CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-455

PHARMACOLOGY REVIEW

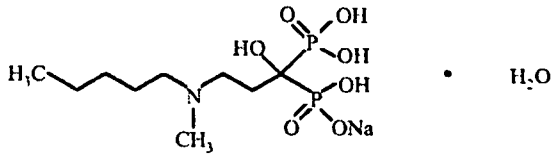
PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number:	**21,455**
Compound:	**Ibandronate sodium**
Submission date:	July 15, 2002
Sequence number:	000
Type of submission:	N
Information to Sponsor:	Yes (x) (Labeling comments)
Sponsor:	Hoffman La Roche, NJ, USA (Previous Sponsor: Boehringer Mannheim)
Manufacturer for drug substance:	F.Hoffman-LaRoche Ltd., Basel, Switzerland
Reviewer name:	Gemma Kuijpers
Division name:	Division of Metabolic and Endocrine Drug Products
HFD #:	510
Review completion date:	April 16, 2003
Review number:	#1

Drug:

Trade name:	Boniva®
Generic name:	Ibandronate sodium
Code name:	BM21.09955 Na H_2O
Chemical name:	[1-hydroxy-3-(methylpentylamino) propylidene] bisphosphonic acid, monosodium salt, monohydrate
CAS registry number:	114084-78-5
Molecular formula:	$C_9H_{22}NO_7P_2Na.H_2O$
Molecular weight:	Mw 359.2 (sodium salt), 319.2 (free acid)
Conversions:	1 mg P= 5.14 mg free acid; 1 g free acid equivalent = 1.125 g ibandronate monosodium salt, monohydrate)

Structure:



Relevant INDs/NDAs/DMFs:

Drug class:	Bisphosphonate (bone resorption inhibitor)
Indication:	Treatment and prevention of postmenopausal osteoporosis
Clinical formulation:	Film-coated tablet, containing 2.813 mg ibandronate monosodum hydrate (2.5 mg free acid), lactose, povidone, cellulose, crospovidone, stearic acid, silicon dioxide, water
Route of administration:	Oral (tablet)

Proposed use:	2.5 mg tablet, orally, once daily
Pivotal clinical study:	MF4411 (oral treatment study, 2.5 mg daily, or 20 mg every other day, for 12 doses at the start of every 3-month cycle; 3-year fracture and BMD study)
Disclaimer:	Tables and Figures from the electronic NDA submission have been copied for use in this review

APPEARS THIS WAY
ON ORIGINAL

Executive Summary

I. Recommendations

A. Recommendation on Approvability
Approval (AP)

Based on the results of nonclinical pharmacology and toxicology studies, Pharmacology/ Toxicology recommends approval of the NDA for ibandronate sodium (Boniva®) for the indication of treatment and prevention of postmenopausal osteoporosis.

B. Recommendation for Nonclinical Studies
No additional nonclinical studies are required.

C. Recommendations on Labeling
Recommmended labeling changes have been appended to this Review.

II. Summary of Nonclinical Findings

The current application (NDA #21-455) is for the use of ibandronate in the treatment and prevention of postmenopausal osteoporosis. The intended dose is a 2.5 mg tablet, taken once daily, with a glass of water, at least 60 minutes before breakfast.

The mechanism of action of ibandronate, a nitrogen-containing bisphosphonate with high affinity for hydroxyapatite, is inhibition of osteoclast-mediated bone resorption. This inhibition indirectly suppresses bone formation and ultimately leads to an inhibition of bone turnover. In postmenopausal women bone loss is accelerated due to increased activation of basic multicellular units (BMU's) and a negative balance between bone formation and resorption in each remodeling cycle. Ibandronate and other bisphosphonates prevent or reverse this bone loss because they reduce the size of the remodeling space at the tissue level, and they increase the degree of mineralization and increase focal bone balance in each newly formed bone unit. This results in an increase in bone volume and bone mass as reflected by an increase in bone mineral density (BMD).

Single dose safety pharmacology studies in mice, rats or dogs showed that ibandronate did not affect CNS, gastrointestinal, cardiovascular or renal function at doses of 100-2000 times the intended human 2.5 mg/day dose, based on body surface area comparison (mg/m2). Ibandronate did not affect in vitro hERG K+ channel currents at >10,000x the human Cmax at the oral 2.5 mg/day dose.

Nonclinical efficacy pharmacology studies were carried out to investigate the effect of ibandronate in animal models of nonstimulated or stimulated bone turnover. Data from rat studies showed that ibandronate inhibited bone resorption, and is approximately 10x, 50-100x, and 500x more potent than alendronate, pamidronate and clodronate, respectively. In the intact growing rat, ibandronate had a prolonged inhibitory effect and increased cancellous bone volume and density with an optimal dose ≥ 0.001 mg/kg. Mineralization was not affected at doses 1000-5000x times higher than the doses optimally inhibiting bone resorption and turnover, based on data from the intact growing rat and the aged ovariectomized rat.

Long-term pharmacology studies on the effects of ibandronate on bone quality in estrogen-deficient animals were the most relevant studies for the postmenopausal osteoporosis indication. In rats and monkeys, continuous or intermittent dosing for 12 to 16 months prevented the loss of bone induced by ovariectomy (OVX) through inhibition of bone resorption and turnover. At optimal dose levels vertebral bone strength was preserved in parallel with BMD, histologic bone volume and trabecular structure. In the OVX rat, ibandronate also protected femoral cortical bone BMD and strength. In OVX monkeys, ibandronate fully preserved BMD at the ulna and femoral neck,

but bone strength at those sites was not significantly protected. This may have been due to relative inefficacy of ibandronate to protect against cortical thinning or other structural effects resulting from estrogen deficiency not reflected by BMD, or to methodological variability. In the monkey study, significant positive correlations between BMD and strength of vertebrae and femoral neck and between BMC and strength of ulna diaphysis were demonstrated. There was no indication that the relation between BMD and bone strength was eliminated when dosing was carried out in intermittent fashion. Although the efficacy of ibandronate to preserve bone strength at cortical or mixed cortical/cancellous sites appeared to be limited, the animal data do not predict an adverse effect on bone strength. There were no deleterious effects on bone histology or mineralization in all animal species tested.

In rats and dogs, ibandronate was poorly absorbed after oral administration (1% of dose or less) and food markedly suppressed oral bioavailability. After oral administration, Tmax is 0.5-1 h and compound is rapidly cleared (within hours) from plasma by uptake in bone and renal excretion. $T_{1/2}$ (oral or i.v.) is approximately 56 hours in the dog. Bioavailability after s.c. administration is 100% in the rat. Uptake in the bone compartment is reflected by a high volume of distribution (10L/kg in dogs). Approximately 40-50% of an absorbed dose is taken up and stored by bone, and approximately 50% is eliminated unchanged via the kidney. Uptake in bone is linear and related to total dose rather than treatment schedule. Bone levels attained after even a single dose remain high for several months, and $T_{1/2}$ for bone tissue in the rat is 400-500 days. Ibandronate is accumulated in spleen, kidney and liver tissue, but does not cross the blood-brain barrier. In pregnant rats, ibandronate is distributed to the placenta and the fetus, and in lactating rats it is excreted in the milk. Binding to plasma proteins is similar for rat, dog and human (80-99%). There is no evidence for metabolism in rats or dogs, and no evidence for hepatic or renal drug-drug interaction.

In oral toxicity studies of up to 12 months duration in rats and dogs, target organs identified were kidney, liver, lung, esophagus, stomach, thymus and testes. Renal tubular integrity was particularly sensitive to ibandronate in rats and dogs. Safety margins were calculated as the ratio between the animal AUC at the NOAEL and the human AUC at the 2.5 mg oral dose. Safety margins for kidney toxicity were ≥4-7x and ≥27-50x based on data from rats and dogs, respectively. Safety margins for GI toxicity were 27-50x (vomiting, emaciation) and 100-200x (esophagitis and stomach irritation) based on data from dogs, and 34-62x (stomach irritation and hemorrhage) based on data from rats. Liver (rat, dog), and lung, thymus and testicular (dog) toxicities were associated with safety margins ≥25x. Pharmacodynamic effects of ibandronate on bone were observed in all rat and dog toxicity studies at low human exposure multiples. This lead to secondary effects of decreased bone marrow space and increased extramedullary hematopoiesis and at higher doses to anemia. Therapeutic margins calculated as the ratio between the AUC at the NOAEL for kidney toxicity and the AUC at the optimal pharmacologically effective dose were 8x-16x for rats and dogs.

Ibandronate had no mutagenic or clastogenic potential, as demonstrated by negative results in in vitro and in vivo genotoxicity assays. In a carcinogenicity study mice dosed via the drinking water for 90 weeks, an increase in the incidence of adrenal subcapsular adenoma was observed at high human exposure multiples (220-400x). Carcinogenicity studies in rats and mice dosed via oral gavage for 18-24 months did not show an increased incidence of tumors. Exposure multiples achieved in the oral gavage studies were 12x-7x for male and female rats, and 475x-70x for male and female mice, respectively.

In reproductive toxicity studies in the rat, ibandronate caused severe maternal dystocia and maternal and fetal periparturient mortality at doses in the range of human exposure, given either before or during delivery. This effect has been observed with other bisphosphonates and is believed to be result of hypocalcemia due to suppression of skeletal calcium mobilization required for delivery in the rat. Effects on fertility and a fetal kidney anomaly (RPU syndrome) were observed at relatively high human exposure multiples (45x and 30x). An effect of treatment on a pup behavioral developmental parameter (cliff avoidance) was observed when dams were dosed from 14 days before gestation at high human exposure multiples (45x). In the rabbit no

teratogenic effects were identified at human dose (mg/m^2) multiples of 8x-170x. In rats, ibandronate is transferred across the placenta and excreted in milk.

In conclusion, the data from pharmacology and toxicology studies suggest adequate safety of long term use of ibandronate for the treatment or prevention of osteoporosis in postmenopausal women at an oral dose of 2.5 mg/day.

III. Administrative

A. Reviewer signature: Gemma Kuijpers /S/

B. Supervisor signature: Concurrence - _____

Non-Concurrence - /S/
(see memo attached)

C. CC: list:
NDA Arch
HFD-510
HFD-510/Kuijpers/Davis-Bruno/Hedin

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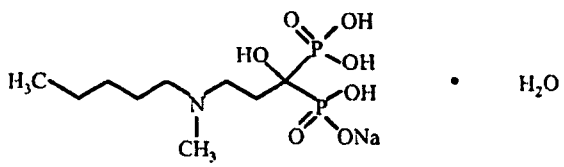
PHARMACOLOGY/TOXICOLOGY REVIEW

INTRODUCTION

Ibandronate (BM 21.0955.Na.H2O) is a bisphosphonate that targets bone and inhibits osteoclasts. The substance was discovered and developed by Boehringer Mannheim GmbH, Germany. This NDA is for the oral use of the compound in the treatment and prevention of osteoporosis in postmenopausal women. BM 21.0955.Na.H_2O (ibandronate) is the "laboratory code" for [1-hydroxy-3- (methylpentylamino) propylidene] bisphosphonic acid, monosodium salt, monohydrate (CAS nomenclature). The approved USAN is ibandronate sodium. The compound has a core P-C-P bond with a nitrogen-containing side chain:



During the development of ibandronate, the basis of dose calculations and the nomenclature of the drug changed. However, the dry substance was ibandronate monosodium salt, monohydrate in all cases. In toxicology studies, the doses of ibandronate are expressed as either weighed drug substance (WDS; ibandronate monosodium salt, monohydrate) or free acid equivalents (FAE; ibandronic acid). Ibandronic acid is the active ingredient. The conversion factor in the studies ranges from 1.125 to 1.15 (1g free acid equivalent = 1.125-1.15g monosodium salt). In clinical studies, doses are expressed as free acid equivalents.

The proposed oral dose for treatment and prevention of postmenopausal osteoporosis is 2.5 mg per day. The pivotal Phase 3 clinical study was a multicenter, randomized, placebo-controlled Phase 3 fracture study performed in North America and Europe. The doses used were either placebo, 2.5 mg/day continuously, or 20 mg given every other day for 12 doses at the beginning of every 3 months, for a total of 3 years (Study MF4411). Sponsor proposes to market only the 2.5 mg/day oral dose. Due to poor oral absorption (1% of dose) and the inhibition of absorption by food or milk, dosing is prescribed in the morning, with 6 to 8 oz of water, at least 1 hour before the first food or drink. Patients are advised to remain upright. Absorption is rapid and maximum plasma levels are obtained with 0.5-2 hours.

To demonstrate the potential efficacy, and to support the safety of ibandronate in humans, *in vitro* and *in vivo* nonclinical pharmacology, toxicology, and pharmacokinetic (ADME) studies were conducted by or for Boehringer Mannheim. All toxicology studies with ibandronate were conducted according to Good Laboratory Practice (GLP). The Tables of protocol summaries and results from pharmacology, toxicology, and ADME studies provided in the NDA indicate the GLP status of each study.

Ibandronate is currently marketed actively in 17 countries for the treatment of hypercalcemia of malignancy.

BACKGROUND

Ibandronate belongs to the class of bisphosphonates, which are compounds taken up in bone preferentially where they inhibit osteoclastic bone resorption in a direct and/or indirect manner. The pharmacologic action of the bisphosphonate ibandronic acid is based on binding of the compound to the bone matrix and bone cells (osteoblasts and osteoclasts). This results in an inhibition of osteoclastic bone resorption and the coupled process of bone formation. The effects on osteoclastic bone resorption are thought to be mainly indirect through cellular actions of the

bisphosphonate on the osteoblast. Bisphosphonates are known to act at the molecular, cellular and tissue level. N-substituted compounds such as ibandronate have also been reported to inhibit the mevalonate pathway in the osteoclast, inhibiting normal osteoclast function and reducing the osteoclast's lifespan.
In postmenopausal osteoporosis, bone turnover is generally accelerated due to estrogen deficiency. This causes a decrease in bone mass because of the increase in skeletal remodeling space. Postmenopausal decreases in bone mass are also believed to result from a negative bone balance (resorption>formation) in each microscopic bone remodeling unit (BRU) leading to microarchitectural deterioration of bone tissue. These events lead to a macroscopic decrease in bone mineral density (BMD), which is inversely related to an increase in fracture risk. This has been demonstrated most convincingly for the vertebrae/spine.

Maintenance or increase of bone mass (BMD) with a bisphosphonate is believed to result from both a reduction in remodeling space due to inhibition of the coupled processes of resorption and formation (bone turnover), and a reversal of the negative bone balance. The latter may occur as a consequence of increased bone formation and mineralization accompanying the bisphosphonate-induced decrease in activation frequency and increase in life time of the BRU, or as a result of other direct positive effects on the bone-forming osteoblast. Bisphosphonates can reduce the risk of osteoporotic vertebral and appendicular skeletal fractures in postmenopausal women.
Currently marketed bisphosphonates for the osteoporosis indication in the US are alendronate and risedronate (daily or weekly dosing). Bisphosphonates marketed for other indications are pamidronate, etidronate, zoledronate, tiludronate. Other compounds used for osteoporosis prevention and treatment are estrogen, SERMs, calcitonin, and PTH. Only the latter is an anabolic agent whose action is based on stimulation of new bone formation.

Known adverse effects of bisphosphonates are GI and renal events. The renal toxicity is believed to be related to the renal excretion of these compounds. GI events (esophageal, gastric, intestinal irritation, ulceration, perforation) have been observed with other bisphosphonates. The mechanism of GI toxicity is unclear. In animal studies, GI toxicity has been observed with several bisphosphonates. However, IV dosing can also cause adverse GI events.

