Effects of Treatment with Ibandronate on Bone Mass, Architecture, Biomechanical Properties, and Bone Concentration of Ibandronate in Ovariectomized Aged Rats

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ABSTRACT. ***Objective.*** To investigate the effects of treatment with ibandronate, a highly potent nitrogen-containing bisphosphonate, on bone loss, bone quality, biomechanical properties, and bone concentrations in aged ovariectomized rats.

Methods. Eight-month-old female Wistar rats were ovariectomized (Ovx) or sham-operated. Treatment was started 10 weeks following Ovx with subcutaneous ibandronate in doses of 0.2, 1.0, 5.0, or 25 μg/kg/day for 12 mo. Additional groups received 25 or 125 μg/kg intermittently every 25 days, resulting in the same total dose as compared to 1.0 or 5.0 μg/kg/day, respectively. Bone analyses by x-ray densitometry, peripheral quantitative computed tomography (pQCT), dual energy x-ray absorptiometry (DEXA), histomorphometry, 3-point bending, and compression tests were performed in femora, tibiae, and lumbar vertebrae in separate groups at the beginning and the end of treatment. Ibandronate concentration in tibiae and vertebrae was determined by gas chromatography mass spectroscopy at the end of the study.

Results. Ovariectomy resulted in a significant reduction in bone mass ($p \leq 0.0001$) and strength ($p < 0.05$) by 10 weeks after surgery in long bones, while only a trend was present in vertebrae. When compared to age matched Ovx controls, ibandronate resulted in a dose dependent increase in bone mineral density (BMD), trabecular bone volume and trabecular number, load to failure (F_{max}), and yield load in long bones and vertebrae. The lowest significant dose, which was different from Ovx controls, ranged between 0.2 and 1.0 μg/kg/day, with higher doses not differing from sham controls. Increased trabecular separation ($p \leq 0.0001$) was fully prevented by all doses. Vertebral BMD (pQCT and DEXA) positively correlated with F_{max} by $r = 0.88$ ($p \leq 0.0001$) both; correlation of femoral F_{max} versus cortical BMD was $r = 0.61$ ($p \leq 0.0001$).

Conclusion. Bone concentrations of ibandronate were linear with the dose, suggesting linear kinetics in the applied dose range. In general, the same total cumulative ibandronate dose given provided equivalent results, independent of the administration schedule. (J Rheumatol 2002;29:2200–8)

Key Indexing Terms:
IBANDRONATE BONE MINERAL DENSITY
BONE ARCHITECTURE BONE QUALITY POSTMENOPAUSAL OSTEOPOROSIS

Following menopause, estrogen deficiency results in increased bone turnover with an imbalance between bone formation and bone resorption. Consequently, bone mass decreases and bone microarchitecture deteriorates. The decrease in bone mass and quality increases the risk of fractures and thus morbidity and mortality[1]. Until fractures occur, osteoporosis essentially remains a silent disease that often remains undetected. Thus, major efforts have been directed at identifying women at risk and appropriately initiating therapeutic intervention.

The ovariectomized (Ovx) rat is considered a good model to study this disease as bone loss in rats and postmenopausal women shares many characteristics including pathophysiological aspects, as well as the skeletal response to different therapies[2,3]. Consequently, the Ovx rat is one of 2 models recommended for studies of human osteoporosis by health authorities[4-6].

Bisphosphonates are among the most promising agents for the treatment and prevention of postmenopausal osteoporosis. They inhibit bone resorption, increase bone mineral density (BMD), and reduce the risk of future fractures. The antifracture efficacy of bisphosphonates has been docu-

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mented in a number of clinical[7-11] and animal studies[12-14] for postmenopausal osteoporosis. Although many studies have been published using bisphosphonates in the Ovx rat model, most of them were performed in younger growing rats, and thus the results may have been affected by the animals' growth rate. In addition, little is known about the longterm effects of bisphosphonates in remodeling species, such as the rat, when treatment starts after significant bone loss has already occurred. Only a few studies have been published using this model, and there has not been a direct comparison of continuous versus intermittent treatment[15,16].

Ibandronate, a highly potent nitrogen-containing bisphosphonate currently under development, has proven effective at considerably lower doses than other bisphosphonates in both rats and ovariohysterectomized beagles[17–19]. It has also been shown that in intact growing rats, the total cumulative dose over a given period provides comparable results irrespective of daily (low dose) or intermittent (high dose) administration[20]. Similar results have recently been reported for beagles[19] and for aged estrogen depleted rats treated for prevention of bone loss[21].

This study was designed to determine the effects of daily and intermittent treatment of ibandronate on established bone loss in the Ovx rat with emphasis on the effect on bone mass, architecture, strength, and drug concentration in bones.

MATERIALS AND METHODS

Animals and conditions. Female Wistar rats, obtained from Moellegaard Breeding Center (Schoenwalde, Germany), were 8 months of age with a body weight of 250.8 ± 24.8 g (mean ± SD) when they entered the study. They were kept in single cages and fed a commercial standard chow (Ssniff®, Ssniff Spezialdiäten GmbH, Soest, Germany) containing 1.1 g calcium (Ca) and 0.8 g phosphorus (P) per 100 g dry weight. They were fed *ad libitum* with free access to tap water. The experiments were approved by the local governmental Animals Ethic Committee (Regierungspräsidium Karlsruhe, Germany).

