

Total Administered Dose of Ibandronate Determines Its Effects on Bone Mass and Architecture in Ovariectomized Aged Rats

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ABSTRACT. Objective. Ibandronate is a highly potent nitrogen-containing bisphosphonate that can prevent bone loss in various animal models as well as in clinical trials. We evaluated the effects of different doses and treatment schedules in ovariectomized aged rats, a model of human osteoporosis.

Methods. Eight-month-old female Wistar rats were ovariectomized or sham operated ($n = 15/\text{group}$). Doses of 0.1 to 30 $\mu\text{g}/\text{kg}/\text{day}$ ibandronate were administered subcutaneously over a period of 20 weeks with or without a 5 times higher single loading dose starting one day postsurgery. In a subsequent experiment, the optimal preventive dose, which is the lowest dose that prevented bone loss completely, or a suboptimal preventive dose were administered over the same period, either daily or by 3 cyclical intermittent regimens (on/off weeks = 1/2, 1/4, and 1/6), resulting in the same cumulative total dose.

Results. Ovariectomy induced significant bone loss in the following primary endpoints: femoral radiographic density, dry weight/tissue volume, and calcium content/tissue volume. Histomorphometry in the tibia resulted in reduced trabecular bone mass, thickness, and number, and increased separation. The optimal dose was 1.0 $\mu\text{g}/\text{kg}/\text{day}$, while 0.1 $\mu\text{g}/\text{kg}/\text{day}$ was suboptimal. Higher doses resulted in a plateau. The loading dose had no effect on the results. Cyclical intermittent administration dose-dependently prevented bone loss, providing equivalent results per total dose, irrespective of the administration schedule.

Conclusion. There were no differences between the various regimens, suggesting that it is the total dose of ibandronate rather than the treatment schedule that is important for efficacy, at least within the tested dosing intervals. (J Rheumatol 2002;29:990–8)

Key Indexing Terms:

BONE MINERAL DENSITY
IBANDRONATE

INTERMITTENT TREATMENT

BONE ARCHITECTURE
RATS

Osteoporosis as a consequence of the menopause is characterized by decreased bone mass and strength resulting from a compromise of bone density and/or bone architecture, leading to increased fracture risk. The morphologic hallmarks of this process are a reduction in the number of trabeculae, thinning of the trabeculae, and loss of trabecular connectivity¹. The main reason for this process is the estrogen deficiency after menopause, which in all mammals increases bone turnover and results in an imbalance between bone resorption and bone formation. After ovariectomy, bone loss is detected in women by about one year². Similar

changes occur within weeks of ovariectomy in rats^{3–6}. Ovariectomy induced bone loss in rats and postmenopausal osteoporosis share many characteristics; these include pathophysiological aspects as well as the skeletal response to different therapies^{7,8}. Consequently, the US Food and Drug Administration recommends the ovariectomized rat model as one means to study agents for the prevention and treatment of postmenopausal osteoporosis⁹.

Bisphosphonates, potent inhibitors of bone resorption, are candidates for preventing bone loss and have proven efficacy in a variety of clinical and animal studies^{10–15}. Although many studies have used bisphosphonates in the ovariectomized rat model, most of these were performed in younger, growing rats, and thus the results may be superimposed by the animals' growth rate. In addition, little is known about the longterm effects of this class of drugs in a bone modeling species, such as the rat, or what may be the ideal treatment schedule in estrogen depleted animals. Clinically, bisphosphonates are currently administered daily or intermittently, with varying rationales for the applied intervals. Ibandronate, a potent nitrogen-containing bisphosphonate, has proven effective in inhibiting bone resorption at considerably lower doses than other bisphosphonates in

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both rats and ovariectomized beagle dogs¹⁶⁻¹⁸. We investigated the optimal dose of ibandronate to prevent bone loss and alterations in bone architecture following cessation of ovarian function in aged rats.

In addition, since there are indications in the literature that bone protection by bisphosphonates may be a function of the total effective dose of the compound in younger rats, we also evaluated whether the effects of continuous and cyclical intermittent administration of ibandronate with different regimens that all resulted in the same total dose in aged rats would provide different results^{14,15}. This approach should also give insight to what extent the coherence concept “activation of bone turnover, followed by its depression, free time, and repetition of this regimen” — ADFR, which predicts an increase in bone mass — holds true in rats¹⁹.

MATERIALS AND METHODS

Animals and conditions. Female Wistar rats were obtained from Charles River-WIGA (Sulzfeld, Germany). The animals were 8 months of age when they entered the study. During the experiment they were kept in single cages and fed a commercial standard chow (Ssniff®, Ssniff Spezialdiäten GmbH, Soest, Germany) containing 1.1 g calcium and 0.8 g phosphorus per 100 g dry weight and were fed ad libitum, with free access to water. Their body weight was recorded every week. The experiments were approved by the local animal ethics committee.

