Kidney International, Vol. 63 (2003), pp. 2020–2027

Efficacy of 19-Nor-1,25-$(OH)_2D_2$ in the prevention and treatment of hyperparathyroid bone disease in experimental uremia

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Efficacy of 19-Nor-1,25-$(OH)_2D_2$ in the prevention and treatment of hyperparathyroid bone disease in experimental uremia.

Background. The control of parathyroid hyperplasia and high circulating parathyroid hormone (PTH) levels is crucial in preventing secondary hyperparathyroidism (SH) in renal failure. Parathyroid gland enlargement and elevated levels of PTH are major contributors to increase bone resorption, a feature of renal osteodystrophy.

Methods. These studies assessed the efficacy of the 1,25$(OH)_2D_3$ analog, 19-Nor-1,25$(OH)_2D_2$ (19-Nor), in the prevention (protocol I) and treatment (protocol II) of SH and renal osteodystrophy in uremic rats. In protocol I, normal and uremic rats were fed a high phosphorus diet for 2 months; uremic rats were administered intraperitoneal injections of either vehicle or 19-Nor (200 ng three times a week). In protocol II, normal and uremic rats were fed a high phosphorus diet for 4 months; 2 months after the onset of uremia, rats were administered either intraperitoneal vehicle or 19-Nor (200 ng three times a week). Serum PTH and bone histology were used to assess the degree of SH.

Results. 19-Nor was effective in preventing (protocol I) and suppressing (protocol II) the significant SH induced by uremia and further enhanced by a high phosphorus diet. In protocol I, bone histology in uremic controls showed a threefold increase in the cancellous bone mass compared to normal rats. This expansion in unmineralized bone was accompanied by 5-, 1.5-, and 7-fold increases in eroded surface, mineralization lag time (MLT), and bone formation rate (BFR/BS), respectively. Moreover, cortical bone porosity in untreated uremic rats increased 267-fold compared to normal animals. 19-Nor ameliorated these changes in cancellous and cortical bone. In protocol II, the reported indices worsened even further. In contrast, 2 months of 19-Nor treatment improved bone histology by reducing cortical bone porosity, woven bone formation, MLT, and BFR/BS.

Conclusion. In an experimental model of chronic renal failure (CRF), 19-Nor prevents SH and ameliorates the histomorphometric changes induced by uremia and high phosphorus diet. In addition, 19-Nor suppresses serum PTH and improves bone histology in uremic rats with established severe SH. Further studies in patients with CRF are necessary to define the clinical applicability of 19-Nor on bone histology in humans.

Key words: renal osteodystrophy, vitamin D, bone histomorphometry.

Received for publication May 20, 2002
and in revised form August 5, 2002, December 6, 2002, and January 22, 2003
Accepted for publication February 3, 2003

In patients with chronic renal failure (CRF), parathyroid hyperplasia, and enhanced synthesis and secretion of parathyroid hormone (PTH) characterize secondary hyperparathyroidism (SH) [1–4]. The enlargement of the parathyroid glands and high circulating levels of PTH [5] are major contributors to increased bone resorption, a feature of renal osteodystrophy. Uremia, low calcium intake, high dietary phosphorus, and vitamin D deficiency are the main regulators of SH [6]. Although the mechanisms by which these factors control PTH biosynthesis and secretion are well known, optimal treatments are still being developed.

1,25$(OH)_2D_3$ (1,25D) has been used in the treatment of SH because of its efficacy in suppressing PTH secretion and synthesis [7]. However, both hypercalcemia and hyperphosphatemia, a result of increased intestinal calcium and phosphorus absorption and bone-mineral resorption, may develop in some patients, thus, this may preclude 1,25D use in these uremic patients. In addition, 1,25D-induced enhancement of serum calcium levels promotes an elevated calcium-phosphate product increasing the risk of vascular calcification especially in patients receiving calcium salts as phosphate binders [8, 9].

Previous studies in our laboratory have demonstrated that 19-Nor-1,25$(OH)_2D_2$ (19-Nor), a new analog of 1,25$(OH)_2D_3$ (1,25D), suppresses PTH in uremic rats with SH at doses which do not increase either serum calcium or phosphorus [10, 11]. The mechanisms responsible for the decreased calcemic and phosphatemic activities of 19-Nor are not completely understood. Data from our laboratory in parathyroidectomized rats fed either a calcium- or phosphorus-deficient diet and treated with 1,25D or 19-Nor have shown that this new analog is approximately 10 times less active than 1,25D in promoting calcium and phosphorus resorption from bone [12].

However, both promote bone differentiation of the osteoblast phenotype, which is required for normal bone formation. Furthermore, 19-Nor is currently used in hemodialysis patients as an alternative to 1,25D therapy [13].