Experimental design. Bilateral ovariectomies or sham surgeries (sham) were performed from the ventral approach under general anesthesia (200 mg/kg hexabarbitone intraperitoneally). The rats were divided into 11 different groups (15/group) and treated with the bisphosphonate ibandronate or solvent (isotonic NaCl) in different regimens. All treatments were initiated 10 weeks after surgery. At the beginning of the experiment an additional group of 15 animals was sacrificed on the day of surgery (Day 0) and used as baseline control. Ibandronate [1-hydroxy-3-(methylpentylamino)propylidene] bis-phosphonic acid (monosodium salt monohydrate) was dissolved in isotonic saline and the pH was adjusted to 7.4. The solution was freshly prepared every month and stored in a normal refrigerator (4°C). The doses are expressed as μg/kg of free acid equivalents of ibandronate. The volume of administration was 2 ml/kg.

The animals were treated subcutaneously with ibandronate at daily doses of 0.2, 1.0, 5.0, or 25.0 μg/kg/day for a duration of 12 months. Additional groups received 25.0 or 125.0 μg/kg every 25 days. During the study, longitudinal analyses of BMD were performed in the left tibia of 3–5 animals of each group by peripheral quantitative computed tomography (pQCT). At the end of the experiment, 24 h after the last administration, the animals were sacrificed by exsanguination and various bones were removed and processed either for radiographic analyses (right femur), histomorphometry (left tibia and lumbar vertebra L3), determination of the drug concentration in the bone (right tibia and lumbar vertebra L1), or biomechanical properties and BMD (lumbar vertebra L4 and left femur).

Specimen preparation. The right femora, right tibiae, and vertebrae L1 were cleaned of soft tissue by dermestid beetles (*Dermestes maculatus*) as described[22]. Subsequently the bones were dried to constant weight at 80°C. After adaptation to room temperature, the femora were subjected to x-ray densitometry. The dried right tibiae and vertebrae L1 were analyzed for their concentration of ibandronate. The left tibiae as well as lumbar vertebrae L3 were prepared for histomorphometrical analysis by standard procedures.

For bone quality studies, lumbar vertebrae L4 and left femora were carefully freed of soft tissue and frozen at –20°C in 0.9% NaCl solution until further processing. Vertebrae were analyzed for BMD by dual-energy x-ray absorptiometry (DEXA) and pQCT prior to a compression test of the vertebral bodies. The femora were analyzed for cortical BMD at the midshaft prior to a 3-point bending test.

Bone mass and architecture. pQCT analyses (pQCT 960, Stratec Medizintechnik, Pforzheim, Germany) were done in the proximal metaphysis 6 mm distal from the knee joint using a threshold of 750. The threshold derived from previous investigations and was found to discriminate best between trabecular and cortical bone at this location. The first 5 animals from each group were analyzed longitudinally during the whole study. If animals died, they were not replaced by other animals and thus the group size ranges from 3 to 5 throughout the measurements.

Femoral x-ray densitometry. The cleaned femora were radiographed and analyzed according to the described procedure with slight modifications[23]. In brief, the bones were radiographed together with an aluminum wedge as a reference object. For densitometry purposes, the radiographic film is positioned between the light source of a microscope and a TV camera, which produces a TV image of the radiographed shadow of the bone. The electronic image is then analyzed by a real-color image analyzing system (CBA 8000, Wild Leitz GmbH, Wetzlar, Germany). The density is expressed as mm of aluminum equivalents. The region of interest was the distal metaphyseal area between the 2 cortices of the femur.

Histomorphometry. The excised left tibiae and lumbar vertebrae were cleaned from the attached soft tissues and the fibula removed. A transverse saw cut was performed slightly proximal to the tibial midshaft, and the posterior part of the tibial head was cut off in a frontal plane with a scalpel. Vertebrae were cut laterally by one-third in the sagittal level. These procedures allow better permeation of the bathing solution. The bones were then fixed in neutral 4% formaldehyde (containing 1% of $CaCl_2$). On the next day, the samples were transferred to 70% alcohol for storage. For further processing the bones were dehydrated in alcohol, cleared in xylene, and embedded in methylmethacrylate[24]. Frontal sections of the head of the tibia were cut with a precision saw and subsequently 6 μm thick sections were made on a Jung microtome (model K) in a frontal plane. The sections were stained with the von Kossa-reaction and McNeal's tetrachrome or, in some cases, with Movat's stain. In these sections the histomorphometric analyses were performed.

The tibial sections (proximal metaphyses) were analyzed with a real-color image analyzing system (CBA 8000) at magnification ×70. The metaphyseal area chosen for histomorphometric quantification began 1.0 mm distal of the epiphyseal growth plate-metaphyseal junction to exclude the primary spongiosa. The area of analysis extended distally 1.4 mm with an extension of 2.5 mm resulting in a total tissue area of 3.5 mm^2. Lumbar vertebrae L3 were analyzed in the mid-spine in sagittal sections. The area of analysis was within the vertebral body with the maximal possible rectangle, with an extension of about 6.5 mm in length and 5.0 mm in width, without touching cortical bone. Smaller regions of interest were excluded from analyses. In both tibial and vertebral sections, cancellous tissue area, cancellous bone area, and cancellous bone perimeters were determined automatically by the analyzing system. Trabecular bone volume (Cn-BV/TV in percentage), trabecular thickness according to the plate model (Tb.Th in μm), trabecular number (Tb.N in n/mm), and trabecular

separation (Tb.Sp in μm) were calculated, named, and abbreviated according to the standardization approved by the American Society for Bone and Mineral Research[25].