Experimental design. Two studies were performed: a dose-ranging study and a study in which different administration schedules were applied, all resulting in the same total dose at the end of the study. Bilateral ovariectomies (Ovx) or sham surgeries (sham) were performed from the ventral approach under general anesthesia (200 mg/kg intraperitoneal hexobarbitone). Rats were assigned to the different study groups by stratified randomization according to their baseline body weight ($n = 15/\text{group}$) and treated with the bisphosphonate ibandronate or vehicle (isotonic NaCl) in different regimens. All treatments were initiated one day after surgery. At the beginning of both experiments an additional group of 15 animals were sacrificed on the day of surgery (Day 0) and used as baseline controls.

Ibandronate [1-hydroxy-3-(methylpentylamino)propylidene] bis-phosphonic acid (monosodium salt monohydrate) was dissolved in isotonic saline and the pH value 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 $\mu\text{g}/\text{kg}$ of free acid equivalents of ibandronate. The volume of administration was 2 ml/kg.

Dose dependent protocol. The rats were divided into 7 OvX groups and 7 sham groups ($n = 15/\text{group}$), and treated subcutaneously with ibandronate at daily doses of 0.1, 0.3, 1.0, 3.0, 10.0, and 30.0 $\mu\text{g}/\text{kg}$ or vehicle for 20 weeks (140 days). A 5 times higher loading dose was administered in each group as their first administration. Four additional groups of rats (2 OvX and 2 sham groups receiving 1 or 3 $\mu\text{g}/\text{kg}/\text{day}$) received no loading dose. The optimal preventive daily dose of ibandronate that inhibited development of OvX induced bone loss was found to be 1.0 $\mu\text{g}/\text{kg}/\text{day}$ in the dose dependent experiment.

Dosing schedule protocol. In the subsequent study, rats were divided into 18 different groups ($n = 15/\text{group}$) and treated with ibandronate or vehicle in different regimens (Table 1). The optimal daily dose and treatment time from the dose dependent protocol were then used in the schedule dependent arm in both OvX and sham rats. This resulted in a total dose per animal of 154 $\mu\text{g}/\text{kg}$. Additionally, this total dose was divided and administered in different cyclical intermittent regimens to OvX and sham rats over a period of 20 to 22 weeks. One schedule consisted of a one week “on” treatment with a daily dose of 2.75 $\mu\text{g}/\text{kg}/\text{day}$ followed by a therapy-free interval of

2 weeks as an “off” treatment. A total of 7 cycles plus an additional “on” treatment were applied. The third treatment schedule consisted of a one week “on” treatment with a daily dose of 4.4 $\mu\text{g}/\text{kg}/\text{day}$ followed by a therapy-free interval of 4 weeks as “off” treatment. A total of 4 cycles plus an additional “on” treatment were applied. The fourth treatment schedule consisted of a one week “on” treatment with a daily dose of 5.5 $\mu\text{g}/\text{kg}/\text{day}$ followed by a therapy-free interval of 6 weeks as “off” treatment. A total of 3 cycles plus an additional “on” treatment were applied. The same treatment schedules were applied in OvX and sham rats using one-tenth (1/10) of the doses described above. In the treatment-free intervals, the rats received subcutaneous (SC) administration of isotonic saline. Three control groups were used: one baseline group, which was sacrificed at the beginning of the study, and one sham and one OvX group, which were administered isotonic saline throughout the whole experiment. The dose in the part of the experiment where the high total dose was applied is also denoted the “optimal preventive dose,” while that where the low total dose was applied is denoted the “suboptimal dose.”

Specimen preparation. All animals were sacrificed after receiving their respective total cumulative dose, 140 to 154 days after ovariectomy, by cardiac bleeding under general anesthesia. Success of OvX was confirmed at necropsy by failure to detect ovarian tissue and by uterine atrophy indicated by the weight of the resected uteri. Animals with a uterus weight < 350 mg were regarded as not completely ovariectomized and were excluded from the whole analysis.

The right femurs were removed and cleaned of soft tissue by dermestid beetles (*Dermestes maculatus*)²⁰. This method was adapted in our laboratory and assessed to be reliable in routine use. The subsequent procedures involved the determination of radiographic density, femur length, tissue volume, dry weight, ash weight, and calcium content of the femurs. The right tibias were removed and prepared for histomorphometric analysis.

Bone analyses. Femoral radiographic densitometry. The cleaned femurs were radiographed and analyzed according to the procedure and method as described, with slight modifications²¹. In brief, the bones were radiographed together with an aluminum reference wedge. For densitometry purposes the x-ray film is positioned between the light source of a microscope and a TV camera, which produces a video 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 pixels representing the sum of brightness values of each picture point. The region of interest was the distal metaphyseal area between the 2 cortices of the femur. Cortical bone analyses were performed on the radiographs in the midshaft of the femurs. Bone diameter (B·Dm) and marrow diameter (Ma·Dm) were detected from the cortical density profile⁶. Cortical thickness (Ct·Wi) was calculated as $(B\cdot Dm - Ma\cdot Dm)/2$. The cortical thickness index (CTI) was calculated as: $CTI (\%) = (B\cdot Dm - Ma\cdot Dm)/B\cdot Dm \times 100$.

Femoral length. Femoral length was measured on the axis between the greater trochanter and the top of the external condyle using a digital caliper.

