Does the Phosphate Binder Lanthanum Carbonate Affect Bone in Rats with Chronic Renal Failure?

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Abstract. Adequate control of phosphate levels remains an important issue in patients with chronic renal failure (CRF). Lanthanum carbonate has been proposed as a new phosphate binder. Previous studies have shown a high phosphate binding capacity (>97%) and low gastrointestinal absorption of lanthanum, without serious toxic side effects in the presence of a normal renal function (NRF). Because of lanthanum's physicochemical resemblance to calcium, the possible effects of it on bone have to be considered. The aim of this study was to investigate the effects of lanthanum carbonate on bone histology in NRF and CRF rats after oral administration of the compound with doses of 100, 500, or 1000 mg/kg per d for 12 wk. Bone histomorphometry showed that CRF animals that received vehicle developed secondary hyperparathyroidism. Urinalysis of lanthanum-loaded CRF animals showed a dose-dependent decrease in urinary phosphorus excretion, which was clearly more pronounced in the CRF groups compared with NRF animals. Phosphatemia, however, remained normal. Lanthanum carbonate administration induced a dose-dependent decrease in bone formation rate and increase in osteoid area in CRF animals. Three of seven animals in the CRF-1000 group and one of eight animals in the NRF-100 group were classified as having a mineralization defect. The number of cuboidal osteoblasts, however, was not affected, indicating that bone changes were not due to a toxic effect of lanthanum on the osteoblast. Furthermore, lanthanum concentrations in the femur remained low and did not correlate with histomorphometric parameters. These findings suggest that the administration of high doses of phosphate binder (1000 mg/kg per d lanthanum carbonate), in combination with decreased 25-(OH) vitamin D_3 in the uremic state, resulted in phosphate depletion and followed by an increased mobilization of phosphorus out of bone and/or reduced incorporation into bone. There was no evidence that lanthanum had a direct toxic effect on osteoblasts.

In patients with chronic renal failure (CRF), loss of renal function can lead to various metabolic disturbances. Among these, altered mineral and phosphorus metabolism still remains an important aspect of the treatment of these patients (1). Increased phosphatemia and disturbances in the vitamin D and parathyroid hormone (PTH) metabolism ultimately lead to bone disorders that cannot always be prevented by dialysis treatment. In addition to dietary phosphorus restriction, the use of phosphate binding agents is necessary in the majority of patients.

The most commonly used phosphate binders contain either aluminum or calcium. Although these compounds adequately control the phosphorus status of the patient, severe side effects may arise. Aluminum is a bone-seeking element and has been shown to interfere with normal bone mineralization and osteoblast function, leading to the so-called aluminum-related bone disease, expressed as osteomalacia, adynamic bone, or mixed renal osteodystrophy (2–4). Furthermore, occurrences of potentially lethal neurologic disorders have been associated with aluminum toxicity, as well as more subtle disorders at the level of parathyroid gland function (5), hematopoiesis, and resistance to erythropoietin (6). Calcium-containing phosphate-binders, because of the high doses needed for adequate phosphate binding, may lead to hypercalcemia, which may contribute to the development of extraosseous calcifications, especially in combination with vitamin D therapy (7).

Aiming to find new phosphate-binding agents with fewer side effects, lanthanum carbonate [$La_2(CO_3)_3$; Fosrenol] has recently been proposed. In experimental as well as in clinical studies, lanthanum carbonate has been shown to be a safe and effective phosphate binder (8–12)

Physicochemically, lanthanum has a number of characteristics similar to calcium, leading to a preferential uptake in bone. Some bone-seeking elements, such as aluminum (3,13–15) and strontium (16), have been shown to interfere with bone metabolism in CRF. The aim of this study was to investigate whether the administration of lanthanum carbonate at clinically relevant as well as high doses affects bone remodeling and osteoblast function in a rat model of CRF.

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Materials and Methods

Experimental Design

Male Wistar rats (14 wk at start of study, 400 to 450 g body wt) were randomly allocated to one of eight study groups. Four groups underwent a sham operation, and the other groups underwent a 5/6 nephrectomy by ligation of two of the three branches of the left renal artery, followed 1 wk later by removal of the right kidney.

Treatment started after a 2-wk stabilization period and consisted of daily oral gavage with a suspension of $La_2(CO_3)_3.4H_2O$ in 2% carboxymethylcellulose for 12 wk at doses of 100, 500, and 1000 mg/kg per d. Control groups received vehicle only. Each treatment was given to a group of animals with CRF and a group of animals with normal renal function (NRF). All animals were weighed weekly, and individual treatment doses were adjusted according to the most recently recorded body weight. A constant dose volume of 10 ml/kg was used. The animals had free access to food and water during the whole study period. The diet used contained 1.00% Ca, 0.75% phosphorus, and 2000 IU/kg vitamin D_3. Food and water consumption were monitored daily throughout the treatment period.

Urine and blood samples were taken before installation of the renal failure (baseline; week −4), before the start of dosing (week 0), and at monthly intervals thereafter (weeks 4, 8, and 12). For collection of urine, animals were housed in metabolic cages for 24 h. Blood samples were taken from the tail vein under ketamine/xylazine anesthesia at the end of the urine collection period. Serum, plasma, and urine samples were stored at −80°C until analysis.

At the end of the treatment period, animals were labeled with tetracycline (30 mg/kg) and demeclocycline (30 mg/kg) at 7 and 3 d, respectively, before being sacrificed. Animals were killed by exsanguination through the abdominal aorta, and bone was removed. The tibias were fixed in Burkhardt's solution for 24 h and subsequently stored in 70% ethanol at 4°C until further processing for histomorphometric analysis. Bone marrow was rinsed out from the femurs using saline, and the cortical bone was stored dry at −80°C for lanthanum analysis.

