

Long-term treatment with lanthanum carbonate reduces mineral and bone abnormalities in rats with chronic renal failure

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Abstract

Background. Lanthanum carbonate (FOSRENOL[®], Shire Pharmaceuticals) is an effective non-calcium, non-resin phosphate binder for the treatment of hyperphosphataemia in patients with chronic kidney disease (CKD). In this study, we used a rat model of chronic renal failure (CRF) to examine the long-term effects of controlling serum phosphorus with lanthanum carbonate treatment on the biochemical and bone abnormalities associated with CKD–mineral and bone disorder (CKD–MBD).

Methods. Rats were fed a normal diet (normal renal function, NRF), or a diet containing 0.75% adenine for 3 weeks to induce CRF. NRF rats continued to receive normal diet plus vehicle or normal diet supplemented with 2% (w/w) lanthanum carbonate for 22 weeks. CRF rats received a diet containing 0.1% adenine, with or without 2% (w/w) lanthanum carbonate. Blood and urine biochemistry were assessed, and bone histomorphometry was performed at study completion.

Results. Treatment with 0.75% adenine induced severe CRF, as demonstrated by elevated serum creatinine. Hyperphosphataemia, hypocalcaemia, elevated calcium × phosphorus product and secondary hyperparathyroidism were evident in CRF + vehicle animals. Treatment with lanthanum carbonate reduced hyperphosphataemia and secondary hyperparathyroidism in CRF animals ($P < 0.05$), and had little effect in NRF animals. Bone histomorphometry revealed a severe form of bone disease with fibrosis in CRF + vehicle animals; lanthanum carbonate treatment reduced the severity of the bone abnormalities observed, particularly woven bone formation and fibrosis.

Conclusions. Long-term treatment with lanthanum carbonate reduced the biochemical and bone abnormalities of CKD–MBD in a rat model of CRF.

Keywords: bone disease; chronic kidney disease–mineral and bone disorder; FOSRENOL; lanthanum carbonate; phosphate binder

Introduction

Chronic kidney disease (CKD) affects ~17% of the adult population in the USA [1]. For these patients, death is a more likely outcome than progression to dialysis, primarily because of cardiovascular disease [2]. CKD–mineral and bone disorder (CKD–MBD) describes a triad of inter-related biochemical, bone and vascular abnormalities that have been linked with increased cardiovascular-related morbidity and mortality in patients with CKD [3].

Disturbed phosphorus homeostasis is central to the pathophysiology of CKD–MBD. Excretion of phosphorus declines as CKD progresses, resulting in a positive phosphorus balance and the eventual development of hyperphosphataemia. This state is linked to an increased mortality risk in patients with CKD [4]. A key role of phosphorus is to stimulate synthesis and secretion of parathyroid hormone (PTH) and parathyroid cell proliferation [5–7]. These effects occur both directly via post-transcriptional processes [8], and indirectly through inhibition of 1,25-dihydroxyvitamin D metabolism and via skeletal resistance to the actions of PTH [9].

Secondary hyperparathyroidism and parathyroid gland hyperplasia often develop early during the course of CKD; the latter (nodular hyperplasia) is often irreversible and may be refractory to therapy. Ultimately, these abnormalities lead to a high-turnover form of renal bone disease. Therefore, it is important to prevent parathyroid gland hyperplasia and secondary hyperparathyroidism by controlling the biochemical abnormalities of CKD–MBD, including elevated phosphorus levels, early in the course of disease [10].

The non-calcium, non-resin phosphate binder lanthanum carbonate (FOSRENOL[®], Shire Pharmaceuticals, Basingstoke, UK) is commonly used in clinical practice to reduce serum phosphorus in patients with CKD stage 5 who are undergoing dialysis. Sustained reduction of serum phosphorus has been demonstrated in clinical trials [11];

further studies have also suggested improvement in bone parameters in some patients [12–14].

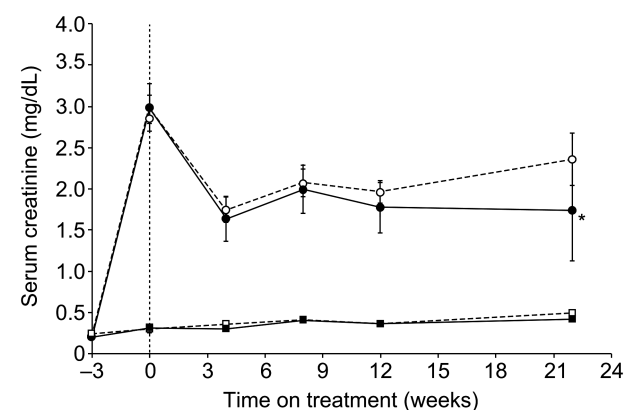
In rats, severe hyperparathyroidism occurs when chronic renal failure (CRF) is chemically induced by addition of adenine to the diet [15,16]. Short-term dietary supplementation of adenine has been used to examine the effects of phosphate binders in studies of up to 12 weeks in duration [17,18]. Adenine is metabolized to 2,8-dihydroxyadenine, which precipitates as crystals in the microvilli and apical region of the proximal tubular epithelia, thus inducing severe CRF [19]. The resulting CRF is characterized by increased levels of serum creatinine and phosphorus, and decreased levels of serum calcium [15]. This model provides a rapid and reliable method of inducing severe secondary hyperparathyroidism with rapid deterioration of bone within 4 weeks, and is considered to be compatible with clinical observations in dialysis patients [16].

The objective of this study was to evaluate the long-term effect of controlling serum phosphorus with lanthanum carbonate on biochemical and bone parameters in a rat model (adenine-induced) of severe secondary hyperparathyroidism. We also examined the effects of long-term treatment with lanthanum carbonate on tissue lanthanum deposition and liver function.

Materials and methods

Animals and diets

Male Wistar rats (Hsd:WI) were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN, USA). At the start of the study, rats were 12 weeks old and weighed ~250–275 g; body weight was measured weekly throughout the study. Rats were housed one per polycarbonate cage with a 12-h light–dark cycle and were given free access to water. During 1 week of acclimatization, animals were fed Teklad standard diet containing 0.8% calcium, 0.6% phosphorus, 21% protein and 2 IU/g vitamin D. Subsequent experimental diets are described below. The study protocol was approved by the Institution of Animal Care and Use Committee at MDS Pharma Services (Bothell, WA, USA).