Femoral tissue volume. The femurs were submerged in distilled water and hydrated for 24 h. To ensure proper degassing, this step was performed under low vacuum in an exsiccator. After blotting with moistened blotting paper, the tissue volume of the bone (bone plus bone marrow volume) was measured by volume displacement of distilled water using a pressure transducer (TSE 2000, TSE, Bad Homburg, Germany).

Femoral dry weight. The bones were dried to a constant weight at 80°C in an incubator and then weighed when they were adapted to room temperature.

Femoral ash analysis. Bones were ashed in a muffle furnace (Heraeus M110, Heraeus, Hanau, Germany) at 600°C, and the ash was dissolved in 5 ml of 5 M HCl.

Calcium determination. For calcium determination, serum or dissolved bone ash were diluted with a lanthan nitrate solution (1% lanthan nitrate in 0.1 M HCl) for atomic absorption spectrophotometry (Perkin Elmer 2100, Perkin Elmer Corp., Ueberlingen, Germany).

Table 1. Groups in the dosing schedule protocol. Doses are expressed as free acid equivalents of ibandronate.

Group (n = 11–15)	Status	Compound	Daily Dose, $\mu\text{g/kg SC}$	Total Dose/Animal, $\mu\text{g/kg}$	Cycle on-off, weeks	Day of Sacrifice
1	—	—	—	—	—	0
2	Sham	NaCl	—	—	—	154
3	Ovx	NaCl	—	—	—	154
4	Sham	Ibandronate	0.1	15.4	Cont	154
5	Ovx	Ibandronate	0.1	15.4	Cont	154
6	Sham	Ibandronate	1.0	154	Cont	154
7	Ovx	Ibandronate	1.0	154	Cont	154
8	Sham	Ibandronate	0.275	15.4	1–2	154
9	Ovx	Ibandronate	0.275	15.4	1–2	154
10	Sham	Ibandronate	2.75	154	1–2	154
11	Ovx	Ibandronate	2.75	154	1–2	154
12	Sham	Ibandronate	0.44	15.4	1–4	147
13	Ovx	Ibandronate	0.44	15.4	1–4	147
14	Sham	Ibandronate	4.4	154	1–4	147
15	Ovx	Ibandronate	4.4	154	1–4	147
16	Sham	Ibandronate	0.55	15.4	1–6	154
17	Ovx	Ibandronate	0.55	15.4	1–6	154
18	Sham	Ibandronate	5.5	154	1–6	154
19	Ovx	Ibandronate	5.5	154	1–6	154

Ovx: ovariectomized, Cont: continuous, Sham: sham operated.

The primary endpoints for femoral bone mass were radiographic density, femoral dry weight/tissue volume, and calcium content/tissue volume. All other measures were secondary.

Histomorphometry. The excised right tibias were cleaned from the attached soft tissues and the fibula removed. A transverse saw cut was performed slightly proximal to the midshaft, and the posterior part of the tibial head was cut off in a frontal plane with a scalpel. This procedure allows 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 methylmethacrylate²². Frontal sections of the head of the tibia were cut with a precision saw and then 6 μm sections were made on a Jung microtome (model K) in a frontal plane. Sections were stained with von Kossa reaction and McNeal's tetrachrome stain. Histomorphometric analyses were performed in these sections.

The sections were analyzed with a real-color image analyzing system (CBA 8000) at 70 \times magnification. 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 mm². Cancellous tissue area, cancellous bone area, and cancellous bone perimeter were determined automatically by the analyzing system. Trabecular bone volume ($\text{Cn} - \text{BV/TV}$ as percentage), the primary histomorphometric measure, and trabecular thickness according to the plate model ($\text{Tb}\cdot\text{Th}$ in μm), trabecular number (Tb N in n/mm), and trabecular separation ($\text{Tb}\cdot\text{Sp}$ in μm) as the secondary measures were calculated, named, and abbreviated according to the standard of the American Society for Bone and Mineral Research²³. Previous investigations have shown that similar results were obtained measuring either a single field at a magnification of 70 \times or 10 fields at a magnification of 160 \times resulting in approximately the same total tissue area.

Statistics. The primary endpoints were multiplicity adjusted using the Bonferroni approach. 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 were performed²⁴. Based on an equivalence threshold of 25%, therapeutic equivalence was investigated by one sided Fieller's confidence intervals for the ratio assuming approximated Gaussian distribution as well as variance homogeneity. No multiplicity adjustment was used, simply 2 sample comparisons. The SAS program for estimation of Fieller's confidence intervals was used.

RESULTS

Effects of ibandronate on femoral bone mass and size. The radiographic density of the proximal metaphyses of the tibias, a primary endpoint, underwent a dramatic decrease after ovariectomy ($p < 0.001$). Ibandronate dose dependently inhibited this bone loss (Figure 1, Table 2). Ovariectomy induced a significant reduction in the primary endpoints, dry weight/tissue volume and calcium content/tissue volume ($p < 0.001$). Ibandronate dose dependently inhibited this bone loss, with 1 $\mu\text{g/kg/day SC}$ being the optimal dose (except for calcium content/tissue volume, which was 0.1 $\mu\text{g/kg/day}$). The optimal dose is defined as the lowest dose, which was not statistically different from sham solvent controls. The optimal dose is also the minimum equivalent dose compared to the sham solvent control, as well as the highest effective dose step, i.e., higher doses resulted in a plateau. The results were independent of whether a 5 times higher loading dose was applied or not (Table 2). Ovariectomy or the test compound did not significantly influence the tissue volume.