Biochemical Analysis

Creatinine, calcium, and phosphorus were determined in the serum samples with an automated Vitros 750 XRC system. PTH levels were measured using a rat PTH IRMA kit (Immutopics), performed according to the manufacturer's instructions. Alkaline phosphatase was measured according to IFCC 1983/4 (17) using 4-nitrophenol-phosphate as substrate and intestinal alkaline phosphatase as standard. Osteocalcin was measured using a rat osteocalcin ELISA kit (DRG Instruments, Germany), performed according to the manufacturer's instructions.

Urinary phosphorus and glucose were determined with an automated Vitros 250 system. Creatinine was measured by a modified Jaffé method. Total protein was assessed according to Bradford (18). Urinary pH, bilirubin, and urobilinogen were measured with Combur 10 dipsticks (Roche). Urinary calcium was determined by flame atomic absorption spectrophotometry using a Perkin-Elmer model 372 AAS (19). 25-(OH) vitamin D_3 and 1,25-$(OH)_2$ vitamin D_3 were assessed using methods previously described (20,21).

Lanthanum in plasma, urine, and bone was measured by means of inductively coupled plasma emission spectrometry at the Centre for Analytical Sciences (University of Sheffield, Sheffield, UK) according to an in-house developed, good laboratory practice-validated method.

Bone Histomorphometry

The tibias were dehydrated in increasing ethanol concentrations and impregnated in methyl-methacrylate for 6 d. Afterwards, polymerization was allowed to proceed for 48 h under N_2-atmosphere at 4°C. After polymerization, 5-μm sections were Goldner stained for visualization of osteoid and mineralized bone. Ten-micrometer sections were mounted unstained in 100% glycerol for fluorescence visualization of tetracycline labels.

The sections were analyzed using a KS-400 image analysis system. Calibration of image pixel size was performed before each measurement cycle by using a calibration grid. Bone area, osteoid area, osteoid perimeter, eroded perimeter, and quiescent perimeter were measured by manually tracing the mineralized and osteoid area and marking erosion, osteoblasts, and osteoclasts on the computer screen, after which the system calculated the areas and perimeters. Double-labeled perimeter and total perimeter were measured in a similar way on unstained sections. Interlabel distance was measured by tracing the labels, after which the system measured the distances between the labels at regular intervals, perpendicular to the labels. Out of these primary measurements, the following derived parameters were calculated according to standardized procedures (22): mineral apposition rate, bone formation rate (BFR), osteoid width, and mineralization lag time. Because in rats, non-specific single labeling is common, only the double-labeled perimeter was included in the calculation of the BFR. In the absence of published histomorphometric reference values for defining renal osteodystrophy in the rat, histologic assessment of bone lesions was based on a comparison with the concurrent NRF group receiving vehicle only. So, the bone turnover was increased when the BFR was >95th percentile of the NRF group receiving vehicle (3147 $\mu m^2/mm^2$ per d); impaired mineralization was present when the BFR was <5th percentile of the NRF control group (570 $\mu m^2/mm^2$ per d) in combination with an osteoid area >95th percentile (1.86%).

Statistical Analyses

Results are shown as either mean ± SEM or individual measurements. For each time point and variable, a Kruskall-Wallis test was performed to test for differences between groups, followed by a Mann-Whitney *U* test with Bonferroni correction when significant differences between groups were found. Differences between groups were considered significant at $P < 0.05$.

For calculation of the mean urinary phosphorus levels and further statistical analysis, results below the detection limit of the assay (4.4 mg/dl) were replaced by half the detection limit (2.2 mg/dl). The more conservative approach of replacing these results by the detection limit itself did not influence the statistical results and conclusions. Correlations between histomorphometric results and renal function were calculated using the nonparametric Spearman Rho test and results were considered significant at $P < 0.05$.

Results

Biochemical Analysis

Food and water consumption did not differ significantly between treatment groups. Body weight evolution was similar in all groups. Average weight at the start of the study (mean ± SD) was 421.76 ± 40.12; average weight at the end of the study was 525.26 ± 60.63 g. Average daily food intake was 27.2 g per animal.

In the NRF groups, serum creatinine remained normal throughout the study period. In CRF groups, a statistically significant increase in serum creatinine level was seen throughout the treatment period (Table 1). No differences between treatment doses were observed. Similarly, proteinuria remained normal in all NRF animals, whereas CRF animals showed a

Table 1. Serum creatinine levels (mg/dl) in the various study groups (mean ± SEM)[a]

Treatment	*N*	Baseline (Week −4)	Start of Treatment (Week 0)	End of Treatment (Week 12)
NRF				
vehicle	9	0.38 ± 0.04	0.47 ± 0.02	0.47 ± 0.04
100 mg/kg per d	8	0.44 ± 0.02	0.45 ± 0.02	0.46 ± 0.03
500 mg/kg per d	9	0.41 ± 0.01	0.47 ± 0.03	0.50 ± 0.02
1000 mg/kg per d	10	0.49 ± 0.03	0.46 ± 0.03	0.43 ± 0.02
CRF				
vehicle	9	0.43 ± 0.02	1.37 ± 0.12[b]	3.20 ± 0.94[b]
100 mg/kg per d	7	0.41 ± 0.01	1.13 ± 0.06[b]	1.57 ± 0.23[b]
500 mg/kg per d	8	0.43 ± 0.03	1.26 ± 0.09[b]	1.85 ± 0.31[b]
1000 mg/kg per d	7	0.41 ± 0.02	1.29 ± 0.11[b]	1.80 ± 0.24[b]

[a] NRF, normal renal function; CRF, chronic renal failure.
[b] $P < 0.05$ *versus* NRF receiving same treatment dose. No statistically significant differences were noted between different treatments within the NRF and CRF groups.

statistically significant increase after induction of CRF (week 0, 29 ± 26 mg/24 h; week 4, 114 ± 91 mg/24 h; week 12, 176 ± 85 mg/24 h).

