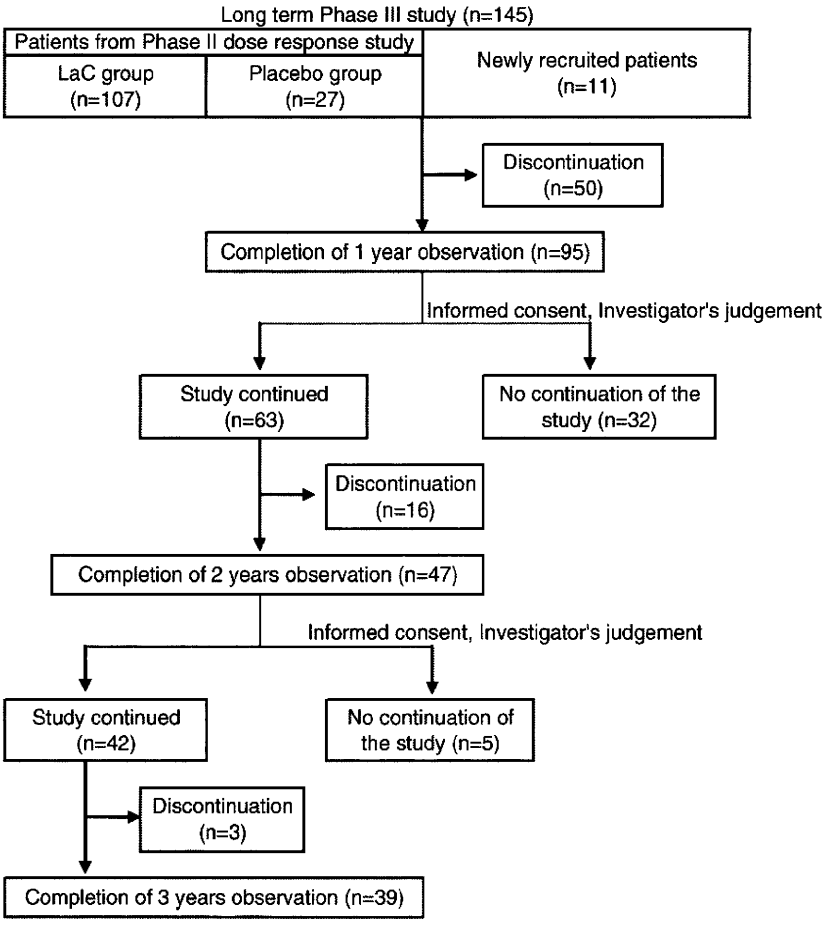

Fig. 1 Disposition of the patients

previously [22]. The total maximum treatment periods were 150 and 156 weeks for patients in the placebo and active arms, respectively. In order to avoid problems with interpreting the data, the start of open-label treatment when all patients were receiving 750 mg/day was defined as week 0.

Endpoints

Patients were followed up every 4 weeks during the extension study. The efficacy of LaC therapy was assessed based on the rate that the the target serum Pi ($\geq$3.5 mg/dL and $\leq$5.5 mg/dL) was achieved. The serum Pi level, the serum-corrected calcium level, the serum calcium × phosphate product, and the serum intact parathyroid hormone (iPTH) level were also summarized descriptively.

For the safety evaluation, the incidence of treatment-emergent drug-related adverse events, concomitant therapy, and compliance with treatment were evaluated. Safety-related endpoints included vital signs, laboratory data, electrocardiographic (ECG) data, and the serum lanthanum level.

Laboratory tests

Blood samples before hemodialysis were collected for routine biochemistry, hematology tests and bone turnover markers. The plasma concentration of lanthanum was measured by inductively coupled plasma mass spectrometry. All measurements were carried out at central laboratories.

Statistical analysis

All variables were analyzed by calculating descriptive statistics: frequency tables for categorical variables and the mean, standard deviation (SD), minimum, median, quartiles, and the maximum values for continuous variables. Statistical analyses were performed using Student's t test. The level of statistical significance was defined as $P < 0.05$.