PHARMACOLOGY/TOXICOLOGY PROGRAM

The nonclinical pharmacology/toxciology program was conducted using relevant animal models and drug was administered i.v., p.o., s.c. and by other routes. Since the compound is highly polar it is absorbed poorly. I.v. and s.c. administration were used in the majority of pharmacology studies. In the rat these routes have comparable bioavailability. Toxicology studies (toxicity, reproductive toxicity, genotoxicity, carcinogenicity) were carried out using both oral and i.v. routes. ADME studies were carried out to determined pharmacokinetics and bioavailability, absorption, distribution, excretion, metabolism, protein binding and drug interactions. Compound was measured using radioactive labeling, [redacted] Toxicokinetic assessments of non-radioactive compound in plasma were part of several toxicity studies. Local tolerance studies evaluating i.v., s.c., p.v., and i.a. routes were carried out in rabbits, rats and guinea pigs. These studies have not been reviewed for this NDA, since the proposed clinical administration route is oral (2.5 mg tablet).
When relating pharmacodynamic skeletal effects of the compound to plasma levels it needs to be kept in mind that the drug exerts its intended action by binding to the target tissue (bone and bone cells that adhere to bone). Thus, plasma levels are not direct determinants of the pharmacologic action. This is reflected in the (pre)clinical finding that a single dose administration can be effective for several months. However, with regard to non-skeletal toxicities as well as for the purpose of species comparison, systemic exposure as measured by plasma levels (C_{max}, AUC) is an adequate parameter to relate dose to adverse effect.

I. PHARMACOLOGY

Preclinical pharmacology studies were submitted to the NDA to address the efficacy and safety of ibandronate. Pharmacologic activity was evaluated in a wide variety of animal models and in some *in vitro* studies. Effects of ibandronate on bone resorption and bone structure and/or quality were assessed in estrogen-deficient rats, dogs, and monkeys. Bone studies were carried out using the iv or sc administration routes, since the compound was originally developed for intermittent iv administration. Other pharmacology studies included in vitro studies, and safety pharmacology studies.

As recommended by the FDA Guidelines for the Evaluation of Preclinical and Clinical Agents used in the Prevention or Treatment of Postmenopausal Osteoporosis, long term studies in ovariectomized animals were carried out in rats (s.c.) and in monkeys (i.v.). Various techniques were used to provide information on bone efficacy and safety of treatment (bone mass by DEXA or QCT, bone architecture by static and dynamic histomorphometry, and bone strength by mechanical testing). Since the monkey study is considered the most clinically relevant study it is discussed in detail in this NDA review.

Primary pharmacologic activity

In the primary pharmacology studies doses are expressed as mg P(phosphorus)/kg, as active ingredient (ibandronate sodium, WDS) or free acid equivalent (FAE).

Table 1 Conversion Table for mg Drug, mg Free Acid Equivalent, and mg Phosphorus for Four Bisphosphonates

Drug	MW[a]	MW[a] of Free Acid	1 mg P[b] = x mg drug (MW:62)	1 mg P[b] = x mg free acid	1 mg drug = x mg P[b]	1 mg drug = x mg free acid	1 mg free acid = x mg drug
Clodronate	360.9	244 9	5 82	3 95	0 17	0 678	1 474
Pamidronate	257 0	235 0	4.14	3 79	0 24	0 914	1.094
Alendronate	271 0	249 0	4.37	4 01	0 22	0.919	1.088
Ibandronate	359 2	319.2	5 79	5.14	0 19	0 888	1 125

[a] MW = molecular weight; [b] P = phosphorus
Clodronate (Cl_2MBP): dichloromethylene bisphosphonic acid, disodium salt, tetrahydrate
Pamidronate (AHPrBP): 3-amino-1-hydroxypropylidene bisphosphonic acid, monosodium salt
Alendronate (AHBuBP): 4-amino-1-hydroxybutylidene bisphosphonic acid, monosodium salt
Ibandronate (BM 21.0955 $Na\text{-}H_2O$): [1-hydroxy-3-(methylpentylamino)propylidene] bisphosphonic acid, monosodium salt, monohydrate.
NOTE: Molecular weight depends on water and sodium content; data for mg P and mg free acid are not affected.

Pharmacology studies were carried out in different animal models to demonstrate the efficacy of the compound to inhibit bone resorption or bone turnover. Part of these studies were reviewed when the original IND was submitted, as non-GLP data (Ron Steigerwalt, Review December 16, 1994, Study Nrs. D1-D11, Addendum to Original IND review).

Effects on retinoid-induced bone resorption

In the thyroparathyroidectomized (TPTX) rat, retinoid-induced hypercalcemia was blocked by ibandronate administered by i.v., s.c. or p.o. routes. Ibandronate was more potent than other bisphosphonates and its ED50 was 0.0007 mg P/kg.(D1). Thus, ibandronate appears to be 5-10x more potent than alendronate.

Table 2 Comparison of the Effective Doses of Four Bisphosphonates Administered s.c. to TPTX Rats

Bisphosphonate	ED_{50} (mg P/kg)	Potency in Comparison to Clodronate	ED_{100} (mg P/kg)	Potency in Comparison to Clodronate
Clodronate	0 3	1	1 0	1
Pamidronate	0.03	10	0 1	10
Alendronate	0 003	100	0 02	50
Ibandronate	0 0007	400	0.002	500

In the TPTX rat model, i.v. and s.c. doses appeared equally effective and the effect was related to total dose rather than dose regimen (within limits) or parenteral route (D3). Experiments with the TPTX rat model showed that a single sc injection (0.003-0.01 mg P/kg) has sustained inhibitory effects on bone resorption (D4). A biologic half life of 5 days was determined. Oral doses appeared ca. 100 times less effective than sc doses suggesting a 1% bioavailability (D2).

Effects in tumor-related bone loss

Malignancy is often associated with increased bone resorption and hypercalcemia. These events result from the invasion and lysis of bone by tumor cells or from the production of factors (eg PTHrP) by tumor cells that increase osteoclastic bone resorption and/or renal calcium reabsorption. Ibandronate (dose range 0.001 to 0.01 mg P/kg) inhibits tumor-related bone loss and hypercalcemia (D7).

Effects on non-stimulated bone turnover

Some bisphosphonates can inhibit mineralization, ie, calcification in bone at lower doses than in cartilage. The woven bone in primary spongiosa is a very sensitive indicator of mineralization delay. Primary spongiosa is the bone that is deposited initially as a network of immature (woven) trabeculae in the growth plate. This primary spongiosa is either replaced by secondary bone, or removed to form bone marrow or converted into primary cortical bone by filling of spaces between the trabeculae. This bone normally calcifies so rapidly that osteoid seams are barely detectable, but they appear upon mineralization delay.

In the growing male Wistar rat (s.c. doses 0.01, 0.1, 1 mg P/kg for 7 days) (Muhlbauer et al, JBMR, 1991, Vol. 6, 1003-101) all doses were effective in inhibiting bone resorption. Effectiveness was histologically evident as an inhibition of resorption of calcified cartilage septa leading to an increased metaphyseal bone density in the tibia. The highest s.c. dose of 1 mg P/kg did not induce osteoid seams in the primary spongiosa or widening of osteoid seams lining the endosteal surface of metaphyseal cortical bone. There was also no delay or inhibition of cartilage calcification at this dose. Compound increased tibial BV/TV at all doses, and increased Oc.N and Oc.S/BS (2x) at 0.01 and 0.1, but reduced osteoclast # and surface at 1 mg P/kg. Thus, at doses effective in inhibiting bone resorption osteoclast recruitment is not inhibited.
Conclusion of the study by Muhlbauer et al (1991) was that mineralization was not affected by ibandronate at doses up to 1 mg P/kg neither in woven or lamellar bone nor in cartilage.

In a study in growing male Wistar rats (Schenk assay) at a range of sc doses from 0.0003 to 10 mg P/kg, for 7 days (part of the data reported by Muhlbauer et al, 1991), ibandronate inhibited bone resorption, increased cancellous bone volume, and increased number of osteoclasts at doses of 0.001 mg P/kg or more. The highest dose of 10 mg P/kg was lethal and the data at that dose were not considered. Ibandronate was 10, 100, 600 times more potent than alendronate, pamidronate, clodronate. At the 1 mg P/kg dose, there was no inhibition of mineralization or longitudinal bone growth as measured in the tibial metaphysis (Fleisch H, BM Report D8; Muhlbauer et al, 1991). This was 1000x the anti-resorptive dose in this model.

Data from ^{45}Ca kinetic studies in the rat showed that endogenous bone resorption was inhibited (more than formation) by ibandronate at doses ranging from 0.0001 to 0.01 mg P/kg sc (similarly when given daily for 10 days, or as a single dose). The effects persisted for 1 month (D11).

In a 104-week term carcinogenicity study with 3,7,15 mg/kg/day (oral) ibandronate increased BMD, compressive strength and stiffness in vertebral and femoral bone (D17). Correlation between BMD and maximal strength was significant for vertebral bodies, but not for long bones.

In adult dogs, long term s.c. administration for 34-36 weeks had no effect on bone quality in terms of histomorphometry, fracture healing and bone strength, regardless of treatment schedule (0.001 mg/kg/day, or 0.003 mg/kg/day for 7 days followed by 14 days off-treatment for 11 cycles, or 0.006 mg/kg/day for 7 days followed by 42 days off-treatment for 5 cycles; total dose was 0.252 mg/kg/animal in all cases) (D12, D20, D21).

In dogs treated for 12 months orally (toxicology study D16), doses up to 10 mg/kg/day had no adverse effects on mechanical properties of vertebral cancellous bone or humeral cortical bone or on density of vertebral bone, immediately after treatment or after 6-mo recovery.

In retired breeder rats, 20 weeks of s.c. administration of ibandronate at 0.001 to 0.03 mg/kg/day caused an increase in bone mass at doses ≥0.01 mg/kg/day, which was reversed after 20 weeks of recovery (D28). In intact rats, alendronate and ibandronate at equipotent doses were equally effective in preventing age-dependent changes in bone mass and structure (D29).

Effects on estrogen-deficiency bone loss

STUDIES

Species	Report	Study description	Prevention/ Treatment	Publication
MONKEY	D30	16 month study in OVX cynomolgus monkeys. Treatment started at day OVX, with i.v. doses of 10, 30, 150 ug/kg, once monthly.	Prevention	Smith et al (2003), Bone Vol. 2, pp45-55
	D31	Micro-tomographical imaging and biomechanical testing of bones from cynomolgus monkeys treated for 16 months (Study D30)		Muller, R. et al (2001), 47th Annual Meeting of Orthop. Res. Soc., February 2001, CA
RAT	D14	20-week study in aged Wistar rats. Treatment started 1 day after OVX. Doses 0 0001, 0 0003, 0 001, 0 003, 0.01, 0.03 mg/kg/day s c.	Prevention	
	D15 D22	20-22 week treatment in aged Wistar rats, starting after OVX. Doses 0.0001, 0.001 mg/kg/day s.c. or higher intermittent doses resulting in similar cumulative doses	Prevention	
	D25 D26 D27	12-month treatment of aged Wistar rats with 0.0002, 0.001, 0.005, 0.025 mg/kg/day, or 0.025 and 0.125 mg/kg every 25 days, starting 10 weeks after OVX	Treatment	Bauss, F et al (2002) J.Rheumatology
DOG	D9	4-week study in OHX dog, doses 0.0001, 0 0003, 0.001, 0.01, 0.1 mg/kg were given s.c. for 6 out of 7 days per week.	Prevention	Monier-Faugere et al (1993), JBMR 8 (11), 1345-1355
	D13	16-month study with 12-month treatment starting 4 months after OHX in dogs, doses of 0.0008, 0.0012, 0 0041, 0.014 mg/kg (5 days/wk), or 0 065 mg/kg for 14 days followed by 11 wks off	Treatment	Monier-Faugere et al (1999), JBMR 14 (10), 1768-1778

RAT

In aged female Wistar rats, daily sc administration (0.0001 to 0.03 mg/kg/day) for 20 weeks starting 1 day after surgery prevented OVX-induced bone loss as assessed by femoral X-ray density, Ca content/tissue volume, and tibial cancellous bone volume (D14). When compared to sham-operated rat, 0.001 mg/kg/day (1 ug/kg/day) was the optimal dose to prevent OVX-induced bone loss. OVX caused increased trabecular separation (Tb.Sp) which was prevented by ibandronate. Ibandronate did not affect the slight reduction in cortical width caused by OVX (radiographical data).

In the same model, it was investigated whether there was a difference in the efficacy of a total (cumulative) doses when given either daily or cyclical intermittently (D15, D22). OVX rats were treated for a 20-22 week treatment period (daily with a suboptimal dose of 0.0001 mg/kg/day or an optimal dose of 0.001 mg/kg, or by three different cyclical regimens of 1 wk on and 2, 4 or 6 wks off with either dose). The 4 different dosing regimens resulted in the same total dose of 0.154 and 0.0154 mg/kg/animal. The drug-free periods (2 wks, 4 wks, 6 wks) reflected roughly 1, 2, 3 times the bone remodeling period in aged OVX rats.
The high dose completely prevented OVX-induced bone loss when given daily or with 2-wk or 4-wk intervals (indicated by X-ray density, weight and ash analysis of femur, and BV/TV of tibia). With a 6-wk interval, efficacy was less only in terms of bone X-ray density. Concomitant with the prevention of bone loss, ibandronate prevented OVX-induced trabecular separation at the tibia, independent of treatment schedule. Thus, the different treatment schedules had similar efficacy within a range of dosing intervals lasting from one day up to 4-6 weeks. Cortical bone strength of the femoral shaft (3-pt bending) was not affected by OVX or ibandronate despite changes in bone mass at that site (D22).
The drug-free period of 4-6 weeks is approximately 2-3 bone remodeling periods in aged rats (assuming a remodeling period in the aged rat of approximately 2 weeks ie 14 days). Thus, extrapolation of these data to humans would suggest that treatment daily would be equivalent with the same cumulative dose given once every 10-15 weeks (assuming remodeling period in humans is 2-3 longer than in rat).

EFFECTS OF DIFFERENT DOSING SCHEDULES OF BM21.0955 ON BONE MASS AND MORPHOLOGY IN AGED OVARIECTOMIZED RATS AFTER 12 MONTHS OF INTERVENTIONAL SC TREATMENT (February, 1999) (Study D25)
In an interventional (treatment) study in the aged female Wistar rat, the effect of ibandronate was investigated when given for 12 months starting at 10 weeks after OVX.

Study design

Group No (n=15)	Status	Substance	Daily Dose (µg/kg)	Administration s.c.	Week of Sacrifice
1	baseline	—	—	—	0 (wk 50/94)
2	Sham	—	—	—	10
3	Ovx	—	—	—	10
4	Sham	solvent	—	daily	62
5	Ovx	solvent	—	daily	62
6	Ovx	BM 21 0955	0 2	daily	62
7	Ovx	BM 21.0955	1	daily	62
8	Ovx	BM 21 0955	5	daily	62
9	Ovx	BM 21.0955	25	daily	62
10	Ovx	BM 21.0955	25	intermittent single adm. every 25 days	62
11	Ovx	BM 21 0955	125	intermittent single adm. every 25 days	62

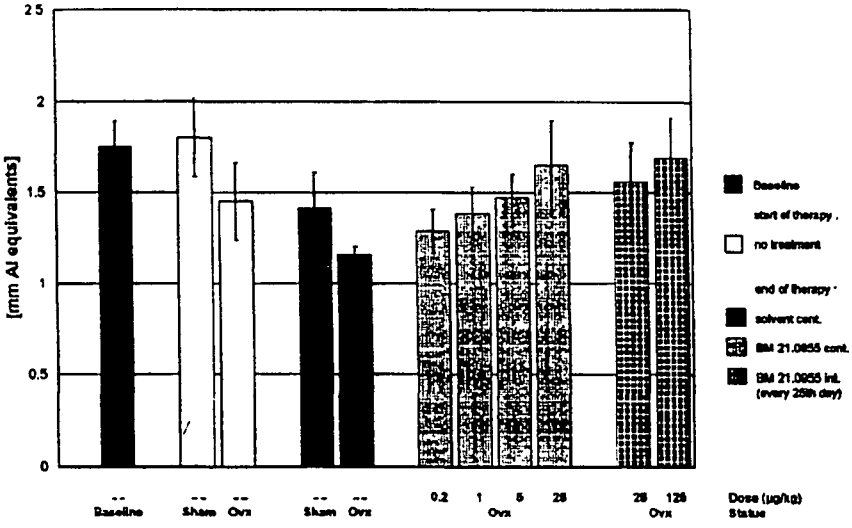
Doses are expressed as free acid equivalents

S.c. doses of 0.0002, 0.001, 0.005, 0.025 mg/kg/day (FAE) (N=15/grp) were given for 12 months starting 10 weeks after surgery. Measured parameters included femoral bone mass (X-ray density, mineral analysis), histomorphometric parameters of trabecular bone in tibia and L3 (Cn-BV, Tb.Th, Tb.N and Tb.Sp), proximal tibial BMD (pQCT), and vertebral L1-L4 BMD (DEXA). Bone strength was tested in femur neck, femur midshaft and lumbar vertebrae. As in the other rat studies, bone turnover markers were not evaluated.