—○— NRF + vehicle —○— CRF + vehicle
—■— NRF + lanthanum carbonate —●— CRF + lanthanum carbonate

Fig. 1. Changes in serum creatinine in rats with normal renal function or chronic renal failure during 22 weeks of treatment with vehicle or lanthanum carbonate. * $P < 0.05$ vs all other groups. Data are presented as mean \pm standard error of the mean. NRF, normal renal function; CRF, chronic renal failure. Dashed vertical line indicates initiation of vehicle or lanthanum carbonate treatment.

Induction and maintenance of chronic renal failure

Animals were randomized into treatment groups based on body weight. CRF was induced by feeding rats an adenine-supplemented diet [0.75%

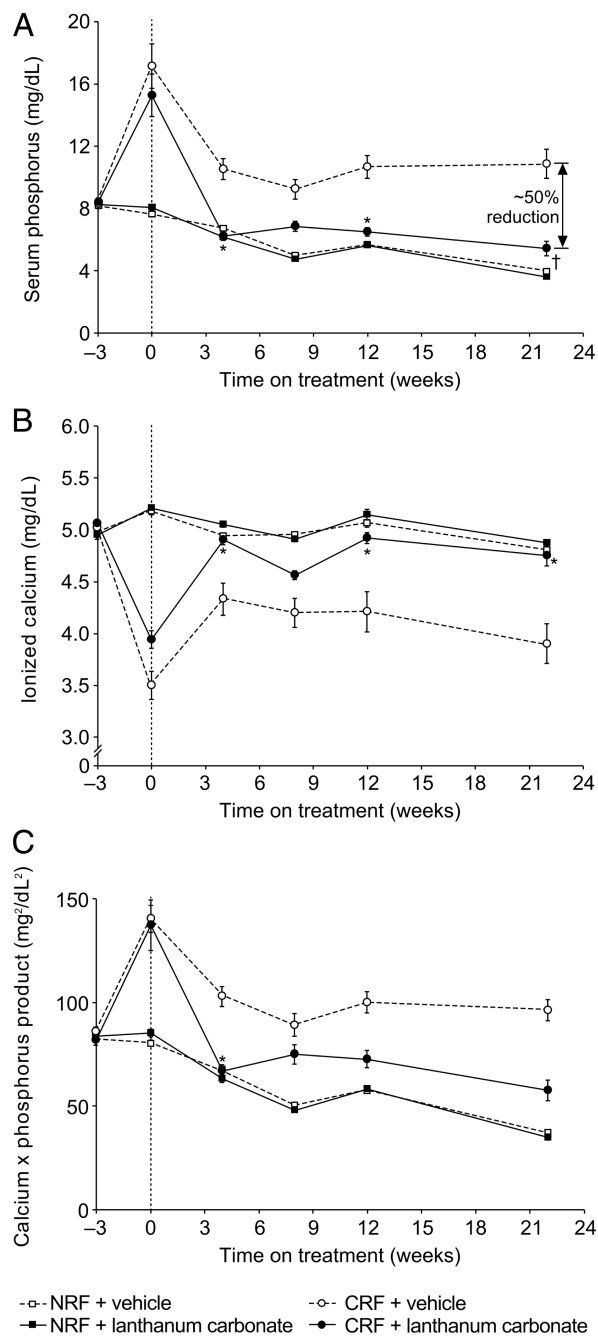


Fig. 2. Changes in (A) serum phosphorus, (B) ionized calcium and (C) calcium \times phosphorus product in rats with normal renal function or chronic renal failure during 22 weeks of treatment with vehicle or lanthanum carbonate. A. * P = not significant, CRF + lanthanum carbonate vs NRF groups. $\dagger P < 0.05$ vs all other groups. B. * P = not significant, CRF + lanthanum carbonate vs NRF groups. $P < 0.05$, CRF + lanthanum carbonate vs CRF + vehicle, Week 0–22. C. * P = not significant, CRF + lanthanum carbonate vs NRF groups. $P < 0.05$, CRF + lanthanum carbonate vs CRF + vehicle, Week 4–22. Calcium \times phosphorus product was calculated using total calcium. Data are presented as mean \pm standard error of the mean. NRF, normal renal function; CRF, chronic renal failure. Dashed vertical line indicates initiation of vehicle or lanthanum carbonate treatment.

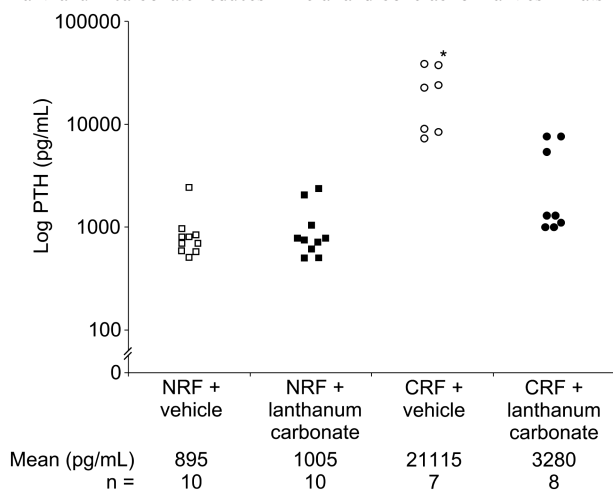


Fig. 3. Serum PTH levels in rats with normal renal function or chronic renal failure after 22 weeks of treatment with vehicle or lanthanum carbonate. * $P < 0.05$ vs all other groups. NRF, normal renal function; CRF, chronic renal failure.

(w/w) adenine] for 3 weeks according to the protocol described by Yokozawa *et al.* [15]. Control rats with normal renal function (NRF) were fed a similar diet excluding adenine.

After the initial 3-week disease-induction period, rats were divided into four different treatment groups ($n = 10$ per group). NRF animals were fed a normal diet supplemented with 2% cellulose as vehicle or a diet supplemented with 2% (w/w) lanthanum carbonate (1% w/w elemental lanthanum, supplied by DHP Clinical Trial Supplies, Powys, UK). CRF animals were fed a diet containing 0.1% (w/w) adenine and vehicle or the adenine diet supplemented with 2% (w/w) lanthanum carbonate. Weekly food consumption was recorded for each animal. The aim was to assess the effects of long-term treatment with lanthanum carbonate on the mineral and bone abnormalities associated with CKD-MBD; this was possible over 22 weeks of treatment.

Biochemical parameters

Blood samples were collected from the retro-orbital sinus under isoflurane anaesthesia prior to the induction of CRF (Week -3) and after 0, 4, 8, 12 and 22 weeks of treatment. At the end of the study, rats were anaesthetized with isoflurane for final blood collection. Animals were then sacrificed, and tissues were carefully collected and preserved for measurement of lanthanum content or histomorphometric analysis. Serum samples were stored on ice before assessment of blood biochemistry.