Results

One hundred and forty-five patients from 15 centers were enrolled, and all of them were included in the safety analysis. Two patients were excluded from the efficacy analysis because the duration of LaC treatment was less than 14 days.

Thirty-nine patients completed the entire 3 years of treatment. Table 1 shows the reasons for patient discontinuation during years 1, 2, and 3 of the extension study.

Table 1 Discontinued patients

	1st year[a]	2nd year[b]	3rd year[c]
Total number of discontinued patients	50	16	3
Adverse events	32	6	1
Consent withdrawn	10	1	0
Investigator judgement	6	4	1
Insufficient therapeutic effect	1	1	0
Protocol definition	0	2	1
Protocol violation	0	2	0
Death	1	0	0

[a] Number of patients who discontinued before the completion of 1 year

[b] Number of patients who discontinued from 1 to 2 years

[c] Number of patients who discontinued from 2 to 3 years

Table 2 Demographic data

	Total (N = 145)
Age (mean ± SD, years)	57.4 ± 9.8
Sex	
Male	85 (58.6%)
Female	60 (41.4%)
Weight (mean ± SD, kg)	59.0 ± 10.8
Years on dialysis (mean ± SD)	9.3 ± 5.7
Underlying disease causing renal failure	
Chronic glomerulonephritis	74 (51.0%)
Diabetic nephropathy	27 (18.6%)
Others	44 (30.3%)

Discontinuation was generally because of adverse events. Demographic data are summarized in Table 2.

LaC dosage

Figure 2 shows the changes in the dose of LaC during the 3-year study period. With weekly dose adjustment based on the serum Pi value, there was an increase in the dose (mean ± SD) from 750 ± 0 mg/day in week 0 to 1,875 ± 750 mg/day at the end of treatment. The dose of LaC reached a plateau in week 7, and dose changes were similar in the subgroups with and without vitamin D therapy (final LaC doses for these subgroups were 1,875 ± 750 and 1,350 ± 689 mg/day, respectively).

Efficacy

The mean serum Pi level was 8.03 ± 1.51 mg/dL at baseline, and it decreased to 5.33 ± 1.27 and 5.33 ± 1.04 mg/dL after 1 and 3 years of treatment, respectively. The mean