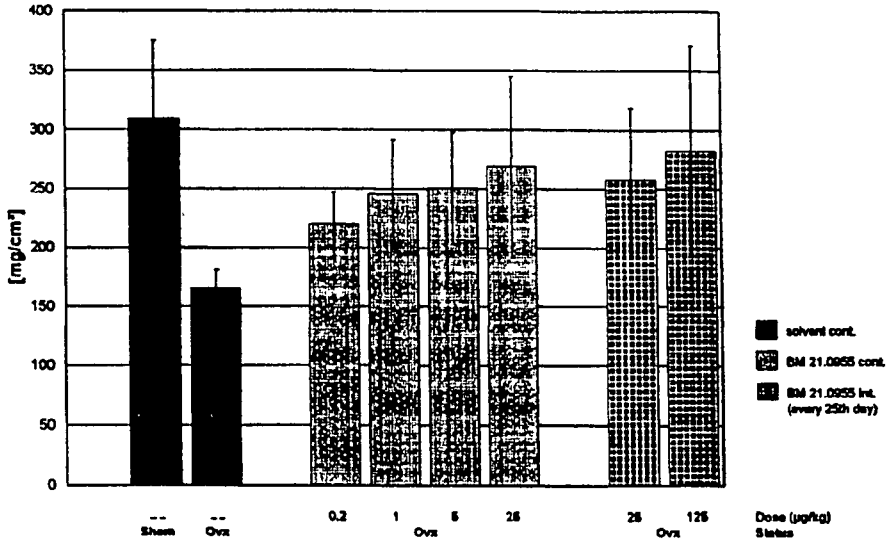
Results
OVX caused significant decreases in femoral X-ray density, proximal tibial trabecular BMD (QCT), (Cn-)BV/TV (hmm) and cortical thickness (QCT), and vertebral BMD (DEXA). There was no effect on tibial cortical BMD (QCT). Ibandronate dose-dependently prevented the OVX-induced changes in bone mass and bone architecture. The preventive effect occurred in both long bones and vertebrae, and was near maximal by 4 months of treatment.

The optimal dose range leading to reversal of OVX bone effects to sham levels or above was 0.001-0.005 mg/kg/day. The dose of 0.025 mg/kg was supra-optimal. Prevention was also obtained when doses of 0.025 and 0.0125 mg/kg were given once every 25 days (this gives same total dose as daily 0.001 and 0.005 mkd doses). This indicated that within these time limits (1 day-1 month) total treatment dose is the determining factor for efficacy.

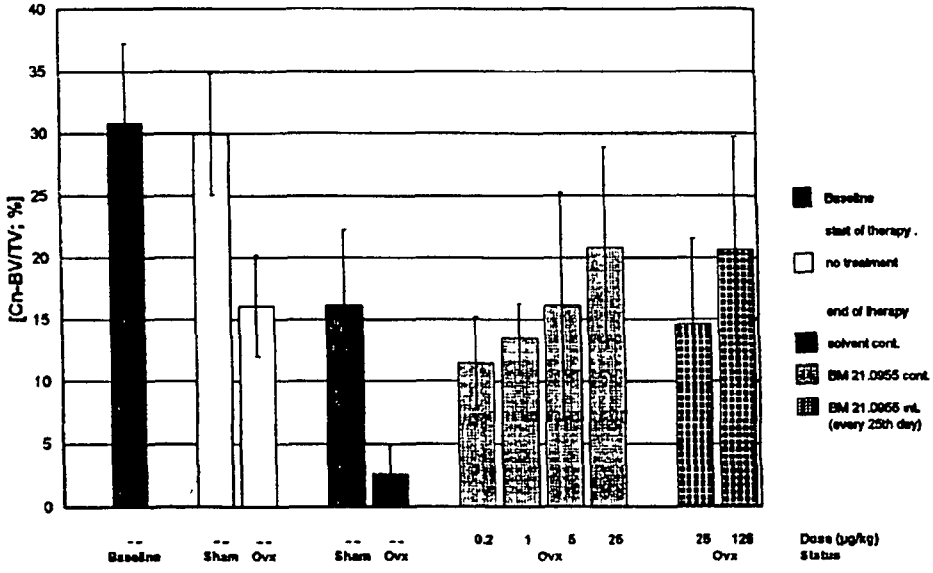
X-ray density [mm aluminium equivalents] ; right femurs of 22-month old ovariectomized (Ovx) or sham-operated (Sham) Wistar rats treated s.c for 12 months continuously or intermittently with BM 21 0955 beginning 10 weeks after surgery (Mean+/- SD , n=9-15/group)



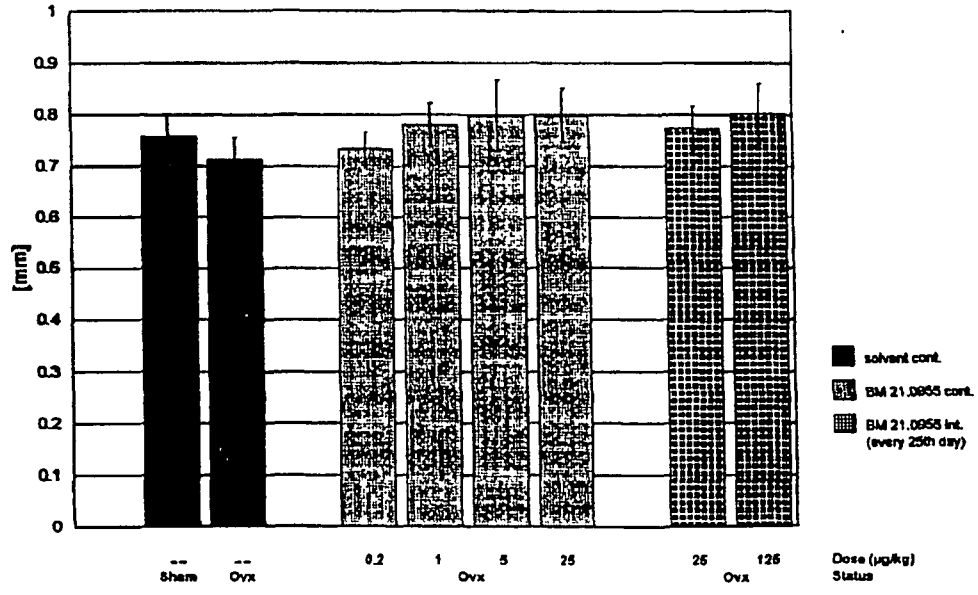
Trabecular density [pQCT, mg/cm³] ; right proximal tibia (6mm distal to knee joint) of 22-month old ovariectomized (Ovx) or sham-operated (Sham) Wistar rats treated s.c. for 12 months continuously or intermittently with BM 21.0955 beginning 10 weeks after surgery (Mean+/- SD , n=9-13/group)



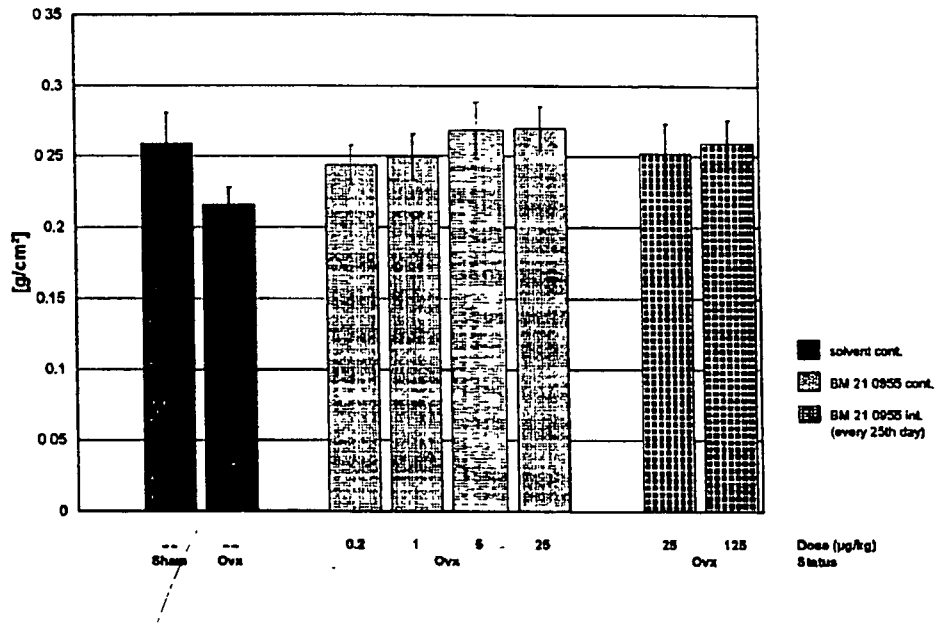
Bone volume per tissue volume [Cn-BV/TV; %] of the proximal metaphysis; left tibias of 22-month old ovariectomized (Ovx) or sham-operated (Sham) Wistar rats treated s.c. for 12 months continuously or intermittently with BM 21.0955 beginning 10 weeks after surgery (Mean+/- SD , n=8-15/group)



Cortical thickness [pQCT, mm] ; right proximal tibia (9mm distal to knee joint) of 22-month old ovariectomized (Ovx) or sham-operated (Sham) Wistar rats treated s.c. for 12 months continuously or intermittently with BM 21.0955 beginning 10 weeks after surgery (Mean+/- SD , n=9-13/group)



Lumbar bone mineral density [DEXA, g/cm²] ; L1-L4 of 22-month old ovariectomized (Ovx) or sham-operated (Sham) Wistar rats treated s.c. for 12 months continuously or intermittently with BM 21.0955 beginning 10 weeks after surgery
(Mean+/- SD , n=9-13/group)



Histomorphometry

Cancellous bone volume in the proximal tibia was reduced by OVX and preserved by ibandronate (shown above). Trabecular parameters (TbN, TbSp) were markedly changed by OVX as expected and reversed by all doses of ibandronate, significantly by doses of ≥0.2 or ≥1 ukd (vs OVX). Thus, trabeculae lost connectivity due to OVX. However, Tb.Th was not significantly affected.

Proximal tibia metaphysis

Group	4	5	6	7	8	9	10	11	
	Sham	OVX							
	0	0	0.2	1	5	25	"1"	"5"	
Cn-BV/TV	16	2.6*	11.5	13.5	16.2	20.8	14.6	20.7	
Tb.N (mm-1)	3.7	0.62*	2.8	3.3	3.5	3.9	3.3	4.1	
Tb.Sp (um)	245	2966*	332	269	285	230	320	236	
Tb.Th (um)	43	37	41	41	43	52	42	49**	

*statistically significantly different from Sham (p<0.0001); ** from OVX

Lumbar vertebra L3

Group		4	5	6	7	8	9	10	11	
Cn-BV/TV		22.5	12.7*	18.4	18.7	23.0	27.0	21.5	23.7	
Tb.N (mm-1)		3.9	2.6*	3.8	3.8	4.1	4.1	3.8	3.8	
Tb.Sp (um)		204	371*	218	216	192	182	214	204	
Tb.Th (um)		58	49	49	49	57	66	57	62**	

*statistically significantly different from Sham (p<0.0001); ** from OVX

Bone strength

Biomechanical testing showed that femoral neck shear test parameters were not affected by OVX or drug treatment (D26). However, femoral shaft strength (3-point bending) and lumbar vertebral strength (compression test) were decreased by OVX, and ibandronate prevented this decrease in a dose-dependent manner with the 0.0002 mg/kg/day dose being optimal (D27).

Femoral neck strength

Effects of a 1-year Treatment of Ibandronate on Femoral Neck Strength

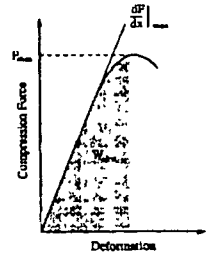
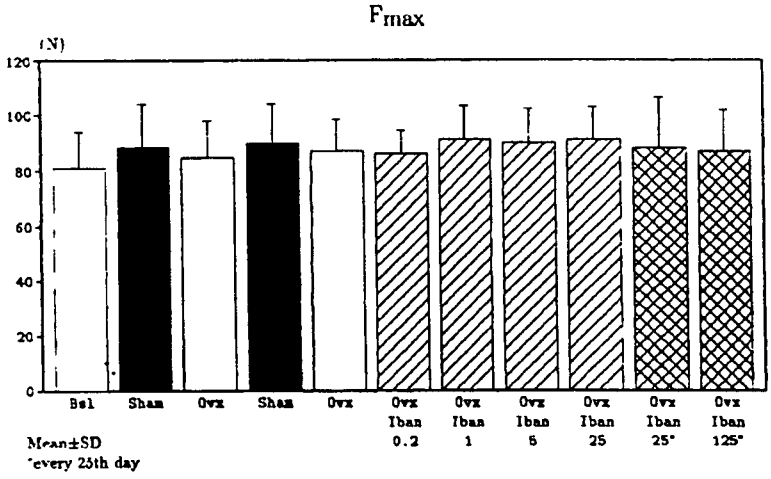
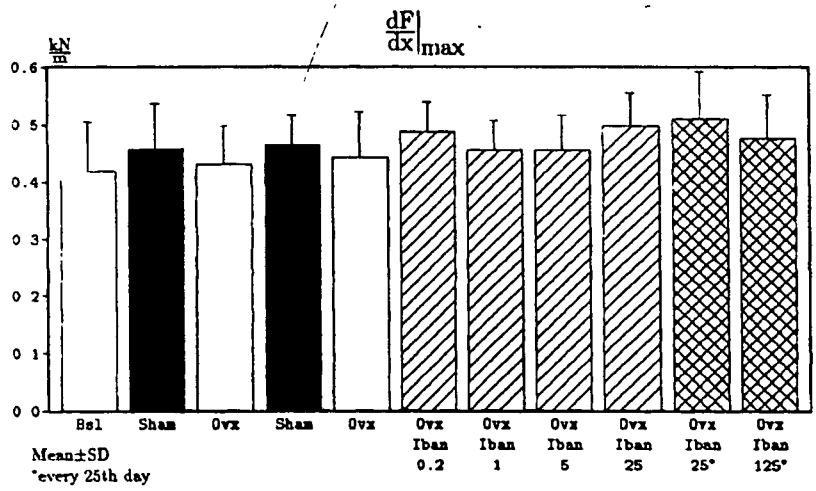
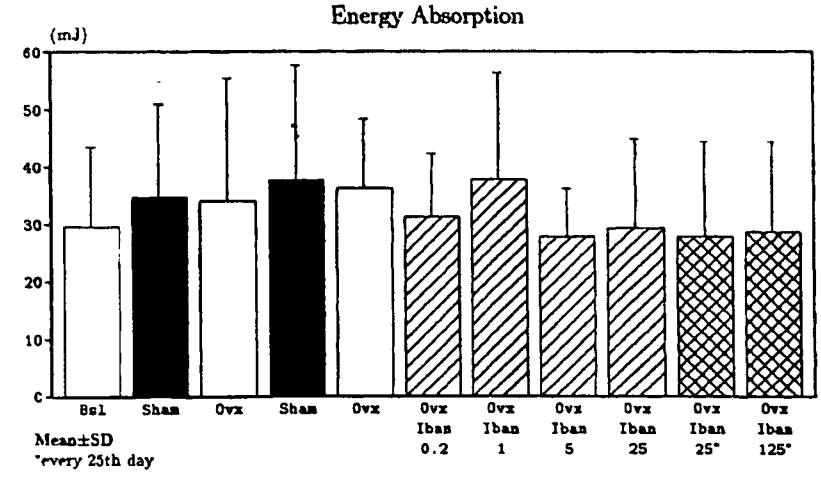


Figure 1.1 Schematic representation of the load-deformation curve



Effects of a 1-year Treatment of Ibandronate on Femoral Neck Strength





These findings could have been caused by:

- An increase in cortical thickness at the femoral neck site due to long term Ovx.
- A large interindividual variation at this site, due to different loading patterns in these aged rats.
- Low sensitivity of the test-procedure.