In NRF animals, serum PTH concentrations did not change *versus* baseline during the treatment period and were not affected by administration of lanthanum carbonate (Table 2). In CRF animals, serum PTH was consistently higher than in the corresponding NRF group. Furthermore, in all CRF groups, there was a steady increase in PTH levels after surgery, pointing to the development of hyperparathyroidism (Table 2; intermediate time points not shown).

Serum calcium did not differ between treatment groups or renal status. However, a significant increase in urinary calcium excretion was noted in all CRF animals when compared with NRF animals, at the start of dosing (week 0; NRF, 0.57 ± 0.05 mg/24 h; CRF, 4.83 ± 0.38 mg/24 h; $P < 0.01$), which lowered but was still significantly higher by the end of the observation period (week 12; NRF, 0.36 ± 0.03 mg/24 h; CRF, 1.04 ± 0.16 mg/24 h; $P < 0.01$). No effect of treatment dose on urinary calcium excretion was seen.

Serum phosphorus levels did not show any statistically significant differences over time or between groups (Figure 1A). Urinary phosphorus excretion was significantly lower in the CRF animals that received the highest treatment dose when compared with the vehicle group at weeks 4, 8, and 12 (Figure 1B). Indeed, phosphate levels were below the detection limit of the assay (4.4 mg/dl) in the urine of 14% of the CRF animals that received the 1000 mg/kg per d dose already after 4 wk of treatment. By the end of the treatment period, urinary phosphorus was undetectable in 57% of the CRF animals that were treated with 1000 mg/kg per d. In the NRF groups, a similar trend of lower urinary phosphate excretion with increasing treatment dose was visible, but the effect was much less marked and did not reach statistical significance. Urinary phos-

Table 2. Serum PTH levels (pg/ml) in the various study groups (mean ± SEM)[a]

Treatment	*N*	Baseline (Week −4)	Start of Treatment (Week 0)	End of Treatment (Week 12)
NRF				
vehicle	9	60 ± 7	64 ± 9	92 ± 29
100 mg/kg per d	8	61 ± 5	73 ± 8	89 ± 25
500 mg/kg per d	9	58 ± 5	75 ± 8	191 ± 115
1000 mg/kg per d	10	58 ± 9	66 ± 8	77 ± 14
CRF				
vehicle	9	57 ± 5	261 ± 42[b]	979 ± 351[b]
100 mg/kg per d	7	65 ± 4	198 ± 20[b]	580 ± 154[b]
500 mg/kg per d	8	86 ± 23	220 ± 18[b]	402 ± 84[b]
1000 mg/kg per d	7	65 ± 10	219 ± 39[b]	427 ± 290

[a] PTH, parathyroid hormone.
[b] $P < 0.05$ *versus* NRF receiving same treatment dose. No statistically significant differences were noted between different treatments within the NRF and CRF groups.

