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Treatment of a murine model of high-turnover renal osteodystrophy by exogenous BMP-7

Esther A. González, Richard J. Lund, Kevin J. Martin, John E. McCartney, M. Mehrdad Tondravi, T. Kuber Sampath, and Keith A. Hruska

Division of Nephrology, Saint Louis University School of Medicine, and Renal Division, Department of Medicine, and Department of Pathology, Washington University School of Medicine, St. Louis, Missouri; and Curis, Inc., Hopkinton, Massachusetts, USA

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Background. The secondary hyperparathyroidism of chronic kidney disease (CKD) produces a high turnover osteodystrophy that is associated with peritrabecular fibrosis. The nature of the cells involved in the development of peritrabecular fibrosis may represent osteoprogenitors expressing a fibroblastic phenotype that are retarded from progressing through osteoblast differentiation.

Methods. To test the hypothesis that osteoblast differentiation is retarded in secondary hyperparathyroidism due to CKD producing bone marrow fibrosis, we administered bone morphogenetic protein 7 (BMP-7), a physiologic regulator of osteoblast regulation, to C57BL6 mice that had CKD produced by electrocautery of one kidney followed by contralateral nephrectomy two weeks later. Following the second surgical procedure, a subgroup of mice received daily intraperitoneal injections of BMP-7 (10 μg/kg). Three to six weeks later, the animals were sacrificed, blood was obtained for measurements of blood urea nitrogen (BUN) and parathyroid hormone (PTH) levels, and the femora and tibiae were processed for histomorphometric analysis.

Results. The animals had significant renal insufficiency with BUN values of 77.79 ± 22.68 mg/dL, and the level of renal impairment between the CKD untreated mice and the CKD mice treated with BMP-7 was the same in the two groups. PTH levels averaged 81.13 ± 51.36 and 75.4 ± 43.61 pg/mL in the CKD and BMP-7 treated groups, respectively. The animals with CKD developed significant peritrabecular fibrosis. In addition, there was an increase in osteoblast surface and osteoid accumulation as well as increased activation frequency and increased osteoclast surface consistent with high turnover renal osteodystrophy. Treatment with BMP-7 eliminated peritrabecular fibrosis, increased osteoblast number, osteoblast surface, mineralizing surface and single labeled surface. There was also a significant decrease in the eroded surface induced by treatment with BMP-7.

Conclusions. These findings indicate that BMP-7 treatment in the setting of high turnover renal osteodystrophy prevents the development of peritrabecular fibrosis, affects the osteoblast phenotype and mineralizing surfaces, and decreases bone resorption. This is compatible with a role of osteoblast differentiation in the pathophysiology of osteitis fibrosa.

Key words: chronic kidney disease, renal osteodystrophy, osteitis fibrosa, bone morphogenetic protein 7, secondary hyperparathyroidism.

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The term renal osteodystrophy is used to describe the wide spectrum of skeletal abnormalities found in patients with chronic kidney disease (CKD) that ranges from states of high bone turnover to states of low bone turnover [1, 2]. Histologically, high-turnover renal osteodystrophy, often referred to as osteitis fibrosa, is characterized by increased numbers of osteoblasts as well as increased bone formation rate and osteoid accumulation. Similarly, there are increased number of osteoclasts and increased bone resorption [3]. In addition to these findings, there is fibrosis in the peritrabecular bone marrow space, the etiology of which has remained elusive [4, 5]. Clinically, the accumulation of fibrous tissue in the bone marrow in patients with secondary hyperparathyroidism is a well recognized cause of anemia and may significantly impair the response to erythropoietin [6–8].

In normal circumstances, bone marrow fibroblasts may develop from pluripotential mesenchymal stem cells, which can give rise to a number of committed and restricted cell lineages including adipocytes, myocytes and osteoblasts as well as fibroblasts [9, 10]. However, in high-turnover renal osteodystrophy, there may be dysregulation of the pathways leading to osteoblast and fibroblast differentiation in a direction that favors the accumulation of collagen secreting cells in the peritrabecular bone marrow [11, 12]. In this regard, we focused on one of the key regulators of osteoblast development, the bone morphogenetic proteins (BMPs), which belong to the transforming growth factor-β (TGF-β) superfamily and have a wide variety of biological functions [13, 14]. The bone morphogenetic proteins were originally iso-

lated from bone extracts that were capable of inducing new bone formation at ectopic sites [15]. In vitro, the BMPs have been demonstrated to favor the development of osteoblastic cells from undifferentiated precursors [16–19]. The actions of the bone morphogenetic proteins are mediated by a transmembrane receptor complex consisting of a BMP type I and a type II receptor that have serine threonine kinase activity [13, 20]. The binding of ligand to the BMP receptor results in phosphorylation of Smad proteins, which are then translocated to the nucleus where they regulate the expression of BMP responsive genes [13, 21].

One of the bone morphogenetic proteins, BMP-7, also known as osteogenic protein 1 (OP-1) is highly expressed in the adult kidney, and circulates in the bloodstream. Therefore, it is possible that decreases in renal mass could result in decreased BMP-7 production [22, 23]. As is the case with several of the BMPs, BMP-7 has been shown to induce osteoblast growth and differentiation in vitro [24–26]. In experimental animals, BMP-7 has been shown to induce ectopic bone formation as well as to heal bone defects [26–29]. The osteogenic properties of this protein have been explored in the orthopedic arena, and studies in humans have confirmed that exogenous BMP-7 can generate new bone and aid in the healing of fractures [28]. BMP-7 deficient mice have developmental defects of the skeleton, kidneys and eyes, and die shortly after birth [30–32]. The effects of BMP-7 deficiency in adult skeletal remodeling have not been clarified. As mentioned above, in the normal situation, BMP-7 favors the differentiation of osteoblasts from mesenchymal stem cells. Considering that BMP-7 is expressed in the kidney and is important in osteoblast development and function, in CKD one may expect an accumulation of osteoblast precursors as a result of parathyroid hormone (PTH) stimulation of osteoprogenitors. These progenitors may be unable to differentiate into mature osteoblasts because of BMP-7 deficiency. Alternatively, in the setting of decreased BMP-7 production by the kidney, the mesenchymal stem cells may be constrained from differentiating along the osteoblast pathway, and therefore may differentiate along an alternate pathway, the fibroblast pathway. In either situation, the subsequent accumulation of fibrous cells could then offer an explanation for the marrow fibrosis observed in secondary hyperparathyroidism in the setting of CKD. The present studies examined the effect of BMP-7 on the development of peritrabecular fibrosis in a murine model of CKD.

