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Prevention of Bone Loss in Ovariectomized Rats by Combined Treatment With Risedronate and 1α,25-Dihydroxyvitamin D_3

REINHOLD G. ERBEN,[1] LIS MOSEKILDE,[2] JESPER S. THOMSEN,[2] KARIN WEBER,[1] KERSTIN STAHR,[1] ALYSON LEYSHON,[3] SUSAN Y. SMITH,[3] and ROGER PHIPPS[4]

ABSTRACT

Bisphosphonates inhibit bone loss through inhibition of osteoclast-mediated bone resorption. At low doses, vitamin D metabolites can prevent bone loss in models of osteopenia in rats by an antiresorptive effect, while at high doses they also stimulate osteoblast activity and show an anabolic effect. Therefore, combined therapy with bisphosphonates and vitamin D analogs might be expected to be more effective than either treatment alone. It was the aim of this study to compare the efficacy of risedronate and of the naturally occurring vitamin D hormone 1α,25-dihydroxyvitamin D_3 (calcitriol), alone and in combination, for the prevention of ovariectomy-induced bone loss in rats. One hundred ten female 4-month-old Sprague–Dawley rats were used for this experiment. Ninety rats were bilaterally ovariectomized (OVX), 10 rats were sham-operated (SHAM), and 10 rats were killed at the time of surgery as a baseline control. Groups of rats (10 rats/group) received vehicle or daily doses of 0.1 mg or 0.5 mg of risedronate or 0.05 μg or 0.1 μg of calcitriol/kg body weight, alone and in combination. Both compounds were administered orally via gavage, commencing on the day after surgery. Although estrogen deficiency–induced bone loss was prevented by individual prophylactic administration of risedronate or calcitriol, OVX rats treated with a combination of risedronate and calcitriol had higher bone mineral density (BMD), cancellous bone area (B.Ar), and bone strength in long bones and vertebrae compared with rats receiving risedronate alone. Furthermore, calcitriol enhanced the suppressive effects of risedronate on osteoclast number and partially counteracted the suppressive effects of risedronate on bone formation and histomorphometric indices of osteoblast team performance. Risedronate did not reduce the anabolic effect of calcitriol, and at the high dose it normalized hypercalcemia in calcitriol-treated OVX rats. Therefore, this study in OVX rats suggests that combined therapy with bisphosphonates and vitamin D analogs may offer advantages over the treatment with bisphosphonates or vitamin D analogs alone. (J Bone Miner Res 2002;17:1498–1511)

Key words: bisphosphonates, vitamin D, osteoporosis, histomorphometry, ovariectomy

INTRODUCTION

NEWLY DEVELOPED bisphosphonates such as risedronate and alendronate are potent inhibitors of the bone resorptive capacity of mature osteoclasts and also may induce osteoclast apoptosis.[1] Bisphosphonates prevent estrogen deficiency–induced bone loss in animals,[2,3] and these drugs also are very effective in the prevention and treatment of postmenopausal osteoporosis.[4,5] However, although bisphosphonates increase bone mineral density (BMD) at several bone sites and significantly reduce spine and hip fracture rates, they do not appear to increase cancellous bone area (B.Ar) measured by histomorphometry in patients with osteoporosis.[6,7]

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[1]Institute of Physiology, Physiological Chemistry, and Animal Nutrition, Ludwig Maximilians University, Munich, Germany.
[2]Department of Cell Biology, Institute of Anatomy, University of Århus, Århus, Denmark.
[3]ClinTrials BioResearch, Senneville, Quebec, Canada.
[4]Procter & Gamble Pharmaceuticals, Miami Valley Labs, Cincinnati, Ohio, USA.

Therefore, a combination of an antiresorptive and a bone anabolic drug may be a powerful treatment option for osteoporosis. However, data to support this notion are conflicting. A study in ewes has shown that the bisphosphonate tiludronate blocked the bone anabolic actions of parathyroid hormone (PTH) in this animal model.[8] Similarly, when rats were pretreated with alendronate for 2–4 months, the bone anabolic response to subsequent treatment with high doses of a PTH analog was diminished in the tibia and femur compared with animals that did not receive the bisphosphonate pretreatment.[9] On the other hand, the anabolic activity of PTH is not hampered when antiresorptive agents are coadministered in rodents,[10–13] and a pilot study in a small number of postmenopausal women has suggested that alendronate does not block the bone anabolic response to PTH in humans.[14]

The role of vitamin D metabolites in the treatment of osteoporosis is still controversial. Some clinical studies have suggested that therapy of patients with osteoporosis with active vitamin D metabolites increases BMD in the spine and forearm and reduces vertebral fractures.[15–18] However, other studies have provided negative results.[19,20] In addition, the use of vitamin D metabolites in patients with osteoporosis, especially at higher doses, has been hampered by the major side effects of this compound class, hypercalcemia and hypercalciuria, because of excessive stimulation of intestinal calcium absorption. Although the results of clinical trials have been ambiguous, studies in rats have shown clearly that active vitamin D metabolites can fully prevent estrogen deficiency–induced loss of cancellous and cortical bone by suppression of bone resorption.[21–24] At higher doses, vitamin D metabolites are bone anabolic and increase osteoblast team performance by augmenting osteoblast activity and proliferation of osteoblast precursors.[23–27]

The aim of this study was to compare the efficacy of risedronate and of the naturally occurring vitamin D hormone 1α,25-dihydroxyvitamin D_3 (calcitriol), alone or in combination, for the prevention of ovariectomy-induced bone loss in rats. The data provided by this experiment indicate that combined therapy with bisphosphonates and calcitriol may offer advantages over the treatment with bisphosphonates or calcitriol alone.

MATERIALS AND METHODS

Animal procedures

All animal procedures were conducted in accordance with the United States Department of Agriculture (USDA) Animal Welfare Regulations. One hundred ten female ~4-month-old Sprague–Dawley CD rats (Charles River Laboratories, Raleigh, SC, USA) weighing 250–310 g were used for this experiment. Ninety rats were ovariectomized (OVX) bilaterally by dorsal approach under isoflurane anesthesia, 10 rats were sham-operated (SHAM), and 10 rats were killed at the time of surgery to serve as a baseline control. The animals were housed individually at 22°C with a 12-h light/12-h dark cycle and were fed a standard laboratory chow (PMI 5002; PMI, Montreal, Canada) containing 0.8% calcium and 0.6% phosphorus. Food was available ad libitum to all rats except for a period from 4 h before dosing until 2 h after dosing with risedronate. Water was available ad libitum.

TABLE 1. STUDY DESIGN

Group	N	*Status*	*Treatment*	*Dose*
BL	10	Intact	BL	—
1	10	SHAM	Vehicle	—
2	10	OVX	Vehicle	—
3	10	OVX	Risedronate	0.1 mg/kg per day
4	10	OVX	Risedronate	0.5 mg/kg per day
5	10	OVX	Calcitriol	0.05 μg/kg per day
6	10	OVX	Calcitriol	0.1 μg/kg per day
7	10	OVX	Risedronate	0.1 mg/kg per day
			Calcitriol	0.05 μg/kg per day
8	10	OVX	Risedronate	0.1 mg/kg per day
			Calcitriol	0.1 μg/kg per day
9	10	OVX	Risedronate	0.5 mg/kg per day
			Calcitriol	0.05 μg/kg per day
10	10	OVX	Risedronate	0.5 mg/kg per day
			Calcitriol	0.1 μg/kg per day

BL, baseline.

Animals were randomized into the groups (10/group) shown in Table 1. Animals received daily doses of both vehicles (risedronate and calcitriol, groups 1 and 2) or 0.1 mg/kg or 0.5 mg/kg of risedronate and calcitriol vehicle (groups 3 and 4) or 0.05 μg/kg or 0.1 μg/kg of calcitriol and risedronate vehicle (groups 5 and 6) or a combination of risedronate and calcitriol (groups 7–10). Starting the day after surgery, both compounds and their vehicles were administered orally via gavage daily for 12 weeks at a dose volume of 10 ml/kg. Risedronate was dissolved in sterile saline, and calcitriol was dissolved in 0.1% Tween 20. The dosing solution for calcitriol was prepared freshly each day from a stock solution of calcitriol dissolved in 100% ethanol. To prevent interference with risedronate absorption, calcitriol or calcitriol vehicle was administered in the morning and risedronate or risedronate vehicle was administered in the afternoon.