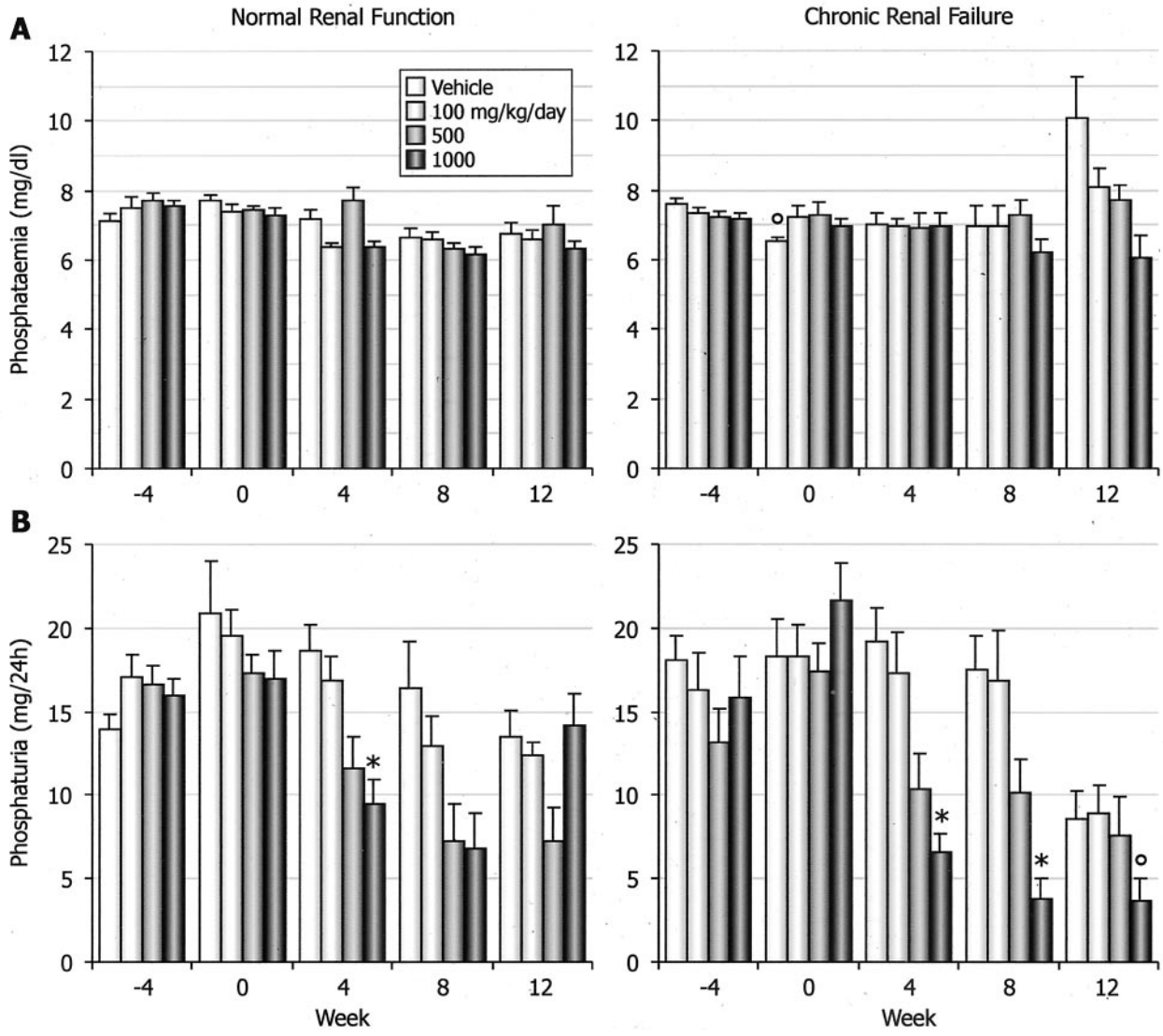

Figure 1. Phosphorus status of the study animals. (A) Serum phosphorus (mg/dl). (B) Urinary phosphorus (mg/24 h). A statistically significant dose-dependent decrease in urinary phosphorus excretion was observed in animals that had chronic renal failure (CRF) and were treated with lanthanum carbonate already after 4 wk of treatment. In animals with normal renal function (NRF), the decrease was less pronounced and not statistically significant. Phosphatemia showed a trend toward a dose-dependent decrease after 8 wk of treatment, but no statistically significant differences were found. *$P < 0.05$ *versus* vehicle; °$P < 0.05$ *versus* NRF, receiving same lanthanum carbonate dose.

phorus excretion remained above the detection limit in all NRF animals throughout the study period. No statistically significant differences in urinary volume were noted between any of the treatment groups.

No effects of treatment dose on serum osteocalcin or alkaline phosphatase levels were seen in animals with either NRF or CRF. CRF animals, however, had higher osteocalcin levels as compared with NRF animals that received the same treatment dose ($P < 0.05$).

Vitamin D measurements indicated that 1,25-$(OH)_2$ vitamin D_3 levels in CRF rats did not to differ from those found in rats with NRF, and there was no difference between animals that received either lanthanum carbonate or vehicle. 25-(OH) vitamin D_3 levels in CRF rats, however, were significantly lower than those measured in rats with NRF in all treatment groups, independent of the treatment dose (Figure 2).

Urinary pH, bilirubin, and urobilinogen did not differ between treatment groups throughout the study period.

Bone lanthanum content showed a dose-dependent increase (Figure 3) in both NRF and CRF animals *versus* their respective vehicle groups, reaching statistical significance in the NRF animals that received 500 mg/kg per d or more and in CRF animals that received 1000 mg/kg per d. Comparing bone lanthanum levels in CRF *versus* NRF animals, a statistically significant difference was noted only in the 1000 mg/kg per d groups.

Plasma lanthanum levels showed no significant differences between the various treatment groups, and they did not depend