METHODS

Male C57/BL6 mice were purchased from Harlan (Indianapolis, IN, USA). BMP-7 was provided by Creative BioMolecules, Inc. (Hopkinton, MA, USA). Xylazine, ketamine and calcein were obtained from Sigma-Aldrich Company (St. Louis, MO, USA). The study protocol was approved by the institutional animal care committee.

Induction of chronic kidney disease and treatment protocol

Four-week-old male C57/BL6 mice were allowed to acclimatize for one week. CKD was induced by the procedure previously described by Gagnon and Gallimore [33]. Briefly, CKD is achieved after two surgical procedures. The first procedure involved electrocautery of the right kidney. The mice were anesthetized by administering ketamine (150 mg/kg) and xylazine (7.4 mg/kg) IP. A 2-cm right flank incision was made and the right kidney was identified. The perirenal fat and adrenal gland were separated from the kidney by blunt dissection and the kidney was exposed. The entire cortex of the right kidney was cauterized except for a 2 mm area around the hilum. The kidney was returned to the renal fossa and the subcutaneous tissues were sutured with 3-0 silk. The skin was closed using surgical clips. The mice were fed regular chow (Harlan Teklad, Madison, WI, USA) and were allowed water ad libitum for two weeks and were then subjected to a left nephrectomy. The left kidney was exposed by the same procedure described above, the hilum was ligated using 6-0 silk and the kidney was excised. The wound was closed as described above. Following the second surgical procedure, the animals were randomized into two groups. One group received daily intraperitoneal injections of BMP-7 at a dose of 10 μg/kg (CKD + BMP-7), and the second group received no treatment (CKD). Normal mice were used as controls at the request of the Animal Care Committee, since pilot studies demonstrated no difference between normal and sham operated/vehicle injected controls. The animals received regular mouse chow and water at libitum for the duration of the studies. Animals were sacrificed after 3, 4 or 6 weeks of CKD. Blood samples were obtained by intracardiac puncture at the time of sacrifice for blood urea nitrogen (BUN) and PTH levels in each group of animals. Five animals were sacrificed after 3 weeks and were used for histological analysis. Eighteen CKD animals (10 CKD untreated, 8 CKD + BMP-7 and 3 controls) were sacrificed 4 weeks post-nephrectomy after receiving calcein labeling (see histomorphometry) and were analyzed for all histomorphometry parameters except fibrous volume. Ten CKD mice (5 CKD treated and 5 CKD + BMP-7) were sacrificed 6 weeks post-nephrectomy for analysis of fibrous volume. Results obtained from each group are shown and indicated in the text and Figures as appropriate.

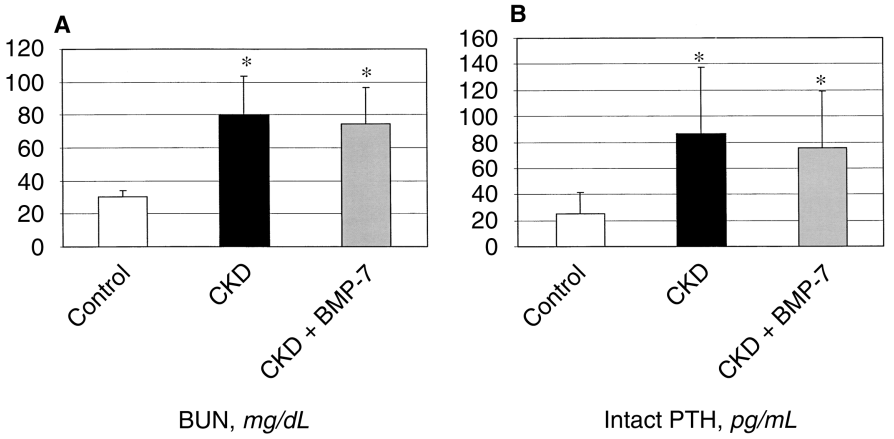

Fig. 1. Blood urea nitrogen (BUN) and intact parathyroid hormone (PTH) levels in mice with chronic renal insufficiency. Chronic kidney disease (CKD) was induced in male C57/BL6 mice by electrocautery of one kidney followed by contralateral nephrectomy two weeks later. The mice were 7 weeks old at the time of induction of renal failure and were sacrificed 3 to 6 weeks later. Blood urea nitrogen (*A*) and intact PTH levels (*B*) were determined in samples obtained by cardiac puncture at the time of sacrifice ($N = 8, 15$ and 11 for control, CKD and CKD + BMP-7 groups, respectively). Values represent mean ± SD. $*P < 0.01$ for CKD groups as compared to controls.

Measurements of parathyroid hormone and blood urea nitrogen

Blood samples were obtained by intracardiac puncture at the time of sacrifice and transferred to heparinized tubes. After centrifugation (400 × *g* for 10 min), plasma was removed, aliquoted and frozen at −80°C. Intact PTH levels were measured by two-site immunoradiometric assay (IRMA) using a commercially available kit (Immutopics, San Clemente, CA, USA). BUN was measured using a commercially available kit (INFINITY™ BUN Reagent; Sigma Diagnostics, St. Louis, MO, USA).