The cortical bone size of the femur, detected from density profiles on radiographs, was expressed by bone diameter

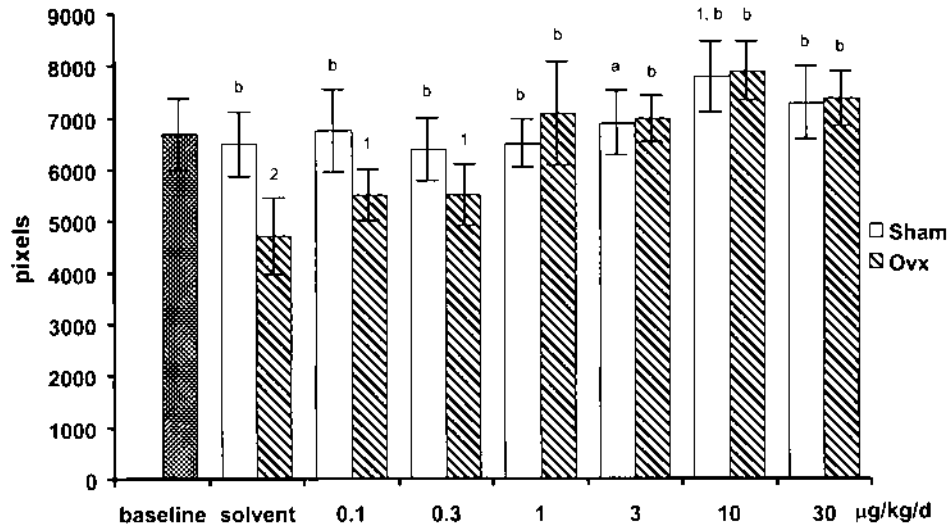


Figure 1. Radiographic density (pixels); right femurs of ovariectomized (Ovx) or sham operated (Sham) rats after 20 weeks of daily SC treatment with ibandronate (mean \pm SD; n = 12–15). A 5-times higher loading dose was administered in each group as their first administration. Significance versus sham solvent controls: ¹p \leq 0.05, ²p \leq 0.001; significance versus Ovx solvent controls: ^ap \leq 0.005, ^bp \leq 0.001.

Table 2. Results of femoral analysis and histomorphometry in the proximal metaphysis in the right tibias of ovariectomized (Ovx) or sham operated (Sham) rats after 20 weeks of daily SC treatment with ibandronate or vehicle in the dose dependent protocol. Doses are expressed as free acid equivalents of ibandronate.

Group (n = 9–15)	Status	Daily Dose, μ g/kg SC	Loading Dose, +/-*	Right Femur Dry Weight/Tissue Volume, mg/ml	Right Femur Calcium/Tissue Volume, mg/ml	Tb·N, no. \times mm ⁻¹	Right Tibia Tb·Th, μ m	Tb·Sp, μ m
1	base	—	—	1143 (37)	272 (16)	4.58 (0.51)	49.8 (4.7)	171 (26)
2	Sham	—	—	1144 (32) ^c	283 (12) ^c	3.96 (0.87) ^b	48.9 (4.7) ^c	216 (64) ^b
3	Ovx	—	—	1087 (28) ³	245 (15) ³	0.82 (0.77) ³	35.2 (16.2) ³	2144 (2270) ^c
4	Sham	0.3	—	1189 (41) ^{c,2}	294 (20) ^{c,1}	4.64 (0.98) ^c	48.3 (5.2) ^b	174 (37) ^c
5	Ovx	0.3	—	1159 (40) ^c	287 (13) ^c	3.52 (0.79) ^c	41.2 (4.6)	257 (73) ^c
6	Sham	1	—	1211 (28) ^{c,3}	308 (17) ^{c,3}	4.88 (0.99) ^{c,1}	50.0 (5.8) ^a	163 (52) ^c
7	Ovx	1	—	1238 (39) ^{c,3}	323 (19) ^{c,3}	4.35 (0.87) ^c	47.3 (7.1) ^a	192 (55) ^c
8	Sham	0.1	+	1199 (47) ^{c,3}	314 (9) ^{c,3}	4.99 (0.78) ^{c,2}	43.9 (4.6)	162 (36) ^c
9	Ovx	0.1	+	1154 (40) ^c	300 (10) ^{c,2}	2.29 (0.89) ^{c,3}	35.9 (7.0) ²	490 (284) ^c
10	Sham	0.3	+	1207 (33) ^{c,3}	318 (11) ^{c,3}	4.09 (0.91) ^c	43.0 (7.2)	218 (86) ^c
11	Ovx	0.3	+	1176 (31) ^c	305 (10) ^{c,3}	3.53 (0.86) ^c	39.1 (5.1) ¹	267 (110) ^c
12	Sham	1	+	1235 (29) ^{c,3}	318 (10) ^{c,3}	4.46 (1.07) ^c	47.5 (9.2) ^a	191 (72) ^c
13	Ovx	1	+	1228 (26) ^{c,3}	318 (13) ^{c,3}	4.40 (0.96) ^c	46.4 (9.8) ^a	193 (72) ^c
14	Sham	3	+	1194 (74) ^{c,2}	313 (22) ^{c,3}	4.92 (0.66) ^{c,1}	54.7 (16.3) ^c	152 (37) ^c
15	Ovx	3	+	1203 (25) ^{c,3}	313 (10) ^{c,3}	4.71 (0.77) ^c	47.8 (6.7) ^b	171 (49) ^c
16	Sham	10	+	1211 (36) ^{c,3}	317 (11) ^{c,3}	4.81 (0.67) ^{c,1}	58.8 (19.9) ^{c,1}	154 (41) ^c
17	Ovx	10	+	1227 (27) ^{c,3}	323 (13) ^{c,3}	5.15 (0.61) ^{c,1}	45.3 (7.2)	152 (26) ^c
18	Sham	30	+	1224 (33) ^{c,3}	324 (19) ^{c,3}	5.45 (0.91) ^{c,3}	58.4 (16.5) ^c	130 (29) ^c
19	Ovx	30	+	1258 (31) ^{c,3}	327 (11) ^{c,3}	4.74 (0.79) ^c	50.3 (6.1) ^b	166 (39) ^c