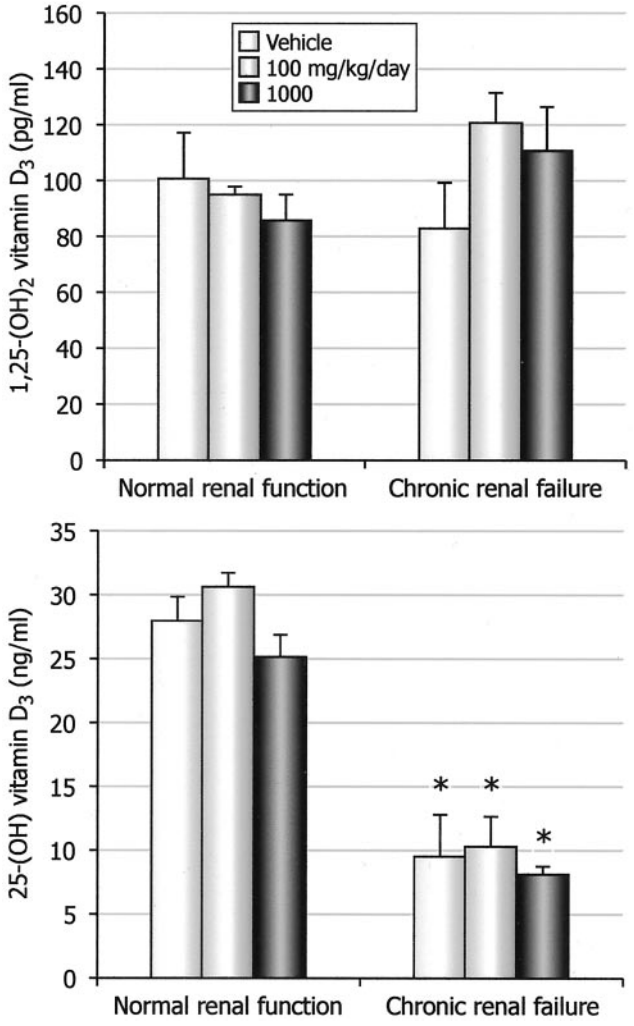

Figure 2. Vitamin D levels at sacrifice. After 12 wk of dosing, 25-(OH) vitamin D_3 (bottom) shows a significant decrease in all CRF groups when compared with NRF animals that received the same treatment dose. Different lanthanum carbonate doses did not influence 25-(OH) vitamin D_3 levels. 1,25-$(OH)_2$ vitamin D_3 (top) remains normal in all groups. Data shown are measurements from three random animals in each NRF group and five animals in each CRF group. $*P < 0.05$ *versus* NRF, receiving same lanthanum carbonate dose.

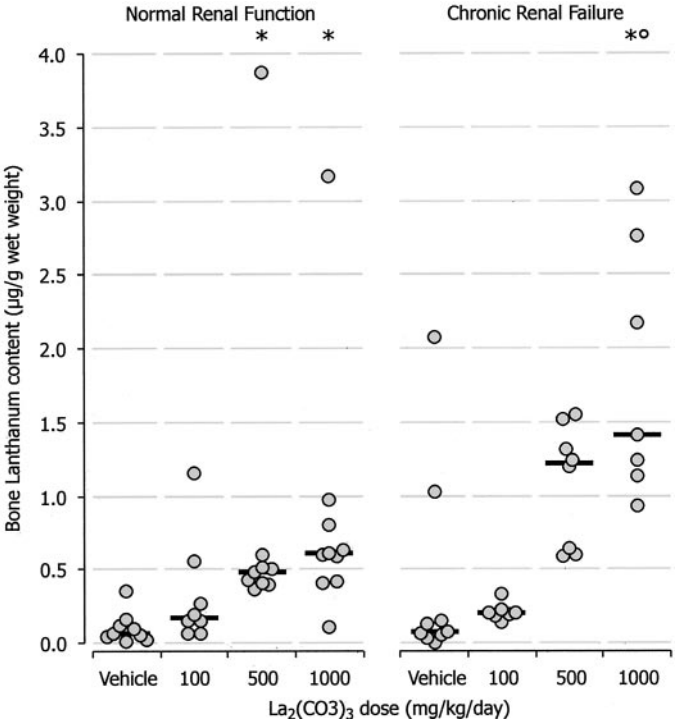

Figure 3. Femur lanthanum content (in μg/g wet wt). Each dot represents an individual animal; bold lines indicate group median values. A clear dose-dependent increase can be seen in NRF and CRF animals, indicating some accumulation of lanthanum in bone. $*P < 0.05$ *versus* vehicle; $°P < 0.05$ *versus* NRF, receiving same lanthanum carbonate dose.

on renal status of the animals. The average baseline plasma lanthanum level was 0.44 ± 0.07 ng/ml, and after 4 wk of treatment, this increased to 2.43 ± 0.54 ng/ml. It is interesting that from this time point onward, no further rise in plasma lanthanum content was seen. At the end of the treatment period (12 wk), plasma lanthanum levels averaged at 1.50 ± 0.16 ng/ml.

Bone Histomorphometry

Bone histology of several treatment groups is shown in Figure 4. In the NRF groups, no statistically significant differences were found between the vehicle- and lanthanum carbonate–treated groups for any of the bone parameters (Figure 5). Vehicle-treated animals with CRF showed a significantly increased BFR and mineral apposition rate when compared with NRF animals and a tendency toward higher eroded perimeter, in combination with an increased osteoid area, osteoid perimeter, and osteoid width. This indicates the development of hyperparathyroid bone in the vehicle-treated CRF animals, further supported by the increased osteoblast perimeter ($P < 0.01$, CRF vehicle *versus* NRF vehicle; Figure 6). In the highest dose group of the CRF animals, superimposed on the development of hyperparathyroidism, a clear trend toward a lower BFR and mineral apposition rate and higher mineralization lag time was observed. The higher osteoid area and osteoid width further indicate the development of an impaired mineralization at this dose, however, without affecting the number of cuboidal osteoblasts. No differences in bone area were found between treatment groups. Using the proposed criteria, four animals across the groups were categorized as having signs of impaired mineralization (high osteoid area; low BFR): one (13%) of eight of the 100 mg/kg per d NRF group and three (43%) of seven of the 1000 mg/kg per d CRF group.

Discussion

In this study, the classical 5/6 nephrectomy model ("remnant kidney") was used to induce CRF. There were no significant differences in the degree of renal failure between the various groups of lanthanum carbonate–treated animals, as measured by serum creatinine levels and proteinuria. The installation of CRF went along with the development of secondary hyper-