Bone quality analyses. For preparation and testing, the bone specimens had to be frozen and thawed at room temperature a few times. Repeated freeze-thaw cycles have been shown not to influence mechanical properties of trabecular bone[26]. After thawing, the arch of the vertebrae and the transverse processes of lumbar vertebrae L4 were carefully removed using the Minimum Contact Point System (MCP System, Exakt Apparatebau GmbH, Norderstedt, Germany) with a special device for fixation of the vertebrae. Afterwards, the vertebral bodies of all groups were fixed on acrylic glass slides with Technovit 7210 VLC (Kulzer Exakt, Heraeus Kulzer GmbH, Wehrheim, Germany), which photopolymerized under blue light in a precision adhesion press (Exakt Apparatebau). The vertebral bodies were aligned along the dorsal longitudinal ligament. To remove the cortical endplates and ensure planoparallel surfaces, the slide with the vertebral body was fixed to the MCP System and 2 parallel transverse cuts were made, about 1.5 mm away from the cranial and caudal ends. The MCP system was operated under constant irrigation[27].

DEXA and pQCT analyses of lumbar vertebral bodies. DEXA analyses were performed by a Hologic QDR 1000/W (Hologic Inc., Waltham, MA, USA; supplied by Siemens AG, Erlangen, Germany). BMD of all lumbar vertebral bodies was measured after removal of the endplates. Analyses were performed using the high resolution software for small animals.

pQCT was used to measure a single 1 mm thick slice in the middle of each vertebral body. Tissue area and BMD was measured using a resolution with a voxel size of $0.148 \times 0.148 \times 1.0$ mm.

pQCT analyses of left femora. The area of the left femora located under the upper support during the 3-point bending test was scanned by pQCT in a 1 mm slice using a voxel size of $0.148 \times 0.148 \times 1.0$ mm, and cortical BMD was determined. The caudal surfaces of the femora faced downwards.

Compression testing of lumbar vertebral bodies. The central parts of the lumbar vertebral bodies were compressed along the cephalocaudal axis. The mechanical tests were performed using a screw driven mechanical material testing machine (Zwick, Model Z010, Ulm, Germany). Displacement was registered by an extensometer for compression bending tests. The bone specimens were compressed at a nominal deformation rate of 0.5 mm/min. Before testing, a preload of 5 N was applied. Ultimate load (N), deformation at ultimate load (mm), and stress (N/mm^2; maximum compressive force per unit cross sectional area of the vertebral body) were measured or calculated using the provided Zwick standard test program for tensile, flexure, and compression tests. To normalize tissue area in relation to BMD, the following calculation was used: normalized tissue area = (total density $\times$ tissue area)/mean for total density of the control group. The modulus of elasticity (MPa, slope of the stress – % deformation curve within the elastic region, which is the linear part of the curve constrained between 30 and 60% of the maximum stress value) was calculated.

Three-point bending of left femora. The femora were tested by 3-point bending until failure using the screw driven mechanical testing machine described above. Displacement was registered by an extensometer. The caudal surfaces of the femora were placed on the lower supports of the bending apparatus, which were 16 mm apart. Before testing, a preload of 5 N was applied. The actuator was displaced at a rate of 0.5 mm/min. Calculations of the mechanical variables were performed by the provided software.

Concentration of ibandronate in bones. The dried bones were ground to pieces and dissolved in 2 M HCl at 50°C for 18 h. By this procedure, 100% of ibandronate is recovered from the bone matrix without any chemical degradation. To each solution, N-trideuteromethyl ibandronate was added as an internal standard. Subsequently, ibandronate was coprecipitated with calcium-phosphate and separated by a cation exchange procedure. After oxidation with chlorine, ibandronic acid was converted to N-pentyl-N-methyl-ß-alanine, which was measured as the ethyl ester by gas chromatography mass spectroscopy. The accuracy and precision of this method are 107.5 and 12.9%, respectively.

Statistical analysis. All statistical evaluations were performed by the SAS system (Version 6.10). Assuming approximate Gaussian distribution as well as variance homogeneity, 2 sided t tests and the many-to-one comparisons procedure according to Dunnett within the ANOVA model were performed[28] and the multiplicity adjusted p values are reported. The dose response relationship is characterized by the lowest significant dose with respect to control. Based on an equivalence threshold of 25%, therapeutic equivalence was investigated by one sided Fieller's confidence intervals (CI) for the ratio assuming approximated Gaussian distribution as well as variance homogeneity. No multiplicity adjustment was used. Parametric correlation analysis was used for characterization of the dependency between the respective endpoints.

RESULTS

Ibandronate was well tolerated by all animals. Weight gain was normal in sham rats and was increased with Ovx as expected (Table 1). No treatment effects could be detected. The animal death rate was evenly distributed among all groups and reflects that of aged Wistar rats. The animals that died prematurely and those lost for technical reasons were excluded from all analyses.

Bone mass and architecture. The effect of ovariectomy in comparison to sham surgery was analyzed at the beginning of therapy (10 weeks after Ovx) and at the end of therapy (62 weeks after Ovx). At the beginning of therapy, significant changes ($p \leq 0.0001$) were present in Ovx animals regarding femoral x-ray density, tibial trabecular bone mass and density, while no morphological changes occurred in lumbar vertebrae L3.