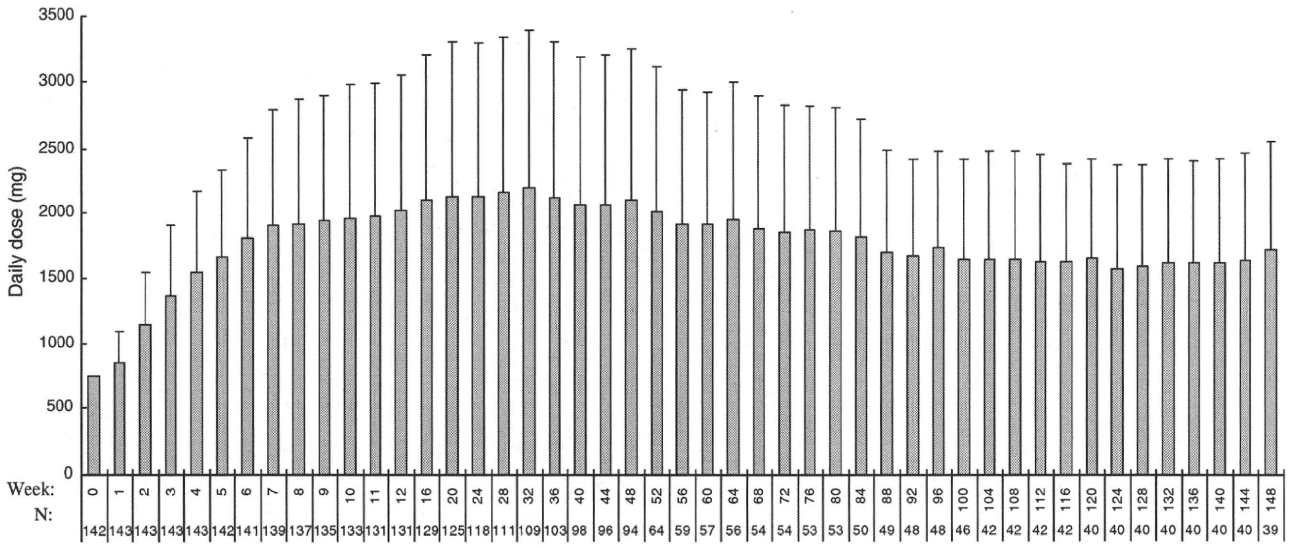

Fig. 2 Daily dose of lanthanum carbonate. The daily dose of lanthanum carbonate is shown as the mean ± SD. Twenty-six and 106 patients entered the open-label treatment phase (week 0) at an initial dose of 750 mg/day from the placebo and lanthanum carbonate arms of the Phase II dose-finding study, respectively. Eleven new patients started open-label treatment from week 0 at 750 mg/day

reduction of Pi from baseline was within the range of −1.51 ± 1.48 mg/dL in week 1 (95% CI −1.76, −1.27) to −3.08 ± 1.76 mg/dL in week 128 (95% CI −3.69, −2.47) (Fig. 3a). The reduction in Pi was significant ($P < 0.05$, vs. baseline) at all evaluated time points. Figure 4 shows the percentages of patients whose serum Pi levels were within the target ranges of the guidelines of KDOQI (3.5–5.5 mg/dL, Fig. 4a) and the Japanese Society for Dialysis Therapy (JSDT 3.5–6.0 mg/dL, Fig. 4b). After week 10, ca. 50–85% and ca. 70–90% of the patients were within the target ranges of the respective guidelines.

The mean corrected serum calcium level was generally stable and remained within the target range according to the guideline for 3 years (Fig. 3B).

The mean Ca × P value showed similar behavior to the serum Pi. The reduction in the Ca × P value was significant ($P < 0.05$, vs. baseline) at all evaluated time points (Fig. 3C).

The iPTH level was generally stable throughout the treatment period, with a median value of 262.0 pg/mL at baseline and 283.8 pg/mL at 3 years (Fig. 3D).

Safety

Almost all patients (99.3%, 144/145) experienced at least one adverse event during the 3 years of the study, and 60.0% (87/145) had adverse events related to the study drug. However, most of the drug-related adverse events were mild (87.3%, 76/87), with 8 and 3 patients having moderate and severe drug-related adverse events, respectively. The severe events were unstable angina, constipation, and breast cancer ($n = 1$ each). The incidences and profile of the drug-related adverse events during the present 3-year study were similar to those observed during the previous study [22]. The most common drug-related adverse events were gastrointestinal disorders, such as vomiting (32.4%), nausea (29.0%), stomach discomfort (16.6%), upper abdominal pain (9.7%), and diarrhea (9.0%) (Table 3).

Serious drug-related adverse events were reported in six patients. There was one death due to acute myocardial infarction during this study, but it was not related to the study drug according to the reporting investigator.

During the 3-year observation period, there were no consistent patterns of change in the mean values of laboratory parameters, vital signs, and ECG parameters.

Table 4 shows the means and standard deviations of bone turnover markers; that is, alkaline phosphatase (ALP), osteocalcin, bone-specific alkaline phosphatase (BAP), and crosslinked *N*-telopeptide of type I collagen (NTx).

Plasma lanthanum level

Figure 5 shows the plasma lanthanum level over the 3 years of the study. Lanthanum levels increased slightly from 2 weeks to 7 months and then were almost unchanged from 7 months to 3 years. The baseline plasma lanthanum level was also measured, but the concentration was below the lower limit of quantification (<0.03124 ng/mL) in all subjects.