1,25D not only potently stimulates bone resorption but also promotes bone formation. The effects of 1,25D on osteoblasts and their precursors, which are multiple and complex, include (*1*) decreasing the rate of cell proliferation, while enhancing the process of mineralization; (*2*) increasing both gene and protein expression of a number of matrix proteins, including type 1 collagen, osteocalcin, and osteopontin; and (*3*) stimulating production of the ectoenzyme alkaline phosphatase and the growth factors, transforming growth factor-β (TGF-β) and insulin-like growth factor-1 (IGF-1), as well as the family of binding proteins that modulates the activity of the latter molecule [14]. Studies in patients with CRF demonstrated that, in some patients, the administration of calcitriol induced adynamic bone disease [15]. Concern that prolonged 19-Nor therapy might have negative side effects on the bone metabolism of hemodialysis patients led us to examine two markers of bone formation, alkaline phosphatase and osteocalcin in MG-63 cells, an osteoblastic-like cell line [16]. These in vitro studies suggested that 19-Nor is as active as 1,25D in promoting bone formation.

In the present studies, we examined the in vivo effects of 19-Nor administration on bone. In our experimental model of CFR, 19-Nor therapy not only prevents SH and ameliorates the histomorphometric alterations induced by uremia and high dietary phosphorus, but also suppresses serum PTH and improves bone histology in uremic rats with established severe SH.

METHODS

Experimental protocols

Normal and uremic (5/6 nephrectomized) female Sprague-Dawley rats, 8 to 10 weeks old and weighing 225 to 250 g, were studied. The 5/6 nephrectomy involved the ligation of several branches of the left renal artery and excision of the right kidney. All animals were fed a high phosphorus diet containing 0.9% phosphorus and 0.6% calcium, purchased from Dyets, Inc. (Bethlehem, PA, USA).

Protocol I. During the studies, normal rats (control group) ($N = 9$) received 100 μL of propylene glycol as vehicle, three times a week for 2 months. Immediately after the onset of renal failure, uremic rats received intraperitoneal injections of either 100 μL of propylene glycol as vehicle ($N = 8$) or 19-Nor (200 ng) ($N = 9$) three times a week for 2 months.

Protocol II. Normal rats ($N = 8$) received 100 μL of propylene glycol as vehicle, three times a week for 4 months, uremic rats received either vehicle ($N = 8$) or 19-Nor ($N = 8$) as in protocol I, except that the injections were begun 2 months after the induction of renal failure and continued for an additional 2 months. For both protocols all rats received subcutaneous injections of a tetracycline derivative, demeclocycline (#D-614) (Sigma Chemical Co., St. Louis, MO, USA), 20 mg/kg, 12 days before sacrifice. In addition, both 2- and 4-month uremic and normal animals were injected subcutaneously with calcein (#C-0875) (Sigma Chemical Co.), 10 mg/kg, 2 days before sacrifice (10 days after the demeclocycline administration).

At the end of each study, normal and uremic rats were anesthetized and sacrificed by exsanguination. Arterial blood was drawn by aortic puncture for analytic determinations. After sacrifice, tibiae were surgically removed, stripped of all muscle, and saved for histomorphometric analysis of cancelleous and cortical bone.

All experimental protocols were approved by the Animal Studies Committee at Washington University School of Medicine.

Analytic determinations

Plasma phosphorus and creatinine were determined using an autoanalyzer (COBAS-MIRA Plus, Branchburg, NJ, USA). Ionized calcium was measured by an ionized calcium–specific electrode (Model ICA-1; Radiometer, Copenhagen, Denmark). Intact PTH levels were measured by an immunoradiometric assay specific for rat intact PTH (Immutopics, San Clemente, CA, USA).

Tetracycline derivative preparation

Thirty minutes prior to injection, demeclocycline powder was mixed with bacteriostatic water, stirred, and adjusted for a pH between 7.2 and 7.4. Calcein powder was slowly added to a sodium bicarbonate saline solution and stirred until dissolved. This solution was filtered through a 0.2 μ millipore filter, mixed, and used for the injections.

Bone histomorphometry

Immediately after removal of all muscles, tibiae were fixed in 70% alcohol. The proximal portion and the middle third of the right tibiae were stained with Villanueva stain, dehydrated in graded concentrations of ethanol, defatted in acetone, and embedded in methyl methacrylate (Fisher Scientific, Fairlaw, NJ, USA). Longitudinal sections of the proximal portion of the tibiae and cross sections at the tibiofibular junctions of the tibial shafts were cut to a thickness of 230 μm using a low speed metallurgic saw and then ground to thicknesses of 20 μm (proximal portion of the tibiae) and 30 μm (tibiofibular junctions of the tibial shafts) for histomorphometric measurement. Histomorphometry was done using a semiautomatic image analysis system (OsteoMeasure, OsteoMetrics, Inc., Decatur, GA, USA) linked to a microscope equipped with transmitted and fluorescence light. All

Table 1. Abbreviations of bone histomorphometric indices

Parameters	Abbreviations
Cancellous bone	
Total bone volume	TBV/TV
Woven bone volume	WB/TV
Eroded surface	ES/BS
Mineralization lag time	MLT
Bone formation rate (bone surface based)	BFR/BS
Bone formation rate (bone area based)	BFR/BV
Cortical bone	
Total cross-sectional area	TAr
Marrow area	MaAr
Cortical bone width	CtWi
Cortical bone area	CtAr
Porosity area	PoAr
Periosteal-bone formation rate	Ps-BFR
Osteoid perimeter	Ec-OPm

parameters were calculated according to the recommendations of the Histomorphometry Nomenclature Committee of the American Society of Bone and Mineral Research [17, 18].