Body weights were taken weekly. All animals were given subcutaneously a single tetracycline label (25 mg/kg) on day 1 of the study and a calcein double label (8 mg/kg) 12 days and 2 days before the rats were killed. After an overnight period of food deprivation, all rats were killed by exsanguination from the abdominal aorta under isoflurane anesthesia 12 weeks postovariectomy. Blood samples for PTH measurements were obtained from the tail vein immediately before necropsy. Serum samples were stored at −80°C until assayed. Success of ovariectomy was confirmed by lack of ovarian tissue at necropsy and a marked reduction in uterine weight.

Serum analysis

Serum samples were analyzed for calcium, phosphorus, creatinine, urea, alkaline phosphatase, and PTH. Serum calcium, alkaline phosphatase activity, blood urea nitrogen

(BUN), creatinine, and phosphorus were analyzed on a Hitachi 717 autoanalyzer (Boehringer, Mannheim, Germany). Serum PTH was measured with a rat-specific immunoradiometric assay reacting with both N-terminal and intact PTH (Nichols, San Juan Capistrano, CA, USA). The intra- and interassay variabilities of this assay were below 10%.

BMD measurements

BMD of the left tibias and of the L3 vertebrae was measured by DXA using a Hologic QDR 2000 plus bone scanner (Hologic, Inc., Waltham, MA, USA). The scans of the tibias were analyzed for BMD of the whole tibia and of subregions that included the proximal, central, and distal portions of the bone. The scans of the L3 vertebra were analyzed for BMD of the whole bone and of a central subregion that excluded the majority of cortical bone.

Biomechanical testing

Biomechanical testing of the fifth lumbar vertebral body, the femoral neck, the femoral diaphysis, and the distal femoral metaphysis was performed in a blind fashion with a materials testing machine (Alwetron TCT 5; Lorentzen & Wettre, Stockholm, Sweden) on bones that had been frozen at −20°C in saline-soaked gauze. All specimens were kept in Ringer's solution after thawing. For all tests a constant deformation rate of 2 mm/minute was used. Load-deformation curves were recorded and analyzed by a computer. Biomechanical parameters evaluated include ultimate load (F_{max}), maximum stress ($\sigma_{max} = F_{max}$/cross-sectional area), stiffness, energy absorption (W_{abs}), and Young's modulus (maximum slope of the stress-strain curve).

The L5 vertebra was mounted on a wooden block, and a central specimen with a height of approximately 3.9 mm was prepared using a precision parallel saw (Exakt, Norderstedt, Germany). All remaining parts of the process were removed using a fine electric saw (Minimot 40 E; Proxxon, Niersbach, Germany). The length of the specimen was measured with a micrometer, and the volume was determined by Archimedes principle with an electronic balance (Mettler AG245; Mettler-Toledo, Nänikon-Greifensee, Switzerland). The vertebral bodies were tested along the cephalocaudal axis. After biomechanical testing, the specimens were ashed, and the ash content was expressed as milligrams of ash per cubic millimeter of specimen volume (ash density).

The right femur was used for a three-point bending test of the diaphysis, followed by compression tests of the distal metaphysis and the femoral neck. Biomechanical testing of the femoral neck was performed by applying vertical load onto the femoral head in a direction parallel to the axis of the femoral diaphysis. A 4-mm-thick section with planoparallel ends consisting of metaphyseal bone was sawed from the distal part of the femora just over the patellofemoral joint cartilage. Measurements of the section volume and biomechanical testing along the proximodistal axis then were performed as described for the vertebral bodies. After biomechanical testing, the distal femoral metaphyseal specimens were ashed.

Histology

At necropsy, the fourth lumbar vertebrae and the proximal right tibias were defleshed and fixed in 70% ethanol at room temperature. After fixation, the bones were embedded undecalcified in methylmethacrylate as described previously.[28] Five-micrometer-thick undecalcified sections were prepared with an HM 360 microtome (Microm, Walldorf, Germany). The sections were sampled in the median plane of the vertebrae and the midsagittal plane of the tibias and were stained with von Kossa[29] and toluidine blue at acid pH.[30] For demonstration of cement lines the surface staining technique described by Schenk et al.[28] was used.

Histomorphometry

All measurements were performed on the secondary spongiosa of the proximal tibial metaphysis and of the fourth lumbar vertebral body. The measurements were made in a blind fashion and are presented as two-dimensional histomorphometric terms. Histomorphometric parameters were calculated and expressed according to the recommendations made by the American Society for Bone and Mineral Research (ASBMR) nomenclature committee.[31]

Structural histomorphometric measurements were made with an automatic image analysis system (VIDAS; C. Zeiss, Oberkocken, Germany) connected to a Zeiss stereo microscope (C. Zeiss) via a TV camera (Bosch, Stuttgart, Germany). All measurements with the automatic image analysis system were performed on sections stained with von Kossa at a magnification of ×2.0. A measuring field (~18 mm^2 in the tibias and ~12 mm^2 in the vertebrae) encompassing most of the cancellous bone in the L4 vertebrae and the tibias was used. The cancellous bone within 0.5 mm from the growth plates was excluded from the measurements in both the tibias and the vertebrae. All values for individual animals are the mean of two measurements made on two different sections that were generally ~100 μm spaced from each other. The image analysis system automatically determined the measuring area (tissue area [T.Ar]), B.Ar, and bone perimeter (B.Pm). From these data, the structural parameters B.Ar (B.Ar/T.Ar), B.Pm (B.Pm/T.Ar), trabecular width (Tb.Wi), trabecular number (Tb.N), and trabecular separation (Tb.Sp) were calculated.

Cellular, dynamic, and bone structural unit (BSU)-related histomorphometric measurements were made using a semiautomatic system (Videoplan; C. Zeiss) and a Zeiss Axioskop microscope with a drawing attachment. In the cancellous bone of the fourth lumbar vertebra and the proximal tibial metaphysis ~30 fields (7–8 mm^2) were evaluated in each section. At least 20 mm of cancellous B.Pm was sampled in the tibia and the vertebra of each animal. The area within 0.5 mm from the growth plates was excluded from the measurements in tibial and vertebral cancellous bone. Static histomorphometric parameters were measured in sections stained with toluidine blue, and dynamic fluorochrome-based parameters were measured in unstained sections.

The following primary parameters were determined at magnification ×200: B.Pm, osteoid perimeter, osteoblast

perimeter, eroded perimeter, osteoclast perimeter, number of osteoclasts, calcein double-labeled perimeter, and tetracycline-labeled perimeter. Osteoid seams with a width <2 μm were excluded from the measurements. Osteoblasts were defined as mononuclear, basophilic cells in contact with osteoid. Osteoclasts were defined as large, irregularly shaped cells with a foamy, slightly metachromatic cytoplasm containing one or more nuclei and located within Howship's lacunae. Osteoid width was determined directly at magnification $\times$400, sampling each osteoid seam every 50 μm. Osteoid seams with a width <2 μm were not included. For the measurement of eroded perimeter, only erosions with a depth of at least 3 μm were included into the measurements.

The interlabel width between calcein double labels was measured at magnification $\times$400, sampling each double label every 50 μm. The mineral apposition rate (MAR) was given by the arithmetic mean of the width measurements, divided by the interlabel period (10 days). Bone formation rate (BFR/B.Pm) was calculated by multiplying mineralizing perimeter (double-labeled B.Pm) by the MAR. The wall width of completed cancellous bone structural units was measured at magnification $\times$200. The distance between the reversal line and the bone surface was measured at four equidistant points in at least 15 remodeling sites in each animal. Values for MAR, osteoid width, and wall width were not corrected for obliquity of the plane of sectioning.

Statistical analysis

The biochemical and BMD data for the baseline control and the vehicle-treated SHAM (group 1) and OVX (group 2) groups were analyzed using one-way ANOVA. When the ANOVA performed over all groups indicated a significant ($p < 0.05$) difference among the groups, statistical differences between individual groups were subsequently evaluated using Tukey's multiple comparison test as a post hoc test. Because histomorphometry was not performed on the baseline group, statistical comparisons between the SHAM + vehicle group and the OVX + vehicle group were made using a two-sided *t*-test for unpaired samples.

The biochemical, histomorphometric, and BMD data for the OVX groups (groups 2–10) were analyzed by ANOVA followed by Tukey's multiple comparison test. Results for Tukey's test are reported for the comparison between individual treatment groups and the OVX vehicle control, as well as for the comparison between combination treatments and the respective individual treatment components, for example, 0.1 mg/kg of risedronate plus 0.05 μg/kg of calcitriol (group 7) versus 0.1 mg/kg of risedronate (group 3) and versus 0.05 μg/kg of calcitriol (group 5).

Because most of the biomechanical data were not distributed normally, these data were analyzed by the nonparametric Kruskal-Wallis test, including a multiple comparison procedure.