At the end of treatment, 62 weeks after surgery, the effect of ovariectomy was also tested in the respective age matched sham and Ovx groups. A significant decrease in bone mass was detectable in femoral x-ray density, trabecular bone volume, trabecular number in long bones as well as in vertebrae, with the exception of trabecular thickness (tibiae and vertebrae) and distal cortical BMD. Trabecular separation, which reflects the connectivity of trabecular bone, was significantly increased ($p \leq 0.0001$) in both types of bone (Table 1).

Effects of aging. Analyses on the effect of aging were performed between the respective Ovx or sham groups 10 and 62 weeks after surgery. There was a further decrease in BMD and endpoints for bone mass with time in both long bones and vertebrae, which means that the effects on bone mass and structure were more pronounced with time in both sham and Ovx groups. However, there were no significant changes in trabecular thickness even 62 weeks after Ovx.

Effects of treatment. At the end of the treatment period the effects of all treatment doses were compared with the age matched Ovx control. There was a clear dose dependent increased femoral BMD. The lowest dose different from solvent treated Ovx controls (lowest significant dose) ranged between 0.2 and 1.0 μg/kg/day (Figure 1). Higher doses were not different from sham controls at the end of treatment, which means that the optimal dose is in the same dose range.

Table 1. Effect of ibandronate on architecture in tibia and lumbar vertebrae L3 and body weight.

Status	Baseline	Sham	Ovx	Sham	Ovx	Ovx	Ovx	Ovx	Ovx	Ovx	Ovx
Week of Sacrifice	0	10	10	62	62	62	62	62	62	62	62
Dose, μg/kg	—	—	—	—	—	0.2	1	5	25	25i	125i
Left tibia											
Tb.N (n × mm^{-1})	6.56	6.28	3.81[a]	3.71	0.61[a]	2.82[4]	3.28[4]	3.51[4]	3.93[4]	3.32[4]	4.08[4]
	± 0.70	± 0.65	± 0.85	± 1.05	± 0.44	± 0.63	± 0.45	± 1.41	± 1.12	± 1.21	± 1.37
Tb.Sp (μm)	107.0	113.2	233.5	245.2	2965.9[a]	331.8[4]	268.9[4]	284.9[4]	230.5[4]	320.4[4]	235.7[4]
	± 17.3	± 17.9	± 64.7	± 74.7	± 3046.9	± 90.8	± 44.1	± 138.8	± 117.2	± 215.8	± 146.7
Tb.Th (μm)	47.12	47.63	42.01	43.30	37.43	40.22	40.82	43.42	51.66[2]	42.13	48.80[1]
	± 9.47	± 5.93	± 3.81	± 7.23	± 13.10	± 8.51	± 4.87	± 9.49	± 10.07	± 8.07	± 9.87
Vertebra L3											
Cn-BV/TV (%)	24.1	23.9	22.0	22.5	12.8[a]	18.4	18.7[1]	23.0[3]	26.9[4]	21.5[2]	23.7[4]
	± 3.7	± 3.6	± 3.7	± 6.4	± 4.6	± 3.2	± 3.5	± 5.6	± 5.4	± 8.0	± 6.9
Tb.N (n × mm^{-1})	4.55	4.14	4.02	3.87	2.56[a]	3.80[4]	3.79[4]	4.05[4]	4.09[4]	3.80[4]	3.78[4]
	± 0.47	± 0.32	± 0.48	± 0.51	± 0.64	± 0.42	± 0.24	± 0.35	± 0.52	± 0.57	± 0.29
Tb.Sp (μm)	168.8	184.6	197.5	204.4	370.8[a]	217.5[4]	215.6[4]	192.3[4]	182.0[4]	213.6[4]	204.0[4]
	± 21.4	± 14.1	± 33.9	± 38.61	± 139.6	± 26.4	± 19.4	± 27.0	± 30.6	± 58.0	± 30.2
Tb.Th (μm)	53.49	58.10	54.44	58.38	48.66	48.62	49.22	56.61	66.40[2]	56.51	62.37[1]
	± 9.89	± 10.43	± 6.10	± 16.25	± 8.38	± 8.13	± 8.26	± 11.90	± 14.44	± 19.33	± 16.17
Body weight											
Weight (g)	227	250	275	336	379	348	357	377	377	388	359
	± 16	± 18	± 41	± 58	± 50	± 52	± 45	± 38	± 43	± 53	± 33

Doses are daily doses; i: doses administered intermittently every 25 days. Data are expressed as a mean ± SD (n = 9–15). Significance between Ovx versus sham at the beginning of treatment (Week 10) or end of treatment (Week 62):[a] $p \leq 0.0001$; Ovx control versus doses at the end of treatment:[1] $p \leq 0.05$, [2] $p \leq 0.01$, [3] $p \leq 0.001$, [4] $p \leq 0.0001$.