*5-times higher dose was administered on Day 1. Data are expressed as mean (SD) (n = 11–15). Statistical significance vs Ovx solvent control (group 3), multiplicity adjusted p values: ^ap < 0.05, ^bp < 0.01; ^cp < 0.001. Significance vs sham solvent control (group 2), multiplicity adjusted p values: ¹p < 0.05, ²p < 0.01, ³p < 0.001. base: baseline value.

(B·Dm) and marrow diameter (Ma·Dm) and was nearly unchanged, whereas cortical width (Ct·Wi) and cortical thickness index (CTI) were slightly but not statistically significantly reduced in Ovx solvent controls. All doses of ibandronate prevented these Ovx induced changes, and in

addition had no detrimental effects on cortical width or CTI. Femoral tissue volume and length were not influenced by either ovariectomy or by the treatment. Only the results of the baseline group were slightly below all the other groups' results (data not shown).

Effect of dosing schedule on bone mass and mineral content. All endpoints (radiographic density, dry weight/tissue volume, and ash weight/tissue volume) revealed a clearly significant effect of ovariectomy by 56%, 5%, and 12%, respectively (each $p < 0.0001$). All treatment schedules, irrespective of the total dose per schedule, were effective with respect to the Ovx solvent controls, except calcium/tissue volume with cyclic administration in suboptimal dose. For the optimal dose, all endpoints measured in the Ovx groups in all administration schedules are at least equivalent with respect to the sham solvent controls (Figure 2). Continuous and intermittent administration were equivalent for all primary endpoints, except radiographic density, which was inferior in the 1–6 cycle. For the suboptimal dose (except radiographic density and Ca content, which were at least equivalent, both continuously), all endpoints measured in the Ovx groups were inferior with respect to the sham solvent control for all administration schedules. Continuous versus cyclical intermittent administrations were equivalent for all primary endpoints.

Effects of dose on histomorphometry. Histomorphometry of the tibias was performed in the proximal metaphyses. Trabecular bone volume (Cn – BV/TV), the primary histomorphometric endpoint, decreased significantly by 80% in the solvent group after ovariectomy ($p < 0.001$). This reduction in bone mass was prevented dose dependently by ibandronate with an optimal dose of 1 $\mu\text{g/kg/day}$ (Figure 3). Higher doses resulted in a plateau, meaning that there is no further increase in bone mass. Trabecular number (Tb-N) and thickness (Tb-Th) decreased after ovariectomy, and

trabecular separation (Tb-Sp), an indicator for trabecular connectivity, was increased (Table 2). All ovariectomy induced changes were dose dependently prevented by ibandronate.

Effects of dosing schedules on histomorphometry. Similar to the first study, in the second experiment, all measures for trabecular bone mass and architecture were consistently changed by ovariectomy. Ibandronate prevented these changes optimally by the optimal total dose and suboptimally by the suboptimal total dose, providing equivalent results per total dose, both irrespective of the applied schedules (Figure 4). In particular, trabecular separation, representing the connectivity of cancellous bone, was prevented accordingly (data not shown).

Serum calcium levels were in the normal range at the end of both experiments in all groups.

DISCUSSION

Ibandronate was evaluated in the ovariectomized rat model to determine its efficacy and the optimal dose for the prevention of bone loss due to cessation of ovarian function. Primary efficacy variables were radiographic density of the distal metaphyses of the femur, femoral dry weight/tissue volume, calcium content/tissue volume, and trabecular bone volume (Cn – BV/TV) of the proximal tibia. Daily administration of ibandronate over a period of 140 days produced a dose dependent inhibition of ovariectomy induced bone loss as revealed in these variables. Comparison of doses in the Ovx solvent and sham solvent controls indicated that the optimum dose was 1 $\mu\text{g/kg/day}$ at bone sites of predomi-