Vitamin D therapy

The numbers of patients on oral vitamin D treatment, parenteral vitamin D, or no vitamin D therapy were,

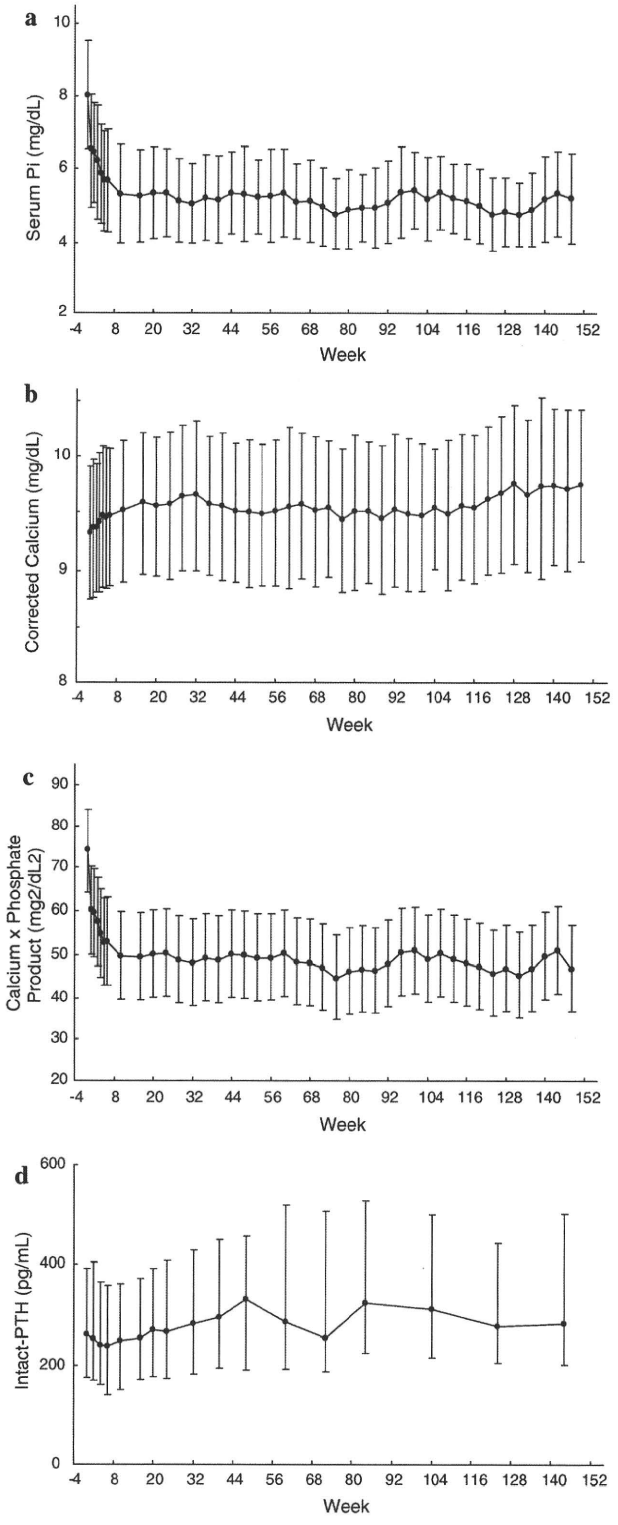

Fig. 3 Changes in serum phosphate and calcium-related variables: **a** serum inorganic phosphate (Pi); **b** corrected calcium; **c** calcium × phosphate product; **d** intact parathyroid hormone (intact-PTH). Mean ± SD values are shown for **a–c** and median ± 25th percentile for **d**

respectively, 68 (47.6%), 28 (19.6%), and 47 (32.9%) at baseline versus 66 (46.2%), 55 (38.5%), and 22 (15.4%) at the last observation. The median (min, max) value of the maximum dose of vitamin D (regardless of the route of administration) in week 0 was 0.375 (0.15, 15) μg for the 96 treated patients, and it increased slightly to 0.500 (0.25, 15) μg in the last week for the 121 patients treated at that time.

Discussion

In this study, LaC therapy maintained a reduction in the phosphate level for up to 3 years in Japanese dialysis patients. Hyperphosphatemia is directly linked to increased morbidity and mortality in CKD patients on dialysis [1–3]. Therefore, long-term control of phosphate by safe and effective phosphate binders is necessary to improve the prognosis of these patients.