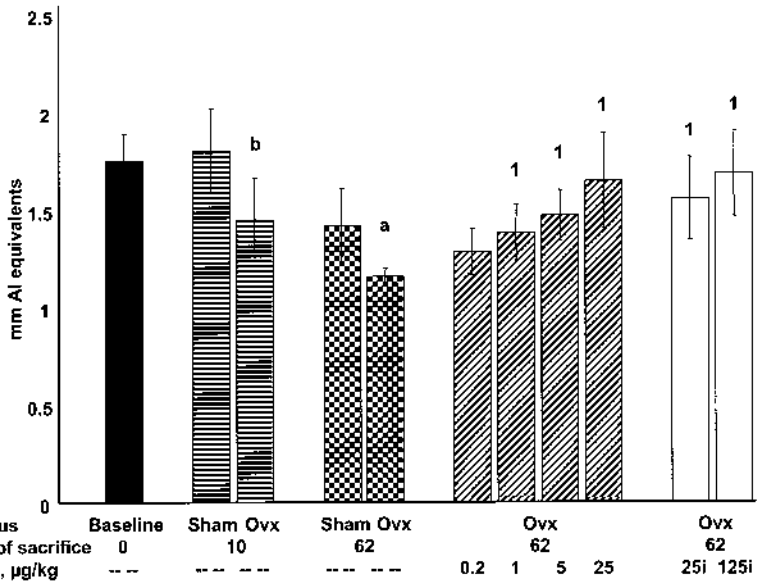

Figure 1. X-ray density of the distal right femora. Doses are daily doses; i: doses administered intermittently every 25 days. Data are expressed as mean ± SD, n = 9–15. Significance between Ovx versus sham at the beginning of treatment (Week 10) or end of treatment (Week 62): [a]$p \leq 0.01$, [b]$p \leq 0.0001$; Ovx versus doses at the end of treatment: [1]$p \leq 0.0001$.

In the tibiae, trabecular bone volume measured by histomorphometry showed similar results compared to trabecular pQCT analyses at the end of the study (Figures 2 and 3, and Table 2). Histomorphometric analyses in tibiae and vertebrae revealed the lowest dose (0.2 μg/kg/day) being already the lowest significant dose and the optimal dose (the exception was trabecular bone volume, where the optimal dose is 1 μg/kg/day in both bones).

The treatment schedules (daily administration vs intermittent administration with the same total dose) were equivalent for nearly all of the endpoints in all bones irrespective of the analytical method. The only exception: the high inter-

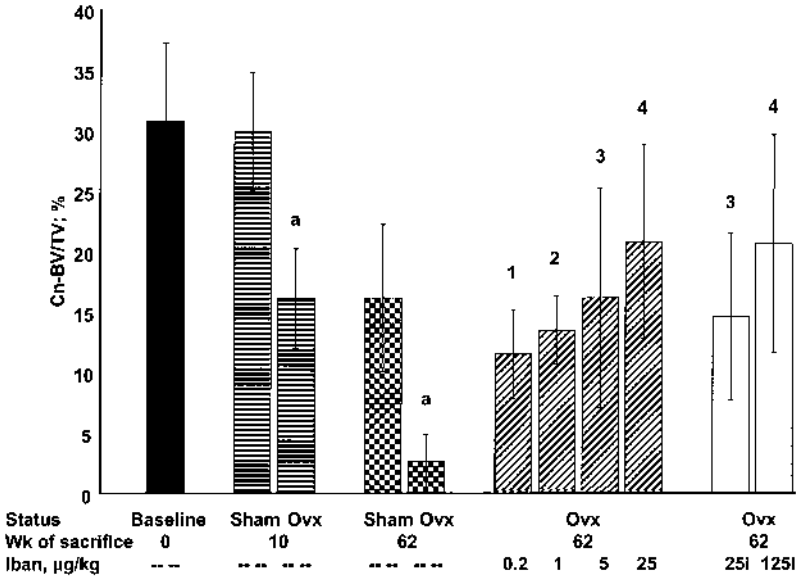

Figure 2. Cancellous bone volume in the proximal metaphysis of left tibiae. Doses are daily doses; i: doses administered intermittently every 25 days. Data are expressed as mean ± SD, n = 9–15. Significance between Ovx versus sham at the beginning of treatment (Week 10) or end of treatment (Week 62): $^{a}p \leq 0.0001$; Ovx versus doses at the end of treatment: $^{1}p \leq 0.05$, $^{2}p \leq 0.01$, $^{3}p \leq 0.001$, $^{4}p \leq 0.0001$.

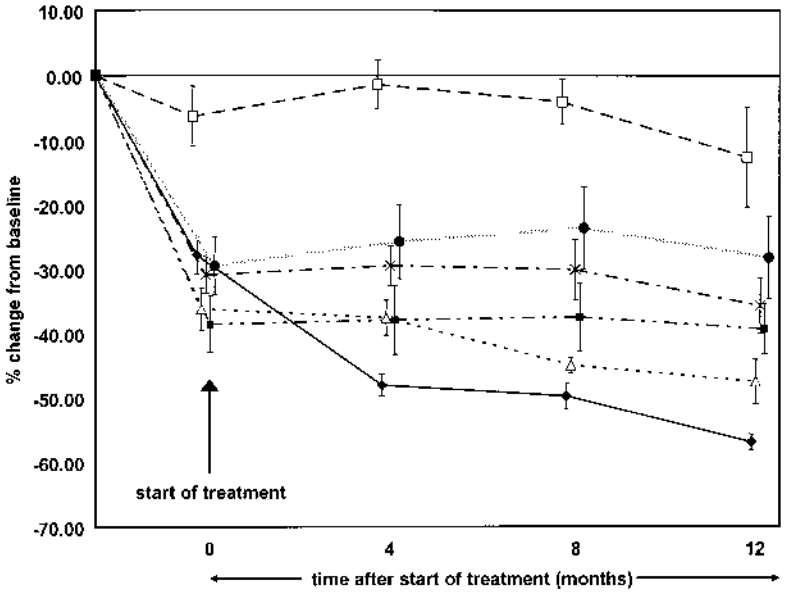

Figure 3. Time course of trabecular bone density (pQCT) in the right proximal tibiae. Data are expressed as mean ± SEM, n = 3–5. Symbols: sham controls (□), Ovx controls (◆), daily doses (µg/kg): 0.2 (△), 1.0 (×), 5.0 (■), 25.0 (●).

mittent dose (125 µg/kg every 25 days) was superior to the corresponding daily dose (5 µg/kg) with respect to the prevention of trabecular separation. However, because trabecular separation was fully prevented by all treatment doses and schedules, the superiority of the cyclical regimen did not appear to have biological relevance, especially as no difference in biomechanical properties occurred.