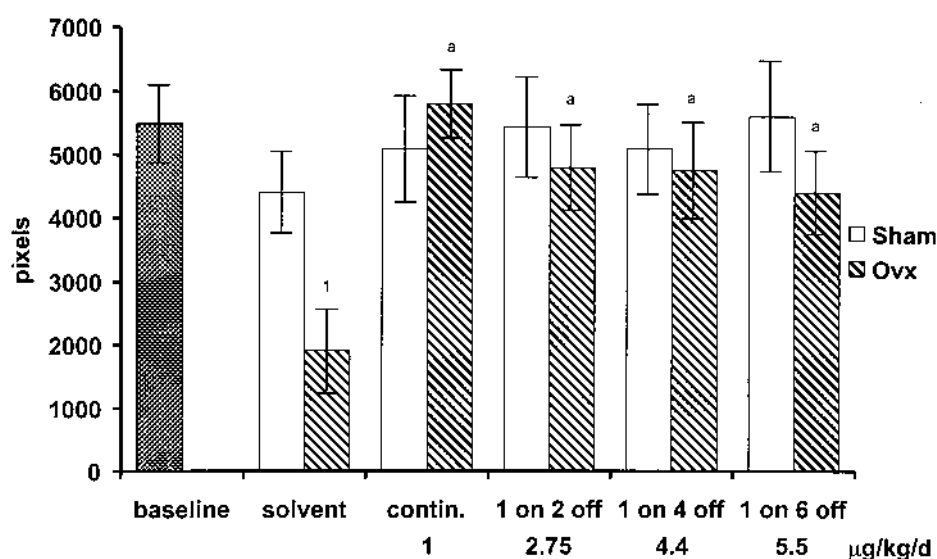


Figure 2. Radiographic density (pixels); right femurs of 54 week ovariectomized (Ovx) or sham operated (Sham) rats following different SC administration schedules (20–22 weeks) with ibandronate (total amount 154 $\mu\text{g/kg}$; mean \pm SD; $n = 11$ –15). Significance between sham solvent versus Ovx solvent controls: ¹ $p \leq 0.0001$; dose schedules versus Ovx solvent controls: ^a $p \leq 0.0001$. Results of all dosing schedules in Ovx groups are inferior to sham solvent controls, while all discontinuous regimens provide equivalent results in comparison to continuous dosing (except 1–6 cycle, which is inferior in Ovx rats).

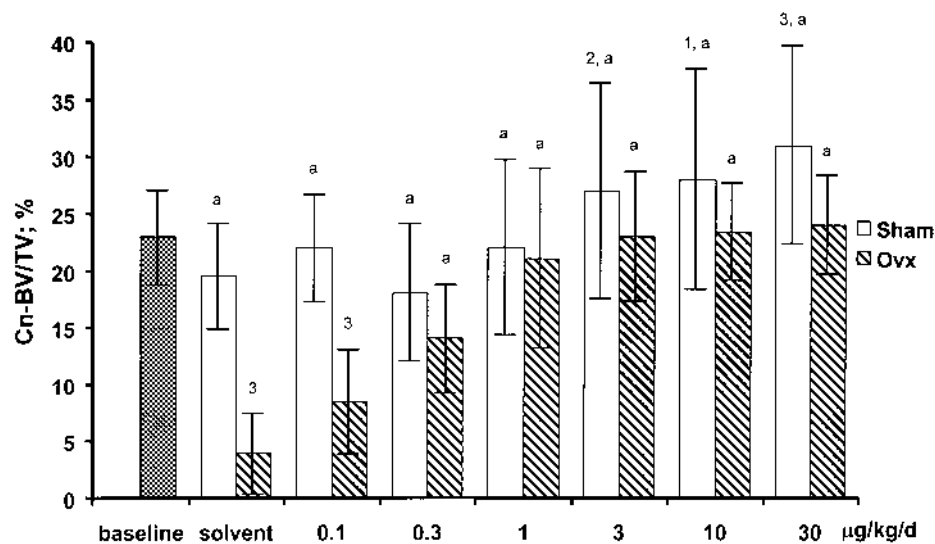


Figure 3. Bone volume per tissue volume (Cn - BV/TV; percentage) of the proximal metaphysis; right tibiae of ovariectomized (Ovx) or sham operated (Sham) rats after 20 weeks of daily SC treatment with ibandronate (mean \pm SD; n = 11–15). A 5 times higher loading dose was given in each group as their first administration. Significance versus sham solvent controls: ¹p \leq 0.005, ²p \leq 0.001, ³p \leq 0.001; significance versus OvX solvent controls: ^ap \leq 0.001.

nantly trabecular bone, which was the metaphyseal area of femurs and tibiae. Although analyses of the total bone mass (dry weight/tissue volume and calcium/tissue volume) revealed a lower optimal dose for calcium/tissue volume, the highest effective dose step and the average optimal dose always remains 1 μ g/kg/day. The difference between the more sensitive trabecular bone area and the total bone mass may be related to the obviously weaker OvX effect on the latter variable taking into consideration the lower bone turnover in cortical bone. Since half of the bone resorption antagonizing effect of a single dose of ibandronate is diminished within the first 5 days in the rat model for retinoid induced bone resorption, an immediate steady-state condition was expected to be reached by administration of a 5 times higher loading dose¹⁶. However, in our study the loading dose, which corresponded to < 3% of the total dose and thus is negligible with respect to the total dose, had no effect on the results.