Serum samples from all time points were assayed for creatinine, inorganic phosphorus, total calcium, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and CO_2 using an Olympus AU400 Clinical Chemistry Analyzer (Olympus America Inc., Center Valley, PA, USA). Ionized calcium was measured in whole blood using a Bayer Rapidlab 865 (Bayer, Pittsburgh, PA, USA). The serum samples collected before

sacrifice were immunoassayed for 25-hydroxyvitamin D (radioimmunoassay, DiaSorin Inc., Stillwater, MN, USA), 1,25-dihydroxyvitamin D (radioimmunoassay, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) and PTH (enzyme-linked immunosorbent assay, Immotopics International, San Clemente, CA, USA). Whole blood pH was measured using an electrochemical half-cell (Bayer Rapidlab 865).

Urine samples were collected at Week -3 and after 0, 4, 8, 12, 18 and 22 weeks of treatment. Animals were housed in metabolic cages for 18 h. Thymol was added to preserve samples, and the volume and pH were measured. Samples were then centrifuged (3000 rpm, 10 min, 4°C) before being analysed for calcium and phosphorus using an Olympus AU400 Clinical Chemistry Analyzer. Only urine phosphorus was assessed at Week 18 of treatment.

Tissue lanthanum analysis

Care was taken at autopsy to avoid cross-contamination of lanthanum between tissues because transfer of lanthanum present at high concentrations in the gastrointestinal tract and on the skin could result in misleading results [20]. Samples of soft tissue (250 mg) and bone (60 mg) were immediately frozen for tissue lanthanum analysis. Samples were analysed using previously validated methods [20] by inductively coupled plasma-atomic emission spectrometry (ICP-AES; Spectro CircoTM CCD ICP-Atomic Emission Spectrometer, 408.672 nm line) or inductively coupled plasma-mass spectrometry (ICP-MS; Agilent HP4500 ICP Mass Spectrometer), using praseodymium as an internal standard. ICP-AES was used to quantify lanthanum in the range 0.01–10 $\mu\text{g/mL}$ and ICP-MS for tissues containing lanthanum in the range 0.021–10 ng/mL, with accuracy and precision in the range 98.5–103.5%. The lower limit of quantitation varied dependent on the weight of the tissue samples: 1.5–13.7 ng/g wet weight for soft tissues and 33 ng/g wet weight for bone.

Bone histomorphometry

Bone specimens for histomorphometric analysis were fixed in 10% buffered formalin immediately after collection. The marrow cavity of the left proximal tibia was exposed using a low-speed diamond wheel saw; the cut was made in a frontal plane at an angle of 90° to the tibial tuberosity to create a flat surface for positioning and embedding. A second cut was made ~1.5 cm away from the proximal end, and the resulting section of proximal tibia was processed for histology using polymethyl methacrylate as an embedding medium. Following processing and embedding, tibial samples were sectioned using a rotary microtome equipped with a tungsten carbide microtome knife. After blocks were trimmed and faced to the sampling site, 5- μm sections were cut and stained with Goldner's trichrome for bright-field microscopy.

Following staining, bone histomorphometry was performed using an OsteoMeasure software program (version 4.00c, OsteoMetrics, Inc., Atlanta, GA, USA) interfaced with a Nikon Eclipse E400 light/epifluorescent microscope and video subsystem. Static histomorphometry of the secondary spongiosa of the proximal tibia was performed in a 1.4-mm \times 2.1-mm area. The region of interest was determined by locating the upper edge of the growth plate and scanning down by ~1.4 mm. Osteoblast, osteoclast and osteoid parameters were measured using a 20 \times objective. All other parameters were measured with a 10 \times objective.

Table 1. Blood biochemistry in rats with normal renal function or chronic renal failure after 22 weeks of treatment with lanthanum carbonate or vehicle

Parameter	NRF + vehicle ($n = 10$) ^a	CRF + vehicle ($n = 8$) ^b	NRF + lanthanum carbonate ($n = 10$) ^c	CRF + lanthanum carbonate ($n = 8$) ^d
1,25-dihydroxyvitamin D (pg/mL)	437.20 \pm 45.53	318.55 \pm 59.67	333.97 \pm 22.41	346.97 \pm 44.23
25-hydroxyvitamin D (ng/mL)	21.53 \pm 1.04	28.47 \pm 2.43 ^a	22.66 \pm 0.72	31.30 \pm 3.89 ^{a,c}
Alkaline phosphatase (U/L)	51.10 \pm 2.07	146.38 \pm 8.54 ^{a,c}	56.30 \pm 3.43	125.25 \pm 44.28 ^{a,c}
Alanine aminotransferase (U/L)	31.40 \pm 5.53	50.13 \pm 5.61	39.60 \pm 9.41	45.75 \pm 16.18
Aspartate aminotransferase (U/L)	97.10 \pm 10.13	107.13 \pm 10.77	89.00 \pm 5.43	95.88 \pm 33.90
pH	7.42 \pm 0.01	7.35 \pm 0.03 ^a	7.39 \pm 0.01	7.38 \pm 0.02
Serum CO_2 (mEq/L)	22.50 \pm 0.34	21.71 \pm 0.42	23.50 \pm 0.37 ^b	22.25 \pm 0.56

Data are presented as mean \pm standard error of the mean. Superscript letters indicate a significant difference to corresponding group. NRF, normal renal function; CRF, chronic renal failure.