Bone histology and histomorphometry

For in vivo fluorescent labeling, the mice received IP calcein 20 mg/kg seven and two days prior to sacrifice four weeks after nephrectomy. Tibiae and femora were dissected at the time of sacrifice and placed in buffered formalin. The specimens were dehydrated in a series of graded ethanol solutions, and implanted undecalcified in a plastic embedding kit H7000 (Energy Beam Sciences, Inc., Agawam, MA, USA). Bones were sectioned longitudinally through the frontal plane in 5 μm sections with a JB-4 Microtome (Energy Beam Sciences, Inc.). Tissue was stained with Goldner's trichrome stain for trabecular and cellular analysis. TRAP staining was used to identify osteoclasts and define osteoclast surfaces. Unstained 10 μm sections were used for calcein-labeled fluorescence analysis. A reticulin stain was used in the animals sacrificed six weeks after nephrectomy for analysis of peritrabecular fibrosis. Slides were examined at ×400 magnification using a Leitz microscope attached to an Osteomeasure Image Analyzer (Osteometrics, Atlanta, GA, USA). Ten contiguous 0.0225 mm^2 fields of the proximal tibia, 150 μm distal to the growth plate (to exclude secondary spongiosum), were examined per animal. Primary, derived and kinetic measures of bone remodeling were calculated and reported per the guidelines of the American Society of Bone and Mineral Research [34].

Calculations

Statistical analyses were performed using the Student *t* test. Differences between groups were considered significant when $P < 0.05$. Data are expressed as means ± standard deviation.

RESULTS

Evaluation of renal insufficiency

Blood urea nitrogen levels were used to assess renal function. The levels in normal mice averaged 30.53 ± 3.52 mg/dL and in CKD mice averaged 77.79 ± 22.68 mg/dL ($P < 0.05$). As shown in Figure 1A, BUN levels in animals with CKD treated with BMP-7 ($N = 11$) were not different from animals with CKD that had not received treatment ($N = 15$), (BUN = 74.26 ± 22.59 and 80.38 ± 23.17 mg/dL in BMP-7 treated vs. untreated CKD mice, $P = 0.51$). There was also no difference in the BUN between CKD animals sacrificed at different time points (data not shown). Consistent with previous studies, we found that this model of CKD results in growth retardation [33]. Thus, prior to the induction of CKD, the weights averaged 15.53 ± 1.25 g, and 4 weeks later there was no significant change in the CKD mice (16.02 ± 1.55 g, P = NS), whereas a significant increase in weight was noted in the control animals during the same time period (18.37 ± 0.96 g, $P < 0.005$). Treatment with BMP-7 did not have an effect on body weight (15.33 ± 1.60 g vs. 16.58 ± 1.34 g in CKD mice treated with BMP-7 vs. CKD untreated mice, respectively, P = NS).

Assessment of secondary hyperparathyroidism

As shown in Figure 1B, intact PTH levels in normal mice averaged 25.00 ± 16.20 pg/mL. Animals with CKD developed significant secondary hyperparathyroidism with average intact PTH levels of 81.61 ± 50.62 pg/mL ($P = 0.0025$ vs. controls). Treatment with BMP-7 did not have a significant effect on the levels of intact PTH