As the study showed no effect of Ovx, one would not expect an effect of the antiresoptive agent Ibandronate

It should be noted that other skeletal sites are more sensitive to Ovx-changes and also to treatmen response - also with antiresorptive agents Such skeletal sites include: The vertebral bodies and the dista femoral metaphyses. Additionally, 3-point-bending test of the femoral diaphyses might be more sensitiv than femoral neck testing as the standard deviation for the 3-point-bending test procedure is relativel low

Thus, there was no information on femoral neck strength from this rat study.

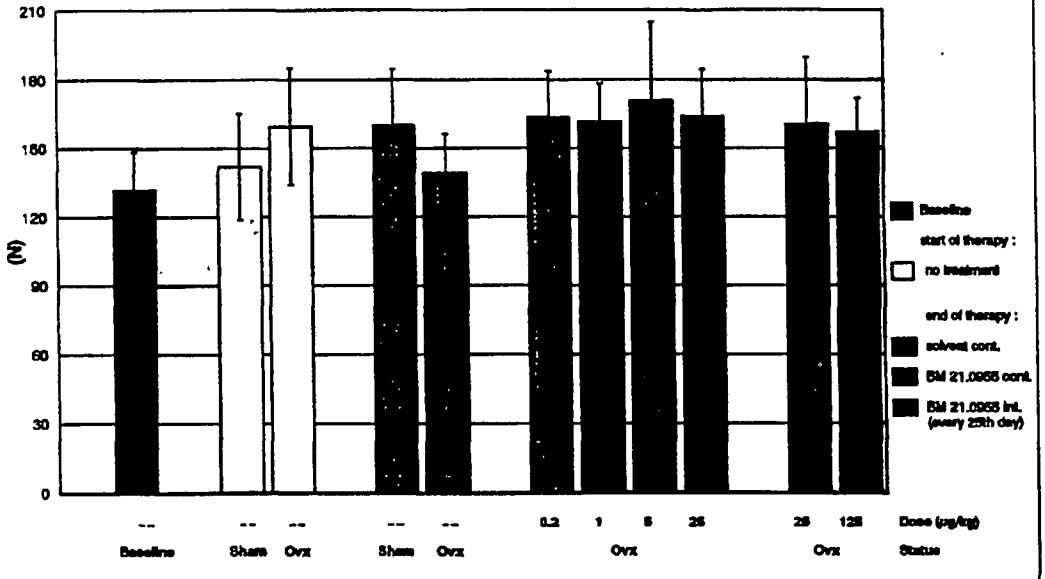
Femur midshaft bone strength (3-pt bending)

At end of treatment, OVX had caused reduction in Fmax, ult stress, cortical BMD and cortical thickness. OVX also caused an increase in femoral marrow diameter, ie., cortical internal diameter. The effects on strength and thickness were prevented completely by all treatment doses including the lowest 0.0002 mkd dose. Yield stress (N/mm2) was not reversed completely at any dose but BMD was completely reversed at 0.005 mkd. Paradoxally, external cortical bone diameter was increased by OVX prior to treatment (possibly an attempt to compensate for internal diameter increase and preserve moment of inertia). This external diameter remained unchanged during treatment. The data show that ibandronate can prevent cortical thinning in the femur, as was observed in the proximal tibia. These findings also illustrate how strength is dependent on macroscopic bone geometry in addition to intrinsic material bone properties such as BMD.

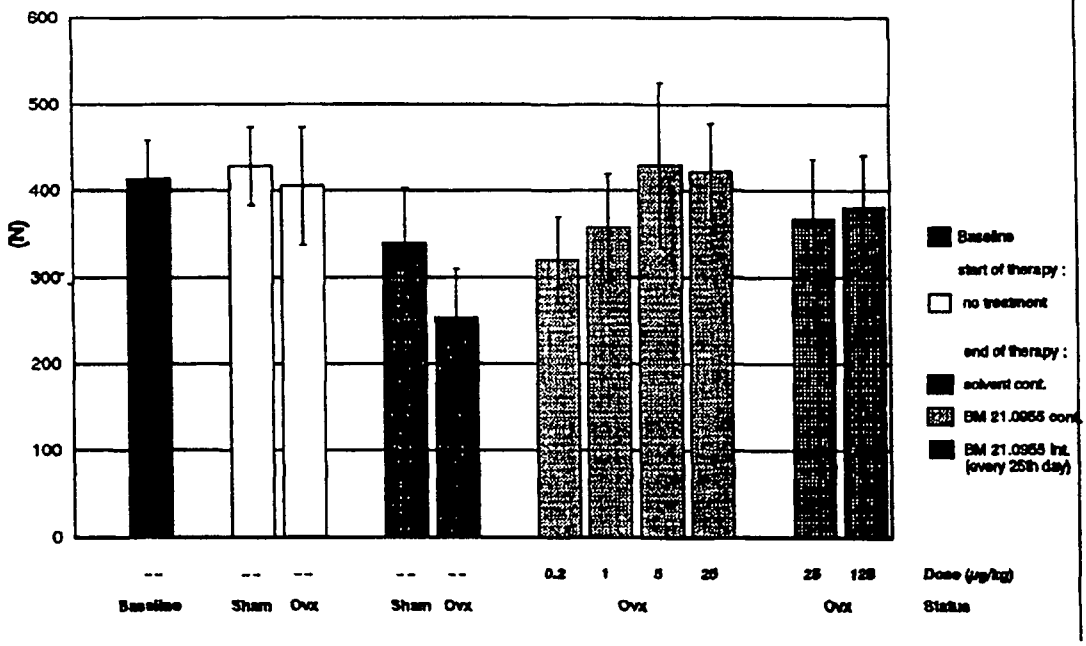
Vertebral strength: (compression)

OVX caused reduction in Fmax of vertebrae L4-L5. BMD of vertebrae was also reduced at end of treatment. Ibandronate prevented these changes dose-dependently, and completely at the 0.001 mkd dose. The 0.005 and 0.025 mkd doses were supraoptimal and increased bone strength to levels above sham.

Ultimate load (N) of left femora of 22-month old ovariectomized (Ovx) or sham-operated (Sham) Wistar rats treated s.c. for 12 months continuously or intermittently with BM 21.0955 beginning 10 weeks after surgery (mean +/- SD, n = 9-15/group).



Ultimate load (N) of lumbar vertebral bodies L4 of 22-month old ovariectomized (Ovx) or sham-operated (Sham) Wistar rats treated s.c. for 12 months continuously or intermittently with BM 21.0955 beginning 10 weeks after surgery (mean +/- SD, n = 9-15/group)



Correlations between bone mass and strength

Lumbar BMD (DEXA and pQCT), pooled over all groups, correlated strongly and positively with Fmax of vertebrae with correlation coefficient r= 0.88. Correlation between DEXA and pQCT of spine was r= 0.89. Correlation between femoral Fmax vs. cortical midshaft BMD (pQCT) was r=0.61. For the pooled groups all correlations were statistically significant.

Study design

Group No. (n=15)	Status	Substance	Daily Dose (µg/kg)	Administration s.c.	Week of Sacrifice
1	baseline	—	—	—	0 (wk 50/94)
2	Sham	—	—	—	10
3	Ovx	—	—	—	10
4	Sham	solvent	—	daily	62
5	Ovx	solvent	—	daily	62
6	Ovx	BM 21 0955	0.2	daily	62
7	Ovx	BM 21 0955	1	daily	62
8	Ovx	BM 21.0955	5	daily	62
9	Ovx	BM 21.0955	25	daily	62
10	Ovx	BM 21 0955	25	intermittent single adm. every 25 days	62
11	Ovx	BM 21.0955	125	intermittent single adm. every 25 days	62

Doses are expressed as free acid equivalents

Comparison of correlations in the individual treatment groups (as opposed to pooled groups) can give information about differences in the relation between BMD and strength in ibandronate vs. sham or OVX control groups.
The correlation between Fmax and BMD at the femur and vertebrae was similar for all groups, except Group 9 (HD of 25 ug/kg/day), where the coefficients were somewhat lower. In Group 9, for L4, BMD (DEXA) vs. Fmax was r=0.65. For femur, cortical BMD (pQCT) vs. Fmax was only r=0.16.
It is unclear why the correlation between femoral midshaft BMD and strength was weak in HD group 9. A low r and r^2 means that only a small part of the variance in strength was contained in the BMD. Both BMD and strength were increased vs. OVX to similar degrees as at the lower doses of 1 and 5 ug/kg/day. Variances in femoral parameters (BMD, thickness, diameter and strength) were similar in this group as in the others. Apparently, factors other than BMD such as spatial bone distribution dominate diaphyseal strength. Nevertheless, those factors were not adversely affected by the high dose because bone strength was intact.

Doses and multiples (12-month rat study)
According to Sponsor the optimal dose in this rat study was 0.0002 mkd-0.001 mkd. Reviewer concluded that the optimal dose was 0.001-0.005 mkd with regard to BMD and bone structure and vertebral bone strength. This dose is equivalent to a human (sc or iv) dose of 0.0002-0.0008 mg/kg/day (based on mg/m^2 BSA comparison). The tested/recommended oral daily dose in humans is 2.5 mg/day, or 0.04 mg/kg/day, which is equivalent to an IV dose of ca. 0.00025 mg/kg/day (assuming human BA = 0.6%). Thus the optimal sc dose in the rat is approximately 1-3x the recommended human dose based on mg/m^2 BSA comparison.
With regard to femoral cortical bone strength Reviewer agrees with Sponsor that the optimal dose was 0.0002 mkd.
The HD tested in the 12-month rat study of 0.025 mg/kg/day was 15x the human dose 2.5 mg oral dose based on mg/m2 comparison, and 35x the human dose based on AUC comparison. When we correct the multiples for species differences in length of the remodeling cycle, the cumulative HD of 0.025 mg/kg/day in the rat over a period of 1 month (1 cycle) is a 5x multiple of the cumulative human 0.04 mg/kg/day dose over a period of 3 months (1 cycle).

Margin between effect of ibandronate on bone resorption and mineralization based on rat data
In the long term studies in the OVX rat bone loss is optimally prevented by 0.001 mg/kg/day (D14, D15) or 0.001-0.005 mg/kg/day (D25). The dose of 0.001 mg/kg/day is approximately 1/5000 times the dose of 1 mg P/kg (5.14 mg/kg FAE) - the highest tested dose that did not interfere with bone mineralization (Muhlbauer et al, 1991, and Study D8). Thus, Sponsor concluded that "In growing rats there was no evidence of impaired mineralization even at doses greater than 5000 times the dose required for osteoporosis treatment" Label: Animal Pharmacology section). It should be noted that this statement, albeit correct, relates to data from the rat only.

DOG
Effects of ibandronate were also investigated in ovariohysterectomized (OHX) female dogs (D9). In a 4-week study, doses of 0.0001, 0.0003, 0.001, 0.01, 0.1 mg/kg were given s.c. for 6 out of 7 days per week. Measured was histomorphometry of the iliac crest. OHX caused increased OS/BS, ES/BS, Oc/BS, reduced bone volume, reduced Tb.Th and increased Tb.Sp. The dose of 0.001 mg/kg prevented the OHX-induced decrease in bone volume. However, bone turnover was not affected by 0.001 mg/kg. Bone turnover as assessed by histomorphometric bone formation and resorption parameters was reduced by 0.01 and 0.1 mg/kg. There were no adverse effects on mineralization (MAR) at all doses up to 0.1 mg/kg (0.1 mg/kg sc ≈ 10 mg/kg oral ≈ 5 mg/kg oral in human ≈ 300 mg = 120x recommended 2.5mg daily dose). Osteocalcin or serum PTH were not affected within the 4 week treatment time.
A long term study was performed in dogs, with continuous or intermittent dosing (D13). Ibandronate treatment was started 4 months after OHX for 12 months, at doses of 0.0008, 0.0012, 0.0041, 0.014 mg/kg (5 days/wk), or 0.065 mg/kg for 14 days followed by 11 wks off therapy. Cumulative dose was 3.64 mg/kg/animal at the intermittent dose and the highest

"continuous" dose. Measured were BMD, histomorphometry of iliac crest and mechanical bone parameters of L3 and long bones.
BMD of the cancellous core of L3, femur and tibia were not affected by OHX or drug treatment. Bone volume (BV/TV) was however reduced by OHX and reversed by all doses. Bone turnover parameters and Ac.F were suppressed significantly at the 0.014 mg/kg dose. Mlt was increased 6-fold at 0.014 mg/kg. Several histomorphometric parameters were not clearly affected by OHX particularly 16 months after surgery. Apparently, changes in bone occurring early after OHX were reversed in the long term. Mechanical properties of L3 and long bones were similar to those of Sham controls in OHX and OHX-ibandronate groups. Thus, histologically determined bone volume was not correlated to BMD and bone strength in this model.

The increase in Mlt mentioned above was not accompanied by an increase in O.Th. The increased Mlt was accompanied by higher crystal size, higher mineral-to-matrix ratio and more uniformly mineralized matrix in treated dogs. Sponsor claims this may indicate an increase in secondary mineralization with ibandronate, which would contribute to the BMD increase seen with BP's. Reviewer agrees that an increased Mlt can accompany and increase in the formation period (FP) of the individual remodeling sites (increase in FP skews data towards longer Mlt's) and is not necessarily an indication of osteomalacia.

NOTE: Mlt = O.Th/Aj.Ar (osteoid thickness/adjusted appositional rate). Aj.AR = MARx (LS/OS). The Mlt is the mean interval between deposition and mineralization of any (infinitesimal) volume of matrix, averaged over the entire life span of the osteoid seam. It can be shown that OV/BV=BFR/BV*Mlt. The Mlt is in contrast to Osteoid Maturation Time (Omt) which is the mean time interval between the onset of matrix deposition and the onset of mineralization at each bone forming site.

In conclusion, ibandronate appears to suppress bone turnover and increase bone volume and mineralization in the OHX dog. However, the data from D9 and D13 show that in the dog OHX has no persistent effects on bone BMD, histomorphometry or strength. Thus, the OHX dog is not an adequate long term animal model for osteoporosis and increased skeletal fragility. In consultation with the Division, Sponsor therefore decided to perform a long term preclinical bone study in another large animal model (OVX monkey).