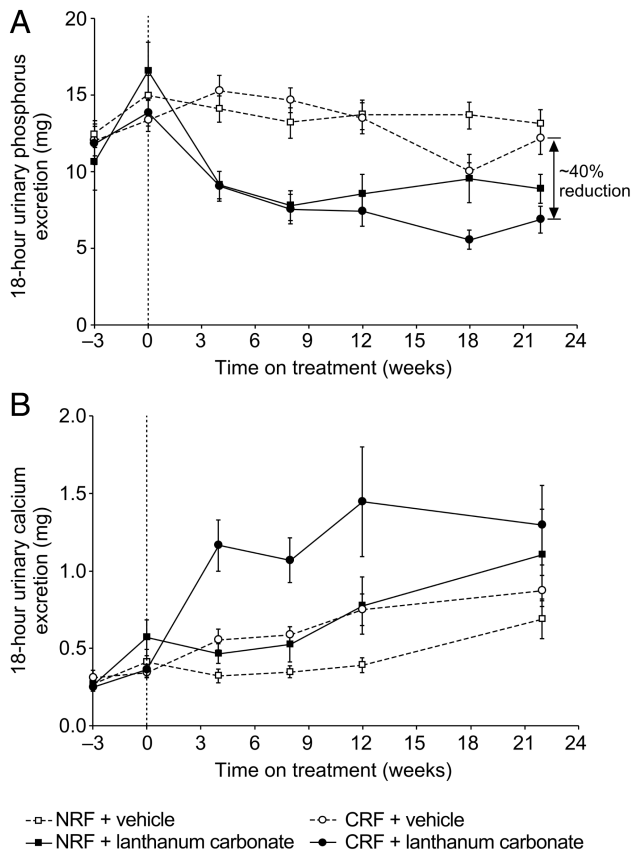


Fig. 4. Changes in (A) urinary phosphorus and (B) urinary calcium excretion in rats with normal renal function or chronic renal failure during 22 weeks of treatment with vehicle or lanthanum carbonate. A. $P < 0.05$ NRF + lanthanum carbonate vs NRF + vehicle, Week 4–22. $P < 0.05$ CRF + lanthanum carbonate vs CRF + vehicle, Week 4–22. B. $P < 0.05$ CRF + lanthanum carbonate vs CRF + vehicle, Week 4–12. Data are presented as mean \pm standard error of the mean. NRF, normal renal function; CRF, chronic renal failure. Dashed vertical line indicates initiation of vehicle or lanthanum carbonate treatment.

Total tissue area, trabecular bone area, trabecular bone perimeter, woven bone area, fibrosis tissue area, osteoblast and osteoid perimeters, osteoid area, osteoid thickness, and osteoclast perimeter were measured. Bone surface/volume, trabecular bone volume, woven bone volume, fibrosis tissue volume, osteoid volume, osteoid surface, osteoblast surface and osteo-

clast surface were calculated according to standardized formulae defined by Parfitt *et al.* [21].

Statistical analyses

All statistical analyses were conducted using SAS statistical software (SAS Institute, Cary, NC, USA). Multiple group comparisons were conducted using one-way (group) analysis of variance by either Duncan or Dunnett's procedure using the vehicle-fed CRF group as reference. Data collected at different time points in the study were analysed separately. Results are reported as mean \pm standard error of the mean (SEM), unless otherwise stated.

Results

Body weight, food intake and survival

NRF animals continued to gain weight during the study; addition of lanthanum carbonate to the diet did not affect weekly food consumption but resulted in slight increases in body weight gain ($P < 0.05$ at Week 14–15). Animals receiving the 0.75% adenine-supplemented diet consumed less food than their NRF counterparts and did not gain weight during the 3-week disease-induction period. Moderate body weight gain occurred after CRF animals were switched to the 0.1% adenine maintenance diet; this gain was unaffected by treatment with lanthanum carbonate.

After 18 weeks, adenine was removed from the maintenance diet owing to observation of significant clinical signs of ill health in CRF rats. After 22 weeks, survival of rats was 8/10 in both the vehicle-treated and lanthanum-carbonate-treated CRF groups; all NRF rats survived. The study was terminated at this point to ensure that there were sufficient numbers of survivors for statistical analyses. Lanthanum carbonate had no adverse effects on the behaviour of animals or on tissue histology. Examination of the CRF rats that died during the study revealed that severe renal failure was the cause of death.

Blood biochemistry

Blood biochemistry was assessed before induction of CRF (Week -3), at the end of disease induction (Week 0) and

Table 2. Tissue lanthanum concentrations in rats with normal renal function or chronic renal failure after 22 weeks of treatment with lanthanum carbonate or vehicle

Tissue	Tissue lanthanum concentrations (ng/g)			
	NRF + vehicle ($n = 10$) ^a	CRF + vehicle ($n = 8$) ^b	NRF + lanthanum carbonate ($n = 10$) ^c	CRF + lanthanum carbonate ($n = 8$) ^d
Liver	1.8 (1.4, 2.5)	3.7 (2.0, 7.6) ^a	244.6 (206.3, 372.1) ^{a,b}	1964.5 (953.4, 2995.3) ^{a-c}
Femur head	12.0 (9.9, 17.2)	14.6 (10.6, 18.8)	559.6 (408.6, 730.9) ^{a,b}	1322.5 (1098.5, 2005.1) ^{a-c}
Femur shaft	13.1 (8.2, 18.7)	17.2 (12.6, 21.0)	326.7 (243.3, 421.0) ^{a,b}	927.7 (716.2, 1107.5) ^{a-c}
Skeletal muscle	2.0 (1.7, 23.5)	5.0 (1.1, 64.5)	57.5 (15.5, 115.9) ^{a,b}	137.4 (17.9, 256.2) ^{a,b}
Kidney	1.9 (1.5, 4.5)	1.7 (1.0, 12.0)	215.5 (156.5, 241.4) ^{a,b}	109.9 (88.4, 246.6) ^{a,b}
Heart	2.1 (1.6, 6.2)	5.0 (1.0, 12.3)	11.6 (8.1, 52.2) ^{a,b}	40.3 (16.9, 82.3) ^{a,b}
Brain (cerebellum)	2.1 (1.3, 5.2)	1.6 (0.8, 4.7)	15.2 (5.0, 40.0) ^{a,b}	12.4 (4.6, 62.16) ^{a,b}
Brain (cerebrum)	2.1 (1.6, 3.2)	1.3 (0.8, 3.1)	9.5 (3.0, 47.6) ^{a,b}	8.2 (3.4, 23.22) ^{a,b}
Brain (mid)	1.5 (0.9, 2.3)	1.2 (0.5, 2.2)	9.9 (2.7, 26.9) ^{a,b}	8.0 (3.4, 28.61) ^{a,b}

Data are presented as median wet weight (10–90th percentiles). Superscript letters indicate a significant difference to corresponding group. NRF, normal renal function; CRF, chronic renal failure.