The values of $p < 0.05$ were considered significant for all statistical analyses. The data are presented as means $\pm$ SD.

RESULTS

Unless otherwise specified, effects of ovariectomy are from comparisons of OVX controls versus SHAM controls. Effects of risedronate or calcitriol are from comparisons of OVX treatment groups versus OVX controls.

Body weight and serum biochemistry

OVX rats showed increased body weight by the end of the experiment (Table 2). Treatment with risedronate or calcitriol had no influence on the ovariectomy-induced increase in body weight. Calcitriol given alone produced a decrease in BUN levels, a dose-dependent increase in serum calcium, and a suppression of serum PTH concentrations. Risedronate, especially at the high dose of 0.5 mg/kg, tended to suppress the increase in serum calcium and the subsequent inhibition of PTH secretion induced by calcitriol.

BMD measurements

Ovariectomy reduced BMD in the whole tibia, proximal tibia, whole L3 vertebra, and L3 vertebra core (data only partially shown; Fig. 1). The central tibia and the distal tibia did not show significant loss of BMD in response to ovariectomy in this study (data not shown). Changes in BMD were very similar in the whole tibia compared with the proximal tibia as well as in the whole L3 vertebra compared with the L3 vertebral core. Therefore, effects on BMD in proximal tibia and L3 vertebral core only are presented.

Both risedronate and calcitriol given alone provided dose-dependent protection against ovariectomy-induced loss of BMD at all sites. Generally, combined treatment with risedronate and calcitriol resulted in higher BMD values at all sites when compared with the individual treatment components. In the animals treated with low-dose risedronate (0.1 mg/kg) in conjunction with high-dose calcitriol (0.1 μg/kg), BMD at the whole tibia, proximal tibia, whole L3 vertebra, and L3 vertebral core was significantly greater than that of the group receiving low-dose risedronate alone. On the other hand, the increase in BMD with calcitriol was not significantly augmented by low-dose risedronate at any site. High-dose risedronate (0.5 mg/kg) in combination with low- (0.05 μg/kg) or high-dose calcitriol resulted in a significant increase in BMD in whole and proximal tibia as well as in L3 vertebra and L3 vertebral core compared with the group treated with high-dose risedronate alone. In contrast with the results obtained for combined treatment with low-dose risedronate, combination of high-dose risedronate with low- or high-dose calcitriol significantly enhanced the positive effects of calcitriol on BMD at the whole and proximal tibia but not in the vertebra.

High-dose risedronate and high-dose calcitriol as well as all combination treatments (with the exception of low-dose risedronate plus high-dose calcitriol) also increased BMD in distal tibia relative the OVX controls (data not shown). We did not observe any significant treatment effects in the central tibia (data not shown).

TABLE 2. BODY WEIGHT AND SERUM BIOCHEMISTRY IN OVX RATS TREATED WITH RISEDRONATE AND CALCITRIOL 12 WEEKS POSTOVARIECTOMY

			OVX								
				Risedronate (mg/kg per day)							
				0.1	*0.5*	*0*	*0*	*0.1*	*0.1*	*0.5*	*0.5*
				Calcitriol (μg/kg per day)							
Variable	*BL*	*SHAM*	*Vehicle*	*0*	*0*	*0.05*	*0.1*	*0.05*	*0.1*	*0.05*	*0.1*
Body weight (g)	275 (11)	324* (20)	387 (22)	386 (28)	417 (34)	405 (33)	388 (31)	390 (33)	388 (34)	373 (25)	391 (22)
Serum ALP (U/l)	55.3 (19.3)	28.2* (6.9)	50.7 (16.3)	45.3 (9.2)	48.6 (12.3)	42.7 (10.0)	45.1 (9.7)	42.7 (7.0)	38.3 (6.7)	40.0 (9.5)	51.7 (12.9)
BUN (mg/dl)	16.4 (2.5)	18.3 (4.1)	18.1 (3.1)	15.7 (2.3)	15.0 (2.2)	14.0* (1.7)	14.1* (2.0)	16.2 (2.8)	16.0 (2.8)	16.8 (3.3)	17.1 (2.1)
Serum Crea (mmol/l)	0.58 (0.04)	0.74* (0.05)	0.64 (0.05)	0.65 (0.05)	0.67 (0.05)	0.66 (0.07)	0.65 (0.05)	0.66 (0.07)	0.68 (0.06)	0.70 (0.08)	0.67 (0.07)
Serum Ca (mg/dl)	10.4 (0.4)	9.7 (0.4)	9.6 (0.2)	9.4 (0.2)	9.7 (0.3)	10.4* (0.4)	10.7* (0.2)	10.3*# (0.5)	10.0 (0.2)	9.7Þ (0.4)	9.8¶ (0.4)
Serum P (mg/dl)	8.5 (0.8)	5.3* (1.7)	7.4 (0.8)	6.6 (0.9)	5.8* (0.9)	8.0 (1.2)	7.8 (0.8)	7.2 (1.7)	7.2 (1.0)	6.6 (1.0)	7.4¥ (1.1)
Serum PTH (pg/ml)	— (—)	228 (180)	175 (102)	167 (81)	146 (63)	79* (69)	29* (24)	54*# (43)	38*# (25)	85 (43)	56 (63)

All values are means ± SD (in parentheses).

BL, baseline; Crea, creatinine; Ca, calcium; P, phosphorus.

* $p < 0.05$ versus OVX + vehicle; # $p < 0.05$ versus OVX + 0.1 mg/kg of risedronate; ¥ $p < 0.05$ versus OVX + 0.5 mg/kg of risedronate; Þ $p < 0.05$ versus OVX + 0.05 μg/kg of calcitriol; ¶ $p < 0.05$ versus OVX + 0.1 μg/kg of calcitriol by Tukey's test.

Biomechanics

Ovariectomy did not induce a significant reduction in biomechanical properties of the fifth lumbar vertebral body (Fig. 1), or any other site tested (Table 3). Generally, treatment with calcitriol alone or in combination with risedronate resulted in significantly higher values for maximum load compared with OVX controls in the L5 vertebra (Fig. 1), the femoral diaphysis, and the femoral neck. However, low-dose risedronate tended to diminish the increase in F_{max} in the L5 vertebra seen with calcitriol treatment. Risedronate alone had significant effects on load values only at the femoral diaphysis. Animals receiving high-dose calcitriol together with high-dose risedronate had significantly higher maximum load values compared with individual risedronate treatment in the L5 vertebra, the femoral diaphysis, and the femoral neck. None of the treatment regimens showed a significant effect at the distal femoral metaphysis.

Histomorphometry

Effects of ovariectomy: In comparison with SHAM controls, ovariectomy resulted in a 61% reduction in tibial cancellous B.Ar and a 22% reduction in vertebral cancellous B.Ar (Figs. 2 and 3; Tables 4 and 5). The tibial and vertebral cancellous bone osteopenia in OVX rats was accompanied by increased discontinuity of the trabecular bone structure, but Tb.Wi was unchanged. The ovariectomy-induced vertebral and tibial osteopenia was associated with elevated static and dynamic indices of bone formation. Osteoclast numbers were higher in OVX animals. However, this effect reached statistical significance only in the tibia. Wall width of completed remodeling units was higher in tibial and vertebral cancellous bone of OVX rats relative to SHAM controls (Fig. 4). Furthermore, ovariectomy induced a three- to fourfold increase in activation frequency in tibial and vertebral cancellous bone.

Effects of risedronate treatment: Risedronate significantly increased tibial cancellous B.Ar and prevented deterioration of trabecular bone architecture induced by ovariectomy (Fig. 2). Relative to OVX vehicle controls, however, risedronate reduced Tb.Wi in the tibia. Although risedronate had dose-dependent positive effects on vertebral cancellous bone architecture (Tb.N and Tb.Sp), it did not result in a statistically significant increase in vertebral cancellous B.Ar (Fig. 2). Trabecular thinning due to risedronate treatment was not observed in vertebral bone (Fig. 3).