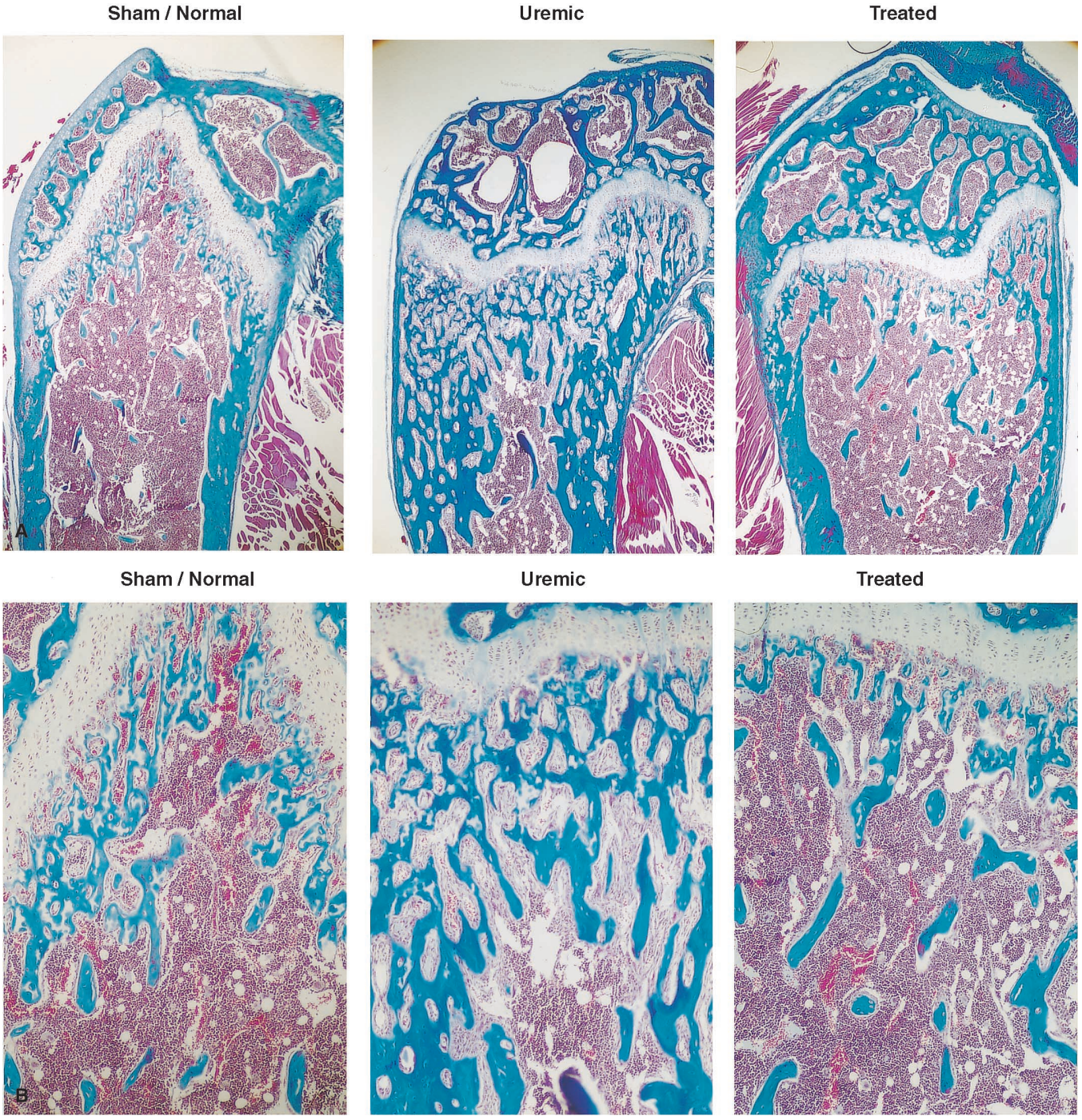

Fig. 2. Effect of BMP-7 on bone histology in mice with chronic kidney disease. Representative histological sections of proximal tibia from control mice, CKD mice and CKD mice treated with BMP-7 (×50 and ×100 magnification in *A* and *B*, respectively). The mice were 7-weeks-old at the time of induction of renal failure and were sacrificed 3 weeks later. Note increased trabecular bone (blue) and decreased marrow space (red) as well as the fibrous appearance of the marrow space in the CKD untreated animals. The histologic abnormalities in the CKD mice treated with BMP-7 are markedly less pronounced.

(75.45 ± 43.61 pg/mL vs. 86.13 ± 51.36 pg/mL in BMP-7 treated vs. untreated mice, $P = 0.58$). There was no significant difference in the intact PTH levels between CKD animals sacrificed at different time points (data not shown).

Effect of treatment with BMP-7 on bone histology

Histologic analysis of bone sections demonstrated that animals with CKD developed significant osteodystrophy as illustrated in Figure 2 (low and high power). In the bones of mice with CKD there was less cortical bone

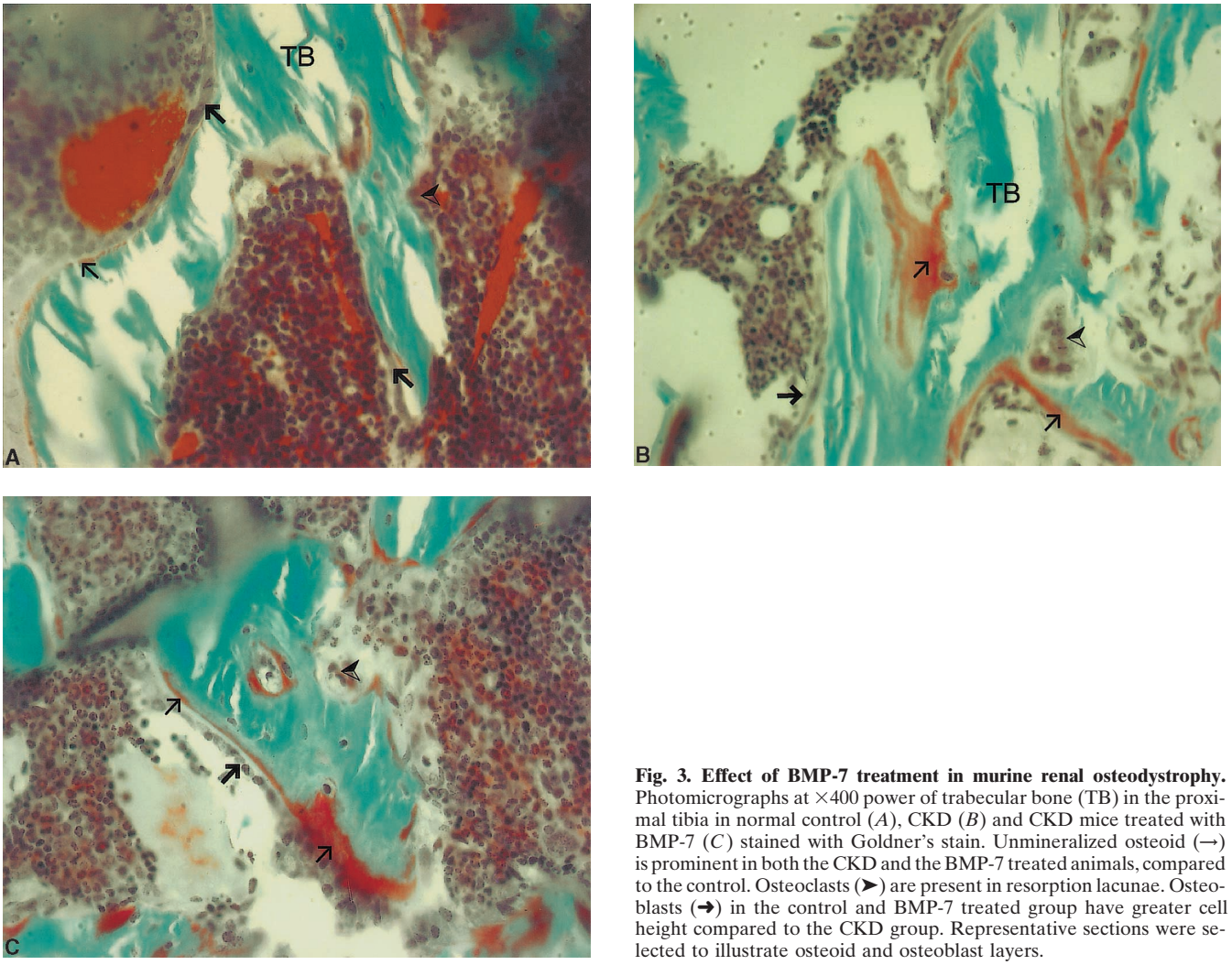

Fig. 3. Effect of BMP-7 treatment in murine renal osteodystrophy. Photomicrographs at ×400 power of trabecular bone (TB) in the proximal tibia in normal control (*A*), CKD (*B*) and CKD mice treated with BMP-7 (*C*) stained with Goldner's stain. Unmineralized osteoid (→) is prominent in both the CKD and the BMP-7 treated animals, compared to the control. Osteoclasts (➤) are present in resorption lacunae. Osteoblasts (➜) in the control and BMP-7 treated group have greater cell height compared to the CKD group. Representative sections were selected to illustrate osteoid and osteoblast layers.