Once the optimum dose was determined, a second part of the study was conducted to determine whether the same total dose of ibandronate per animal, administered daily or cyclically intermittently over a period of 5 months, resulted in different efficacy in the prevention of bone loss. The time of the therapy-free intervals was derived from the 2–3 weeks' duration of the effect of a single dose of ibandronate in a model of retinoid stimulated bone destruction and from the bone remodeling time of rats¹⁶. Taking into account the age dependent and ovariectomy dependent bone turnover rates in rats, the bone remodeling time (sigma) in ovariectomized rats can be regarded as about 5–6 times less than that of humans^{3,5,25,26}. Since the sigma in humans ranges from about 80 to 120 days, the sigma in rats is consequently

calculated to be about 13–24 days. Thus, the therapy-free intervals in our study roughly reflect 1, 2, and 3 sigma times of rats. All measures for the primary endpoints, measured with different and independent analytical procedures, expressed similar and consistent reactions regarding bone loss and its prevention. This indicates that within 2–3 sigma times all the investigated variables reveal that the total dose of ibandronate, and not the treatment schedule, seems to be important for efficacy.

Because doses above the optimal dose of 1 μ g/kg/day result in a plateau, i.e., that no further increase in bone mass can be achieved, it was not likely to detect a potential superiority of the intermittent treatment schedules in comparison to continuous dosing. Thus an additional suboptimal dose (0.1 μ g/kg/day) was administered. However, with the exception of radiographic density and calcium content, which were at least equivalent, both continuously, all endpoints measured in the OvX groups for the suboptimal dose were inferior with respect to the sham solvent control for all administration schedules. Therefore, the intermittent schedules do not demonstrate superiority to the continuous dosing regimens, and these results support the importance of the total dose concept. This clearly argues against the hypothesis that the maximal effect on bone turnover is determined by the bisphosphonate concentration at the bone surface, and not by the cumulative dose, as described for early experiments with pamidronate, because in our study the doses given during the dosing interval are higher than the corresponding continuous dose²⁷. That higher cumulative doses result in a plateau with respect to bone mass is in accord with results from aged ovariectomized beagle dogs, or aged rats in which bone turnover was stimulated by the

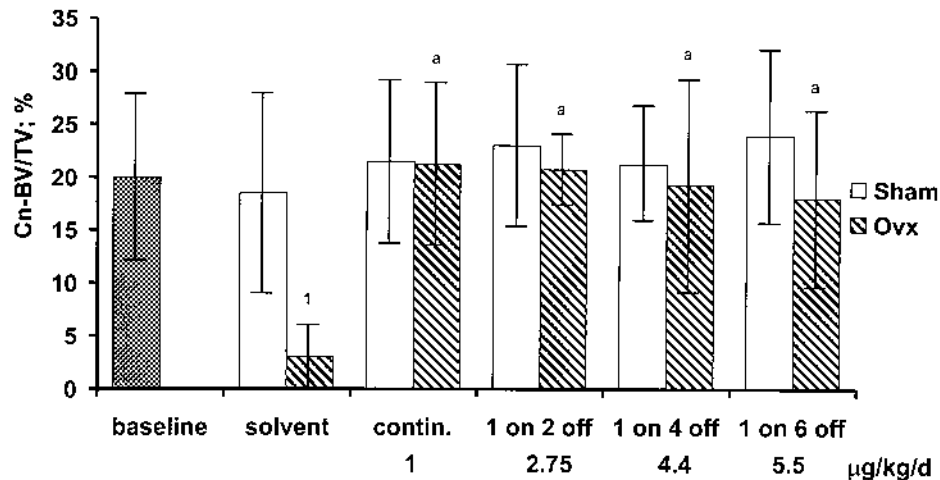


Figure 4A. Effect of optimal dose (total amount 154 µg/kg) on bone volume per tissue volume (Cn – BV/TV; percentage) of the proximal metaphysis; right tibias of 54-week-old ovariectomized (Ovx) or sham operated (Sham) rats following different SC dosing schedules (20–22 weeks) with ibandronate (mean ± SD, n = 9–15). Significance between sham solvent versus OvX solvent controls: ¹p ≤ 0.0001; dose schedules versus OvX solvent controls: ^ap ≤ 0.0001. In OvX groups, the results of all dosing schedules are equivalent to sham solvent controls (optimal dose) and all discontinuous regimens provide equivalent results in comparison to continuous dosing.