The region of the proximal tibial metaphysis (PTM) that was 1 to 4 mm distal to the growth plate–metaphyseal junction was studied and the following measurements taken: total bone volume per total volume (TBV/TV) (%), woven bone volume per total volume (WB/TV) (%), eroded surface per unit of bone surface (ES/BS) (%), mineralization lag time (MLT) (days), bone formation rate per unit of bone volume (BFR/BV) (%/year), and bone formation rate per unit of bone surface (BFR/BS) ($\mu m^3/\mu m^2$/day).

Cortical bone determinations included total cross sectional area (TAr) (mm^2) cortical bone width (CtWi) (μm), cortical bone area (CtAr) (mm^2) marrow area (MaAr) (mm^2), intracortical porosity area (PoAr) (mm^2), osteoid perimeter (OPm) (%), periosteal-bone formation rate (Ps-BFR) (μm/day/100) (Tables 1 and 3).

Statistics

For the biochemical analyses, analysis of variance (ANOVA) was employed to assess statistical differences between all experimental groups tested. Multiple comparisons using the stringent Bonferroni test measured the statistical significance of the differences between every possible two-group comparisons.

For bone histomorphometry, statistical analyses were performed using SPSS for Windows (version 10.0, SPSS, Inc., Chicago, IL, USA). The Bonferroni post-hoc test was selected to analyze differences between groups. $P < 0.05$ was considered significant.

RESULTS

The efficacy of 19-Nor in preventing (protocol I) and treating (protocol II) high phosphorus-induced secondary hyperparathyroidism and bone damage in rats was determined 2 and 4 months after the induction of renal insufficiency by 5/6 nephrectomy. Preliminary studies defined the nonhypercalcemic doses of the vitamin D metabolite employed in the present protocols. Table 1 depicts the abbreviations for the bone histomorphometric indices used in these analyses.

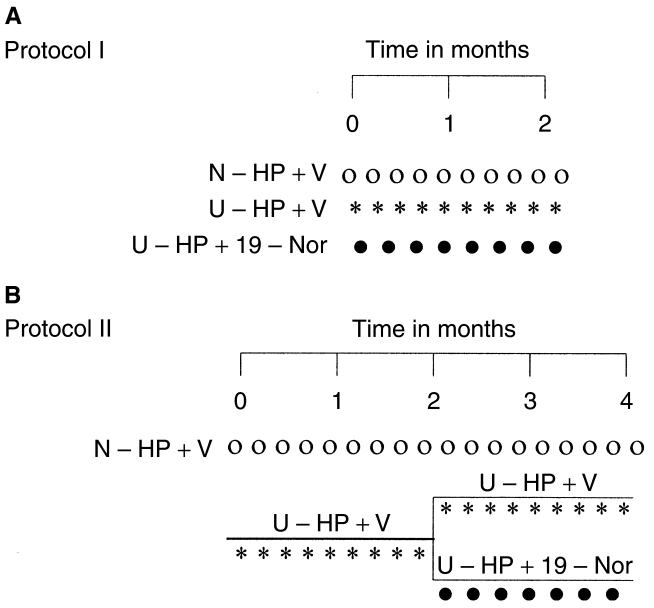


Fig. 1. Experimental design. (*A*) In protocol I, normal (N) and uremic (U) rats underwent one of the following experimental protocols for 2 months: normal control + high phosphorus diet + vehicle (N + HP + V), uremic control + high phosphorus diet + vehicle (U + HP + V), uremic + high phosphorus diet + 200 ng three times a week 19-Nor-1,25-$(OH)_2D_2$ (U + HP + 19-Nor). (*B*) In protocol II, normal (N) and uremic (U) rats underwent one of the following experimental protocols for 4 months: normal control + high phosphorus diet + vehicle (N + HP + V), uremic control + high phosphorus diet + vehicle (U + HP + V), uremic + high phosphorus diet + vehicle for the first 2 months of study and during the last 2 months of study + 200 ng three times a week 19-Nor (U + HP + 19-Nor).

Study design

Figure 1 depicts the experimental design for the protocols for prevention and treatment studies. In protocol I, after the onset of renal failure, the rats were divided into two groups with similar serum creatinine levels. Uremic rats were injected intraperitoneally with vehicle or 19-Nor, 3 times a week for 2 months. In protocol II, after 2 months of renal failure, uremic rats were divided into two groups with similar serum creatinine levels. One group received 19-Nor and the other vehicle three times a week for an additional 2-month period. Normal rats (control group) received 100 μL of propylene glycol as vehicle three times a week for 2 or 4 months in both protocols.