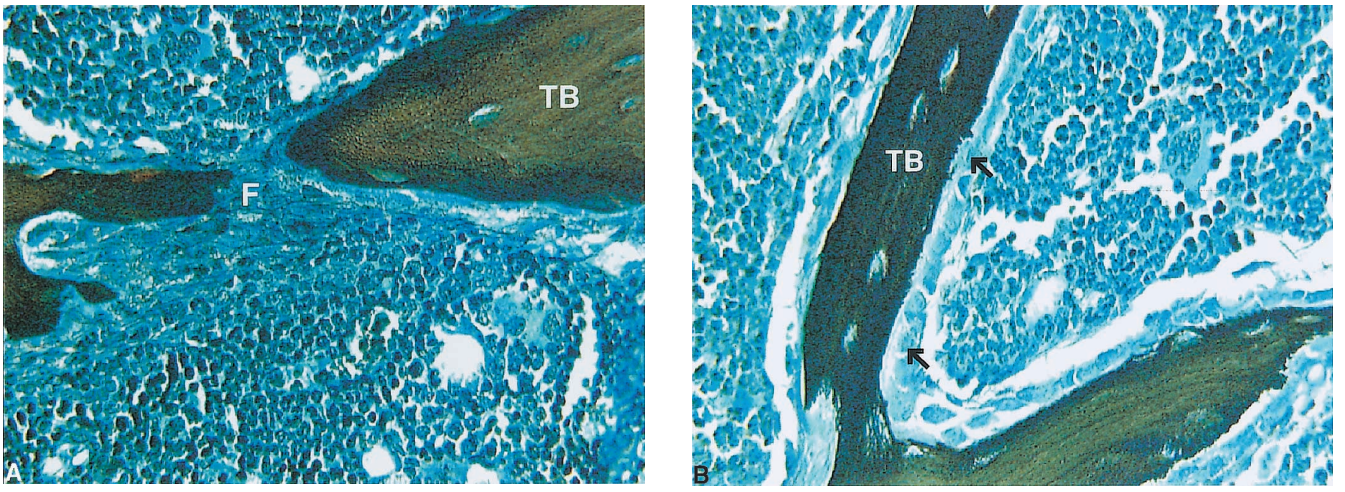

Fig. 8. Effect of BMP-7 treatment on marrow fibrosis in murine renal osteodystrophy. Reticulin stained specimens at ×400 magnification of trabecular bone (TB) in the marrow cavity of the proximal tibia showing significant fibrosis (F) in the marrow cavity of CKD animals (*A*) consistent with osteitis fibrosa. This fibrosis is eliminated in uremic animals treated with BMP-7 (*B*). Osteoblasts (➜) in the BMP-7 treated group have a more cuboidal morphology compared to the CKD group.