Previous placebo-controlled trials have demonstrated that LaC reduces serum phosphate effectively [9], and a large open-label randomized trial indicated that the efficacy of LaC was similar to that of calcium carbonate for controlling serum phosphate [12].

The long-term (12-month) Japanese Phase III study showed that the reduction in the serum Pi from baseline was 2.62 ± 2.12 mg/dL (95% CI 3.05, 2.17) after 1 year of treatment [22], while serum Pi was reduced by 2.34 ± 2.08 mg/dL (95% CI 2.68, 1.99) after 3 years in the present study (Fig. 3a). These results for Pi after both 1 and 3 years are similar to those obtained in a previous short-term (8-week) Phase III double-blind study (decrease of 2.58 mg/dL) [21, 22]. The percentage of patients with serum phosphate within the K/DOQI (3.5–5.5 mg/dL) or JSDT (3.5–6.0 mg/dL) target range was fairly stable throughout the 3-year observation period after it had reached a plateau (Fig. 4).

Besides phosphate, serum calcium is also related to mortality, making the control of this parameter important [1–3]. In the present study, the serum calcium level was relatively constant and was maintained within the normal range throughout 3 years of LaC treatment (Fig. 3b).

Serum iPTH generally remained stable during the 3 years of observation (Fig. 3d). At the start of the study, 96 patients were being treated with vitamin D versus 121 patients at the end of the study. The median dose of vitamin D increased slightly during the 3-year treatment period. Both of these findings imply that vitamin D was required to control the iPTH level. The corrected serum calcium level was maintained over 3 years of treatment in the groups