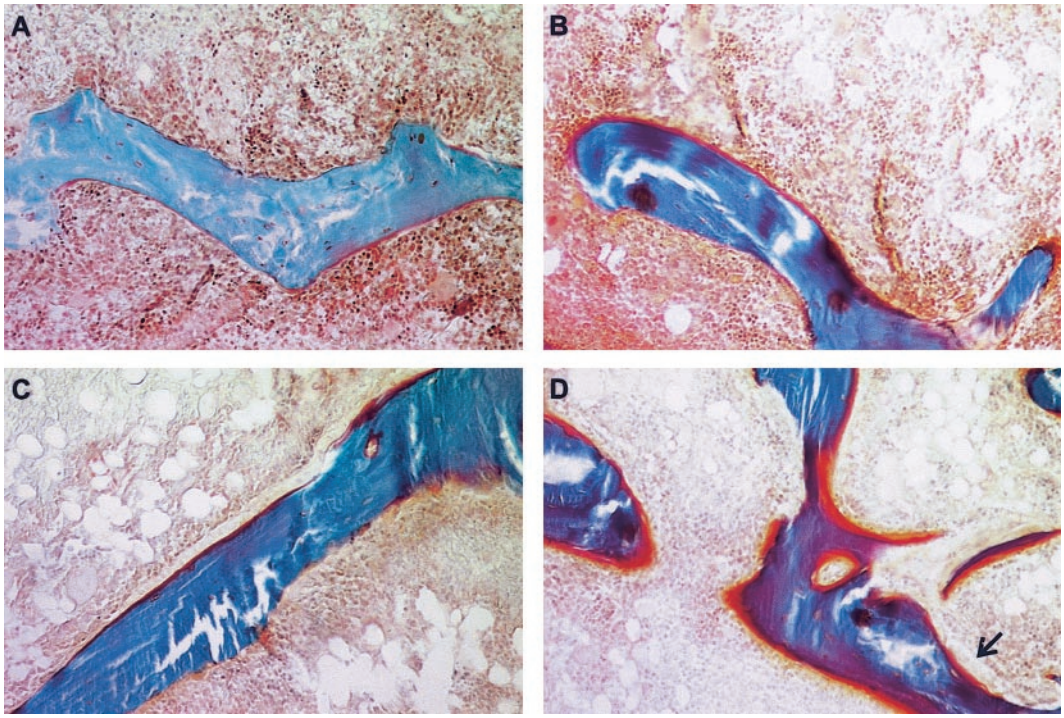

Figure 4. Bone histology. (A) NRF, vehicle. (B) NRF, lanthanum carbonate 1000 mg/kg per d, presenting normal bone histology. (C) CRF, vehicle. Note the slightly elevated osteoid area lined with active osteoblasts, indicating increased bone turnover. (D) CRF, lanthanum carbonate 1000 mg/kg per d. Note the dramatically increased osteoid area, with active osteoblasts still present (arrows). Magnification, ×200.

parathyroidism, expressed by increased serum PTH, increased osteoid area, BFR, eroded perimeter, and osteoblast perimeter. These results are in accordance with previously published data (23–25), evidencing the suitability of 5/6 nephrectomy as a model of stable CRF and renal osteodystrophy.

The administration of lanthanum carbonate at doses <1000 mg/kg per d did not induce any significant changes in bone histology of animals with CRF or NRF. A dose of 1000 mg/kg per d induced a mineralization defect in 43% of animals with CRF, whereas NRF animals that received the same dose presented normal bone histology. In the animals with a mineralization defect, however, seams of cuboidal osteoblasts were still seen.

To explain these results, two main points have to be considered. First, the administration of a powerful phosphate binder induces a dose-dependent phosphate depletion as indicated by the decreased urinary phosphorus excretion. Second, a significant decrease in serum 25-(OH) vitamin D_3 was observed in the CRF animals.

The severe phosphate depletion in the CRF animals that were treated with lanthanum carbonate is evidenced by the dose-dependent decrease in urinary phosphorus excretion, despite elevated PTH levels. Indeed, in all CRF animals that received the highest treatment dose, urinary phosphorus levels were decreased and were even not detectable in 57% of them; this in the presence of a fivefold increase in PTH levels and normal phosphatemia. Phosphate status is known to be a critical factor in bone mineralization and deficiency results in mineralization defects, histologically expressed as osteomalacia. Indeed, osteomalacia as a direct consequence of hypophosphatemia has been reported in dietary phosphate deficiency (26,27), hereditary hypophosphatemia (28), tumor-associated hypophosphatemia (29,30), and drug-induced hypophosphatemia associated with excessive use of phosphate-binding antacids (31,32). Lieuallen *et al.* (27) reported that in CRF rats that were kept on a phosphate-deficient diet, osteomalacia developed within 7 d, this in contrast to NRF animals. The osteomalacia was completely reversed by repleting the animals with phosphorus but not by treatment with exogenous 1,25-$(OH)_2$ vitamin D_3 or 25-(OH) vitamin D_3 (33). We have also reported some preliminary results from a study in 5/6 nephrectomized animals in which sevelamer, a non–metal-containing, nonabsorbed phosphate binder, also induces a phosphate depletion and a mineralization defect similar to that of lanthanum carbonate when given at high doses (34). Furthermore, it was shown recently that systemic phosphate repletion can prevent this phosphate depletion and the development of a mineralization defect in CRF rats (35).