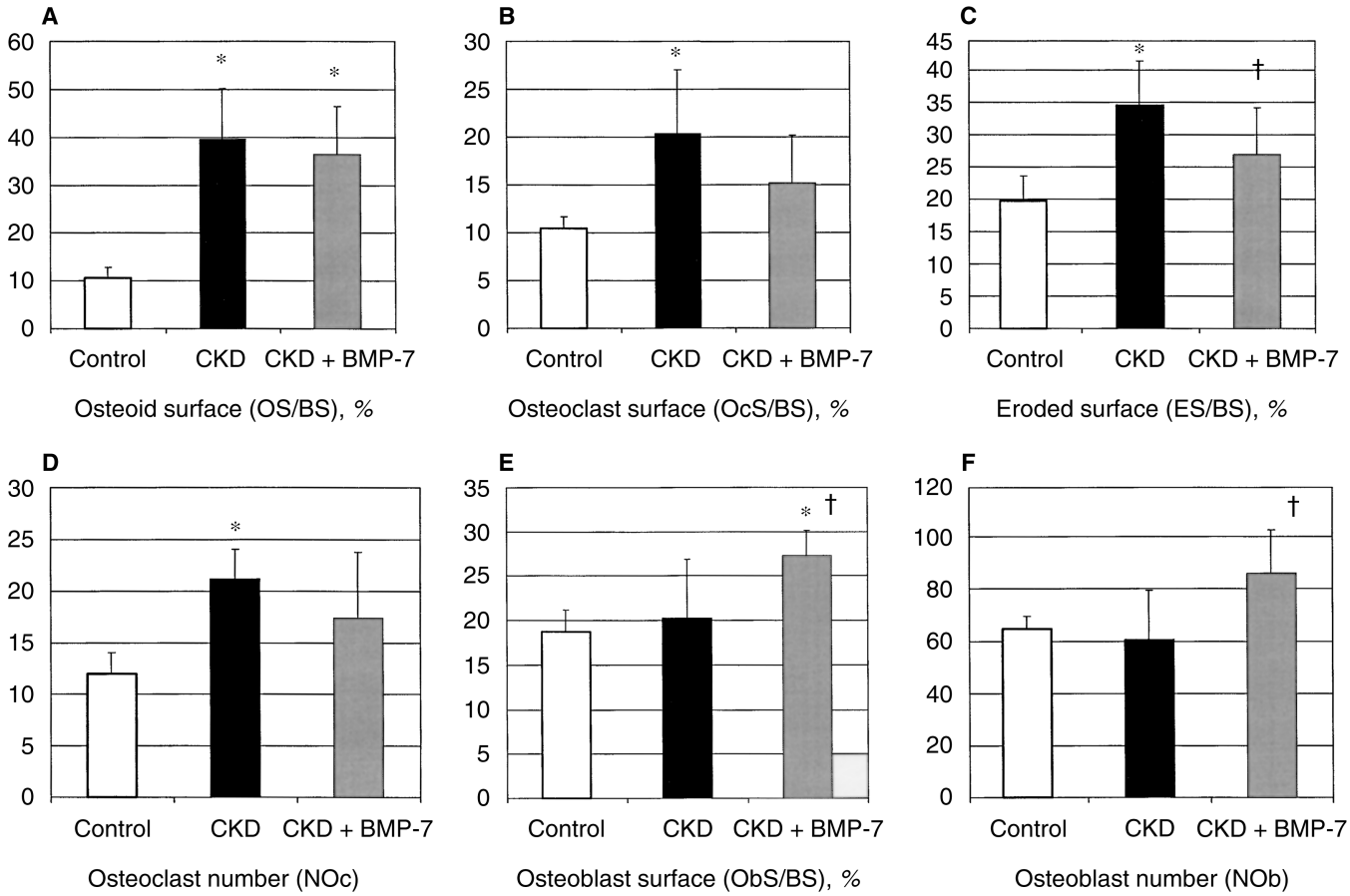

Fig. 4. Effect of BMP-7 treatment on the cellular surfaces of trabecular bone in mice with chronic kidney disease. Histomorphometric analysis in control mice ($N = 3$), CKD untreated mice ($N = 10$), and CKD mice treated with BMP-7 ($N = 8$). The mice were 7 weeks old at the time of induction of renal failure and were sacrificed 4 weeks later. There was an increase in osteoid surface in the CKD animals (A), which was not affected by BMP-7 treatment. Note the increases in osteoclast (B) and eroded surface (C) and osteoclast number (D) in CKD animals. BMP-7 treatment increased the osteoblast surface (E) and osteoblast number (F), and it also decreased the eroded surface (C). Values represent mean ± SD. *Significant ($P < 0.05$) difference between either of the CKD groups and the control group; †Significant difference between the CKD group and the BMP-7 treated group.

as compared to controls; however, there was increased trabecular bone present, and the marrow space was decreased. In addition, there was substantial marrow fibrosis surrounding the trabeculae in the CKD group and the marrow space was occupied by a population of cells with a fibrous appearance. The histologic findings in the bones of the animals with CKD that received BMP-7 differed from those of the untreated animals in that the alterations observed in trabecular bone, cortical bone as well as the fibrous changes were markedly less pronounced.

Effect of treatment with BMP-7 on bone histomorphometry

Representative fields of proximal tibial trabecular bone stained with Goldner's trichrome stain in normal control animals (A), animals with CKD (B) and animals with CKD treated with BMP-7(C) are shown in Figure 3. The animals with CKD had findings consistent with high turnover osteodystrophy as evidenced by significantly increased osteoid surface (OS/BS 10.6% in control vs. 39.7% in CKD mice; Fig. 4A), increased osteoclast surface (OcS/BS 10.5% in control vs. 20.4% in CKD mice; Fig. 4B), increased eroded surface (ES/BS 19.8% in control vs. 34.6% in CKD mice; Fig. 4C) and increased osteoclast number (NOc 12 in control vs. 21.2 in CKD mice; Fig. 4D). In addition to the changes in the cellular surfaces of trabecular bone CKD was associated with decreased mineralizing surfaces (MS/OS 365% in control vs. 103% in CKD mice) (Fig. 5A) and a decreased wall thickness (WTh 6.66 μm in control vs. 4.25 μm in CKD mice; Fig. 5C), resulting in an increased Activation Frequency (Acf 9.93/year in control vs. 17.9/year in CKD mice; Fig. 6). Treatment with BMP-7 increased the osteoblast surface (Fig. 4E), increased the osteoblast number (Fig. 4F) and decreased the eroded surface (Fig. 4C). In addition, BMP-7 eliminated the significant increase in osteoclast surface (Fig. 4B) and osteoclast number (Fig.

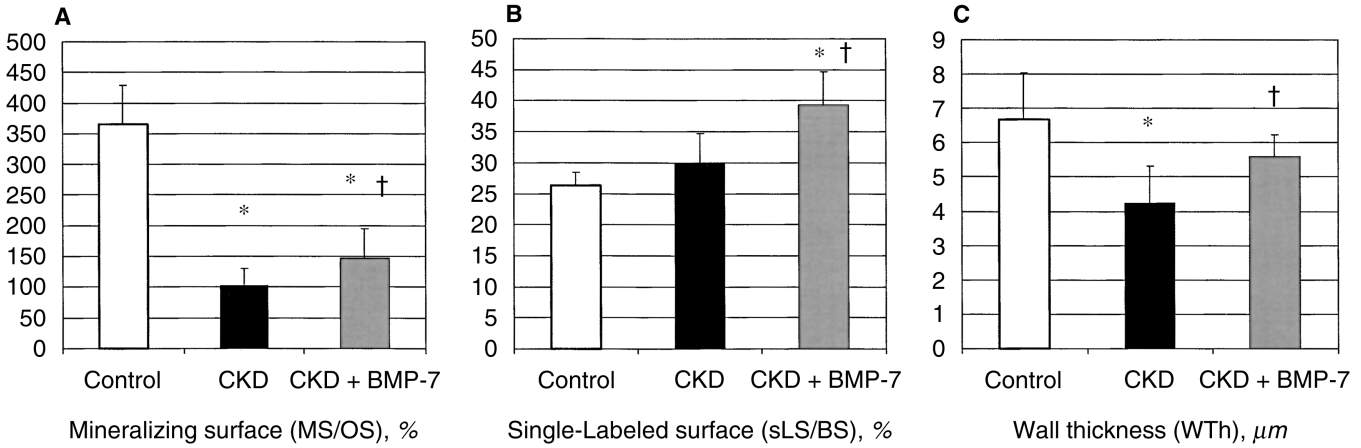

Fig. 5. Effect of BMP-7 treatment on trabecular mineralization in mice with chronic kidney disease. Dynamic histomorphometry in control mice ($N = 3$), CKD untreated mice ($N = 10$), and CKD mice treated with BMP-7 ($N = 8$). The mice were 7 weeks old at the time of induction of renal failure and were sacrificed 4 weeks later. Fluorescent calcein 20 mg/kg was administered intraperitoneally seven and two days prior to sacrifice. Note the decreased mineralizing surface (A) in CKD animals, which was improved with BMP-7 treatment, the significant increase in single labeled surface (B) and the improvement in wall thickness (C) with BMP-7 treatment. Values represent mean ± SD. *Significant ($P < 0.05$) difference between either of the CKD groups and the control group; †significant difference between the CKD group and the BMP-7 treated group.