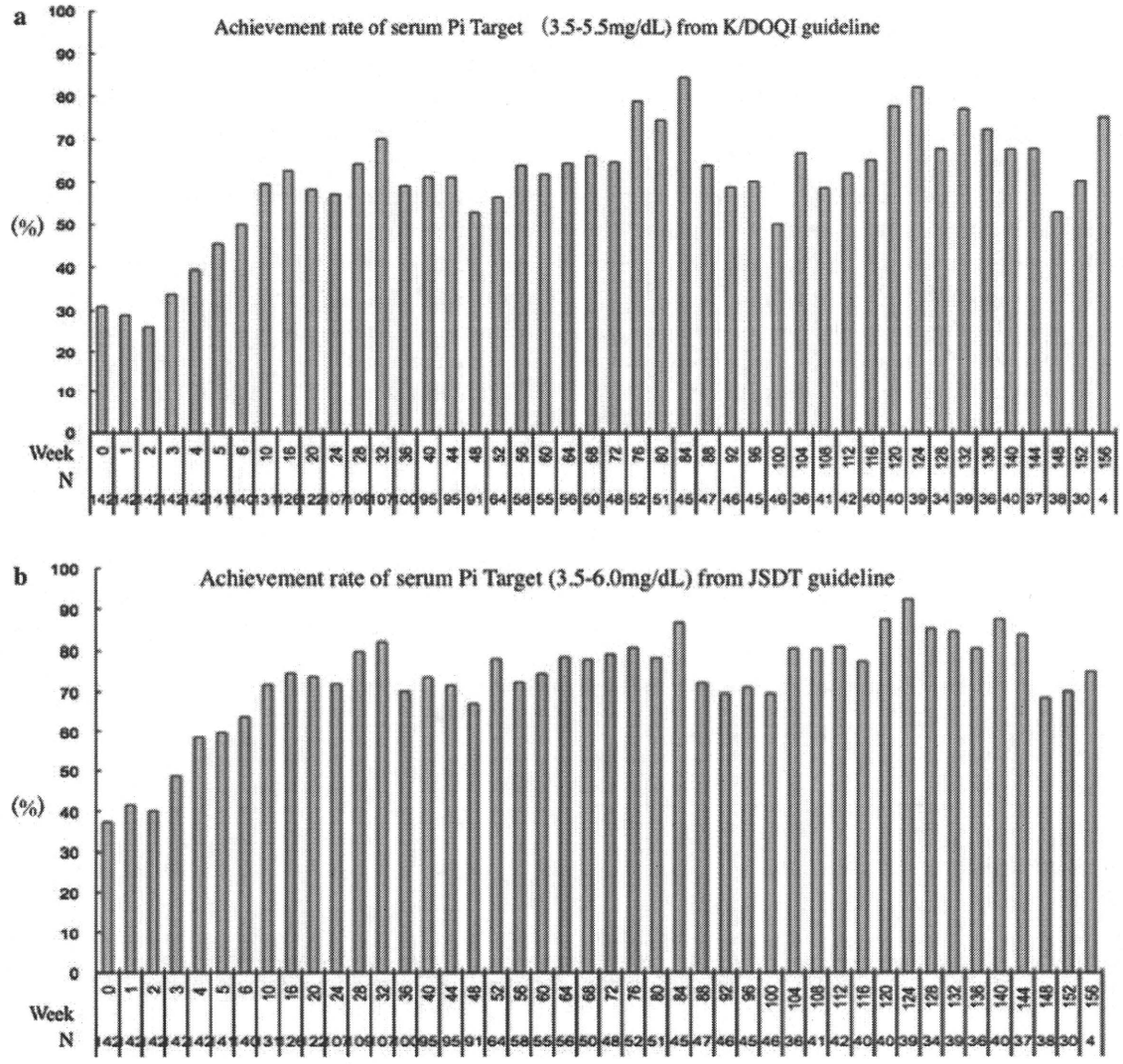

Fig. 4 Achievement rate for the target serum phosphate level. The percentage of patients with serum phosphate levels within the target ranges of the guidelines of KDOQI (3.5–5.5 mg/dL) (**a**) and the Japanese Society for Dialysis Therapy (3.5–6.0 mg/dL) (**b**) during each week is shown

with and without vitamin D treatment (data not shown), as observed in the previous 1-year study [22]. Although caution should be applied when interpreting this result, we would suppose that LaC can be used safely together with vitamin D in hemodialysis patients.

During the present study, the dose of LaC decreased slightly after 1 year and the mean dose was approximately 1,600 mg/day (Fig. 2). Over 3 years of treatment, 60% of the patients experienced drug-related adverse events, but most of them were mild. Gastrointestinal disorders such as vomiting, nausea, and stomach discomfort were the most common drug-related adverse events (Table 3). The frequency and nature of these adverse events were similar to those noted during the first year of treatment in this study, and there were no unexpected or clinically significant abnormalities of laboratory tests, vital signs, and ECG parameters [22]. The percentage of drug-related adverse events in this study was higher than those reported in long term studies in Western countries [18, 19], and their profile was slightly different. Vomiting, nausea and stomach discomfort were the major adverse events in Japanese, but in non-Japanese nausea, diarrhea and flatulence have been reported [19]. However, gastrointestinal symptoms dominated in Japanese and non-Japanese, and the majority of them were mild to moderate in intensity.

Although 39 patients discontinued the study due to adverse events during the 3-year treatment period (Table 1), 16 of them had adverse events that were unrelated to the study drug. Among the 23 patients who discontinued due to study drug-related adverse events, these events were mainly gastrointestinal disorders such as vomiting and nausea. Most of the gastrointestinal disorders

were mild and the patients recovered rapidly after stopping LaC treatment.

No clinically relevant changes were found in the means of bone turnover markers such as osteocalcin, BAP, and NTx. These findings were similar to those noted after 1 year of treatment [22]. In addition, there were no reports of drug-related fractures during 3 years of treatment. This suggests that LaC treatment for up to 3 years is not associated with any undesirable effects on bone.