Risedronate dose-dependently decreased activation frequency as well as static and dynamic histomorphometric indices of vertebral and tibial bone formation (Fig. 4). Moreover, risedronate treatment dose-dependently reduced wall width (Fig. 5) and osteoclast numbers in tibial and vertebral cancellous bone (Fig. 4). High-dose risedronate, alone or in combination with calcitriol, produced changes in osteoclast morphology typical of those seen with bisphos-

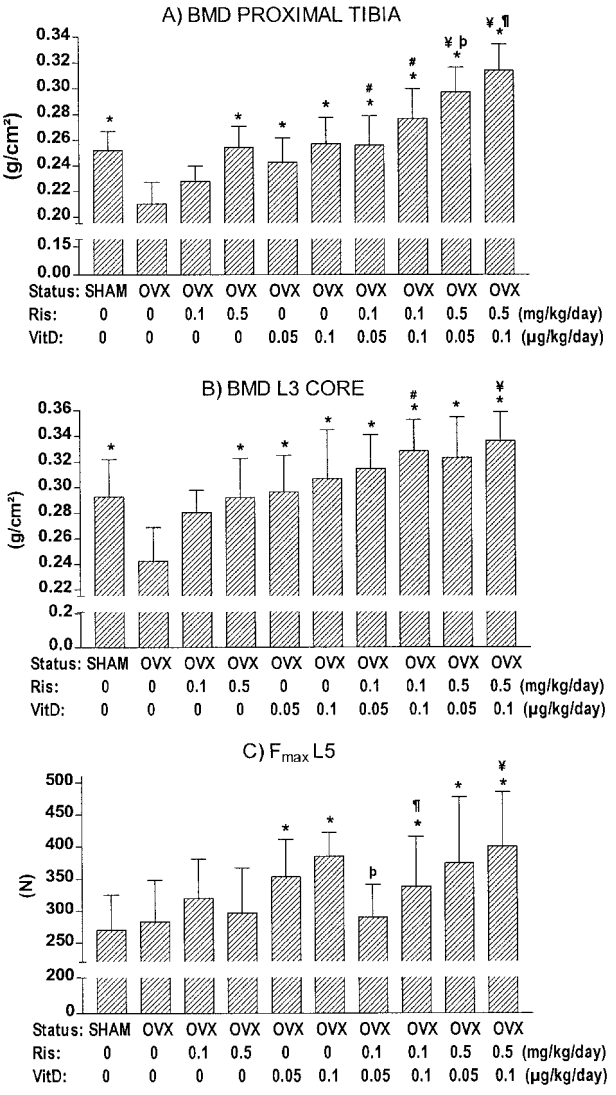

FIG. 1. BMD measured by DXA (A) in the proximal tibia and (B) in the third lumbar vertebral core, and (C) maximum load (F_{max}) values of the fifth lumbar vertebral body measured by a compression test in SHAM and OVX rats orally treated with different doses of risedronate (Ris) or calcitriol (VitD), alone or in combination, 12 weeks post-OVX. Each data point represents the mean ± SD of 10 animals. $^{*}p < 0.05$ versus OVX + vehicle; $^{\#}p < 0.05$ versus OVX + 0.1 of mg/kg risedronate; $^{¥}p < 0.05$ versus OVX + 0.5 mg/kg of risedronate; $^{þ}p < 0.05$ versus OVX + 0.05 µg/kg of calcitriol; $^{¶}p < 0.05$ versus OVX + 0.1 µg/kg of calcitriol by Tukey's test (BMD data) or Kruskal-Wallis test (biomechanical data).

phonates (increased cell size, increased number of nuclei, and altered morphology of nuclei).

Osteoid maturation time, that is, the mean time interval between the onset of matrix deposition and the beginning of mineralization at individual bone-forming sites, showed a slight, dose-dependent increase in both tibial and vertebral bone of risedronate-treated rats. However, risedronate had no effect on osteoid width.

Effects of calcitriol treatment: Calcitriol dose-dependently increased tibial and vertebral cancellous B.Ar (Figs. 2 and 3). The effects of calcitriol on B.Ar were more pronounced in the vertebrae, where high-dose calcitriol increased cancellous B.Ar 21% above the level observed in SHAM controls. The increase in vertebral cancellous B.Ar in calcitriol-treated OVX animals was accompanied by a dose-dependent increase in Tb.Wi and wall width (Figs. 4 and 5). A similar but nonsignificant trend for increased Tb.Wi and wall width was observed in the tibia (Table 4).

At the higher dose, calcitriol significantly reduced osteoclast number in tibial cancellous bone. A similar but nonsignificant trend was observed in the vertebra (Fig. 4). The effects of calcitriol on bone formation and activation frequency were different in the tibia and vertebra. In the vertebra, calcitriol had no significant effect on MAR, mineralizing perimeter, BFR, or activation frequency (Fig. 4). On the other hand, in the tibia, calcitriol treatment induced dose-dependent decreases in MAR, mineralizing perimeter, BFR, and activation frequency. High-dose calcitriol increased osteoid width but had no effect on osteoid maturation time.

Effects of combination treatment: The combination of risedronate and calcitriol therapy had additive effects on cancellous B.Ar in the tibia. With the exception of animals treated with the two low doses (0.1 mg/kg of risedronate and 0.05 µg/kg of calcitriol), combined administration of risedronate and calcitriol to OVX animals resulted in a significantly higher cancellous B.Ar compared with the groups receiving the respective individual risedronate or calcitriol doses alone (Fig. 2). Compared with SHAM controls, tibial cancellous B.Ar was increased by 28% with low-dose calcitriol and high-dose risedronate and by 46% with high-dose calcitriol and high-dose risedronate. Calcitriol coadministration counteracted the decrease in Tb.Wi and also, partially, the decrease in wall width observed with risedronate alone. Furthermore, the combination of calcitriol and risedronate tended to suppress osteoclast numbers more than each of the drugs administered alone. The prevailing influence on histomorphometric parameters of bone formation and on activation frequency in animals cotreated with risedronate and calcitriol was the dose of risedronate administered (Fig. 4). There were no additive effects of the two drugs on these parameters in tibial bone.

The effects of combination therapy in the vertebra differed from those in the tibia. In contrast to the tibia, administration of risedronate together with calcitriol did not augment further cancellous B.Ar in comparison to treatment with calcitriol alone (Fig. 2). Nevertheless, lumbar vertebral cancellous B.Ar was significantly higher in OVX rats receiving low- or high-dose risedronate in combination with high-dose calcitriol relative to treatment with risedronate alone. Moreover, risedronate did not impair the positive effects of calcitriol on Tb.Wi and on wall width of completed remodeling units in the vertebra. Similar to tibial bone, coadministration of risedronate and calcitriol significantly enhanced the suppressive effect on osteoclast number and osteoclast perimeter induced by each compound, especially at the highest doses. This finding of an augmented antiresorptive effect in OVX animals cotreated with risedronate and calcitriol was corroborated by increased preservation of the tetracycline label administered on day 1 of the

TABLE 3. MAXIMUM LOAD VALUES (F_{MAX}) FOR DIFFERENT SKELETAL SITES IN OVX RATS TREATED WITH RISEDRONATE AND CALCITRIOL 12 WEEKS POSTOVARIECTOMY

			OVX							
			Risedronate (mg/kg per day)							
			0.1	*0.5*	*0*	*0*	*0.1*	*0.1*	*0.5*	*0.5*
			Calcitriol (μg/kg per day)							
Site	*SHAM*	*Vehicle*	*0*	*0*	*0.05*	*0.1*	*0.05*	*0.1*	*0.05*	*0.1*
L5 vertebra	270	283	320	297	353*	385*	290^{Þ}	338*¶	374*	400*$^{¥}$
(N)	(55)	(65)	(61)	(70)	(58)	(37)	(51)	(78)	(103)	(85)
Fem diaphysis	151	160	175*	154	171	175*	167	160¶	164	179*$^{¥}$
(N)	(14)	(11)	(18)	(14)	(16)	(18)	(10)	(15)	(25)	(17)
Dist fem metaphysis	524	546	443	466	544	607	502	557	591	536
(N)	(115)	(129)	(109)	(138)	(158)	(159)	(87)	(163)	(217)	(184)
Fem neck	80	73	75	76	83*	87*	90*$^{#}$	85*	92*$^{¥}$	91*$^{¥}$
(N)	(11)	(8)	(11)	(15)	(15)	(12)	(15)	(10)	(9)	(13)

All values are means ± SD (in parentheses).

Fem, femoral; dist, distal.

*$p < 0.05$ versus OVX + vehicle; $^{#}p < 0.05$ versus OVX + 0.1 mg/kg of risedronate; $^{¥}p < 0.05$ versus OVX + 0.5 mg/kg of risedronate; $^{Þ}p < 0.05$ versus OVX + 0.05 μg/kg of calcitriol; $^{¶}p < 0.05$ versus OVX + 0.1 μg/kg of calcitriol by Dunn's test.

study in vertebral cancellous bone. Furthermore, when risedronate was given together with calcitriol, vertebral BFR (Fig. 4), mineralizing perimeter, and osteoblast perimeter were significantly lower relative to treatment with calcitriol alone. However, in comparison with risedronate given alone, high-dose calcitriol partially antagonized the suppressive effect of risedronate on MAR, mineralizing perimeter, BFR, and activation frequency (Fig. 4).