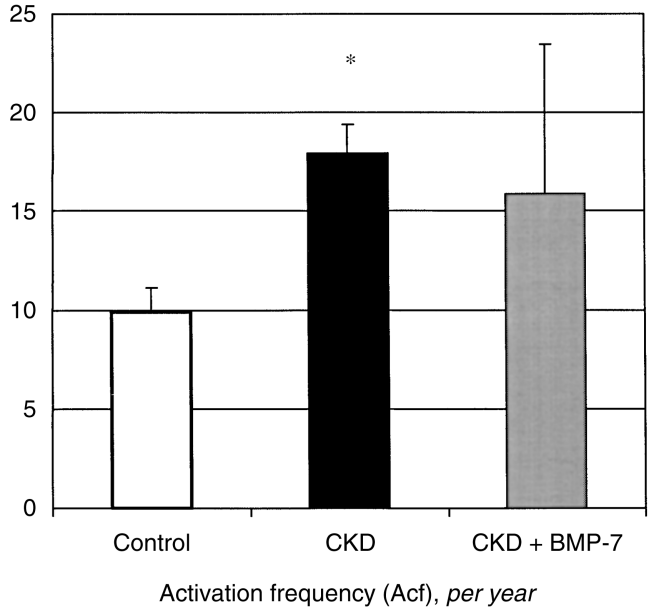

Fig. 6. Effect of BMP-7 treatment on rates of bone turnover in murine renal osteodystrophy. Activation frequency (the probability that a new cycle of remodeling will be initiated at any point on the bone surface) in control mice ($N = 3$), CKD untreated mice ($N = 10$), and CKD mice treated with BMP-7 ($N = 8$). The mice were 7 weeks old at the time of induction of renal failure and were sacrificed 4 weeks later. Note the increased activation frequency in the uremic animals consistent with high turnover renal osteodystrophy. Values represent mean ± SD. *Significant ($P < 0.05$) difference between the CKD group and the control group.

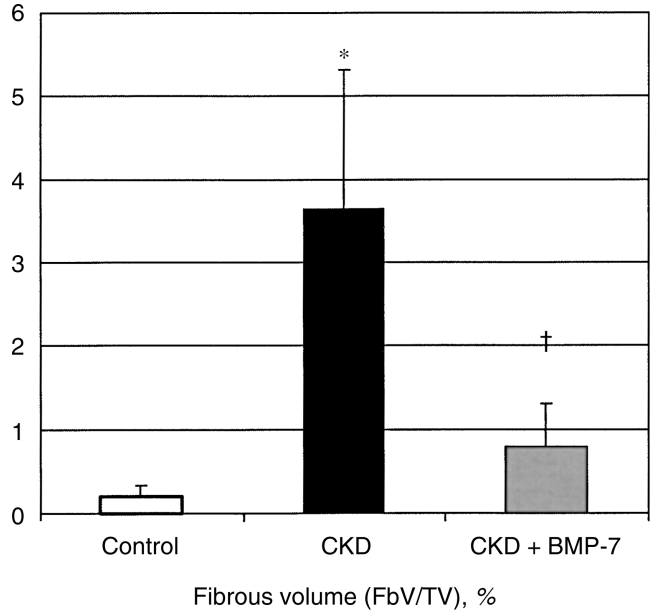

Fig. 7. Effect of BMP-7 treatment on marrow fibrosis in murine renal osteodystrophy. Fibrous volume per total volume (%) was determined in mice treated ($N = 5$) or untreated ($N = 5$) with BMP-7 since the time of induction of CKD. The mice were 7 weeks old at the time of induction of renal failure and were sacrificed 6 weeks later. Note the marked volume of fibrosis in the CKD animals, which was prevented by treatment with BMP-7. Values represent mean ± SD. *Significant ($P < 0.05$) difference between the CKD group and the control group; †Significant difference between the CKD group and the BMP-7 treated group.

4D) observed in the CKD mice compared to the controls; however, the changes induced by BMP-7 were of small magnitude and not statistically significant from the CKD untreated animals. BMP-7 affected trabecular mineralization by increasing mineralizing surface (Fig. 5A), increasing single labeled surface (Fig. 5B) and increasing wall thickness compared to CKD (Fig. 5C). There was no significant change in activation frequency (Fig. 6). As shown graphically in Figure 7 and at ×400 magnification

Table 1. Additional histomorphology data of trabecular bone in the proximal tibia (not included in Figs. 4–7), between normal control mice ($N = 3$), CKD mice ($N = 10$), and CKD mice treated with BMP-7 ($N = 8$) for 4 weeks

			Normal	CKD	CKD + BMP-7
Bone surface	BS/TV	mm^2/mm^3	17.27 ± 1.06	18.74 ± 2.79	17.31 ± 3.33
Trabecular thickness	TbTh	μm	37.34 ± 5.19	41.61 ± 9.12	39.91 ± 5.54
Trabecular number	TbN	/mm	8.63 ± 0.53	9.35 ± 1.37	8.66 ± 1.67
Mineralizing surface	MS/BS	%	37.56 ± 3.2	38.39 ± 5.14	49.24 ± 4.9[ab]
Double labeled surface	dLS/BS	%	11.24 ± 1.46	8.51 ± 3.32	9.95 ± 3.13
Bone formation rate	BFR/BS	$\mu m^3/\mu m^2$/year	66.56 ± 18.51	75.78 ± 17.98	86.86 ± 36.16

Values represent mean ± SD.
[a] Significant ($P < 0.05$) difference between the CKD + BMP-7 group and the control group
[b] Significant difference between the CKD group and the BMP-7 treated group

in Figure 8, a significant peritrabecular fibrosis was present in the bones of animals with CKD, which was largely prevented by treatment with BMP-7 (FbV/TV 3.65% in CKD mice vs. 0.79% in CKD mice treated with BMP-7, $P = 0.0065$). Additional parameters of histomorphometry are shown in Table 1.