Although the absolute bioavailability of LaC is extremely low (0.00127 ± 0.00080%) [24], the plasma lanthanum level increased slightly from 2 weeks to 7 months and then was almost constant from 7 months to 3 years. The measured lanthanum levels showed a wide variation. In an overseas long-term study that continued for up to 6 years, plasma lanthanum levels greater than 2.0 and 4.0 ng/mL were recorded on 15 and 4 occasions, respectively [19]. In the present Japanese long-term study, only 2 lanthanum levels over 2.0 ng/mL were recorded (the values were 2.01 ng/mL at 7 months and 3.05 ng/mL at 2 weeks in different patients without special complications such as liver disorders). The plasma concentration was within the range reported during previous long-term studies from Western countries [18, 19].

Although there are limitations on interpreting our results due to the design of the present study (open label, single arm, flexible vitamin D use, etc.), we did not observe any unexpected adverse events associated with LaC therapy during this 3-year study period.

A further study in Japanese would be required for a longer-term safety evaluation of LaC.

Conclusions

Lanthanum carbonate therapy was able to reduce and maintain the serum phosphate level within the guideline range for up to 3 years in Japanese hemodialysis patients.

Table 3 Drug-related adverse events occurring in at least 3% of all patients

Adverse event[a]	Number of patients (N = 145)
Any event	87 (60%)
Blood and lymphatic system disorders	
Iron-deficiency anemia	8 (6%)
Endocrine disorders	
Secondary hyperparathyroidism	10 (7%)
Gastrointestinal disorders	
Vomiting	47 (32%)
Nausea	42 (29%)
Stomach discomfort	24 (17%)
Upper abdominal pain	14 (10%)
Diarrhea	13 (9%)
Dyspepsia	7 (5%)
Constipation	6 (4%)
Reflux esophagitis	6 (4%)

[a] Adverse events observed during the dose-finding study were excluded

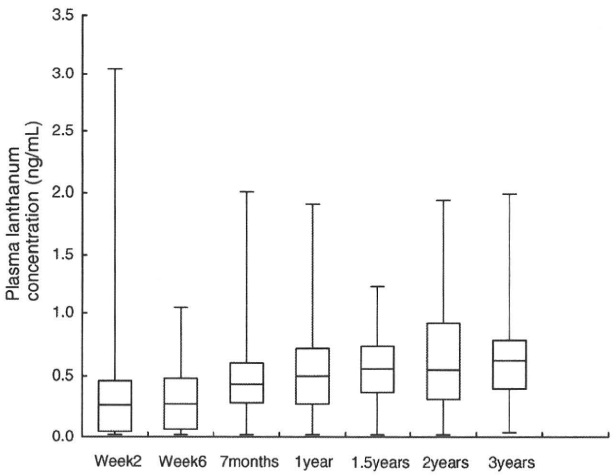

Fig. 5 Plasma lanthanum levels. The *bar in each box* indicates the median value. The *upper* and *lower borders* of the *boxes* are the 25th and 75th percentiles, respectively. Maximum and minimum values are shown by the *error bars*

Table 4 Changes in bone turnover parameters

	Baseline	After 1 year	After 3 years	Normal range
Osteocalcin (mg/L)	86.5 ± 70.4	124.5 ± 86.6	81.8 ± 56.5	3.10–12.70
BAP (U/L)	24.2 ± 11.5	39.1 ± 23.6	38.4 ± 18.0	M 13.00–33.90 F 9.60–35.40
NTx (nmol BCE/L)	168.8 ± 111.4	224.3 ± 141.1	171.5 ± 138.2	N/A
ALP (U/L)	239.1 ± 97.6	318.9 ± 146.1	310.6 ± 163.6	100–325

ALP alkaline phosphatase, *BAP* bone-specific alkaline phosphatase, *BCE* bone collagen equivalent, *NTx* crosslinked *N*-telopeptide of type I collagen, *M* male, *F* female, *N/A* not available

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Conflict of interest This study was carried out by Bayer Yakuhin, Ltd. as a GCP Phase III trial. The author (T. Shigematsu MD, Ph.D.) was the principal investigator of this study. The author also received honoraria for Bayer Yakuhin, Ltd. lecture meetings and a research grant from Bayer Yakuhin, Ltd. However, the author has never had any involvement that may raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

Appendix

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