Combined therapy with risedronate and calcitriol had different effects on bone mineralization in the vertebra compared with the tibia. In the vertebra, high-dose calcitriol reduced the elevated osteoid maturation time in animals receiving high-dose risedronate to approximately OVX vehicle control levels. However, in the tibia high-dose risedronate together with low- or high-dose calcitriol increased osteoid maturation time to a significantly higher level compared with the groups receiving each of the drugs alone.

DISCUSSION

In agreement with a previous long-term study,[2] this experiment has shown that prophylactic administration of risedronate to OVX rats over a 12-week period can prevent estrogen depletion–induced bone loss in the tibia and in the lumbar vertebra by depressing the elevated bone turnover. In both tibial and vertebral bone, risedronate treatment dose-dependently suppressed osteoblast perimeter, mineralizing perimeter, MAR, and wall width, clearly indicating that risedronate decreased osteoblast recruitment and also osteoblast team performance in OVX rats.[32] Recently, it has been suggested that bisphosphonates inhibit glucocorticoid-induced osteoblast and osteocyte apoptosis in vivo in mice.[33] Increased cell survival under the influence of bisphosphonates may enhance the performance of

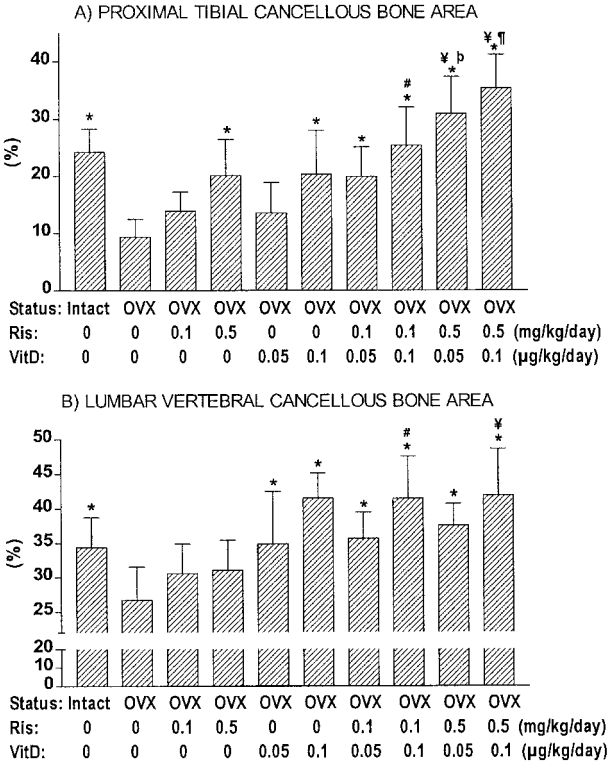

FIG. 2. Cancellous B.Ar in the (A) proximal tibial metaphysis and the (B) fourth lumbar vertebral body in SHAM and OVX rats orally treated with different doses of risedronate (Ris) or calcitriol (VitD), alone or in combination, 12 weeks post-OVX. Each data point represents the mean ± SD of 10 animals. *$p < 0.05$ versus OVX + vehicle; $^{#}p < 0.05$ versus OVX + 0.1 mg/kg of risedronate; $^{¥}p < 0.05$ versus OVX + 0.5 mg/kg of risedronate; $^{Þ}p < 0.05$ versus OVX + 0.05 μg/kg of calcitriol; $^{¶}p < 0.05$ versus OVX + 0.1 μg/kg of calcitriol by Tukey's test.

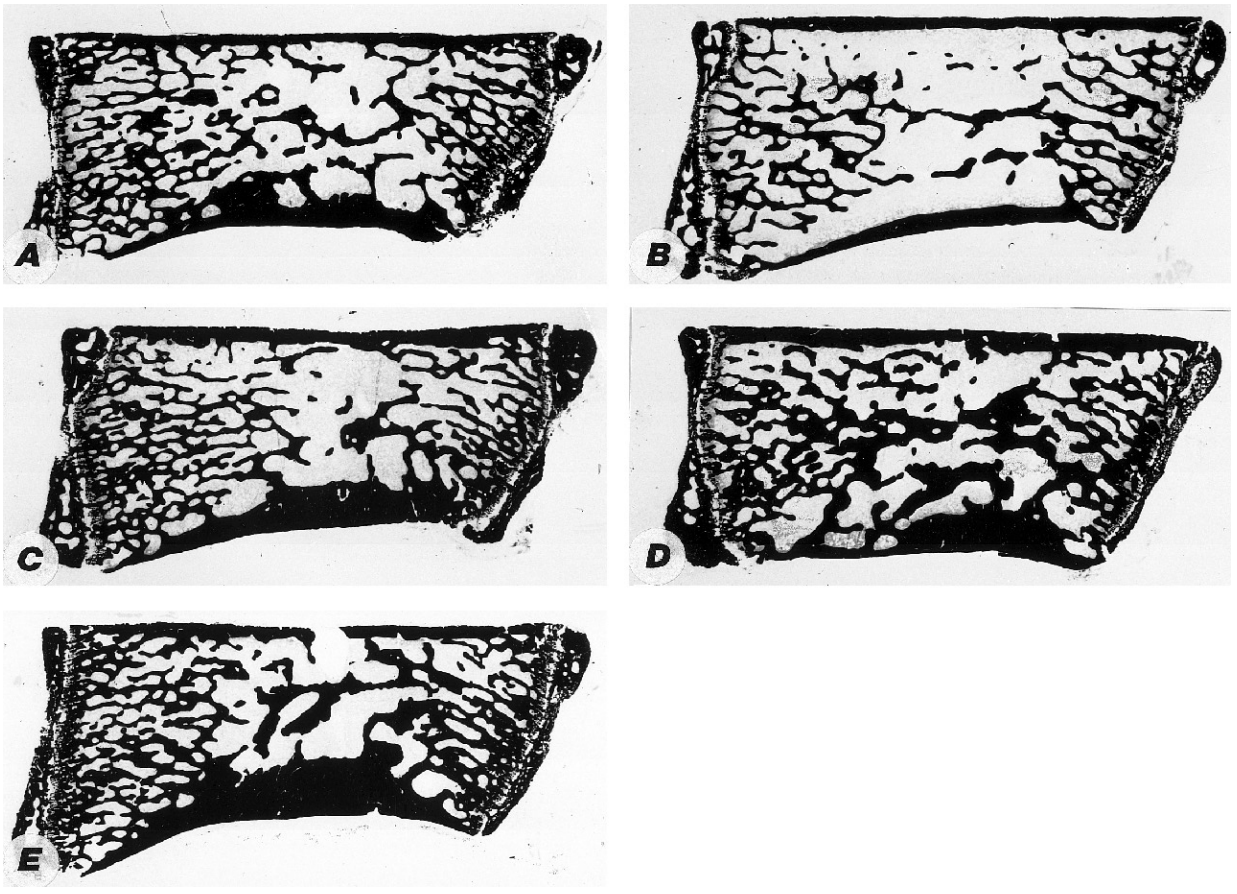

FIG. 3. Fourth lumbar vertebral bodies of (A) a SHAM rat, (B) a vehicle-treated OVX rat, and (C) OVX rats treated with high-dose (0.5 mg/kg per day) risedronate, (D) high-dose (0.1 μg/kg per day) calcitriol, or (E) combined treatment with the high doses of both drugs, 12 weeks post-OVX. (C) Risedronate alone largely protected against estrogen deficiency–induced bone loss and deterioration of cancellous bone structure. (D) High-dose calcitriol had pronounced bone anabolic effects and increased cancellous B.Ar in OVX rats to levels beyond that observed in SHAM animals. (E) Coadministration of risedronate did not impair the bone anabolic effect of calcitriol. Note the increase in Tb.Wi in the OVX animals receiving high-dose calcitriol alone or in combination with risedronate (5-μm-thick undecalcified sections; Von Kossa stain; original magnification $\times$15).

individual osteoblast teams, resulting in increased wall thickness and a positive remodeling balance. In line with this idea, it has been reported that wall thickness and remodeling balance may increase under treatment with risedronate in dogs.[34] However, a 3-year study in dogs treated with high doses of alendronate did not reveal any changes in cancellous bone volume or architecture.[35] In addition, cancellous bone volume and wall thickness were unchanged after 1 year of alendronate treatment in patients with glucocorticoid-induced osteoporosis.[7] In postmenopausal women with osteoporosis, an increase in wall thickness was observed after 2 years but not after 3 years of alendronate treatment.[6] Cancellous bone volume remained unchanged in the latter study. Even in the presence of a down-regulated activation frequency, one would expect a rise in Tb.Wi and cancellous bone volume in long-term studies in the case that bisphosphonates increase remodeling balance. Therefore, neither this study in rats nor the available human data provide evidence to support the hypothesis that bisphosphonates have a positive effect on wall thickness and osteoblast team performance.