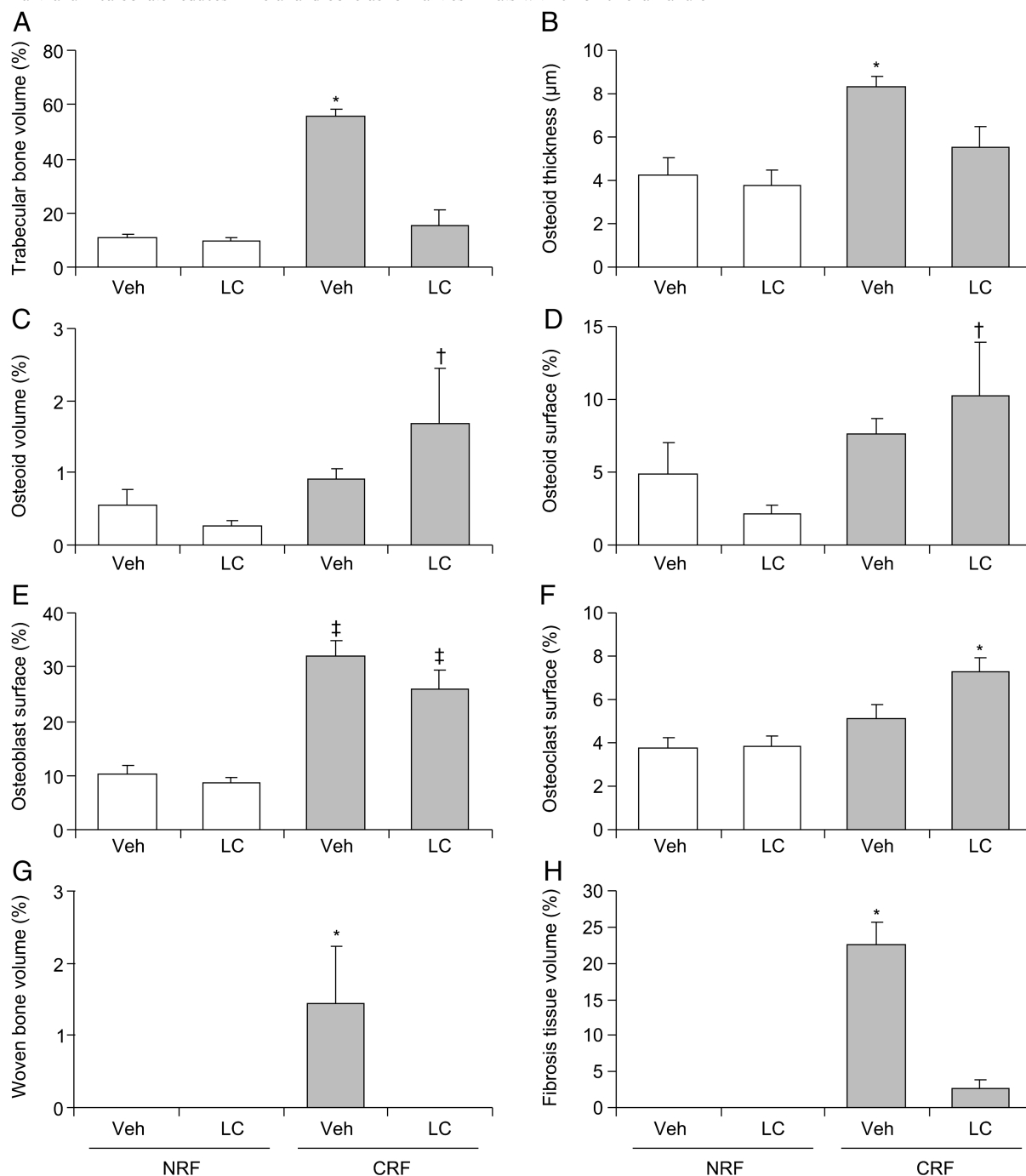
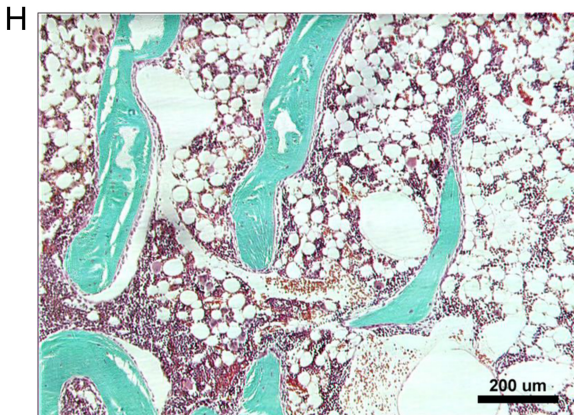
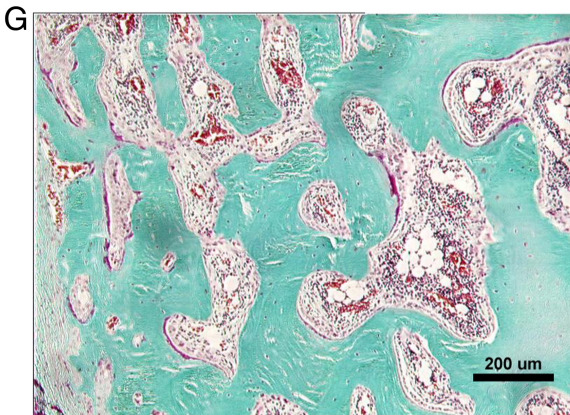
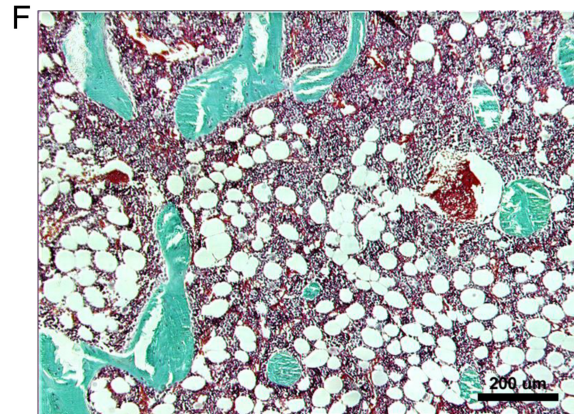
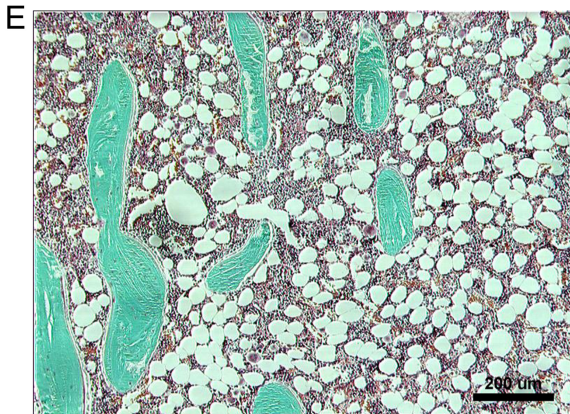
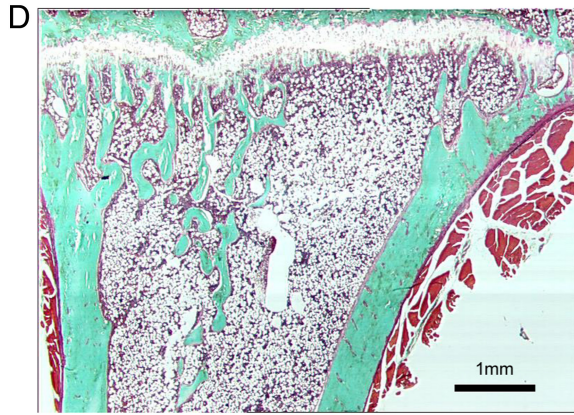
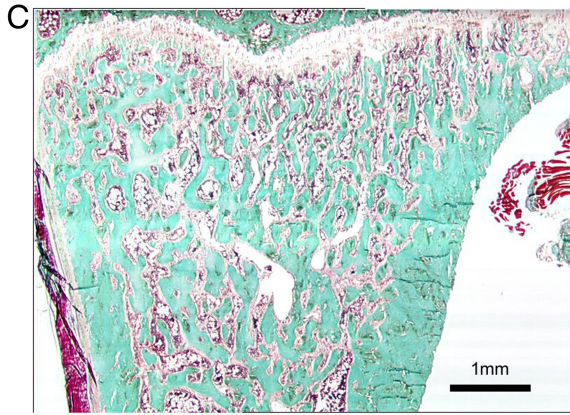
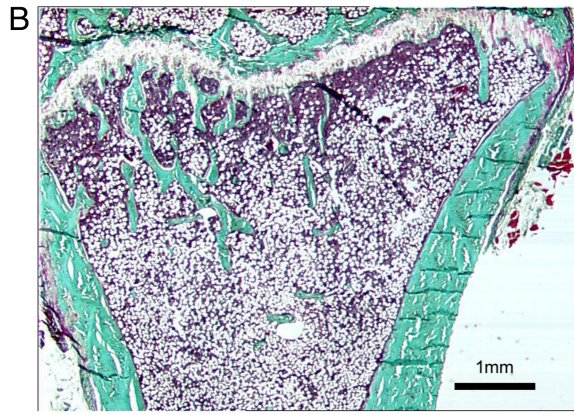
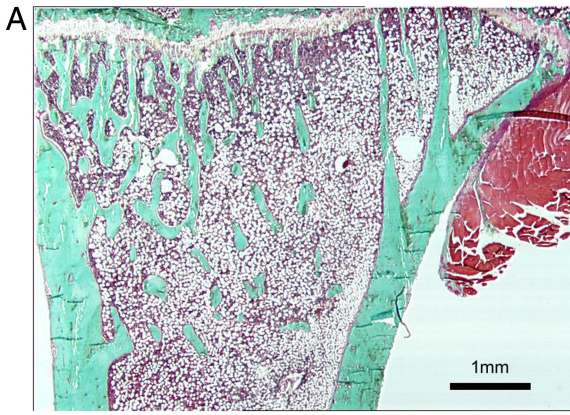