Time course of changes. It was not possible to investigate a time course of changes in bone mass in all animals because of the large number of animals used in this study. However, the first 5 animals per group were analyzed longitudinally during the whole study by pQCT. Since the mean of the endpoints of all animals of the respective groups were similar or close to those of the selected animals at sacrifice

Table 2. Effect of ibandronate on bone mass and biochemical properties in left femur and lumbar vertebrae L4.

Status	Baseline	Sham	Ovx	Sham	Ovx	Ovx	Ovx	Ovx	Ovx	Ovx	Ovx
Week of Sacrifice	0	10	10	62	62	62	62	62	62	62	62
Dose, μg/kg	—	—	—	—	—	0.2	1	5	25	25i	125i
Left Femur											
F_{max} (N)	132 ± 17	142 ± 23	160 ± 25	160[a] ± 24	139[a] ± 17	164[1] ± 20	162[1] ± 17	171[2] ± 34	164[1] ± 21	161 ± 29	157 ± 15
Ultimate stress (N/mm^2)	154 ± 18	157 ± 16	161 ± 12	153 ± 15	135[b] ± 13	147[1] ± 13	146 ± 12	154[3] ± 11	150[2] ± 9	144 ± 7	156[4] ± 12
Yield load (N)	83 ± 14	92 ± 10	92 ± 15	98 ± 16	81[b] ± 16	96 ± 14	98[1] ± 8	99[1] ± 17	101[2] ± 17	98[1] ± 14	97 ± 13
Modulus of elasticity (MPa)	7031 ± 1384	7230 ± 1307	7089 ± 938	6552 ± 1498	5672 ± 969	6377 ± 1368	6191 ± 944	6679 ± 680	6455 ± 1092	6502 ± 1110	6798 ± 1416
Ct-BMD by pQCT (mg/cm^3)	1369 ± 14	1378 ± 22	1374 ± 22	1417 ± 26	1365[d] ± 21	1400[3] ± 18	1415[4] ± 21	1428[4] ± 26	1419[4] ± 17	1414[4] ± 24	1419[4] ± 16
Vertebra L4											
Ultimate stress (N/mm^2)	78 ± 7	78 ± 7	71 ± 8	62 ± 6	65 ± 8	69 ± 8	71 ± 8	70 ± 7	73 ± 7	69 ± 5	72 ± 6
Modulus of elasticity (MPa)	5754 ± 1191	6033 ± 988	5186 ± 641	5493 ± 1151	5341 ± 1231	6112 ± 1176	6114 ± 1221	6679 ± 1306	5980 ± 1151	5525 ± 802	5875 ± 1085
BMD by DEXA (g/cm^2)	0.175 ± 0.010	0.176 ± 0.015	0.174 ± 0.018	0.167 ± 0.021	0.137[d] ± 0.015	0.161 ± 0.013	0.172[2] ± 0.016	0.184[4] ± 0.027	0.185[3] ± 0.012	0.173[1] ± 0.020	0.177[2] ± 0.013
BMD by pQCT (mg/cm^3)	785 ± 51	779 ± 57	763 ± 64	758 ± 73	567[c] ± 87	681 ± 54	709[1] ± 62	793 ± 104	811[1] ± 104	733[1] ± 95	767[2] ± 73

Doses are daily doses; i: administered intermittently every 25 days. Data are expressed as mean ± SD, n = 9–15. Significance between Ovx versus sham at the beninning of treatment (Week 10) or end of treatment (Week 62): [a] $p \leq 0.05$, [b] $p \leq 0.01$, [c] $p \leq 0.001$, [d] $p \leq 0.0001$; Ovx control versus doses at the end of treatment: [1] $p \leq 0.05$, [2] $p \leq 0.01$, [3] $p \leq 0.001$, [4] $p \leq 0.0001$.

(data not shown), the time course can be regarded to be representative for the whole group. It is obvious that all treatment related changes in BMD were already present at 4 months, which was the first time point of analyses after start of treatment (Figure 3). Changes in bone mass progressively declined in Ovx controls, while ibandronate treatment produced a dose dependent retardation or maintenance of this variable in the other experimental groups.

Biomechanical bone strength. At the beginning of the treatment period, only femoral load to failure was significantly increased in Ovx controls ($p \leq 0.05$). One year later, in the Ovx untreated animals, there was a clear and significant reduction in femoral bone strength ($p \leq 0.01$) for the most relevant primary endpoints at the end of the study (ultimate load to failure, ultimate stress, yield load, yield stress, and cortical BMD). Similar results were obtained from vertebrae. The apparent BMD of the vertebral bodies as measured either by DEXA or pQCT analysis was also decreased in the ovariectomized rats at the end of the treatment (Tables 1 and 2).