The daily dose of 0.05 μg/kg of calcitriol provided complete protection against postovariectomy vertebral cancellous bone loss in this study, and 0.1 μg/kg of calcitriol had bone anabolic effects on vertebral bone in OVX animals, increasing cancellous B.Ar and bone strength beyond the level observed in SHAM controls. Thus, at the dosages used in this experiment, calcitriol had more pronounced positive effects on vertebral cancellous bone in OVX rats compared with risedronate. However, both drugs had comparable bone-protective activity in the tibia. Previous experiments[22,25,36] have suggested also that the skeletal effects of vitamin D metabolites are more pronounced in the axial skeleton compared with the appendicular skeleton. The reason for this discrepancy is not known but may be related to a higher extent of remodeling activity in the axial skeleton in the rat.[37]

The major mechanism of the bone-sparing effect of calcitriol in tibial cancellous bone of OVX rats in this study appeared to be a reduction in bone turnover and activation frequency. However, in vertebral cancellous bone, calcitriol did not significantly suppress histomorphometric parame-

TABLE 4. HISTOMORPHOMETRIC DATA IN THE CANCELLOUS BONE OF THE PROXIMAL TIBIAL METAPHYSIS IN SHAM AND OVX RATS TREATED WITH RISEDRONATE AND CALCITRIOL 12 WEEKS POSTOVARIECTOMY

			OVX							
			Risedronate (mg/kg per day)							
			0.1	*0.5*	*0*	*0*	*0.1*	*0.1*	*0.5*	*0.5*
			Calcitriol (μg/kg per day)							
Variable	*SHAM*	*Vehicle*	*0*	*0*	*0.05*	*0.1*	*0.05*	*0.1*	*0.05*	*0.1*
T.Ar (mm^2)	17.8 (1.2)	18.6 (1.5)	19.1 (1.1)	18.3 (0.9)	18.2 (1.9)	19.2 (1.2)	19.9 (1.1)	18.6 (1.4)	19.5 (0.83)	18.8 (1.5)
Tb.Wi (μm)	70 (6)	67 (6)	57 (5)	54* (6)	72 (8)	74 (9)	58Þ (6)	68 (11)	65 (8)	69¥ (10)
Tb.N (no./mm)	4.38* (0.51)	1.77 (0.61)	3.09* (0.65)	4.68* (1.01)	2.34 (0.74)	3.42* (1.03)	4.35*#Þ (0.86)	4.72*#¶ (0.65)	6.05*¥Þ (0.70)	6.51*¥¶ (0.80)
Tb.Sp (μm)	176* (31)	567 (190)	294* (81)	182* (60)	401* (117)	264* (118)	195*Þ (61)	163* (36)	117*Þ (24)	102*¶ (21)
O.Wi (μm)	3.23 (0.34)	3.14 (0.31)	3.27 (0.45)	2.90 (0.30)	3.02 (0.24)	3.26 (0.33)	2.82 (0.44)	3.33 (0.65)	2.84 (0.38)	3.64¥ (0.86)
Omt (days)	5.24* (2.05)	3.60 (1.32)	5.87 (1.59)	7.86* (2.82)	4.09 (0.99)	5.10 (1.17)	6.52 (1.97)	6.91 (2.03)	13.24*¥Þ (4.66)	12.90*¥¶ (4.98)
Ob.Pm/B.Pm (%)	3.41* (2.25)	12.79 (7.21)	4.35* (3.55)	1.42* (0.98)	7.87* (3.32)	6.93* (2.02)	1.82*Þ (1.48)	2.77* (1.31)	0.34*Þ (0.32)	1.09*¶ (1.53)
E.Pm/B.Pm (%)	21.0 (5.1)	21.2 (5.2)	17.7 (2.7)	20.8 (3.7)	22.1 (4.1)	19.9 (5.4)	20.3 (3.1)	17.0 (3.3)	20.4 (4.2)	20.0 (2.8)
N.Oc/Md.Pm (no./mm)	1.84* (0.44)	2.61 (0.69)	1.99 (0.47)	1.67* (0.31)	2.71 (0.76)	1.81 (0.73)	1.63*Þ (0.41)	1.32* (0.50)	1.35*Þ (0.42)	0.97*¶ (0.40)
M.Pm/B.Pm (%)	5.94* (4.25)	21.00 (11.04)	4.91* (3.04)	1.51* (0.72)	13.75* (5.93)	8.71* (3.98)	2.61*Þ (1.78)	3.23* (1.53)	0.63*Þ (0.40)	1.52*¶ (2.19)
MAR (μm/day)	0.68* (0.21)	0.96 (0.33)	0.58* (0.13)	0.40* (0.11)	0.77 (0.14)	0.66* (0.13)	0.45*Þ (0.09)	0.51* (0.16)	0.23*Þ (0.07)	0.33*¶ (0.16)
BFR/B.Pm ($\mu m^2/\mu m$ per day)	0.047* (0.040)	0.231 (0.169)	0.032* (0.030)	0.006* (0.005)	0.109* (0.061)	0.058* (0.030)	0.013*Þ (0.010)	0.016* (0.007)	0.002*Þ (0.002)	0.008* (0.014)
W.Wi (μm)	21.1* (1.5)	25.1 (2.6)	22.1 (1.6)	18.9* (1.2)	25.6 (2.6)	27.5 (2.0)	20.4*Þ (2.2)	23.6¶ (2.0)	21.7*Þ (2.1)	24.0¥¶ (2.2)
FP (days)	35 (18)	28 (8)	39 (7)	51 (15)	35 (8)	43 (10)	46 (9)	49 (13)	100*¥Þ (31)	86*¥¶ (35)
Rm.P (days)	830 (1767)	105 (91)	326 (332)	1141 (1055)	141 (52)	162 (74)	695 (501)	422 (310)	14950*¥Þ (14710)	4364 (5963)
Ac.F (no./year)	0.51* (0.40)	2.16 (1.65)	0.54* (0.60)	0.13* (0.10)	0.93* (0.46)	0.70* (0.22)	0.17* (0.13)	0.30* (0.21)	0.02*Þ (0.02)	0.10* (0.17)

All values are means ± SD (in parentheses).

O.Wi, osteoid width; Omt, osteoid maturation time; E.Pm, eroded perimeter; N.Oc, no. of osteoclasts; Md.Pm, mineralized perimeter; M.Pm, mineralizing perimeter; W.Wi, wall width; FP, formation period; Rm.P, remodeling period; Ac.F, activation frequency.

* $p < 0.05$ versus OVX + vehicle; # $p < 0.05$ versus OVX + 0.1 mg/kg of risedronate; ¥ $p < 0.05$ versus OVX + 0.5 mg/kg of risedronate; Þ $p < 0.05$ versus OVX + 0.05 μg/kg of calcitriol; ¶ $p < 0.05$ versus OVX + 0.1 μg/kg of calcitriol by Tukey's test.

ters of bone formation and bone resorption. Earlier studies have suggested that the bone-preserving activity of low-dose calcitriol in OVX rats is caused by primarily an antiresorptive effect and a reduction in bone turnover,[21] whereas calcitriol at higher doses can increase the recruitment of osteoblast precursor cells from bone marrow and the performance of individual osteoblast teams.[26] Therefore, the anabolic effect of calcitriol in vertebral bone of OVX rats may be based on a positive remodeling balance because of augmented osteoblast recruitment and osteoblast team performance. This hypothesis is supported by the increase in wall width of completed remodeling units and

TABLE 5. HISTOMORPHOMETRIC DATA IN THE CANCELLOUS BONE OF THE FOURTH LUMBAR VERTEBRA IN SHAM AND OVX RATS TREATED WITH RISEDRONATE AND CALCITRIOL 12 WEEKS POSTOVARIECTOMY