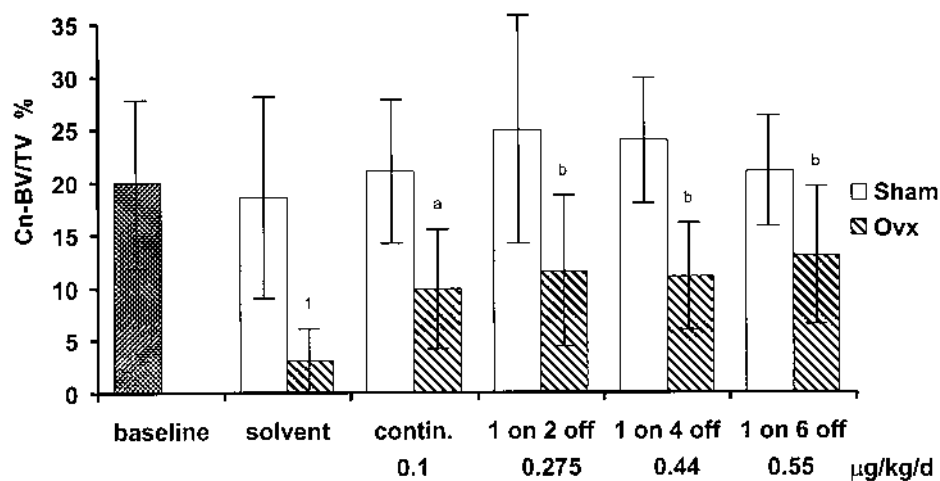


Figure 4B. Effect of suboptimal dose (total amount 15.4 µg/kg) on bone volume per tissue volume (Cn – BV/TV; percentage) of the proximal metaphysis; right tibias of 54-week-old ovariectomized (Ovx) or sham operated (Sham) rats following different SC dosing schedules (20–22 weeks) with ibandronate (mean ± SD, n = 10–15). Significance between sham solvent versus OvX solvent controls: ¹p ≤ 0.0001; dose schedules versus OvX controls: ^ap ≤ 0.05, ^bp ≤ 0.005. In OvX groups, the results of all dosing schedules are inferior to sham solvent controls (suboptimal dose), while all discontinuous regimens provide equivalent results in comparison to continuous dosing.

osteolytic Walker carcinosarcoma 256^{17,28}. In both systems ibandronate, even in high doses, prevented bone destruction only up to values of controls without further increase in bone mass and density. This suggests that, in adult bone, ibandronate only inhibits accelerated (stimulated) bone loss up to the normal physiological range of controls. According to Frost's ADFR concept¹⁹, it was expected that one of the cycles should result in a higher bone mass than continuous dosing. However, this hypothesis could not be verified in our study.

Bone turnover in mature rats is regarded to be 3–5 times higher compared to humans and decreases with age. Thus, the bone turnover of aged rats used in this study (age 8 mo at ovariectomy and 13 mo at termination of study) closely reflects that of adult human bone²⁶. The 5 month treatment period corresponds at least to about 15 to 25 months of treatment in humans if mature intact rats were used. With ovariectomy, bone turnover in rats is increased by a factor of up to 7, although there is an age related decrease in skeletal cellular based metabolic activity in non-ovariectomized

aged rats^{5,25}. In total, these counteracting effects (aging versus ovariectomy) result in increased turnover even in ovariectomized aged rats. Considering the age of the animals at the beginning and the end of the study with the age dependent changes in metabolic activity plus the ovariectomy induced increase in bone turnover, the treatment duration here of 5 months would correspond to a range of 30 to 75 months of treatment in humans.

While bone mass remains the primary determinant of the mechanical strength of bone, bone quality, including biomechanical properties, 2 and 3 dimensional bone structure, histomorphometric analyses, repair healing, and other factors, is also significant^{29,32}. To understand the effect of therapeutic agents on bone it is necessary to analyze not only bone mass (e.g., radiographic density, femoral dry weight/tissue volume, calcium content/tissue volume, and trabecular bone volume), but also bone quality. Since a 3 point bending test of the femoral midshaft did not show significant differences in mechanical properties (ultimate strength, energy to failure, energy absorbed, and stiffness), either between the sham solvent controls and the Ovx solvent controls or any other treatment group, the duration of estrogen depletion seems to be too short to reveal biomechanical differences in such old rats, a result consistent with others' findings^{33,34}. Thus, trabecular separation, as one of the most important histomorphometric indicators of bone quality, was analyzed as well. This variable is a derived structural index defined as the distance between edges rather than between midpoints²³. It is calculated according to the parallel plate model to yield an estimate of the mean distance across the marrow cavity and represents the connectivity of cancellous bone²³. In our study, trabecular separation was preserved by ibandronate, and paralleled bone mass.

In summary, ibandronate prevented ovariectomy induced bone loss in aged rats and maintained bone quality in a dose dependent manner in very low doses, 1 µg/kg/day being the optimal dose. The study further confirms the efficacy and potency of ibandronate. In addition, within 2 to 3 bone remodeling times (sigma), the total dose of ibandronate administered and not the treatment schedule seems to be important for efficacy. This suggests that ibandronate can be administered in a cyclical intermittent fashion with equivalent efficacy, raising the possibility of efficacious alternative dosing schedules. That the 1–6 cycle was of borderline equivalence in some endpoints indicates that the interval time may not be extrapolated longer. First clinical results in postmenopausal women treated with ibandronate (2 month dose-free intervals) or alendronate (weekly intervals) have confirmed that the total effective dose and not the schedule seems to determine the results³⁵⁻³⁷. However, the question whether the dosing interval depends on the bone remodeling time or on the real drug exposure time remains unanswered, and should be investigated in more detail (e.g., dynamic

histomorphometry, determination of microdistribution of drug in bone, or more sophisticated intervals).

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