MONKEY

A STUDY TO DETERMINE THE EFFECTS OF IBANDRONATE ON BONE MASS, STRENGTH AND ARCHITECTURE AFTER 16 MONTHS OF TREATMENT IN THE OVARIECTOMIZED CYNOMOLGUS MONKEY — Project Nr. 87284)

Study period: Dec 1996-March 1998
Report November 30, 1999 (D30)
Batch Nr. 781-449-59Mb

A long term bone quality study was carried out in cynomolgus monkeys (*Macaca fascicularis*). Monkeys were obtained from Monkeys (9-24yrs old), initial weight 2.1-6 kg, were ovariectomized (OVX-ed) and treated from the day of surgery by IV injection, with **0, 0.01, 0.03, 0.15 mg/kg** (0.5 mL/kg) (N=15/group sham and OVX controls; N=12/group treated) every 30 days for 16 months. Acclimation period was 2 months, baseline period 3 months. Doses are expressed as free acid equivalents (FAE). Placebo or test article (BM 21.0955.Na.H_2O) solutions (0 or 1 mg/mL) were diluted with vehicle (0.9% NaCl) to the required concentrations.

Treatment regimen was selected based on the clinical investigations (IV, intermittent dosing) at the time of study. The 16 months in monkeys corresponds to 4 years in humans. The doses selected were the expected optimal dose (MD of 30 ug/kg), a 5x dose (HD of 150 ug/kg) and a 1/3x lower dose (LD of 10 ug/kg). Dose selection was based on rat and dog studies, not on monkey dose range finding study. The doses correspond to human IV doses every 3 months of **0, 0.2, 0.6, 3 mg** (based on mg/m^2 comparison). Clinical studies have been performed with 0.5 and 1 mg/q3 months, but current NDA is for oral administration (2.5 mg/day). The IV studies in humans showed a moderate increase in BMD (spine) of ca. 4%, but no significant vertebral fracture efficacy. This incongruity has not been explained.

Parameters measured:

- BMD of lumbar spine (L1-L4, A/P) and femur (proximal and distal) by DEXA at 0, 4, 8, 12, 16 months, using Hologic densitometer (BMD, mg/area)
- BMD of proximal tibia and distal radius (metaphyseal and diaphyseal) by pQCT, at 0, 4, 8, 12, 16 months (BMC, BMD, geometry), using bone scanner.
- *Ex vivo* BMD of L1-L4 by DXA and pQCT after 16 mo.
- Biochemical markers of resorption (N-telopeptide, pyridinoline, deoxypyridinoline) and formation (osteocalcin, BSAP), at 0, 1, 3, 6, 12, 16 months
- Histomorphometry of iliac crest and rib (at 0, 6, 16 mo), and of radius (distal and central), vertebrae (L3-L4) and proximal femur (16 mo). Sections of bone retained in 70% alcohol and prepared without decalcification for hmm by
- Biomechanical strength of ulna (3-pt bending test), femur (femoral neck shearing test), humerus cortical beams (3-pt bending), vertebrae (L1 and L5) (compression test), at 16 mo. Bone samples were retained frozen (-20°C) and shipped on dry ice to for analysis. Femur was originally planned to be analyzed by 3-pt bending but data were lost and ulna was substituted for this purpose. Data were reported from
- Serum PK, at baseline and 16 mo (predose, 10min, 30min, and 1, 2, 4, 7, 24h postdose)
- Compound in bone (tibia, L6) after 16 mo.
- Whole body radiographs at 0, 16 mo
- Gross necropsy and histopathology (bone,brain, heart, kidney, liver, lung, ovary, thyroid, uterus, abnormalities)

Dose groups

Group No. Identification	Dose Level* (ug/kg/dose)	Dose Volume (mL/kg/dose)	Number of Animals Females
1 Sham Placebo Control	0	0.5	15
2 Placebo Control (OVX)	0	0 5	15
3 Ibandronate (OVX)	10	0.5	12
4 Ibandronate (OVX)	30	0 5	12
5 Ibandronate (OVX)	150	0.5	12

* Dose levels expressed as free acid equivalent

Upon necropsy the following bones were retained for postdosing analysis:

Bone retention is summarized as follows:

BONE	EX VIVO DXA/ pQCT	HISTO-MORPHOMETRY	BIOMECH-ANICAL	IBANDRONATE* ANALYSIS
Femurs		Right whole	Left whole	
Radii		Right distal & central	Left whole (backup)	
Ulna			Left whole	
Humeri		Right midshaft (backup)	Left whole	
Tibia		Right midshaft (backup)		Left whole
Ilium		Ilium		
Rib		Right 9th rib		
Lumbar	L1-L4✦	L3, L4	L1, L2 (backup), L5	L6
Thoracic vertebrae			T11,T12 (backup)	

Bones for biomechanical testing (details):

Bone	Test
Left femur	Cross-sectional cortical area and moment of inertia Proximal regional mineral content, density by DXA Femoral neck test (to failure)
Left ulna	Cross-sectional cortical area and moment of inertia, bone mineral content, density by pQCT 3-point bending (to failure)
Left humerus	Cortical beams, 3-point bending
L1 Vertebra (whole)	Bone mineral content, density by DXA, area by pQCT (with total area) Compression (to failure)
L5 Vertebra (core)	Compression (to failure) Bone mineral density, content, area by pQCT, bone mineral content, density by DXA

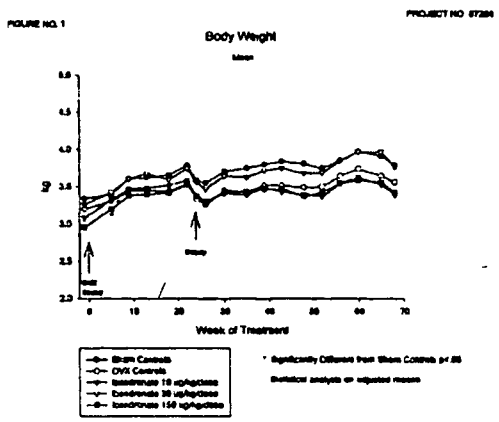
RESULTS

Mortality: Three deaths, 2 in LD, 1 in MD groups (#355, #360, #453), unrelated to treatment. Deaths occurred at Wks 35, 57, 58.

Pathology: No effects of ibandronate on gross necropsy or microscopy. Two OVX animals (#258, #453) were found to have ectopic ovarian tissue. The in vivo data from these monkeys were excluded from analyses, and their bones were excluded from hmm and mechanical testing. Thus N evaluable at 16 months was: 15-13-10-11-12

Serum estradiol levels were reduced from 45-90 pg/mL (at baseline) to 0.0 in OVX animals. Uterine weight reduced by 2/3, from 11.3 in sham controls to 3-3.5g in OVX.

Body weight: no significant effects (terminal weight 3.4-3.8kg)



Hematology: no significant effects

Urine electrolytes, urine creatinine, urinalysis: no significant effects

Serum ALP: Increased in OVX groups due to increased bone turnover by ca. 50% to 300 U/L throughout study. In treated groups, dose related reduction in this increased ALP due to suppression of bone turnover.

Radiography: Minor findings at lumbar spine and right femur, and at proximal tibia and distal radius were not considered to interfere with DXA and pQCT of these sites.

Serum Ca, P: Serum Ca transiently decreased (from ca. 8.9 to 8.4 mg/100dL) at 150 ug/kg at 1 week after 1st dose (Month 1).In response, serum PTH and VitD were increased at that time. Also, trend toward decreased serum Ca and increased PTH1-84 at 150 ug/kg throughout study. Fluctuations in serum Ca, P, PTH and Vit D were in accordance with effects of drug on calcium metabolism.

Biochemical markers of bone turnover:
OVX increased markers of resorption (N-teopeptide, PD and DPD) and later also of formation (OC, sALP). High dose (150) completely prevented the increase, and LD and MD had partial effect.
There appeared to be some reversal of the OVX effect on OC, PD and DPD at 12 and 16 mo as compared to 6 mo.

MARKERS OF BONE FORMATION PROJECT NO 87284

Figure No 2 Osteocalcin
Mean (SD)

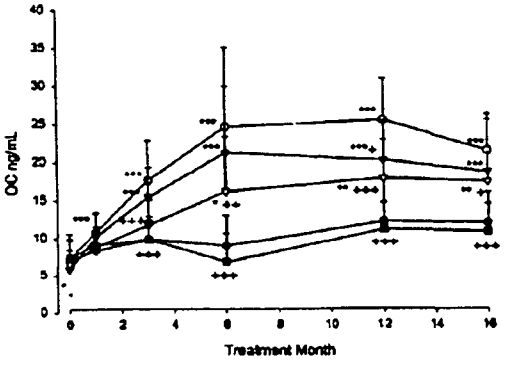
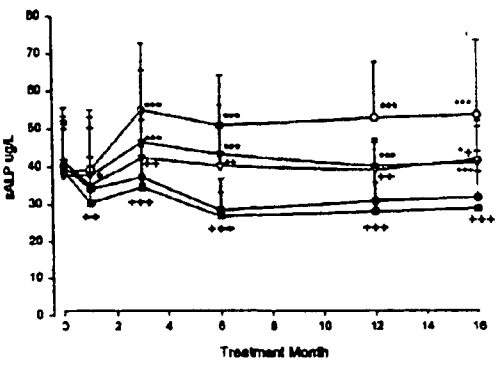


Figure No. 3 Bone Specific Alkaline Phosphatase
Mean (SD)



Sham Controls
OVX Controls
Ibandronate 10 ug/kg/dose
Ibandronate 30 ug/kg/dose
Ibandronate 150 ug/kg/dose

Significantly Different from Sham Controls
* p<.05, ** p<.01, *** p<.001

Significantly Different from OVX Controls
+ p<.05, ++ p<.01, +++ p<.001

Statistical analysis on adjusted means

MARKERS OF BONE RESORPTION PROJECT NO 87284

Figure No. 4 N-Telopeptide
Mean (SD)

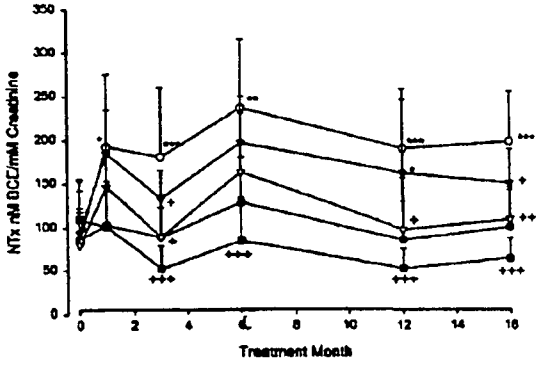
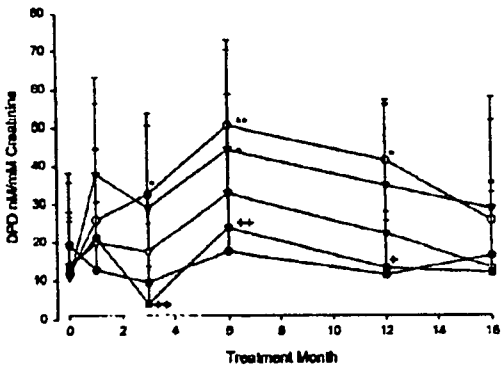


Figure No. 6 Deoxypyridinoline
Mean (SD)



Sham Controls
OVX Controls
Ibandronate 10 ug/kg/dose
Ibandronate 30 ug/kg/dose
Ibandronate 150 ug/kg/dose

Significantly Different from Sham Controls
* p<.05, ** p<.01, *** p<.001

Significantly Different from OVX Controls
+ p< 05, ++ p<.01, +++ p<.001

Statistical analysis on adjusted means

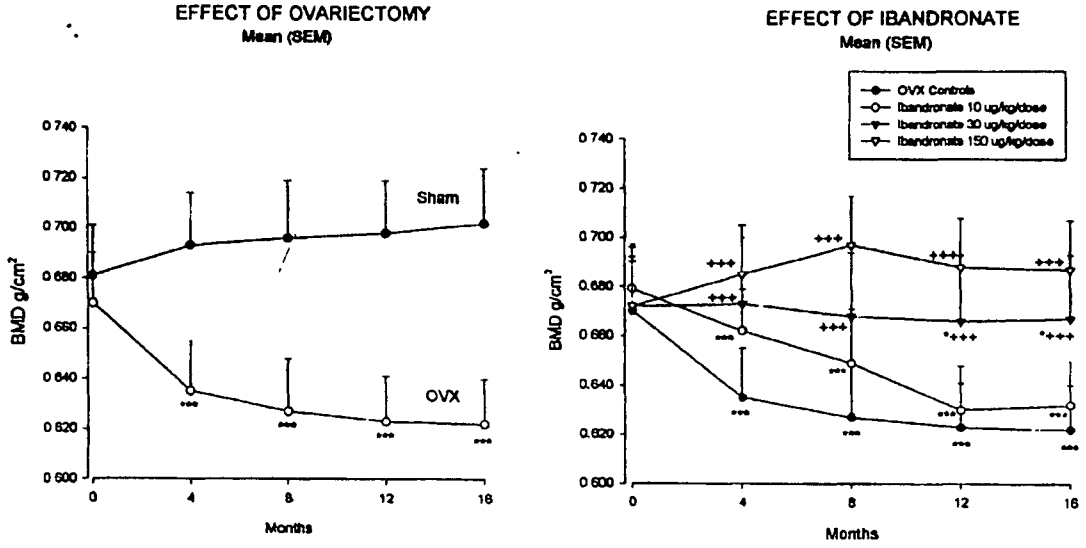
BONE MINERAL DENSITY

FIG. 2 and FIG. 3 (from Smith et al, Bone Vol. 32, 2003) are included below. The figures from the paper are similar as the ones copied from the NDA submission, except that the data are normalized to % of baseline value

Lumbar Spine

DEXA measurements (A/P): decrease in BMD (gram/cm^2) of L1-L4 after OVX. Decrease was progressive, although most was already attained after 4 months. Decrease appeared maximal after ca. 12 months. Sham animals had small increase in BMD over 16 months (3%). Decrease in BMD at 16 months was 7% (OVX vs. baseline) or 11% (OVX vs. sham control). Ibandronate prevented the decrease in BMD in a dose-related manner (20%, 65%, 90% with LD, MD, HD). The effects of OVX and ibandronate were similar when measured *ex vivo* by DEXA (L1-L4) (6%, 65%, 90% prevention of bone loss by LD, MD, HD).

FIGURE NO. 13 AP LUMBAR SPINE L1-L4 BMD BY DXA PROJECT NO. 87284



Significantly Different from Sham Controls
* p< 05, *** p< 001

Significantly Different from OVX Controls
+++ p<.001

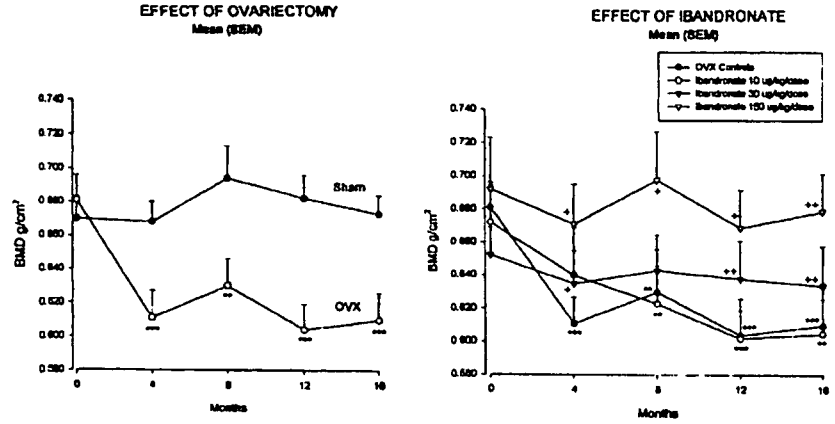
Analyses performed on adjusted means

DEXA was performed either anterio-posterior (AP) or lateral. In the AP scans the spinal (spinous and articular) processes are projected onto the plane and included in the average BMD calculation. In the lateral scans the processes which consist mainly of cortical bone can be eliminated. The effects on lateral and mid-lateral (middle part of vertebral bodies) spine BMD were 11% and 15%, OVX vs. Sham controls, ie, somewhat larger than the A/P effect. The effect of ibandronate on the lateral BMD was similar as the effect on A/P spine.