		OVX							
		Risedronate (mg/kg per day)							
		0.1	*0.5*	*0*	*0*	*0.1*	*0.1*	*0.5*	*0.5*
		Calcitriol (μg/kg per day)							
Variable	*SHAM*	*Vehicle*	*0*	*0*	*0.05*	*0.1*	*0.05*	*0.1*	*0.05*	*0.1*
T.Ar	12.0	12.6	12.0	11.8	12.0	12.2	12.9	11.9	11.4	11.2
(mm^2)	(1.2)	(1.4)	(0.9)	(1.3)	(1.6)	(1.6)	(1.4)	(1.3)	(1.12)	(0.5)
Tb.Wi	87	87	86	80*	95	108*	90	103*#	91	101*¥
(μm)	(6)	(11)	(6)	(7)	(9)	(9)	(7)	(12)	(5)	(12)
Tb.N	4.99*	3.91	4.50*	4.90*	4.64*	4.89*	5.04*	5.11*#	5.26*Þ	5.28*
(no./mm)	(0.41)	(0.47)	(0.44)	(0.39)	(0.70)	(1.03)	(0.32)	(0.30)	(0.34)	(0.32)
Tb.Sp	133*	191	157*	142*	145*	120*	128*	116*#	119*	111*
(μm)	(19)	(32)	(23)	(20)	(39)	(14)	(15)	(17)	(13)	(19)
O.Wi	3.44	3.43	3.48	3.16	3.35	4.07*	3.54	3.69	3.55	4.12¥
(μm)	(0.29)	(0.23)	(0.17)	(0.30)	(0.27)	(0.61)	(0.18)	(0.71)	(0.17)	(0.59)
Omt	4.09	3.99	5.02	5.56*	4.02	4.45	5.16	4.67	6.31*Þ	4.78
(days)	(0.56)	(0.60)	(0.88)	(0.94)	(0.79)	(0.87)	(0.72)	(0.69)	(1.08)	(1.62)
Ob.Pm/B.Pm	2.45*	7.94	3.62*	1.69*	4.93	8.90	2.77*	4.02*¶	1.18*Þ	4.51*¶
(%)	(0.77)	(3.11)	(1.19)	(0.74)	(1.68)	(3.20)	(1.41)	(2.89)	(0.64)	(3.52)
E.Pm/B.Pm	22.2	19.8	21.0	19.5	22.4	17.6	18.8	12.7*#	17.1	9.5*¥¶
(%)	(4.9)	(3.2)	(3.0)	(3.8)	(4.7)	(6.1)	(4.0)	(5.1)	(4.9)	(4.3)
M.Pm/B.Pm	4.73*	12.63	4.52*	2.04*	11.78	14.10	4.07*Þ	5.90*¶	2.15*Þ	5.42*¶
(%)	(2.32)	(5.04)	(1.62)	(1.11)	(3.69)	(3.79)	(1.27)	(2.68)	(0.89)	(3.22)
MAR	0.85	0.88	0.71	0.58*	0.85	0.93	0.70	0.81	0.58*Þ	0.90¥
(μm/day)	(0.12)	(0.15)	(0.11)	(0.09)	(0.14)	(0.12)	(0.09)	(0.23)	(0.09)	(0.16)
Tc.Lb/B.Pm	3.57	3.14	4.01	3.60	5.64	6.35	6.20	7.71*	7.64*	9.35*¥
(%)	(1.69)	(1.44)	(3.37)	(1.92)	(1.76)	(2.16)	(1.72)	(2.45)	(4.49)	(4.72)
FP	26.0	28.0	32.6	37.3*	29.3	30.0	34.3	33.7	41.3*Þ	29.8
(days)	(5.2)	(6.2)	(5.7)	(6.0)	(6.7)	(4.0)	(4.7)	(7.2)	(5.15)	(7.3)
Rm.P	270	135	217	449*	158	100	306	268	675*Þ	120¥
(days)	(126)	(163)	(72)	(207)	(49)	(55)	(177)	(326)	(307)	(115)

All values are means ± SD (in parentheses).

O.Wi, osteoid width; Omt, osteoid maturation time; E.Pm, eroded perimeter; M.Pm, mineralizing perimeter; Md.Pm, mineralized perimeter; Tc.Lb, tetracycline-labeled perimeter; FP, formation period; Rm.P, remodeling period.

* $p < 0.05$ versus OVX + vehicle; # $p < 0.05$ versus OVX + 0.1 mg/kg of risedronate; ¥ $p < 0.05$ versus OVX + 0.5 mg/kg of risedronate; Þ $p < 0.05$ versus OVX + 0.05 μg/kg of calcitriol; ¶ $p < 0.05$ versus OVX + 0.1 μg/kg of calcitriol by Tukey's test.

the concomitant increase in Tb.Wi observed in calcitriol-treated OVX rats. However, it is important to note that the bone anabolic potential of vitamin D analogs is seen only at high doses. Because of the calcemic side effects, such a treatment probably is not feasible in humans with vitamin D compounds of equally calcemic potential as calcitriol.

In vitro, calcitriol is a potent stimulator of bone resorption, increasing the proliferation, differentiation, and fusion of preosteoclasts and augmenting the activity of mature osteoclasts.[38,39] Similarly, acute administration of pharmacologic amounts of calcitriol in vivo results in a transient increase in osteoclast activity and recruitment.[40,41] This acute calcitriol-induced stimulation of bone resorption can be blocked by bisphosphonates.[42] However, chronic calcitriol administration inhibits bone resorption as measured by osteoclast numbers and/or biochemical markers in rats[21,22,27,43] and humans.[18,44,45] In an experiment in which rats were treated with daily injections of high-dose calcitriol, osteoclast numbers were found to be increased in tibial cancellous bone on day 1 but were diminished by day 6 of the study.[41] Under chronic treatment with active vitamin D analogs, the initial rise in bone resorption is thought to be overridden by the suppression of PTH secretion through increased intestinal calcium absorption.[21,27] PTH is the most important hormonal regulator of bone turnover under physiological circumstances. However, calcitriol also may have direct antiresorptive effects independent of PTH suppression. Experiments using parathyroidectomized rats infused with human PTH-related peptide 1-34 as a model of hypercalcemia of malignancy have shown that calcitriol lowers hypercalcemia and bone resorption in a dose-dependent manner in this model, strongly implying an additional direct antiresorptive action of calcitriol on bone cells.[46]

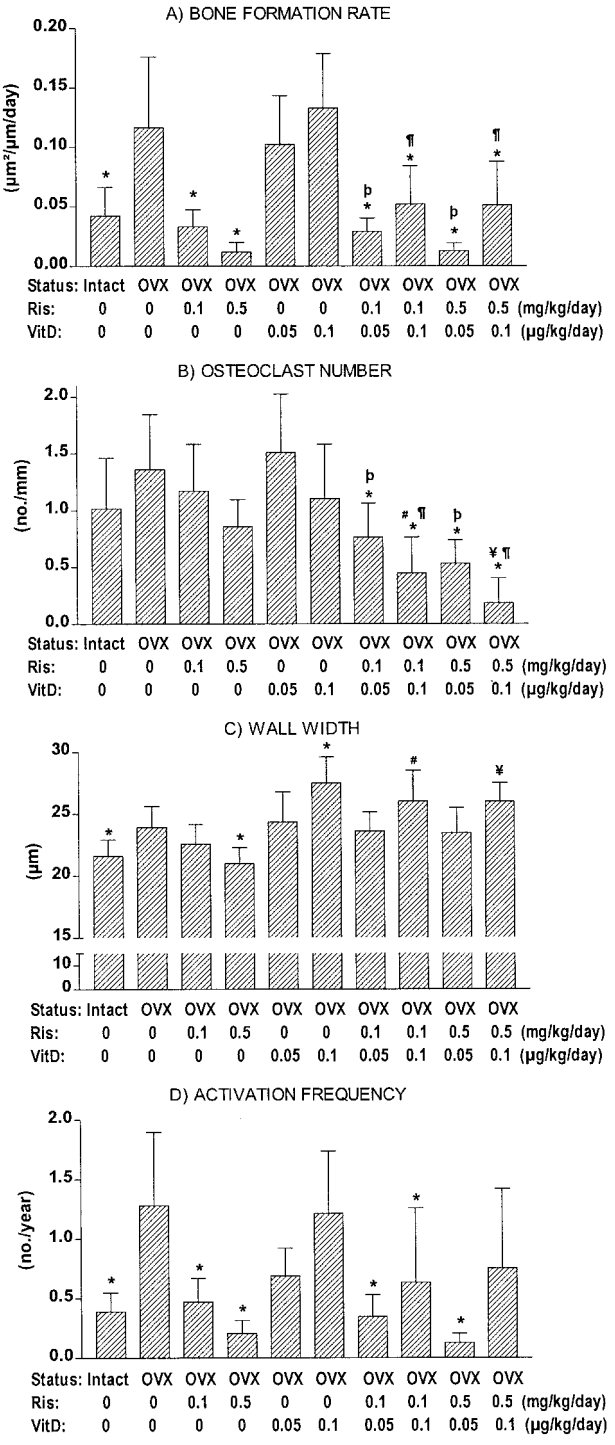

FIG. 4. (A) BFR, (B) osteoclast number, (C) wall width, and (D) activation frequency in vertebral cancellous bone in SHAM and OVX rats orally treated with different doses of risedronate (Ris) or calcitriol (VitD), alone or in combination, 12 weeks post-OVX. Each data point represents the mean ± SD of 10 animals. $^{*}p < 0.05$ versus OVX + vehicle; $^{\#}p < 0.05$ versus OVX + 0.1 mg/kg of risedronate; $^{¥}p < 0.05$ versus OVX + 0.5 mg/kg of risedronate; $^{þ}p < 0.05$ versus OVX + 0.05 μg/kg of calcitriol; $^{¶}p < 0.05$ versus OVX + 0.1 μg/kg of calcitriol by Tukey's test.