Effects of treatment. At the end of the treatment period the effects of all treatment doses were compared to the Ovx control group. There was a clear dose dependent increased bone strength, indicating that even the low dose of ibandronate (0.2 μg/kg/day) is superior to the untreated Ovx controls and thus represents the lowest significant dose (Figure 4). Additionally, no doses were different from the sham control group at the end of treatment, which means that 0.2 μg/kg/day is also the optimal dose. When all groups were pooled, lumbar BMD measured by both DEXA and pQCT positively correlated with F_{max} by 0.88 ($p \leq 0.0001$); the correlation between DEXA and pQCT was 0.89 ($p \leq 0.0001$); and the correlation between femoral F_{max} versus cortical midshaft BMD was 0.61 ($p \leq 0.0001$). The comparison of both treatment schedules (daily administration vs intermittent administration resulting in the same total dose) revealed equivalence.

Concentrations of ibandronate in bones. The results clearly show a dose dependent concentration of ibandronate in the investigated bones (Figure 5). The uptake of the drug by the respective bones was linear with the dose. Additionally, the uptake was related to the total dose administered irrespective whether the dose was administered continuously (daily) or intermittently, provided the same total dose was administered. The mean concentration in the lumbar vertebrae was always about 2 times the mean concentration found in the tibiae, independent of the dose and administration schedule.

DISCUSSION

The bisphosphonate ibandronate was tested in the ovariectomized aged rat model for 12 months using the interventional approach, which means that treatment started after considerable bone loss had already occurred. Our aim was to investigate the effects of ovariectomy and treatment with

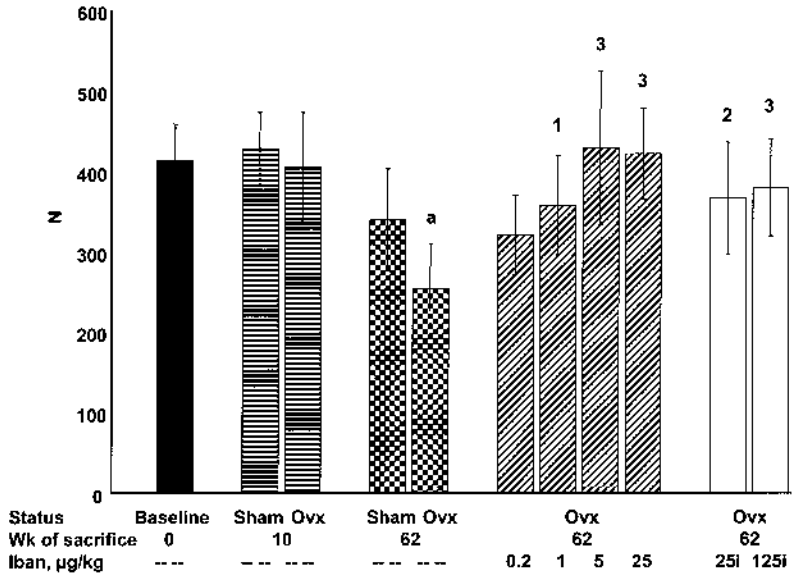

Figure 4. Ultimate load to failure (F_{max}) of lumbar vertebral bodies L4. Doses are daily doses; i: doses administered intermittently every 25 days. Data are expressed as mean ± SD, n = 9–15. Significance between Ovx versus sham at the beginning of treatment (Week 10) or end of treatment (Week 62): $^{a}p \leq 0.01$; Ovx versus doses at the end of treatment: $^{1}p \leq 0.01$, $^{2}p \leq 0.001$, $^{3}p \leq 0.0001$.

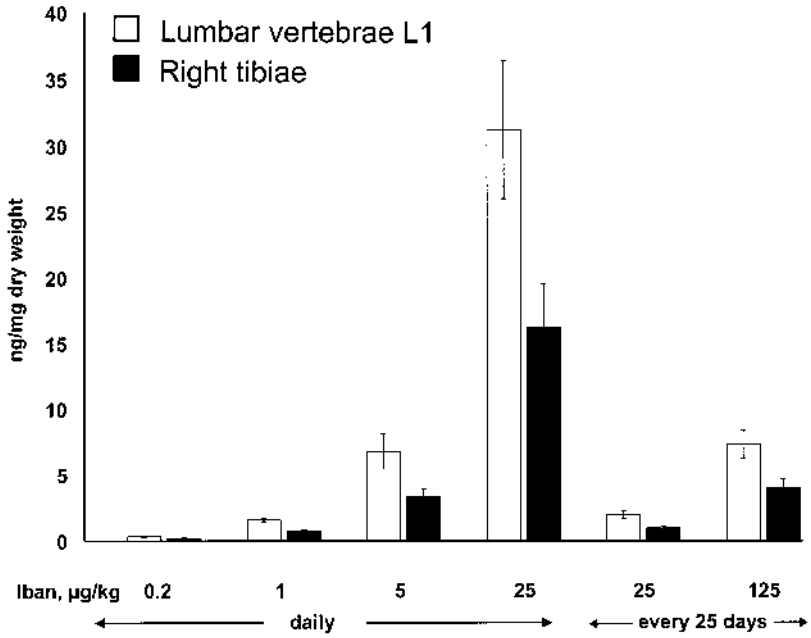

Figure 5. Concentration of ibandronate in lumbar vertebrae L1 and right tibiae after 12 months of treatment.

ibandronate on bone mass, biomechanical bone strength, and architecture of long bones and vertebrae. The concentration of ibandronate in bone was also evaluated.