Effects of 19-Nor on serum chemistry and PTH secretion

Table 2 shows the effects of 19-Nor treatment on serum creatinine, ionized calcium, phosphorus, and PTH

Table 2. Serum chemistries

	Protocol I Prevention			Protocol II Treatment		
	N − HP + V 2 months ($N = 9$)	U − HP + V 2 months ($N = 8$)	U − HP + 19-Nor 2 months ($N = 9$)	N − HP + V 4 months ($N = 8$)	U − HP + V 4 months ($N = 8$)	U − HP + 19-Nor 4 months ($N = 8$)
Creatinine *mg/dL*	0.91 ± 0.07	1.97 ± 0.35[b]	1.68 ± 0.29	1.02 ± 0.11	2.38 ± 0.41[b]	1.62 ± 0.22
Ionized calcium *mg/dL*	4.62 ± 0.46	4.25 ± 0.35	4.81 ± 0.28	4.89 ± 0.37	4.19 ± 0.43	5.02 ± 0.18
Phosphorus *mg/dL*	5.1 ± 0.3	10.5 ± 0.3[a]	7.8 ± 0.2[a,c]	3.8 ± 0.4	11.2 ± 0.3[a]	7.2 ± 0.2[a,c]
Parathyroid hormone *pg/mL*	21 ± 4	908 ± 289[a]	45 ± 17[c]	23 ± 7	2068 ± 354[a]	67 ± 19[c]

Normal (N) and uremic (U) rats underwent one of the following experimental protocols for either 2 or 4 months: normal control + high phosphorus diet + vehicle (N − HP + V), uremic control + high phosphorus diet + vehicle (U − HP + V), uremic + HP diet + 200 ng three times a week 19-Nor-1,25-$(OH)_2D_2$ (U − HP + 19-Nor). Values represent the mean ± SEM.

[a] $P < 0.01$ vs. N − HP + V; [b] $P < 0.05$ vs. N − HP + V; [c] $P < 0.01$ vs. U − HP + V; [d] $P < 0.05$ vs. U − HP + V

levels in uremic rats after either 2 or 4 months of renal failure. Serum creatinine increased in both 2- and 4-month uremic animals fed high dietary phosphorus compared to normal controls. The elevation in serum creatinine levels was lower after 2 and 4 months in uremic rats treated with 19-Nor compared to untreated uremic animals. In addition, as indicated in Table 2, serum phosphorus levels in uremic rats were reduced by 19-Nor administration. As expected, treatment with the vitamin D analog did not significantly increase ionized calcium compared to normal rats fed the same high phosphorus diet.

Untreated uremic controls in both protocols I and II had higher serum PTH levels than normal controls. In contrast, 19-Nor not only prevented the increase in serum PTH induced by high dietary phosphorus after 2 months of uremia, but also reduced the very high PTH levels seen in the 4-month study.

Effects of CRF and 19-Nor on cancelleous and cortical bone (protocol I at 2 months)

For the 2-month study, Table 3 shows the static and dynamic histomorphometric changes in the PTM and the cross sections at the tibiofibular junctions of the tibial shafts. After 2 months of renal failure, total bone mass increased due mainly to increases in woven bone. MLT increased by 48%, while eroded surface and bone formation rate increased by five- to sixfold. In the tibiofibular junctions of the tibial shafts, intracortical porosity area also markedly increased from 0.001 ± 0.000 to 0.267 ± 0.01. In addition, both periosteal and endocortical bone formation increased by at least 30-fold. Compared to the uremic group, treatment with 19-Nor lowered WBV/TV to that of normal controls. MLT, ES/BS and BFR/BS were lowered by 45%, 74%, and 37%, respectively. In uremic rats receiving 19-Nor, PoAr, BFR of the periosteal and BFR of endocortical surfaces were lowered by 96%, 46%, and 57%, respectively.

The effects of 2 months of CRF and preventive 19-Nor administration on both cancellous and cortical bone are illustrated in Figure 2 A and B, respectively. In normal rats very few double labels were observed at either the periosteal or endocortical surfaces. In uremic untreated animals, much more bone, osteoid, double labels, wide interlabeled width, diffuse labels, and bone erosion were seen. In contrast, in 19-Nor–treated rats, more bone of lamellar structure was noted compared to the normal controls. Moreover, we observed less osteoid, double labeling, intracortical porosity and bone erosion in uremic rats receiving 19-Nor.