Fig. 5. Histomorphometry of trabecular bone from rats with normal renal function or chronic renal failure after 22 weeks of treatment with vehicle or lanthanum carbonate. * $P < 0.05$ vs all other groups, † $P < 0.05$ vs NRF + LC, ‡ $P < 0.05$ vs NRF groups. Data are presented as mean \pm standard error of the mean. CRF, chronic renal failure; LC, lanthanum carbonate; NRF, normal renal function; Veh, vehicle. (A) Trabecular bone volume (%) = [trabecular bone area (mm^2)/total tissue area (mm^2)] \times 100. (B) Osteoid thickness (μm). (C) Osteoid volume (%) = [osteoid area (mm^2)/trabecular bone area (mm^2)] \times 100. (D) Osteoid surface (%) = [osteoid perimeter (mm)/trabecular bone perimeter (mm)] \times 100. (E) Osteoblast surface (%) = [osteoblast perimeter (mm)/trabecular bone perimeter (mm)] \times 100. (F) Osteoclast surface (%) = [osteoclast perimeter (mm)/trabecular bone perimeter (mm)] \times 100. (G) Woven bone volume = [woven bone area (mm^2)/total tissue area (mm^2)] \times 100. (H) Fibrosis tissue volume = [fibrosis tissue area (mm^2)/total tissue area (mm^2)] \times 100.

during the subsequent 22 weeks of treatment. At the end of disease induction, serum creatinine was significantly higher in CRF rats than in NRF animals ($P < 0.05$). During the subsequent 22 weeks of treatment, serum creatinine remained elevated in CRF rats, but levels were lower in those treated with lanthanum carbonate than in vehicle-treated

controls ($P < 0.05$ for creatinine at Week 22, Figure 1). The addition of lanthanum carbonate to the diet of NRF animals did not affect creatinine levels.

Serum phosphorus, ionized calcium and calcium \times phosphorus product ($\text{Ca} \times \text{P}$) decreased slightly over time in NRF rats (Figure 2); lanthanum carbonate treatment did



not significantly influence levels of these parameters. The induction of CRF resulted in significant increases in serum phosphorus and $\text{Ca} \times \text{P}$, and a decrease in ionized calcium compared with NRF animals. When CRF rats were switched to the 0.1% adenine maintenance diet, levels improved slightly but remained abnormal. Treatment with lanthanum carbonate significantly reduced serum phosphorus (by ~50% vs vehicle) and increased ionized calcium such that at some time points, levels were not significantly different from those observed in NRF rats. $\text{Ca} \times \text{P}$ was also significantly reduced in CRF rats treated with lanthanum carbonate, compared with vehicle-treated controls ($P < 0.05$).

Levels of PTH (Figure 3) and vitamin D metabolites (Table 1) were assessed at the end of treatment; PTH levels were significantly higher in vehicle-treated CRF animals than in their NRF counterparts (mean 24-fold higher, $P < 0.05$). Treatment with lanthanum carbonate inhibited the increase in PTH levels: at the end of the study, mean PTH values in CRF rats treated with lanthanum carbonate were only 3-fold higher than in the NRF groups treated with lanthanum carbonate or vehicle, and the differences between these groups were not statistically significant. CRF rats had higher levels of 25-hydroxyvitamin D than NRF animals; lanthanum carbonate treatment did not significantly alter levels in CRF or NRF treatment groups. There were no statistically significant differences in 1,25-dihydroxyvitamin D levels between any treatment groups. After 22 weeks of treatment, there were no effects of lanthanum carbonate on the plasma levels of alkaline phosphatase, alanine aminotransferase or aspartate aminotransferase in CRF or NRF rats (Table 1).

Whole blood pH and serum CO_2 were measured at various time points to assess the acidic milieu associated with CRF, and any effects of lanthanum carbonate treatment. Three weeks after initiation, adenine supplementation (0.75%) induced significant reductions in serum CO_2 and whole blood pH suggesting acidosis in CRF rats. Eight weeks after the switch to the maintenance diet (0.1% adenine), serum CO_2 had returned to normal. Treatment of CRF rats with lanthanum carbonate resulted in consistently higher whole blood pH (not significant), but not serum CO_2 , when compared with vehicle-treated CRF animals (Table 1).