In previous studies, with treatment starting immediately after cessation of ovarian function, the lowest significant dose (lowest dose that produced results different from Ovx controls), as well as the optimal dose (dose equivalent to age matched sham controls) was found to be 1 μg/kg/day[21]. In this model, it was shown that the total dose of ibandronate, irrespective of the treatment schedule, is most important. The duration of treatment was 5 months in both studies, with the total optimal cumulative dose being 154 μg/kg/animal. In this study, 12 months' treatment was started after bone loss had already been established. Depending on the under-

lying variable and type of bone, the lowest significant dose and the optimal dose ranged between 0.2 and 1 μg/kg/day, with the lowest dose often being both significant and optimal. Optimal prevention of further bone loss was already present in doses smaller than or equal to the lowest significant dose (0.2–1.0 μg/kg/day corresponds to a cumulative dose of about 180 μg/kg/animal). Thus, the prevention of Ovx induced bone loss seemed to depend on the total cumulative dose over time and not on the daily dose, irrespective of whether treatment started immediately after induction of bone loss or after bone loss had already been established. Short treatment periods require higher daily doses, while longterm treatments allow lower daily doses. Additionally, the total doses, administered either daily or intermittently, produce equivalent results. Consequently, one can conclude that the drug can also be given either in intermittent low doses (longterm) or in intermittent higher doses (short term), provided that the cumulative dose is similar. Alternatively, the intervals between dosing could be shortened, when low doses are administered, or increased, when higher doses are administered. However, it is unlikely that these conclusions can be extrapolated to extremes (e.g., a single high dose administration is sufficient for a very long period), although this might be possible at least within 2–3 remodeling periods in rats[21]. Whether these conclusions hold true in humans or are specific for species such as rats requires further elucidation. However, early clinical results in postmenopausal women treated with ibandronate (2 months' dose-free interval) or alendronate (weekly intervals) have already confirmed that the total effective dose and not the schedule determines the results[29,30].

When the time course of bone loss in untreated controls was considered, trabecular bone loss was detectable earlier in long bones than in vertebrae. This was shown by femoral x-ray density and trabecular bone volume in the tibia, which were already significantly reduced at the start of treatment, while only a trend was present in trabecular bone volume in vertebrae. However, by the end of the treatment period (12 months), the vertebrae in the controls showed significant reduction in trabecular bone. Thus, the time to initiate treatment was selected appropriately. The comparison of BMD in the tibia in the subgroups (n = 3–5/group, Figure 3) and with the entire group at the end of the experiment revealed comparable results, suggesting that the longitudinal followup is representative for the whole group, and the method is suitable even for small group sizes.

Following ovariectomy, bone strength was reduced. This effect was prevented by treatment with ibandronate. The low dose (0.2 μg/kg/day) was found to be the optimal dose, although a dose dependent prevention of Ovx induced decreased bone strength was observed in both long bones and vertebrae. Doses up to 125 times the optimal dose, with corresponding high drug concentration in bones, maintained the normal biomechanical properties of bone. Moreover, endpoints such as ultimate load to failure were even slightly (but not significantly) increased when compared to age matched sham controls. The effects of ibandronate on the biomechanical endpoints of long bones and vertebrae, respectively, were equivalent irrespective of whether the same total dose was given as daily administrations or in cyclical intermittent administrations. The high correlation between bone mass and bone strength was consistent with those obtained from intact rats after 2 years of daily oral treatment with ibandronate in doses far in excess of any therapeutically intended dose[31]. Additionally, even with the heterogeneous ibandronate distribution in bone (layers), which must be postulated after intermittent administration, bone quality remained normal and bone mass paralleled bone strength.

Considering age and ovariectomy dependent bone turnover, the bone remodeling time (sigma) in Ovx rats can be estimated to be about 5–6 times less than that of humans[32-35]. Since the sigma in humans ranges from about 80 to 120 days, the sigma in aged Ovx rats is calculated to be about 13 to 24 days. Thus, the therapy-free interval in this study roughly ranges between 1 and 2 sigma times that in aged Ovx rats.

The results of the drug concentration analysis clearly show that the uptake of the drug by the respective bones was linear in this dose range, an effect that is consistent with other bisphosphonates[36]. Lumbar vertebrae have roughly twice the concentration of ibandronate compared to the tibiae because of the greater ratio of trabecular to cortical bone in the vertebrae compared to that in tibiae. In comparison to cortical bone, a larger bone surface (binding site for bisphosphonates at the apatite surface) is present during the bone remodeling process occurring during the study period. Further, cortical and trabecular bone differ not only in porosity and surface/volume ratio, but also in blood supply and rapidity of turnover[37]. Therefore, the higher concentration of ibandronate in vertebrae is a consequence of normal bone physiology.

In aged Ovx rats, ibandronate prevents further reduction in bone mass, deterioration in architecture, and strength when considerable bone loss has already occurred. The optimal dose is in the range of 0.2 to 1 μg/kg/day. The total cumulative dose rather than the treatment schedule seems to determine efficacy. Comparable results were obtained for biomechanical properties: the optimal dose for the prevention of bone strength is smaller or equal to 0.2 μg/kg/day. The mean concentration of ibandronate in long bones and vertebrae was linear with the dose, suggesting linear kinetics in the tested dose range. These results indicate that ibandronate is a potent, promising bisphosphonate with properties that make it a strong candidate for the treatment and prevention of postmenopausal osteoporosis. Our results confirm the potential to develop different treatment schedules and routes of administration to enhance longterm compliance.

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