Therefore, the available data suggest that the hypercalcemia and hypercalciuria under chronic treatment with vitamin D analogs is caused mainly by augmented intestinal calcium absorption and that dietary calcium is a major modulator of their toxicity. In agreement with this idea, hypercalcemia in a patient with ovarian carcinoma and abnormally high blood levels of calcitriol could not be controlled by treatment with different bisphosphonates.[47] Interestingly, however, high-dose risedronate normalized hypercalcemia and decreased suppression of PTH serum levels in calcitriol-treated OVX rats in this study, suggesting that calcium mobilization from the skeleton may have accounted partially for the hypercalcemia induced by calcitriol. On the other hand, cancellous and cortical bone mass and strength in the axial and appendicular skeleton increased in OVX rats receiving calcitriol alone or in combination with risedronate, indicating that the bone balance is positive under calcitriol treatment. Similar to our results, combined therapy with calcitriol and estrogen corrected the rise in serum ionized calcium seen under treatment with calcitriol alone in a recent 3-year trial in postmenopausal women.[18] Thus, although calcitriol acts as a mild antiresorptive drug, calcium release via osteoclastic bone resorption appears to contribute to the development of hypercalcemia with this compound. In light of the importance of hypercalcemia for the clinical monitoring of patients with osteoporosis treated with vitamin D analogs, it clearly warrants further study whether this side effect could be controlled partially by coadministration of antiresorptive agents such as bisphosphonates or estrogen.

Previous investigations have shown that chronic administration of calcitriol to rats at dosages higher than ~0.1–0.2 μg/kg can result in impaired bone mineralization.[43,48] However, at the dosages used in this study, calcitriol did not adversely affect bone mineralization. Risedronate produced a slight increase in osteoid maturation time, but this was caused by a decrease in MAR and not by an increase in osteoid width. In the absence of an accumulation of osteoid and an increase in osteoid width, these findings are not indicative of defective mineralization. In rats receiving combination therapy, high-dose calcitriol antagonized the increase in osteoid maturation time seen in rats treated with high-dose risedronate in vertebral bone. However, in the tibia calcitriol at both dosages used resulted in a significant increase in osteoid maturation time in animals receiving high-dose risedronate. The reason for the discrepancy between vertebral and tibial cancellous bone is not known.

An interesting finding in this study was that combined treatment of OVX rats with risedronate and calcitriol resulted in a significant enhancement of the suppressive effects on histomorphometric parameters of bone resorption compared with administration of each of the compounds alone. The antiresorptive effects of both drugs are mediated through different pathways. Bisphosphonates inhibit the bone resorptive capacity of mature osteoclasts,[1] and chronically administered calcitriol inhibits osteoclast recruitment mainly through suppression of PTH secretion.[27] Therefore, this investigation suggests that combined treatment with risedronate and calcitriol may result in additive or even overadditive suppressive effects on bone resorption.

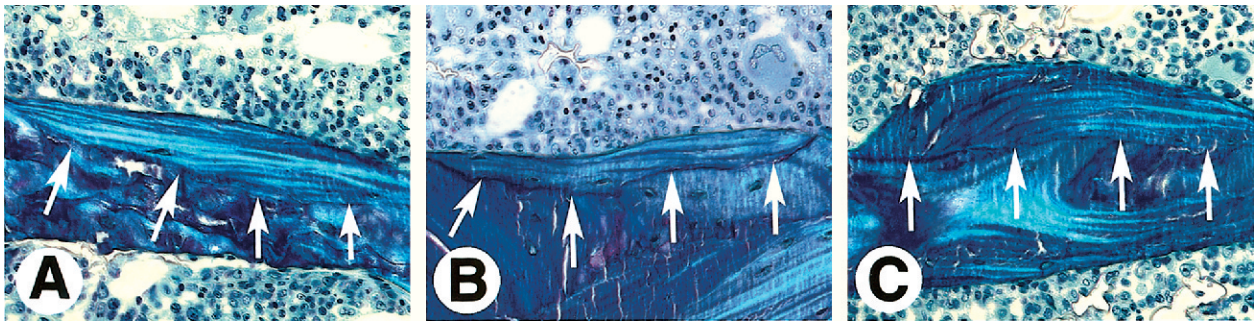

FIG. 5. Representative completed remodeling units in vertebral cancellous bone of (A) a vehicle-treated OVX rat and (B) OVX rats that have been treated for 12 weeks with high-dose (0.5 mg/kg per day) risedronate or (C) with high-dose (0.1 μg/kg per day) calcitriol. The reversal lines are marked with arrows. It is evident that risedronate decreases the wall width of completed remodeling units compared with the OVX vehicle control. In contrast, administration of calcitriol results in a pronounced increase in wall width beyond the level seen in vehicle-treated OVX rats. The convex surface of the remodeling unit shown in panel C is suggestive of a positive remodeling balance caused by overfilling of the resorption cavity. Toluidine blue surface stain for demonstration of cement lines viewed under polarized light (original magnification $\times$400).

Combined administration of risedronate and calcitriol caused additive effects on BMD and cancellous B.Ar in the tibia. However, in the vertebra combination therapy offered no advantage in terms of cancellous B.Ar, BMD, or bone strength compared with calcitriol administration alone. The different effects of combination therapy in the axial and appendicular skeleton may be related to the higher rates of longitudinal bone growth in the proximal tibia as compared with the vertebra.[37] It is well known that antiresorptive drugs increase cancellous bone mass in growing bone sites.[49] Therefore, enhanced suppression of osteoclastic bone resorption by coadministration of risedronate and calcitriol is likely to increase cancellous bone mass in an additive fashion in a fast-growing bone site such as the proximal tibia. In a slowly growing bone site such as the fourth lumbar vertebra, an increase in cancellous B.Ar in OVX rats beyond the level observed in SHAM controls cannot solely be explained by an antiresorptive effect in this 12-week study. Rather, such an effect necessarily involves a remodeling imbalance in favor of bone formation. The data from this experiment suggest that the anabolic actions of calcitriol in vertebral cancellous bone cannot be enhanced by coadministration of risedronate. Previous studies have shown that coadministration of tiludronate in sheep[8] or pretreatment with alendronate in rats[9] blocks or diminishes the anabolic action of PTH. Therefore, it is important to note that coadministration of risedronate did not blunt the bone anabolic effect of calcitriol on vertebral cancellous bone in OVX rats in this experiment. Similar observations have been made in other rat studies in which risedronate was coadministered with PTH[10-13] or prostaglandins.[50]

In conclusion, individual prophylactic administration of risedronate and calcitriol dose-dependently inhibited tibial and vertebral bone loss because of estrogen deficiency in OVX rats. Combination treatment with risedronate and calcitriol increased tibial and vertebral BMD, tibial and vertebral cancellous B.Ar, and vertebral bone strength compared with risedronate alone. Calcitriol enhanced the suppressive effects of risedronate on osteoclast number and partially counteracted the suppressive effects of risedronate on bone formation and histomorphometric indices of osteoblast team performance. Risedronate did not reduce the anabolic effect of calcitriol, and at the high dose it normalized hypercalcemia in calcitriol-treated OVX rats. Therefore, this study in OVX rats suggests that combined therapy with risedronate and calcitriol may offer advantages over the treatment with bisphosphonates or calcitriol alone.

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Address reprint requests to:
Reinhold G. Erben, M.D., D.V.M.
Institute of Animal Physiology
University of Munich
Veterinaerstrasse 13
D-80539 Munich, Germany

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