Urine biochemistry

Lanthanum carbonate treatment resulted in a decrease in urinary phosphorus excretion and an increase in urinary calcium excretion compared with vehicle-treated controls (Figure 4). Indeed, in CRF rats, treatment with lanthanum carbonate reduced urinary phosphorus excretion by ~40%

compared with vehicle-treated animals ($P < 0.05$); this reduction was maintained throughout the study.

Tissue lanthanum concentrations

The distribution of lanthanum in various tissues is shown in Table 2. As expected, lanthanum carbonate treatment was associated with the detection of lanthanum in the tissues of NRF and CRF animals. CRF was associated with increased deposition of lanthanum in liver and bone ($P < 0.05$ vs NRF), but not in the other organs examined.

Bone histomorphometry

Undecalcified histology preparation of the metaphysis of the proximal tibia was performed, and samples were analysed for abnormalities. The parameters described in the Materials and methods section were quantified (Figure 5). There were no statistically significant differences between vehicle and lanthanum carbonate treatment groups in the NRF rats. Vehicle-treated CRF rats had significantly increased trabecular bone volume, osteoid thickness, osteoblast surface, woven bone volume and fibrosis tissue volume compared with NRF animals ($P < 0.05$). Trabecular bone volume, osteoid thickness, woven bone volume and fibrosis tissue volume were significantly lower in lanthanum-carbonate-treated CRF animals, compared with vehicle-treated controls ($P < 0.05$). Despite lanthanum carbonate treatment, osteoid surface and volume, and osteoblast and osteoclast surfaces remained elevated. Representative images are shown in Figure 6.

Discussion

Maintenance of phosphorus balance is essential to limit the progression of CKD–MBD including secondary hyperparathyroidism, renal bone disease and vascular calcification [22]. In this study, using the adenine-induced CRF rat model, long-term treatment with lanthanum carbonate was shown to suppress hyperphosphataemia and secondary hyperparathyroidism with subsequent beneficial effects on bone disease.

Several animal models of CRF have been used to investigate the efficacy of pharmacological agents on parameters associated with CKD–MBD. One such model uses dietary administration of 0.75% adenine to induce CRF. In this model, animals exhibit severe secondary hyperparathyroidism and bone abnormalities, which occur in weeks. Tamagaki *et al.* [16] recently described the pathology associated with adenine-induced CRF in rats, including secondary hyperparathyroidism and bone disease.

Fig. 6. Representative images of the proximal tibia of rats with normal renal function or chronic renal failure after 22 weeks of treatment with vehicle or lanthanum carbonate. Sections (5 μm) were cut and stained with Goldner's trichrome for bright-field microscopy. Representative images (low and high magnification) from each treatment group are presented. (A) NRF + vehicle (low magnification). (B) NRF + lanthanum carbonate (low magnification). (C) CRF + vehicle (low magnification). (D) CRF + lanthanum carbonate (low magnification). (E) NRF + vehicle (high magnification). (F) NRF + lanthanum carbonate (high magnification). (G) CRF + vehicle (high magnification). (H) CRF + lanthanum carbonate (high magnification). CRF + vehicle animals displayed significantly increased trabecular bone volume in the metaphysis of the proximal tibia. At high magnification, increased osteoid and fibrosis tissues were evident in CRF + vehicle (G) but not in CRF + lanthanum carbonate (H) animals. NRF, normal renal function; CRF, chronic renal failure.

They reported that supplementation of the diet with 0.75% adenine for up to 6 weeks resulted in abnormalities, including elevation of serum creatinine and phosphorus, and a decrease in serum calcium. These changes were associated with markedly enlarged parathyroid glands, elevated PTH levels and increased levels of fibrosis in bone tissue. Comparable changes in serum parameters were also described by Nagano *et al.* [17], using a similar model whereby animals received 4 weeks of adenine treatment (2 weeks at 0.75% and 2 weeks at 0.5%) followed by 8 weeks on a normal diet.

This model has been used to examine the effects of a phosphate binder, sevelamer hydrochloride, on renal bone disease parameters over a period of 5 weeks [18]. It was demonstrated that sevelamer hydrochloride treatment suppressed abnormalities in serum phosphorus, calcium and PTH, resulting in normalization of parameters associated with bone disease. Following demonstration of these positive effects of a phosphate binder on bone pathology, we modified the adenine model to investigate the effects of long-term treatment with lanthanum carbonate on abnormal parameters of CKD-MBD and bone pathology.

In our model, dietary supplementation with 0.75% adenine resulted in CRF, including elevation of serum creatinine and phosphorus, and a decrease in ionized calcium. Following the reduction of dietary adenine to 0.1%, creatinine levels stabilized, indicating no further reduction of renal function. As a result, serum phosphorus and ionized calcium also remained more stable. However, PTH levels were extremely high (mean >20 000 pg/mL) following 18 weeks of adenine treatment. In contrast, PTH levels observed in NRF control animals were ~900 pg/mL, which were higher than reported by Tamagaki *et al.* and Nagano *et al.* (both ~100 pg/mL) [16,17]. This variation in PTH data could not be attributed to different methods of PTH measurement because all investigators used similar assays. However, it could be due to the lower concentration of calcium (0.8%) in the diet of our animals, compared with those used in the earlier studies (1.2% and 1.17%, respectively [16,17]). While the values observed were different, the fold increases in PTH associated with adenine treatment were of similar magnitude across these studies. In the study by Nagano *et al.* [17], 4 weeks of adenine treatment (2 weeks at 0.75% and 2 weeks at 0.5%) followed by 8 weeks on a normal diet resulted in an increase in PTH from ~120 pg/mL at baseline to ~6000 pg/mL at the end of the 12-week study (a 50-fold increase). In the study by Tamagaki *et al.* [16], supplementation of the diet with 0.75% adenine for 6 weeks resulted in PTH levels of 1812 pg/mL, compared with 96 pg/mL in normal controls (a 19-fold increase). In our study, administration of adenine for 18 weeks could have resulted in severe parathyroid gland hyperplasia and secondary hyperparathyroidism (represented by a 24-fold increase in PTH levels), driven by conditions of hyperphosphataemia and hypocalcaemia.