Femur

In proximal femur (global proximal femur, femoral neck and trochanter) OVX reduced BMD. In femur neck the decrease in BMD was ca. 11% (OVX vs.,baseline or Sham control). Ibandronate prevented the effect (10% at LD, 40% at MD, 70% at HD). Similar effects were seen in proximal femur, trochanter and distal femur.

FIGURE NO. 16 PROXIMAL FEMUR - GLOBAL AREA BMD BY DXA PROJECT NO 87284

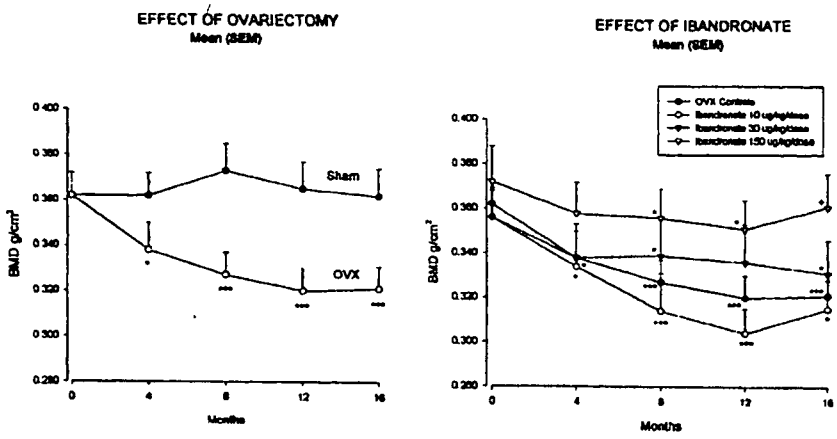


Significantly Different from Sham Controls
** p< 01, *** p< 001

Significantly Different from OVX Controls
+ p< 05, ++ p< 01

Analyses performed on adjusted means

FIGURE NO. 17 FEMORAL NECK BMD BY DXA PROJECT NO 87284



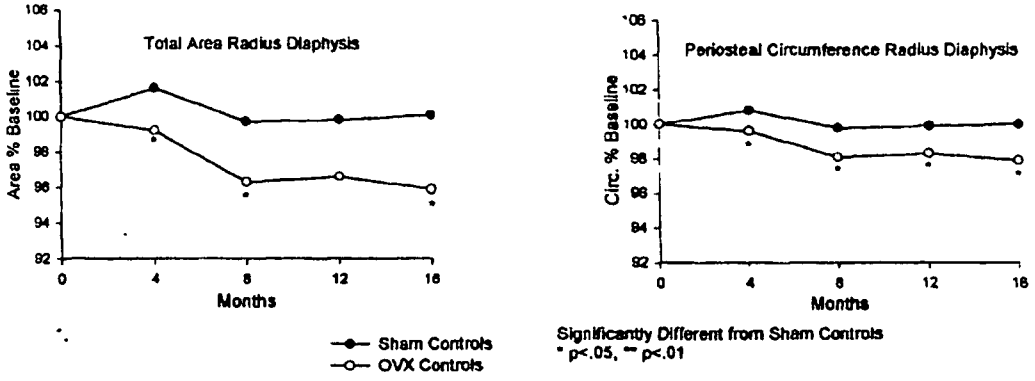
Significantly Different from Sham Controls
* p< 05, *** p< 001

Significantly Different from OVX Controls
+ p< 05

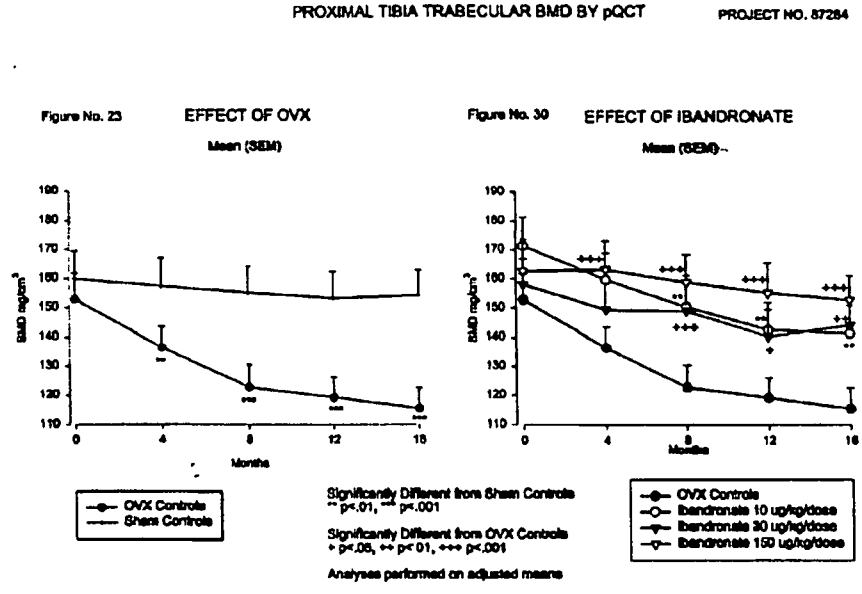
Analyses performed on adjusted means

<u>Proximal tibia and distal radius</u>
<u>pQCT</u>
With this technique a distinction between trabecular and cortical bone can be made. Measurements were performed at a metaphyseal site containing both trabecular and cortical/subcortical areas (3 scans), and a diaphyseal site (1 scan) containing cortical bone. Measured were BMC and BMD of total slice and of trabecular and cortical areas. Also determined were total area, trabecular and/or cortical area, cortical thickness, periosteal and endosteal circumference.

OVX caused a decrease in total diaphyseal bone area (4%) and periosteal circumference (2%) at tibia and radius. Sponsor attributed it to a technical artifact. It seemed a real effect to Reviewer. This did not occur at the metaphysis.



OVX caused decreases in total slice BMC and BMD at both tibia and radius metaphysis (maximal 14% in tibia). Response at the radius was generally less (50% of tibia). In the metaphyseal trabecular bone, there was a large increase in trabecular area (ca. 20%, due to increased resorption at the endosteal surface with more voxels identified as trabecular bone), a decrease in trabecular BMD (ca. 24%), but no change in BMC. The trabecular BMD decrease resulted from increased trabecular bone resorption. Trabecular BMC was unchanged because there was more trabecular bone.



In the metaphyseal cortex, there were decreases in cortical area and thus thickness (20%), and increases in endosteal circumference (accompanying the increase in trabecular area), in both tibia and radius. In diaphyseal cortex, cortical area was decreased at both sites (significant in

tibia), while endosteal circumference was increased in tibia but not radius. Thus, cortical thinning at diaphysis was due to periosteal and endosteal effects in tibia, but was only due to periosteal effects in radius. At metaphyseal end of bone, cortical thinning was only due to endosteal effects at both tibia and radius. Some of these effects were small and not statistically significant.

FIGURE NO 24 EFFECT OF OVARIECTOMY BY pQCT - METAPHYSIS CORTEX PROJECT NO 87284

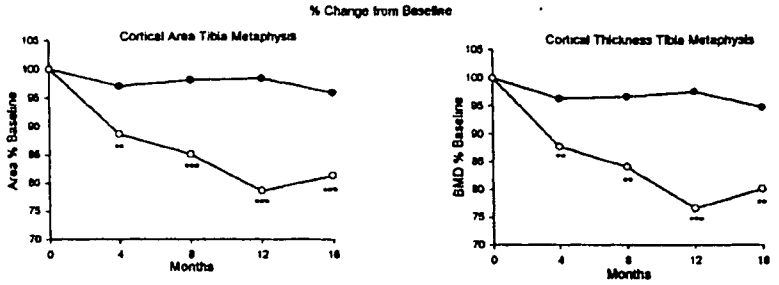
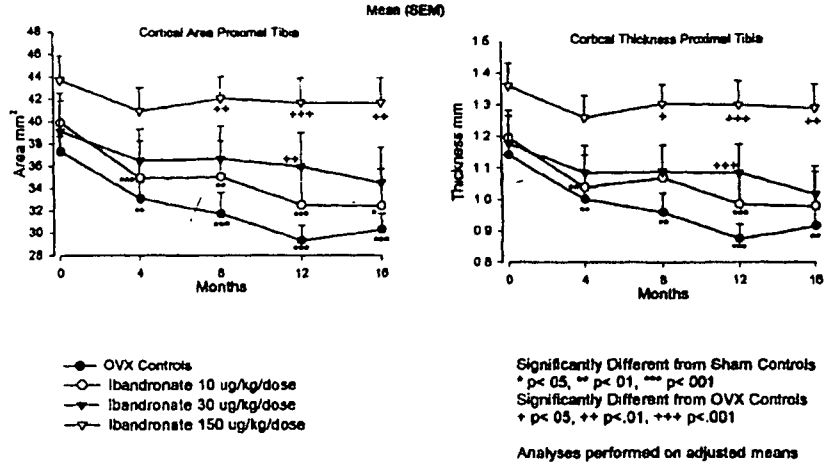
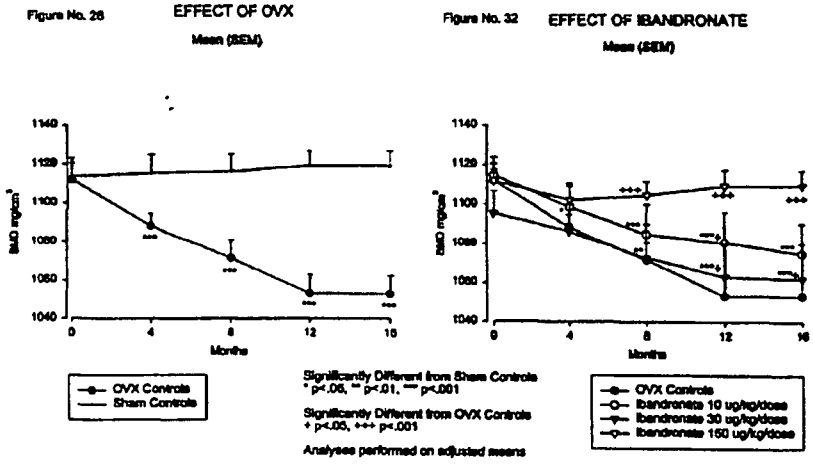


FIGURE NO 31 EFFECT OF IBANDRONATE BY pQCT - METAPHYSIS CORTEX PROJECT NO. 87284



Cortical BMD (diaphysis and metaphysis) was decreased in the tibia but not the radius by ca. 6%. This occurred in addition to the decreased cortical thickness and was probably the result of increased cortical (Haversian) bone turnover.

TIBIA DIAPHYSIS CORTICAL BMD BY pQCT PROJECT NO. 87284



The effect of ibandronate on the tibia and radius clearly showed that the compound prevented the OVX-induced changes in a dose-dependent manner.

In the metaphyseal trabecular bone, the prevention of the BMD decrease (21%) was 40%, 75%, 95%. In the cortical bone the prevention of BMD decrease (6%) was 35%, 40%, 85% (values as compared to sham, not baseline). Cortical thickness was dose-dependently reversed by ibandronate (LD, MD, HD: approximately 25%, 35%, 95%) at both meta- and diaphysis. Changes in periosteal circumference were prevented dose-dependently by treatment at diaphysis, but data for metaphyseal periosteal circumference were not evaluated. Endosteal circumference was preserved dose-dependently at meta- and diaphysis. Apparently, ibandronate was more effective in preventing trabecular bone loss (BMD) than cortical bone loss (BMD and thickness) since dose-response curves for cortical effects appeared shifted to the right.

In *ex vivo* pQCT of the lumbar spine (L1, L2, L3, L4), OVX clearly reduced trabecular and (sub)cortical BMD both by ca. 15%. Ibandronate prevented these decreases optimally at the HD (90-100% prevention). LD and MD were relatively inefficacious (10-30% prevention) with little difference between the two doses. It is unclear why ex vivo pQCT of lumbar vertebrae produced different results for the MD as compared to DEXA, as noted by Sponsor. This was also observed upon ex vivo evaluation of strength-tested vertebrae.

FIG. 2 and FIG. 3 (from Smith et al, Bone Vol. 32, 2003)
BMD of spine and femoral neck (Fig. 2), and BMD of proximal tibia trabecular and cortical bone (Fig. 3). These figures are similar as the ones shown above, except that data are normalized to % of baseline value

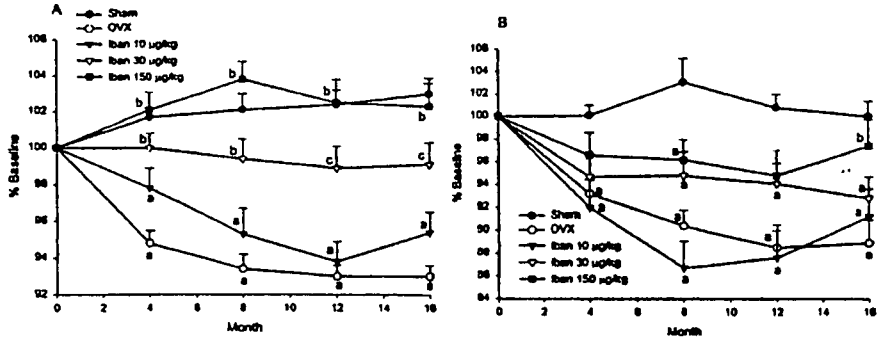


Fig. 2. Effect of OVX and treatment with ibandronate (Iban) on BMD measured by DXA at the lumbar spine L1–L4 (A) and femoral neck (B). Data are presented as mean percentages of baseline and 1 SEM ($n = 10–15$). Significances (relative to change from baseline) ($P < 0.05$): different from sham control, a, different from OVX control, b; different from sham and OVX controls, c.

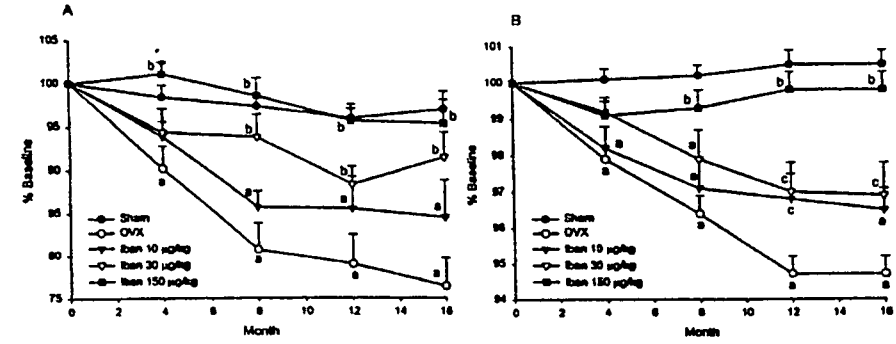


Fig. 3. Effect of OVX and treatment with ibandronate (Iban) on BMD measured by pQCT at the proximal tibia. (A) Metaphysis trabecular vBMD. (B) Diaphysis cortical vBMD. Data are presented as mean percentages of baseline and 1 SEM ($n = 10–15$). Significances (relative to change from baseline) ($P < 0.05$): different from sham control, a, different from OVX control, b; different from sham and OVX controls, c.

HISTOMORPHOMETRY

Tables 1 and 2 from a published paper (Smith et al, Bone Vol. 32, pp45-55, 2003) on this 16-month monkey study are included below.