Lotz *et al.* (36) as early as 1968 reported that antacid-induced phosphorus depletion in humans resulted in a rapid, profound decrease in urinary phosphorus excretion with undetectable urinary phosphorus after 6 d of treatment, followed by a slow and progressive decrease in serum phosphorus over an 80-d treatment period. In that report, urinary phosphorus excretion dropped to undetectable levels, before any significant effects on phosphatemia could be observed. A number of other case reports (37,38) have also shown a dramatic decrease in urinary phosphorus after the administration of high doses of

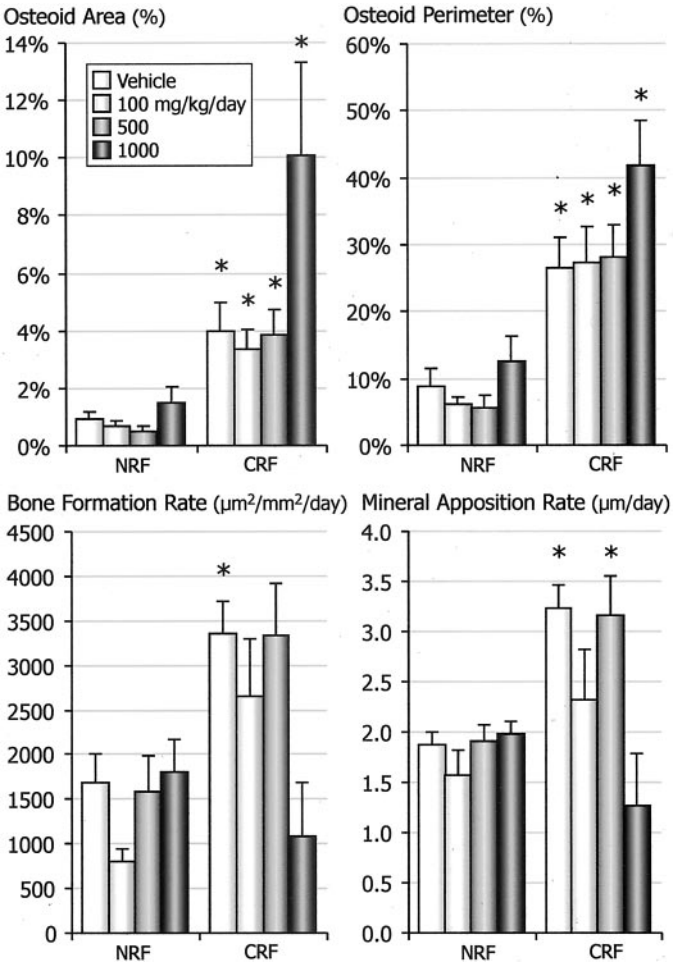

Figure 5. Bone histomorphometric data. All NRF groups present normal bone histology. Compared with the NRF groups, CRF animals that were treated with vehicle show an increased osteoid area and bone formation rate (BFR), indicative of hyperparathyroid bone disease. Osteoid area shows a trend toward a dose-dependent increase, whereas the BFR tends to decrease with increasing treatment doses. $*P < 0.05$ *versus* NRF, receiving same lanthanum carbonate dose.

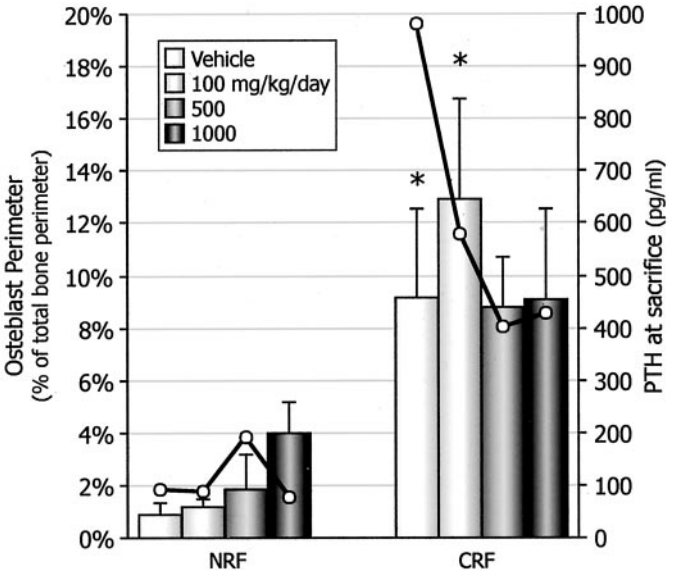

Figure 6. Bars: Osteoblast perimeter (as percentage of total bone perimeter); line graph: serum parathyroid hormone concentrations at sacrifice. For both of these parameters, a significant increase in CRF animals can be seen, consistent with the development of hyperparathyroidism. At the given doses, lanthanum carbonate treatment did not affect osteoblast perimeter. Even in animals that presented an impaired mineralization, cuboidal osteoblasts were still present. $*P < 0.05$ *versus* NRF, receiving same lanthanum carbonate dose.

aluminum-containing antacids. These observations suggest that the first mechanism to compensate for a reduced phosphorus intake consists of a decrease in urinary phosphorus excretion as a result of an increased phosphorus reabsorption by the kidney and a mobilization of phosphate out of bone. This compensatory mechanism will keep serum phosphorus levels within normal limits but may ultimately result in a mineralization defect (39).

In this study, the phosphate depletion was dose dependent, and clear effects on urinary phosphorus excretion were seen at doses of 1000 mg/kg per d, being ~10 times the proposed maximum therapeutic dose in humans. In the vehicle-treated CRF animals, serum PTH levels increased throughout the treatment period, whereas in CRF animals that received lanthanum carbonate, this increase was partially blunted because of the phosphate depletion. Other factors (*e.g.*, ionized calcium levels) may have prevented a further reduction of PTH to levels seen in NRF control animals.