Effects of chronic renal failure and 19-Nor on cancelleous and cortical bone (protocol II at 4 months)

Table 3 shows the static and dynamic histomorphometric changes observed in the 4-month study. After 4 months of CRF, the amount of woven bone was further increased (↑43%), compared to the 2-month study. Elevated bone turnover (BFR/BV) and prolonged MLT were also seen. Moreover, the total cross-sectional area of tibiofibular junctions of the tibial shafts increased by 12%, the MaAr decreased by 22%, and the CtWi increased by 17%. PoAr was also increased compared to 2-month uremic (169%) and age-matched normal rats (700-fold). Importantly, compared to the untreated uremic group, rats receiving 2 months of 19-Nor treatment initiated 2 months after the onset of uremia had lower ES/BS, MLT, and BFR/BV, 79%, 53%, and 88%, respectively. In the tibiofibular junctions of the tibial shafts, porosity area, periosteal bone formation and endocortical bone formation decreased 96%, 33%, and 85% respectively, in 19-Nor treated rats versus untreated uremic controls.

The effects of 4 months of CRF and 19-Nor treatment on both cancellous and cortical bone are showed in Figure 3 A and B, respectively. In uremic untreated rats, more cancellous bone, double labels, osteoid, and erosion were seen. The structure of the cortex was distorted and greater intracortical porosity was present. In contrast, 19-Nor–treated animals had less osteoid, double labeling, intracortical porosity, and bone erosion.

Table 3. Histomorphometric determinations of the proximal tibial methaphyses (PTM) and tibial shaft (TX)

	Protocol I Prevention			Protocol II Treatment		
	N − HP + V 2 months ($N = 9$)	U − HP + V 2 months ($N = 8$)	U − HP + 19-Nor 2 months ($N = 9$)	N − HP + V 4 months ($N = 8$)	U − HP + V 4 months ($N = 8$)	U − HP + 19-Nor 4 months ($N = 8$)
PTM						
TBV/TV %	14.5 ± 3.5	46.3 ± 30.6[a]	8.8 ± 3.7[a,b]	8.0 ± 2.7	59.5 ± 28.5[a]	21.7 ± 7.8[a,b,c]
WB/TV %	0.00 ± 0.00	17.4 ± 15.5	0.00 ± 0.00	0.00 ± 0.00	24.9 ± 13.4	0.00 ± 0.00
ES/BS %	3.2 ± 0.8	16.6 ± 3.6[a]	4.4 ± 2.7[b]	7.9 ± 1.7	20.9 ± 7.7[a]	4.3 ± 1.4[a,b]
MLT *days*	2.7 ± 0.5	4.0 ± 1.1[a]	2.2 ± 0.7[b]	N/A	5.5 ± 3.3	2.6 ± 0.9[b]
BFR/BS $\mu m^3/\mu m^2/d$	11.5 ± 9.1	79.2 ± 24.6[a]	49.6 ± 11.8[a]	0.0 ± 0.0[a]	141 ± 66[a]	26.2 ± 9.7[a,b,c]
BFR/BV %/*y*	143 ± 111	620 ± 159[a]	635 ± 153[a]	0.0 ± 0.0[a]	1782 ± 887[a,c]	220 ± 85[a,b,c]
TX						
TAr mm^2	4.7 ± 0.2	5.0 ± 0.2[a]	5.3 ± 0.2[a,b]	5.2 ± 0.3	5.6 ± 0.3[a]	5.7 ± 0.4[a,d]
MaAr mm^2	0.8 ± 0.1	0.9 ± 0.3	1.0 ± 0.2[a]	1.0 ± 0.1	0.7 ± 0.4[a]	1.0 ± 0.1[b]
CtWi μm	742 ± 60	759 ± 77	766 ± 44	764 ± 40	885 ± 125[a,c]	816 ± 28[a,d]
CtAr mm^2	3.9 ± 0.2	3.8 ± 0.3	4.3 ± 0.2[a,b]	4.2 ± 0.3	4.2 ± 0.3[a,c]	4.7 ± 0.3[a,b,d]
PoAr mm^2	0.001 ± 0.000	0.267 ± 0.01[a]	0.01 ± 0.03[b]	0.001 ± 0.000	0.72 ± 0.43[a,c]	0.03 ± 0.04[a,b]
Ps-BFR $\mu m/d/100$	2.9 ± 2.8	91.1 ± 61.4[a]	49.6 ± 37.1[b]	2.1 ± 3.8	111.2 ± 59.3[a]	74.7 ± 23.6
OPm %	0.44 ± 0.80	48.1 ± 24.7[a]	22.0 ± 13.0[a,b]	11.5 ± 4.4	64.8 ± 26.2[a]	36.8 ± 18.1[a,b,d]

For abbreviations, see Table 1. Normal (N) and uremic (U) rats underwent one of the following experimental protocols for either 2 or 4 months: normal control + high phosphorus diet + vehicle (N − HP + V), uremic control + high phosphorus diet + vehicle (U − HP + V), uremic + HP diet + 200 ng three times a week 19-Nor-1,25-$(OH)_2D_2$ (U − HP + 19-Nor). Values represent the mean ± SD.