Cancellous bone: Iliac crest and rib (0, 6, 16 mo), radius (distal and central), vertebrae (L3-L4) and proximal femur (16 mo) (Table 1)
Cortical bone: Rib, femoral neck, central radius (16 mo) (Table 2)

Trabecular Histomorphometry Variables Analyzed*

File Name	Standardized Nomenclature	Abbreviation	Units
MAR	Mineral Apposition Rate	MAR	µm/d
BV/TV	Trabecular Bone Volume	BV/TV	%
OV/TV	Osteoid Volume	OV/TV	%
WTh	Wall Thickness	W.Th	µm
OsTh	Osteoid Thickness	O.Th	µm
OSBS	Osteoid Surface	OS/BS	%
MSBS	Mineralizing Surface, Double Label	MS/BS	%
MSRSX	Mineralizing Surface, Double + ½ Single Label	MS/BS*	%
ESBS	Eroded Surface	ES/BS	%
OcSBS	Osteoclast Surface	Oc.S/BS	%
MSOSX	Mineralizing Osteoid	MS/OS*	%
BFR/BVX	Bone Formation Rate, Volume Based	BFR/BV*	$mm^3/mm^3/yr$
MlX	Mineralization Lag Time	Ml*	days
FPX	Formation Period	FP*	days
RPX	Resorption Period	Rs.P*	days
AcF	Activation Frequency	Ac.F	#/yr
TbTh	Trabecular Thickness	Tb.Th	µm
TbN	Trabecular Number	Tb.N	mm^{-1}
TbSp	Trabecular Separation	Tb.Sp	µm

*The variable names as they appear in the spread sheet files are in column 1, and the full variable names, the abbreviations, and the units as they appear in the paper by Parfitt *et al* are in columns 2,3 and 4, respectively

Cortical Histomorphometry Variables Analyzed*

File Name	Standardized Nomenclature	Abbreviation	Units
dL.Hs	Haversian Systems with Double Label	H.dL	%
M1.Hs	Haversian Systems with Double or Single Label	H.dL_sL	%
MAR	Haversian Mineral Apposition Rate	H.MAR	µm/d
AHvClAr	Haversian Active Area	H.a.Ar	mm^2
E.MS	Endocortical Mineralizing Surface	Ec.MS	%
P.MS	Periosteal Mineralizing Surface	Ps.MS	%

*The variable names as they appear in the spread sheet files are in column 1, and the full variable names, the abbreviations, and the units as they appear in the paper by Parfitt *et al* are in columns 2,3 and 4, respectively

The effect of OVX was reflected in the histomorphometry parameters. In cancellous bone, as expected, BV/TV was reduced at all sites, but statistical significance was only attained at the radius. Trabecular thickness was variably affected (generally a small decrease). Trabecular separation was minimally and non-significantly increased, Tb.N was minimally and non-significantly decreased. The variability in these parameters was partly due to the relative small power of this microscopic method. The effect of ibandronate on the structural parameters (BV/TV, Tb.Th) was variable and not significant, with only a clear prevention of the Tb.Th decrease in the femur by the HD.

Significant results were obtained for the dynamic parameters such as MS/BS, BFR and Ac.F, assessed by labeling with the fluorochrome xylenol orange. In cancellous bone, MS/BS, BFR and Ac.F were clearly increased by OVX, and the effect was counteracted by ibandronate. The MD of 30 ug/kg was mostly fully efficaceous, while the HD of 150 ug/kg depressed the rates below sham levels. The LD of 10 ug/kg was also fully effective in several cases.

In cortical bone (rib, femur neck, central radius), OVX induced increases in Ac.F as shown by increased fraction of labeled Haversian systems and increased area of active Haversian canals. The increase in Ac.F. was prevented in rib and radius at MD and HD. However, in femoral neck there was no significant treatment effect.
In cortical bone, endocortical mineralizing surface Ec.MS/BS was increased by OVX. Ibandronate prevented these effects dose-dependently at all sites, completely at HD. Thus, cortical bone turnover (AcF) appears less sensitive to treatment than cancellous bone turnover.
Although cortical periosteal bone remodeling (Ps.MS/BS) appeared increased by OVX with suppression by ibandronate, these effects were not significant.

Osteoid thickness was minimally increased by OVX and was decreased to below sham levels at MD and HD at several cancellous sites. W.Th was generally unaffected. Mlt (O.Th/Aj.AR) was increased in iliac crest (6mo) and radius (canc bone). This was accompanied by an increased FP (formation period). It can be understood as an average (over the life span of the osteoid seam) increase of the time between deposition of matrix and mineralization due to the prolonged FP. In conjunction with the lack of an effect on osteoid thickness this does not indicate impaired mineralization. MAR was decreased in iliac crest and L4 upon drug treatment. Probably, this was also due to increased average age of the BRU skewing the value towards slower rates occurring later in the formation period.

Erosion surface(ES/BS) and osteoclast surface (OcS/BS) were increased, in iliac crest (16 mo), and proximal femur (at HD) and in L4 (at LD, MD, or HD). The increases may seem to indicate increased resorption. However, the investigator suggested that it is related to an increase in Rv.P (reversal period) or Rs.P (resorption period) increasing the likelihood of detecting osteoclast covered or eroded bone surface.

There was no evidence of a mineralization defect and the remodeling machinery appeared intact. There was no evidence of microscopically abnormal bone, or abnormal accumulation of unmineralized osteoid on treatment. Bone tissue was normal lamellar bone in all areas at al sites.

Note that in cancellous bone e.g. at L4 the LD completely prevents the OVX-induced increase in turnover as indicated by Ac.F. and BFR but has minimal effects on bone mass (BV/TV or BMD). Apparently turnover and bone mass are correlated, but other factors also determine bone mass. Apart from an increase in turnover rate and remodeling space, OVX may reduce bone mass through e.g. suppression of the osteoblast and a negative bone balance. These other events may be affected by ibandronate according to different dose response characteristics (less potency).

In conclusion, OVX reduced bone volume, increased activation frequency of new remodeling sites and increased bone turnover. Ibandronate suppressed these effects. Effects of ibandronate were sustained over 16 months. Suppression of histomorphometric OVX-effects in cortical bone appeared less sensitive to treatment than the effects in cancellous bone (different dose response). There were no safety issues evidenced by abnormal histologic bone appearance and mineralization was not inhibited.

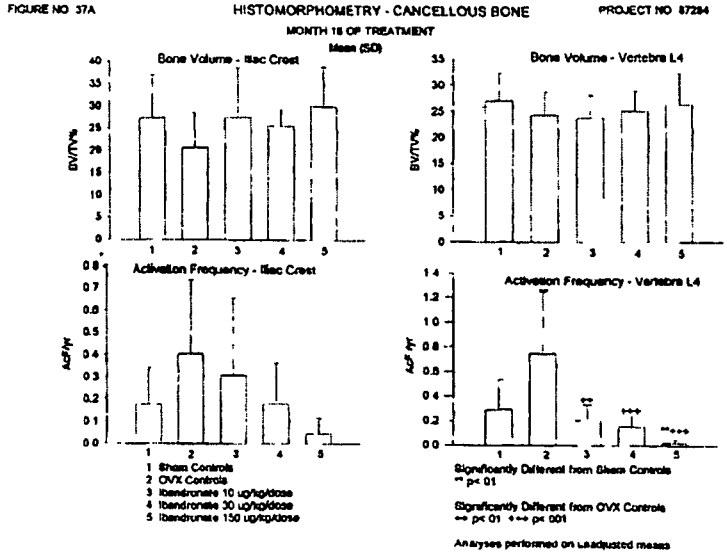


FIGURE NO 37A (cont'd) HISTOMORPHOMETRY - CANCELLOUS BONE PROJECT NO 87284

MONTH 16 OF TREATMENT
Mean (SD)

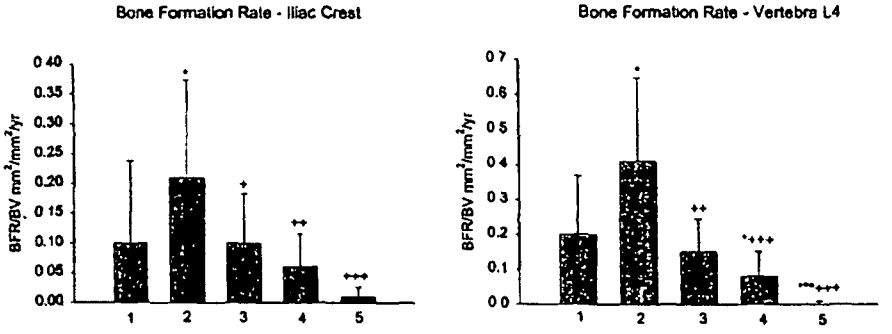


FIGURE NO 38A HISTOMORPHOMETRY - CORTICAL BONE PROJECT NO 87284

MONTH 16 OF TREATMENT
Mean (SD)

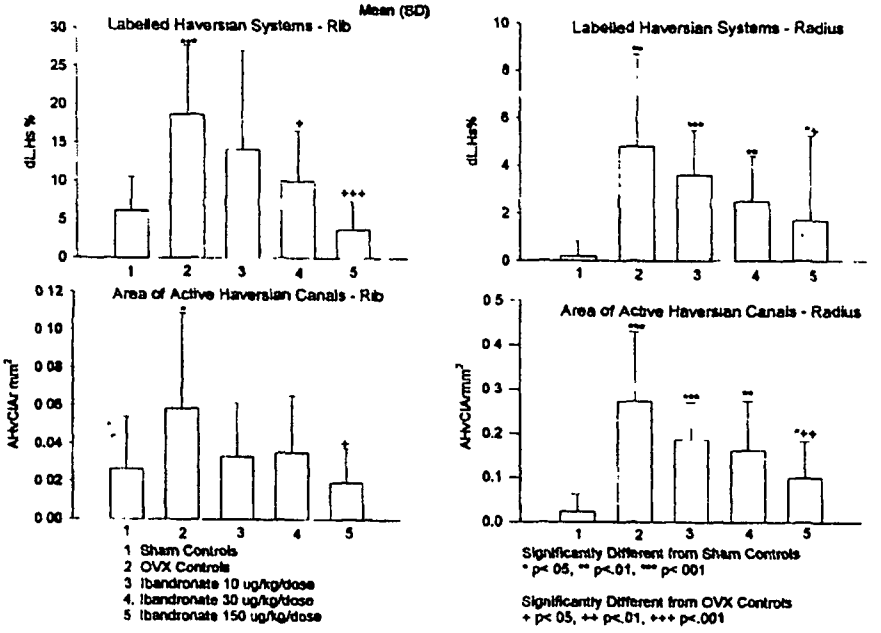


TABLE 1 and TABLE 2 (from Smith et al, Vol. 32, Bone 2003)

Table 1
Histomorphometric parameters of cancellous bone after 16 months treatment with ibandronate

	Sham	OVX			
		Vehicle	Ibandronate (μg/kg/dose)		
			10	30	150
Bone volume/tissue volume (%)					
Iliac crest	27 31 (9 57)	20.70 (7.79)	27.57 (11 11)	25 61 (3 90)	30 07 (8 80)
Proximal femur	26 03 (7.71)	18 84 (6.18)	21 05 (6 86)	22.21 (5 92)	24 72 (9 33)
Distal radius	19 44 (5.54)	12 48 (4 30)[a]	12 92 (5 48)[a]	11 61 (3 81)[a]	14 02 (5 41)[a]
L4	27 01 (5.25)	24 44 (4 34)	23 86 (4.36)	25 19 (3 79)	26.81 (5 85)
Trabecular thickness (μm)					
Iliac crest	173 (33)	171 (46)	198 (62)	170 (30)	192 (32)
Proximal femur	196 (40)	166 (30)	172 (39)	189 (48)	238 (95)[b]
Distal radius	153 (31)	121 (17)[a]	116 (25)[a]	109 (15)[a]	126 (30)[a]
L4	127 (24)	129 (19)	119 (18)	111 (13)	126 (21)
Mineralizing surface/bone surface (%)					
Iliac crest	1.31 (1.44)	4.68 (3.65)[a]	3 45 (3.92)	2.31 (2.70)	0.23 (0 59)[b]
Proximal femur	1.89 (2.68)	6.13 (4.46)[a]	1 43 (1.55)[b]	3 00 (5 79)	0 81 (2.52)[b]
Distal radius	1 40 (1.75)	5.61 (4.02)[a]	5 53 (4.17)[a]	2.57 (2 39)[b]	0 75 (0.86)[b]
L4	2.51 (2.55)	6 77 (4.73)[a]	1 97 (1.20)[b]	0.95 (0 87)[a]	0 05 (0 12)[a]
Bone formation rate/bone volume ($mm^3/mm^3/yr$)					
Iliac crest	0.10 (0 14)	0 21 (0 16)[a]	0 10 (0 08)[b]	0 06 (0 06)[b]	0 01 (0 02)[b]
Proximal femur	0 10 (0.09)	0.36 (0 25)[a]	0 13 (0 16)[b]	0 12 (0 20)[b]	0 04 (0 11)[b]
Distal radius	0.11 (0 13)	0.38 (0 23)[a]	0 28 (0 15)[a]	0 19 (0 14)[a]	0 05 (0 05)[b]
L4	0.20 (0.17)	0.41 (0.24)[a]	0 15 (0 10)[b]	0 08 (0 07)[a]	0 00 (0 01)[a]
Activation frequency (per year)					
Iliac crest	0.176 (0.166)	0.407 (0.331)	0 308 (0.347)	0 178 (0 187)	0 046 (0 071)
Proximal femur	0 237 (0.217)	0 874 (0.579)	0 229 (0.167)	0 655 (0.917)	0 731 (0.903)
Distal radius	0.131 (0 145)	0.377 (0.308)[a]	0 304 (0.257)	0.143 (0.121)[b]	0 049 (0 042)[b]
L4	0 294 (0.240)	0.746 (0.499)[a]	0.206 (0.125)[b]	0 152 (0 92)[b]	0.023 (0 022)[a]
Erosion surface/bone surface (%)					
Iliac crest	1.30 (0.71)	1.88 (0 99)	2 38 (0 97)[a]	3 02 (1 02)[a]	2 73 (0.93)[a]
Proximal femur	2.25 (1.74)	5 65 (3.84)	4 39 (4 73)	4 18 (2.57)	10 03 (7.96)[a]
Distal radius	1 53 (0 98)	3 24 (1.57)[a]	3 41 (1 87)[a]	2 28 (1 42)	2 20 (1 61)
L4	3.37 (1.65)	4 09 (1 27)	7 38 (2.21)[a]	7 78 (3 05)[a]	9 23 (3 83)[a]
Osteoclast surface/bone surface (%)					
Iliac crest	0 79 (0 57)	1 00 (0 67)	1 59 (0 69)[a]	2 20 (0 83)[a]	1 28 (0 74)
Proximal femur	0 17 (0.23)	0.48 (0.42)[a]	0 26 (0 34)	0 28 (0 30)	0 36 (0 55)
Distal radius	0.48 (0.39)	1 40 (0.79)[a]	1 45 (0 79)[a]	0 82 (0 77)	0 60 (0 53)[b]
L4	0.98 (0.42)	1 29 (0.65)	1 88 (0 67)[a]	1 92 (0.75)[a]	0 99 (0 60)

Table 2
Histomorphometric parameters of cortical bone after 16 months of treatment with ibandronate

	Sham	OVX			
		Vehicle	Ibandronate (μg/kg/dose)		
			10	30	150
Area of active Haversian canals (mm^2)					
Rib	0.026 (0 028)	0.058 (0.050)[a]	0 033 (0.028)	0 035 (0 030)	0 019 (0 018)[b]
Femoral neck	0.130 (0 088)	0.299 (0.174)[a]	0 185 (0.094)	0 236 (0 114)	0 214 (0 142)
Central radius	0.023 (0 039)	0.272 (0.158)[a]	0 187 (0.083)[a]	0 161 (0 112)[a]	0 099 (0 083)[a]
Labeled Haversian systems (%)					
Rib	6.1 (4 5)	18.7 (9.1)[a]	14.1 (12.9)	10 0 (6 6)[b]	3 7 (3 6)[b]
Femoral neck	1 9 (1.8)	3.6 (1.5)	2.6 (1.7)	3.5 (3 5)	2 4 (1 9)
Central radius	0.2 (0.6)	4 8 (3.9)[a]	3.6 (1.9)[a]	2.5 (1 9)[a]	1 7 (3 6)[a]
Mineral apposition rate (μm/day)					
Rib	0 85 (0.23)	0.93 (0.18)	0 87 (0.15)	0.97 (0 24)	0 91 (0.33)
Femoral neck	0 76 (0.18)	0.83 (0.16)	0.73 (0.09)	0.74 (0.21)	0 78 (0.11)
Central radius	0.78 (0.45)	1.00 (0.15)	0 80 (0.15)[b]	0.79 (0.23)[b]	0.76 (0.33)[b]
Endocortical mineralizing surface (%)					
Rib	13.24 (13.83)	22.13 (11.90)	17.12 (20.71)	8.58 (6.92)	14 87 (13 87)
Femoral neck	7.10 (8.95)	14.94 (10 10)[a]	5.20 (6.99)[b]	9 19 (8.37)	3 71 (3 92)[b]
Central radius	2 68 (4.90)	13.91 (14.53)[a]	17.87 (12.24)[a]	12.59 (10 92)[a]	3 28 (6 26)[b]

Data are means (SD).
[a] Significantly different from sham controls.
[b] Significantly different from OVX controls.
[c] Significantly different from sham and OVX controls.
$P < 0.05$.