In this study, 1,25-dihydroxyvitamin D levels were not significantly different between NRF and CRF groups after 22 weeks of treatment. This was somewhat surprising given that 1,25-dihydroxyvitamin D deficiency is widely accepted as an important factor contributing to the devel-

opment of secondary hyperparathyroidism in renal failure [23,24]. The finding is consistent with observations in 5/6th nephrectomized male Wistar rats at 14 weeks after surgery [25]. In contrast, Tamagaki *et al.* [16] and Nagano *et al.* [17] reported significant decreases in 1,25-dihydroxyvitamin D after 4–6 weeks of adenine treatment. In rats with moderate renal failure, 1,25-dihydroxyvitamin D production is maintained as PTH levels increase and/or a rise in serum phosphorus levels is prevented [23,26]; reduction of 1,25-dihydroxyvitamin D levels occurs when renal function is markedly decreased. Differences in the phosphorus, calcium and vitamin D₃ content of the diet may partly account for the contrasting observations seen in these studies, but further investigation of fibroblast growth factor-23 levels (unfortunately not possible in this study) could help to elucidate the reason for the lack of differences in 1,25-dihydroxyvitamin D levels observed across the treatment groups in the present study.

Bone histomorphometry revealed a severe form of bone disease in CRF rats, as indicated by significant increases in trabecular bone volume, osteoblast surface, woven bone volume and fibrosis tissue volume. This is likely to have resulted from the severe and prolonged secondary hyperparathyroidism, with excessive production of woven bone responsible for large increases in trabecular bone volume, and explains the osteosclerotic appearance of the trabecular bone in the CRF rats. Similar observations have been made by other investigators [16]. Linear regression analyses (data not shown) demonstrated that the degree of fibrosis significantly correlated with PTH levels, but it was interesting to see that fibrosis correlated to an even greater extent with serum phosphorus levels. The metabolic acidosis evident in CRF rats would also contribute to the severity of the bone disease observed, possibly by directly inducing dissolution of bone, and by effects on osteoblast and osteoclast function.

The bone histology observed is consistent with that reported by Miller *et al.* [27], who demonstrated increased trabecular bone volume, osteoblast number and osteoclast number in 5/6th nephrectomized rats with severe secondary hyperparathyroidism, and with that reported by Tamagaki *et al.* [16], who found increased fibrosis and bone volume in adenine-treated animals. Katsumata *et al.* [18] also observed significant increases in fibrosis volume, porosity ratio and osteoid volume in their adenine-induced model.

In this study, the phosphate-binding effectiveness of lanthanum carbonate was demonstrated by reductions in urinary phosphorus excretion and serum phosphorus levels, compared with vehicle treatment. Treatment with lanthanum carbonate prevented CRF-induced hyperphosphataemia, such that levels of serum phosphorus were similar to those in NRF rats. Normalization of serum phosphorus suppressed the increase in PTH levels observed in vehicle-treated CRF animals, such that levels observed in lanthanum-carbonate-treated rats were not significantly different to those observed in NRF rats. These data are consistent with those reported by Ben-Dov *et al.* [28], using a less severe adenine-induced model of CRF (0.3% adenine). These investigators treated the animals with 3% elemental lanthanum, which resulted in significant reduc-

tion in PTH to levels similar to those observed in control animals. They have also recently demonstrated that lanthanum carbonate, through correction of serum phosphorus levels, destabilizes PTH messenger RNA, thereby decreasing PTH expression [29].

Lanthanum carbonate treatment was associated with an increase in ionized calcium to levels similar to those observed in NRF animals, despite an increase in urinary calcium excretion. As postulated for sevelamer hydrochloride [17], the increase in ionized calcium is likely to have been due, at least in part to a counter-ion effect of reducing serum phosphorus, and could also be due to increased absorption of free calcium made available by the alternative binding of phosphate to lanthanum in the gastrointestinal tract. PTH-regulated mechanisms for renal mineral handling are partially preserved in CRF rats [30]. Therefore, the increase in urinary calcium excretion is likely to be a homeostatic response driven by the reduction of PTH levels associated with lanthanum carbonate treatment. This effect has also been noted previously with sevelamer hydrochloride [30]. Lanthanum carbonate treatment did not affect serum levels of vitamin D metabolites in either NRF or CRF rats, but showed a trend towards correction of CRF-induced acidosis. This is in contrast to reports about sevelamer hydrochloride, which has been shown to exacerbate acidosis in clinical studies [31,32].

Treatment with lanthanum carbonate limited the progression of severe CRF-induced bone disease, particularly fibrosis. Given the significant correlation between levels of fibrosis and serum phosphorus, this was not surprising. The key evidence supporting this finding was lower levels of trabecular bone volume, woven bone volume and fibrosis volume compared with levels observed in vehicle-treated CRF rats. Partial correction of acidosis by lanthanum carbonate may also have had beneficial effects on bone parameters. Differences in osteoid parameters, osteoblast surface and osteoclast surface were not as profound, possibly because PTH levels were still elevated in some of the lanthanum-carbonate-treated rats. It is likely that control of the parameters of CKD-MBD reduced the synthesis of woven bone and fibrosis.

A limitation of this study is that animals were not treated with tetracycline to label bone. As the adenine-induced model results in high-turnover bone disease, at the time of study design, it was not thought necessary to differentiate between high and low bone turnover, as would be permitted by tetracycline labelling. Therefore, analysis of bone mineralization and turnover was not possible, preventing presentation of the data according to the Kidney Disease: Improving Global Outcomes Turnover, Mineralization and Volume (TMV) classification system. There were no effects of treatment on mortality; however, the study was not designed with mortality as an end point.

In this study, CRF was associated with increased deposition of lanthanum in the bone and liver, but not in the other organs examined. Deposition of lanthanum in the bone was 2–3-fold higher in CRF rats than in NRF controls. The levels (~1500 ng/g) were consistent with previous animal and human studies [33,34]. Also consistent with the results of previous studies [25,34–36], there was

no evidence of adverse effects of lanthanum carbonate on bone. In the liver of CRF rats, lanthanum concentrations remained <2000 ng/g wet weight, consistent with the results of previous studies in which levels exceeding 3000 ng/g have never been observed, irrespective of dose or treatment duration [28,37–39]. There was no histological evidence of adverse effects of lanthanum on the liver, and there was no difference in the levels of liver function enzymes between groups treated with lanthanum carbonate or vehicle. The presence of lanthanum in the liver is consistent with biliary excretion being the main route of elimination [40].

This study demonstrates that long-term treatment with lanthanum carbonate to control hyperphosphataemia can reduce the abnormalities of mineral and bone metabolism associated with CRF in rats. Furthermore, this study demonstrates that the modified adenine-induced model may be a useful tool to examine the complex interplay between mineral metabolism and its subsequent effects on bone.

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