[a] $P < 0.01$ vs. N − HP + V; [b] $P < 0.01$ vs. U − HP + V; [c] $P < 0.01$ vs. U − HP + V (2 months); [d] $P < 0.01$ vs. U − HP + 19-Nor (2 months)

DISCUSSION

These studies assessed the effects of the 1,25D analog, 19-Nor, in the prevention and treatment of high phosphorus-induced secondary hyperparathyroidism and bone damage in a model of experimental uremia.

We first studied [protocol I (2 months)] the effect of 19-Nor on the prevention of SH and bone pathology. Second, we studied [protocol II (4 months)] the ability of 19-Nor to reverse SH and correct the alterations in bone histomorphometry. Clearly, after 2 months of uremia, treatment with the vitamin D analog ameliorated the increases in serum creatinine, phosphorus, and PTH levels. Importantly, 19-Nor did not increase ionized calcium significantly above the levels seen in normal rats fed the same high phosphorus diet. However, a minor increase in ionized calcium may also participate in the correction of secondary hyperparathyroidism between the uremic groups.

The control of secondary hyperparathyroidism is an important goal in patients with CRF [19]. 19-Nor is currently being used in the treatment of secondary hyperparathyroidism because it suppresses serum PTH levels without producing significant either hypercalcemia or hyperphosphatemia in uremic rats [10]. Our results confirm that 19-Nor controls SH by reducing serum PTH levels, with little effect on serum calcium and phosphorus levels in uremic rats [16]. Moreover, recent studies in humans have reported a 60% reduction in PTH levels in hemodialysis patients treated with 19-Nor compared to those receiving placebo [13].

It is well known that vitamin D deficiency, an important determinant of SH, is dangerous to the skeleton, particularly the cortical bone [20]. It is also well established that 1-α-hydroxyvitamin-D_3 has a beneficial effect on the histomorphometric changes seen in the bone of CRF patients [21, 22]. Moreover, early treatment with vitamin D during the development of renal failure can prevent not only the biochemical changes but also the histologic bone damage induced by SH in uremic patients [23, 24]. Clearly, improvements in PTH suppression and bone histology were observed in adults and children with renal failure who were treated with alfacalcidol [25, 26]. Recent studies showed the efficacy of the combined therapy with 1,25-dihydroxyvitamin D_3 and 24,25-dihydroxyvitamin D_3 on biochemical and histologic features of renal osteodystrophy in rats with CRF [27]. Moreover,

Fig. 3. Effects of chronic renal failure (CRF) and 19-Nor-1,25$(OH)_2D_2$ (19-Nor) on cancelleous (*A*) and cortical (*B*) bone (protocol II; 4 months). Normal (N) and uremic (U) rats underwent one of the following experimental protocols: normal control + high phosphorus diet + vehicle for 4 months (left panels), uremic control + high phosphorus diet + vehicle for 4 months (middle panels), and uremic + HP diet + vehicle for the first 2 months of study and during the last 2 months of study + 200 ng three times a week 19-Nor-1,25-$(OH)_2D_2$ (right panels). In uremic untreated rats, more cancellous bone, double labels (arrow heads), osteoid and erosion (arrows) were seen (A). The structure of the cortex was distorted, with elevated intracortical porosity (B). In contrast, in 19-Nor-treated animals there was less osteoid, double labeling, intracortical porosity, and bone erosion. 20 mm, Villanueva-stained sections. Abbreviations are: T, trabeculae; M, marrow; O, osteoid; C, cortex; P, porosity. Magnification ×160.