Comparing CRF and NRF animals, a striking difference in bone histology and urinary phosphorus excretion was observed. Rats that have NRF and receive the highest dose of lanthanum carbonate still excrete phosphorus in the urine to a certain extent, and no mineralization defect is observed. A possible explanation for these differences between NRF and CRF groups is the vitamin D status in the different groups. Although 1,25-$(OH)_2$ vitamin D_3 did not differ between animals with CRF or NRF, 25-(OH) vitamin D_3 was significantly lower in all CRF animals, independent of treatment dose. This observation is in line with the data of Clements *et al.* (40) showing an enhanced metabolic clearance of 25-(OH) vitamin D_3 in primary hyperparathyroidism. Although worth being considered, a direct effect of lanthanum on the 25-(OH) vitamin D_3 synthesis in the liver is unlikely, because the 25-(OH) vitamin D_3 levels of the vehicle-treated CRF animals did not differ from those that received lanthanum.

There is growing evidence pointing to a role for 25-(OH) vitamin D_3 in the absorption of calcium and phosphate from the gut, independently from 1,25-$(OH)_2$ vitamin D_3. Indeed, Harrison *et al.* (41) showed that absorption of phosphate from the gut requires not only 1,25-$(OH)_2$ vitamin D_3 but also sufficient amounts of 25-(OH) vitamin D_3. They demonstrated that in rats that received a phosphate- and vitamin D–deficient diet, ^{32}P and ^{45}Ca absorption did not increase in animals with undetectable (<0.5 ng/ml) levels of 25-(OH) vitamin D_3 despite elevated 1,25-$(OH)_2$ vitamin D_3 levels. Animals that received a phosphate-deficient diet in combination with vitamin D repletion showed elevated ^{32}P and ^{45}Ca absorption, with

1,25-$(OH)_2$ vitamin D_3 levels similar to vitamin D–deficient animals, but increased 25-(OH) vitamin D_3. Bordier *et al.* (42) showed comparable effects when in vitamin D–deficient patients [25-(OH) vitamin D_3, 5 ± 3 ng/ml; 18 ± 8 ng/ml in normal controls], administration of 2.5 μg/d 1,25-$(OH)_2$ vitamin D_3 during 8 wk did not correct bone mineralization, Tm_{PO4}/GFR, serum phosphate, and calcium concentrations, whereas 50 μg/d 25-(OH) vitamin D_3 did. In human volunteers, Heaney *et al.* (43) demonstrated an increase in calcium absorption after oral treatment with 25-(OH) vitamin D_3, in the presence of normal 1,25-$(OH)_2$ vitamin D_3 levels. Ghazali *et al.* (44) described low plasma 25-(OH) vitamin D_3 to be a major risk factor for the development of Looser's zones in patients with hyperparathyroidism, independent of plasma 1,25-$(OH)_2$ vitamin D_3. These reports thus suggest that not only 1,25-$(OH)_2$ vitamin D_3 but also 25-(OH) vitamin D_3 plays an active role in the absorption of calcium and phosphate in the gut and in bone metabolism.

In the present study, because of the lower 25-(OH) vitamin D_3 levels, a decreased vitamin D–mediated phosphate absorption from the gut may be expected in CRF animals, thus aggravating the lanthanum-induced decrease in phosphate absorption. To maintain as long as possible a normal phosphatemia, the kidney will maximally increase phosphate reabsorption (even in the presence of increased PTH levels), resulting in a distinct hypophosphaturia and increased mobilization of phosphorus out of bone, as described previously by others (39). In addition, the increased need for phosphate inherent to the CRF-induced high bone turnover may have contributed to the phosphate depletion and further explain the differences in the effects on bone mineralization between NRF and CRF animals.

Various trace elements have already been ascribed a role in the development of bone lesions, *e.g.*, cadmium (45), lithium (46), strontium (16), and, most important, aluminum (2,4,47). Aluminum, in the past also frequently used as a phosphate binder, has been shown to have a direct toxic effect on bone, inhibiting both mineralization (13,14,48) and osteoblast function (3), thus causing the so-called aluminum-related bone disease, expressed as either osteomalacia or adynamic bone disease. Furthermore, a correlation between the bone aluminum content and development/severity of the bone lesions has been observed (2). This is in contrast with the present study, in which no correlation between bone lanthanum levels and the development of a mineralization defect could be demonstrated. The slightly higher lanthanum concentrations in the bone of CRF animals might result from minor differences in gastrointestinal absorption of the element related to the CRF (49) and/or from higher bone turnover in the hyperparathyroid rat. Excretion of lanthanum is known to occur mainly *via* the bile (~63% of 0.3-mg/kg intravenous dose of $LaCl_3$ in NRF rats) and not the kidney (50) (Shire Pharmaceutical Development, unpublished results).

In contrast to aluminum- or strontium-induced osteomalacia, cuboidal osteoblasts were found in all treatment doses in CRF animals, even in those with impaired mineralization. Hence, matrix will still be deposited by the osteoblasts but not adequately mineralized as a result of the lack of phosphate, resulting in a decreased phosphate incorporation in bone. Histomorphometrically, this results in fewer double tetracycline labels and decreased interlabel distance (*i.e.*, reduced BFR) in the presence of an increased amount of osteoid. Such a mechanism was described previously by Lieuallen *et al.* (27).

In conclusion, a dose-dependent mineralization defect occurred in a number of 5/6 nephrectomized rats that received lanthanum carbonate at 1000 mg/kg per d for 12 wk, in contrast to animals that had NRF and received the same treatment dose. Our results suggest that this mineralization defect was a consequence of administering a phosphate binder at high doses rather than being the result of a direct effect of lanthanum on bone. It occurred secondary to phosphate depletion, as evidenced by a pronounced decrease in urinary phosphorus levels, aggravated by decreased 25-(OH) vitamin D_3 levels inherent of the CRF and in the absence of any toxic effect on osteoblasts.

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