DISCUSSION

Renal osteodystrophy is a common complication encountered in patients with chronic kidney disease, and secondary hyperparathyroidism as a result of chronic kidney disease plays an important role in the genesis of high turnover renal osteodystrophy [1, 2, 35]. In addition to increased osteoclastic bone resorption and osteoblastic bone formation, the skeletal lesion of hyperparathyroidism, osteitis fibrosa, is characterized by the presence of bone marrow fibrosis, the etiology of which remains poorly understood. We hypothesized that the marrow fibrosis of hyperparathyroid bone disease in CKD may result from the lack of osteoblast differentiation factors possibly due to their decreased production by the failing kidney. Thus, we studied the effects of BMP-7 administration (a factor made in the kidney known to regulate osteoblast differentiation) to mice with chronic kidney disease. The degree of renal impairment created by the electrocautery and contralateral nephrectomy model is clinically stable over time (Hruska, unpublished data), and not a progressive model of renal disease. Similarly, the degree of hyperparathyroidism remained stable in these studies; however, we do not have information on the progression of secondary hyperparathyroidism beyond six weeks of CKD. We found that mice with CKD developed secondary hyperparathyroidism and high turnover osteodystrophy with peritrabecular fibrosis. Administration of BMP-7 virtually prevented the development of marrow fibrosis and produced significant increases in mineralizing osteoblast surfaces, osteoblast number and morphology.

Parathyroid hormone excess has long been recognized to be associated with the development of marrow fibrosis in humans with either primary or secondary hyperparathyroidism. Studies in experimental animals exposed to continuous PTH administration also have described the presence of fibroblastic cells in trabecular bone [36]. The mechanisms involved in the accumulation of fibroblastic cells in states of parathyroid hormone excess have remained poorly understood. Recent studies by Calvi et al [12] with mice expressing a constitutively active PTH/PTHrP receptor in cells of the osteoblastic lineage, as described in patients with Jansen's metaphyseal chondrodysplasia [37], demonstrated features of hyperparathyroid bone disease including marrow fibrosis. The fibrous cells were found to express a number of osteoblast markers including osteopontin, alkaline phosphatase and collagenase, thus suggesting that these cells represent osteoblast precursors unable to fully differentiate into mature osteoblasts. Marrow fibrosis is also a feature of fibrous dysplasia of bone, a condition resulting from activating mutations of the gene encoding the α subunit of the stimulatory G protein [38–40]. The cells associated with this lesion also appear to be constrained from differentiation into mature osteoblasts [11]. The mechanism for the failure of these osteoblast progenitors to progress normally through the osteoblast differentiation program is not clear, although PTH has been shown to be insufficient in directing osteoblast differentiation in vitro [41, 42].

The present studies demonstrate that exogenous administration of an osteoblast differentiation factor, BMP-7, to animals with CKD and secondary hyperparathyroidism prevents the development of marrow fibrosis and the accumulation of fibrous cells in the bone marrow. Since there is evidence to suggest that the cells that accumulate in the bone marrow in osteitis fibrosa represent poorly differentiated osteoblasts [11, 12], the accumulation of these cells in the setting of secondary hyperparathyroidism due to CKD may be the result of two abnormalities related to the uremic setting. On the one hand, excess PTH has been suggested to increase the proliferation of osteoprogenitors [41, 42] and on the other hand, lack of BMP-7, as a result of decreased renal mass, may inhibit the differentiation of osteoprogenitors into mature osteoblasts. Thus, it appears that by adminis-

tering exogenous BMP-7, the osteoprogenitors fail to accumulate because they are now capable of differentiating into mature osteoblasts. An alternative explanation for the accumulation of fibrous cells in high-turnover renal osteodystrophy may be that in a state of BMP-7 deficiency, the pluripotential mesenchymal stem cells are inhibited from differentiating into osteoprogenitors, and therefore enter an alternative differentiation pathway and give rise to cells with a fibroblastic phenotype.

The present studies clearly demonstrate that CKD and secondary hyperparathyroidism produce abnormalities in osteoblast differentiation reflected by accumulation of fibrous tissue producing cells as well as increased osteoclast activity. BMP-7 eliminated the fibrous producing cells and increased osteoblast number demonstrating improved osteoblast differentiation in CKD. The effects of BMP-7 treatment on osteoclasts were unexpected and the mechanisms involved remain to be determined.

In summary, using an animal model of high-turnover renal osteodystrophy, the present studies demonstrate that treatment with BMP-7 prevents the development of marrow fibrosis. These findings suggest that the bone marrow fibrosis characteristic of secondary hyperparathyroidism in chronic kidney disease may represent the actions of cells constrained from osteoblast differentiation, perhaps as a consequence of BMP-7 deficiency. Additional studies on the cellular abnormalities involved in the generation of marrow fibrosis in high-turnover renal osteodystrophy will further clarify the mechanisms involved in this process, and aid in the development of therapeutic interventions. The prevention of marrow fibrosis is likely to have important clinical implications in the management of patients with chronic kidney disease, since marrow fibrosis in this setting is well known to cause anemia and to render the bone marrow resistant to the actions of erythropoietin [7, 8].

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Reprint requests to Esther A. Gonzalez, M.D., Department of Nephrology, Saint Louis University, 3635 Vista Ave., St. Louis, Missouri 63110, USA.
E-mail: gonzalea@slu.edu

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