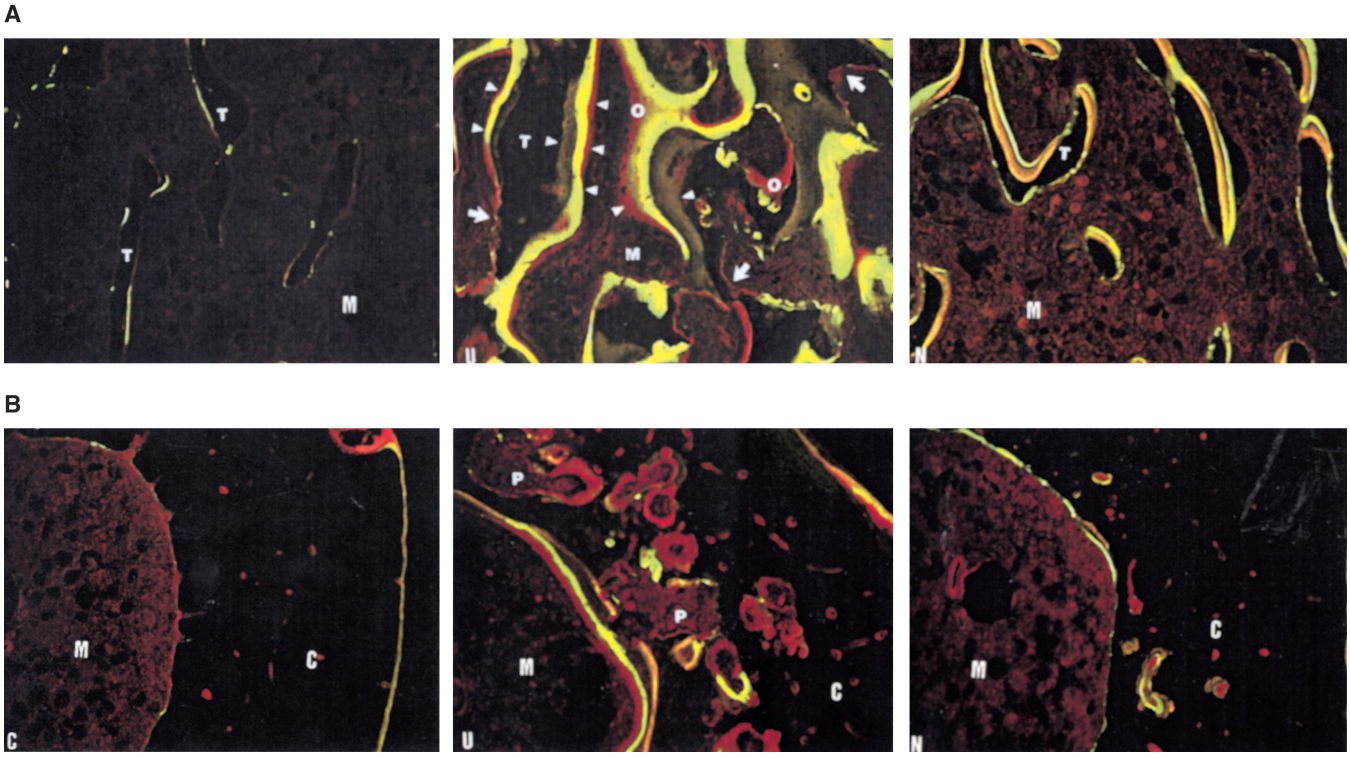
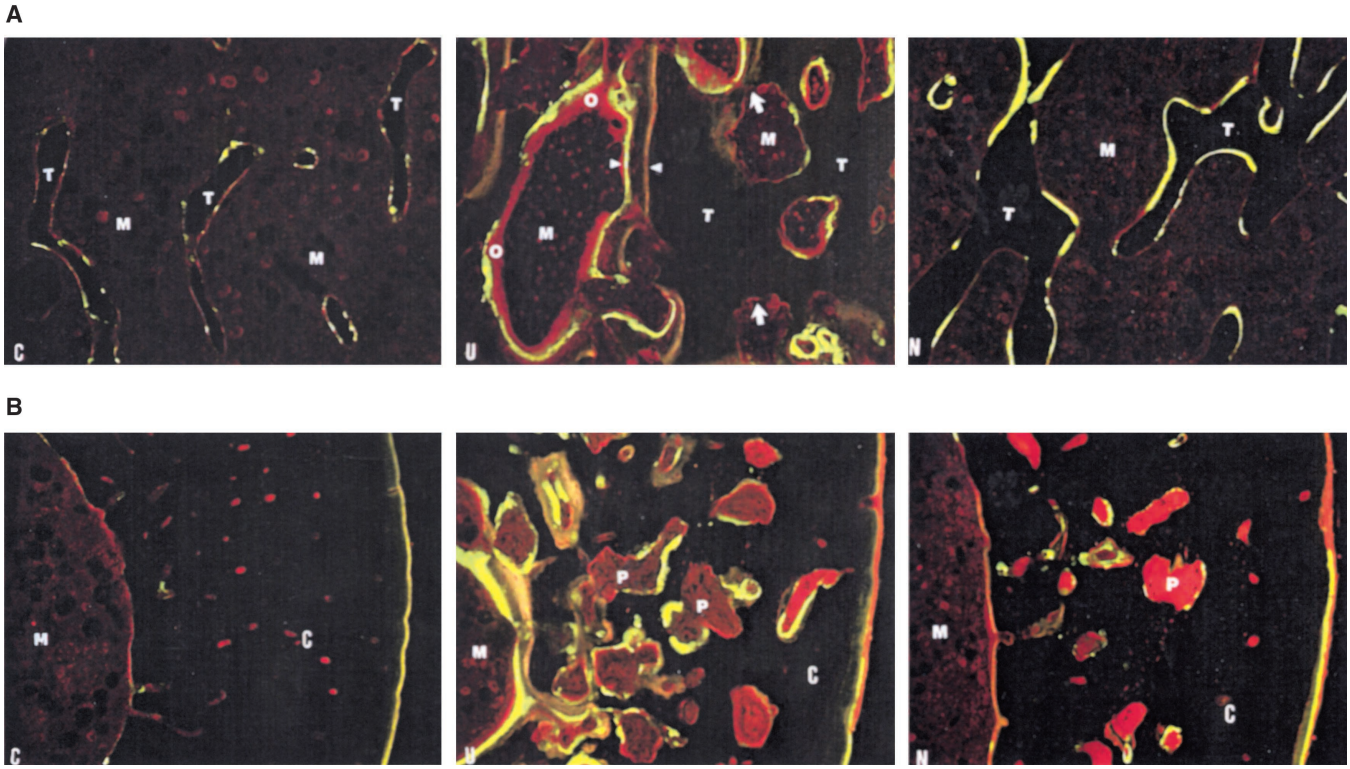


Fig. 2. Effects of chronic renal failure (CRF) and 19-Nor-1,25(OH)$_2$D$_2$ (19-Nor) on cancelleous (*A*) and cortical (*B*) bone (protocol I; 2 months). Normal (N) and uremic (U) rats underwent one of the following experimental protocols for 2 months: normal control + high phosphorus diet + vehicle (left panels), uremic control + high phosphorus diet + vehicle (middle panels), and uremic + high phosphorus diet + 200 ng three times a week 19-Nor-1,25-(OH)$_2$D$_2$ (right panels). Uremic animals had massive osteoid formation, wide interlabel width (arrow heads), bone erosion (arrows) in the proximal tibial metaphysis (A) and had large porosity in the cortex (B). 19-Nor–treated rats had more cancellous bone compared to normal rats, consisting of lamellar bone structure. Moreover, less osteoid, double labeling, intracortical porosity, and bone erosion was seen. 20 μm, Villanueva-stained sections. Abbreviations are: T, trabeculae; M, marrow; O, osteoid; C, cortex; P, porosity. Magnification ×160.



the effect of the combined administration of 24,25-dihydroxyvitamin D_3 and 1-α-hydroxyvitamin-D_3 was studied in patients with chronic renal insufficiency [28]. Finally, another vitamin D analog, 22-oxacalcitriol, has been shown to significantly decrease serum PTH levels and reverse abnormal bone resorption without induction of adynamic bone disease in dogs with renal failure [29].

Because no long-term studies have evaluated the efficacy of 19-Nor in preventing and treating bone disease in renal failure, we studied the effects of this compound on reducing the bone structure modifications that are induced by CRF and further enhanced by high dietary phosphorus in uremic rats.

To assess the role of 19-Nor on bone in vivo, we performed bone histomorphometry on rat tibiae from each experimental condition. Two months of uremia induced significant histomorphometric changes of both the PTM and the cross sections of the tibiofibular junctions of the tibial shafts. The increase in total bone mass was due mainly to an increase in woven bone. In addition, abnormal bone mineralization was the most obvious change following 2 months of uremia. MLT increased by 48%. Whereas increased bone formation was seen at both the periosteal and endocortical surfaces, bone resorption increased only at the endosteal surface. Importantly, preventive treatment with 19-Nor improved bone mineralization. In fact, trabecular and cortical bone mass were maintained at levels similar to normal control rats. Dynamic changes (bone formation, resorption, and turnover) were also similar to normal controls.

After 4 months of uremia, we observed a continued increase in woven bone area, intracortical bone remodeling, porosity area, and defective bone mineralization. Treatment with 19-Nor ameliorated the elevations in bone turnover and bone formation. More important, bone resorption was significantly decreased.

Our studies show the long-term effects of 19-Nor administration on bone histology in uremic rats. By suppressing PTH secretion, 19-Nor decreased the histomorphometric manifestations of secondary hyperparathyroidism on bone [30]. In fact, 19-Nor ameliorated and decreased the uremia-induced abnormal formation of woven bone and decreased bone resorption.

Many in vivo studies on suppression of secondary hyperparathyroidism by calcitriol in patients with CRF have shown similar results [31–34]. Moreover, previous in vitro studies by our group and others [16, 35] have demonstrated that 19-Nor controls osteoblastic growth as potently as 1,25D. Indeed, similar to 1,25D, 19-Nor has been shown to up-regulate vitamin D receptor expression and increase alkaline phosphatase activity in MG-63 cells [16].

CONCLUSION

19-Nor was shown to improve mineralization and prevent the formation of abnormal woven bone. Most important, this vitamin D analog suppressed serum PTH levels without inducing low bone turnover [36].

These studies in rats with CRF demonstrate that 19-Nor prevents secondary hyperparathyroidism and ameliorates the histomorphometric changes induced by uremia and high dietary phosphorus. In addition, 19-Nor suppresses serum PTH and improves bone histology in uremic rats with established severe secondary hyperparathyroidism. Because of its ability to suppress PTH and treat renal osteodystrophy in our experimental model of renal failure, 19-Nor could be considered a tool for therapeutic intervention in secondary hyperparathyroidism in CRF. These results in uremic rats should be interpreted with caution and cannot be extrapolated to patients with CRF. Further studies are critical to evaluate the role of 19-Nor in humans.

ACKNOWLEDGMENTS

This study was supported in part by U.S. Public Health Service, the National Institute of Diabetes and Digestive and Kidney Diseases (grants DK-07126, DK-09976 and DK-30178), and a grant provided by Abbott Pharmaceutical.

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