BIOMECHANICAL TESTING

Table 3 from Smith et al, Bone Vol. 32, pp45-55, 2003 (L1, ulna, femoral neck data) is included.
Bone strength is reflected by the breaking load (ultimate load, N), ie force needed to break the bone. This is the most relevant parameter when assessing bone strength. It depends on the material properties of the bone ("intrinsic strength") and the macroscopic bone geometry. Intrinsic strength depends on the bone material/composition and the micro-architecture of the bone. Since geometry can be assessed in the bone strength measurements, intrinsic strength (N/area) is also a frequently calculated and evaluated parameter. In the current study the tested bone was assessed by DXA or pQCT scans to measure BMC, bone area and BMD. In that way, the scan parameters could be correlated to strength for the individual animals.

Vertebrae: OVX decreased BMD and bone strength (ultimate load, N, and compressive strength, N/mm2) in L5 cores and L1 whole vertebrae. The OVX effect was clearly prevented by ibandronate. In the L5 core a near maximal effect was obtained at the MD of 30 ug/kg. Strength (and BMD) in vertebral cores treated with the HD of 150 ug/kg exceeded strength (and BMD) of the sham control group. In the whole vertebrae L1, ibandronate also prevented the decrease in strength in a dose-related manner. However, at the MD the effect was partial (with regard to both BMD and strength) and at the HD strength was restored to sham control levels. The effect on strength at the HD was significant. The HD did not cause a reduction in bone strength (N or N/mm2).

Humerus: The data on the processed humerus specimens are hard to interpret. Load (N) is not relevant since the specimens were excised and the width and thickness were artificial. Although OVX decreased strength (N/mm2) and all doses of ibandronate appeared to prevent this reduction. The effects were not statistically significant.

Ulna: The data on the ulna (whole cortical bone) suggest that cortical area (QCT) and A/P diameter were decreased by OVX which was not prevented by ibandronate. However, bone density (g/cm3) was decreased significantly by OVX, and this was reversed by treatment with significant effect at the HD. The ultimate load was also decreased by OVX. This effect was for a minor part, and not significantly, reversed by MD and HD ibandronate. Compared to cancellous bone (vertebrae) the decrease in BMD was much less (4.5% vs. 16%). Extrinsic strength (ult load) in ulna was reduced markedly and similarly as in vertebrae (ca. 25%), but intrinsic strength was reduced much less (9%). This indicates that a major part of the effect of OVX on ulna cortical bone is an effect on bone geometry and part of it an effect on bone micro-structure. A decreased cortical area and thickness were also observed in the proximal tibia pQCT assessments and were due to increased inner, not outer cortical diameter. However, in proximal tibia this cortical thinning was completely prevented at the HD.

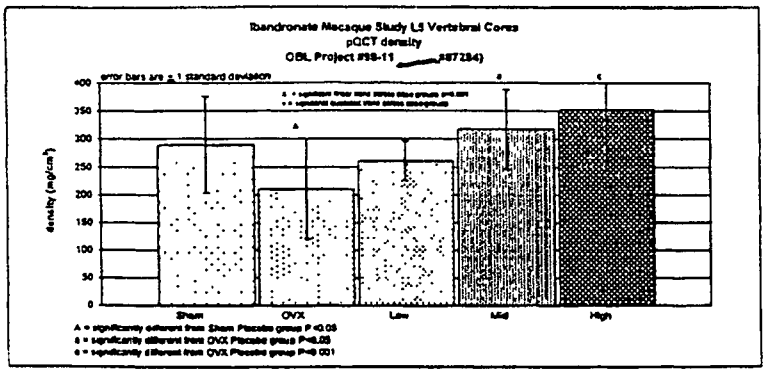
Femoral neck: OVX caused decreases in ultimate load (shearing test) and BMD. Treatment prevented the BMD reduction at all proximal femoral areas including neck, partially at the MD and completely at the HD. Strength was partially reversed at MD and HD but the effect was not significant.

Correlations between BMD and strength were determined for the vertebrae (core and L1), ulna and femoral neck. The correlation was strongest for vertebral cores, and also reasonable for whole vertebrae and femoral neck. For ulna cortical bone correlation between BMD and strength (N/mm2) was modest. The coefficient indicates the degree to which two variables are associated, ie, how much the variation in one contributes to the variation in the other.

Correlation coefficient (r)

Site	r	BMD- ult load(N)	BMD –strength(N/mm2)	BMC-ult load(N)
L5 core	0.76	x		
L1 vertebra	0.66	x		
Ulna	0.23	x		
	0.51		x	
	0.96			x
Fem neck	0.71	x		

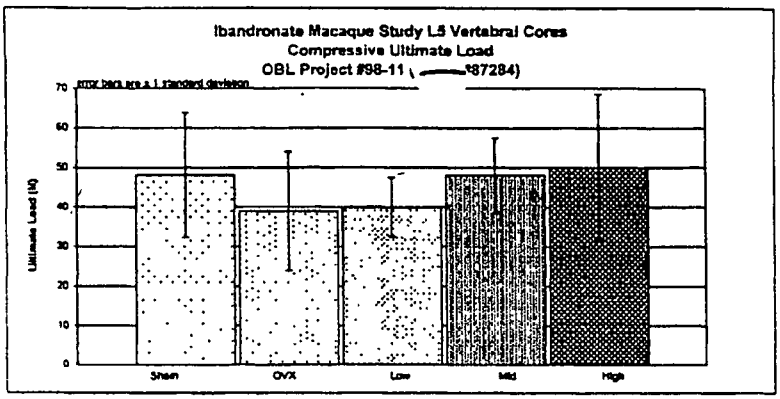
Vertebral cores



Group Differences in percent	from Sham	from OVX	Group COV
Sham	-	+ 38%	30%
OVX	- 28%	-	43%
Low Ibandronate	- 10%	+ 24%	13%
Mid Ibandronate	+ 9%	+ 51%	22%
High Ibandronate	+ 21%	+ 68%	31%

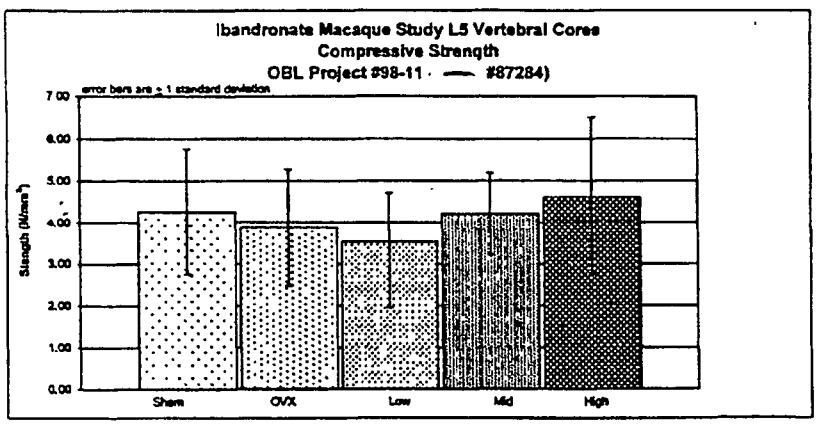
* = significant difference

Figure 6: L5 Vertebral Cores pQCT density



Group Differences in percent	from Sham	from OVX	Group COV
Sham	-	+ 23%	33%
OVX	- 19%	-	38%
Low Ibandronate	- 17%	+ 3%	19%
Mid Ibandronate	0%	+ 23%	30%
High Ibandronate	+ 4%	+ 28%	37%

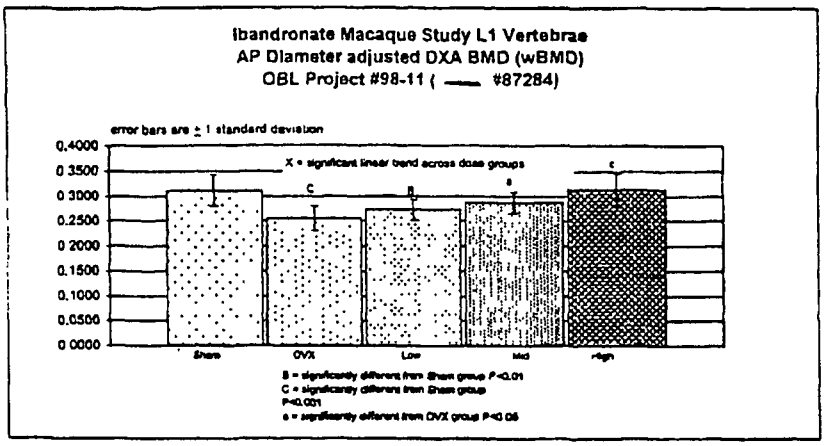
Figure 7: L5 Vertebral Cores Compression Testing - Ultimate Load



Group Differences in percent	from Sham	from OVX	Group COV
Sham	-	+ 10%	15%
OVX	- 9%	-	38%
Low Ibandronate	- 17%	- 9%	33%
Mid Ibandronate	- 1%	+ 9%	23%
High Ibandronate	+ 8%	+ 19%	41%

Figure 9: L5 Vertebral Cores Compression Testing - Strength

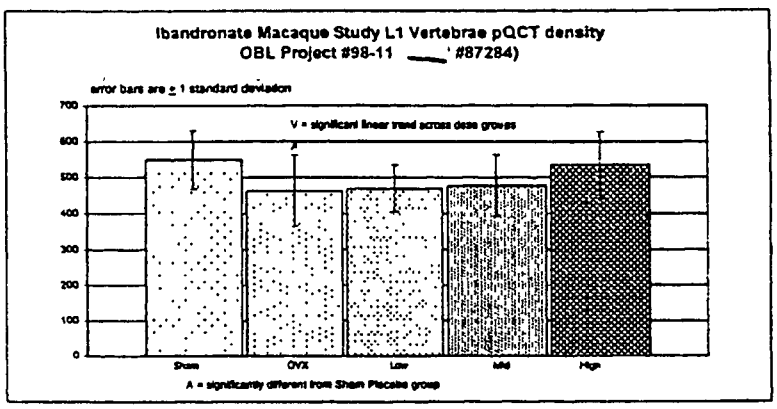
L1 vertebrae



Group Differences in percent	from Sham	from OVX	Group COV
Sham	-	*+ 22%	10%
OVX	*- 18%	-	10%
Low Iband	*- 13%	+ 7%	8%
Mid Iband	- 8%	*+ 12%	8%
High Iband	+ 1%	*+ 23%	11%

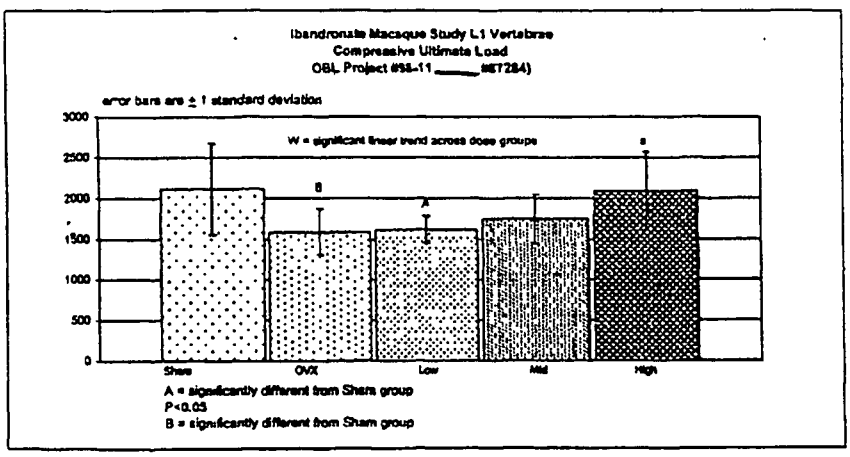
* = significant difference

Figure 15: L1 Vertebrae AP Diameter Adjusted BMD (wBMD)



Group Differences in percent	from Sham	from OVX	Group COV
Sham	-	*+ 19%	13%

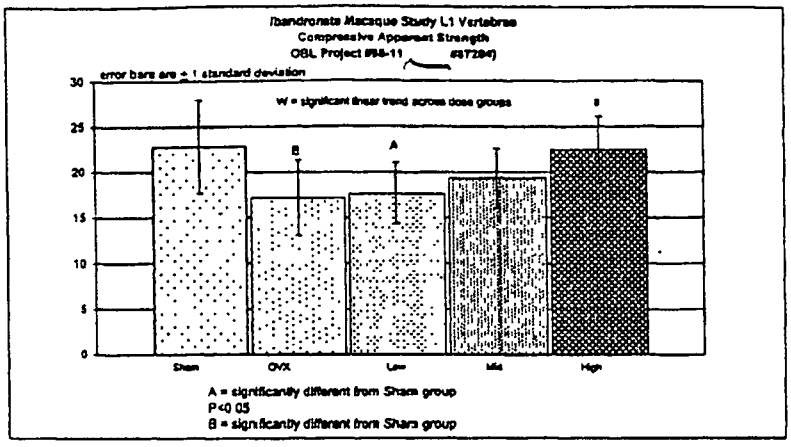
* = significant difference



Group Differences in percent	from Sham	from OVX	Group COV
Sham	-	*+ 33%	26%
OVX	*- 25%	-	18%
Low Iband	*- 24%	+ 2%	10%
Mid Iband	- 14%	+ 10%	17%
High Iband	+ 1%	*+ 32%	23%

* = significant difference

Figure 19: L1 Vertebrae Compression Test - Ultimate Load

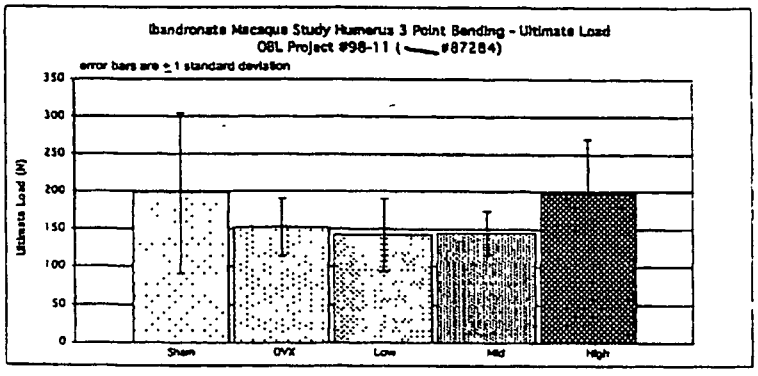


Group Differences In percent	from Sham	from OVX	Group COV
Sham	-	+ 33%	22%
OVX	- 25%	-	24%
Low Band	- 23%	+ 3%	19%
Mid Band	- 15%	+ 13%	17%
High Band	- 2%	+ 31%	18%

* = significant difference

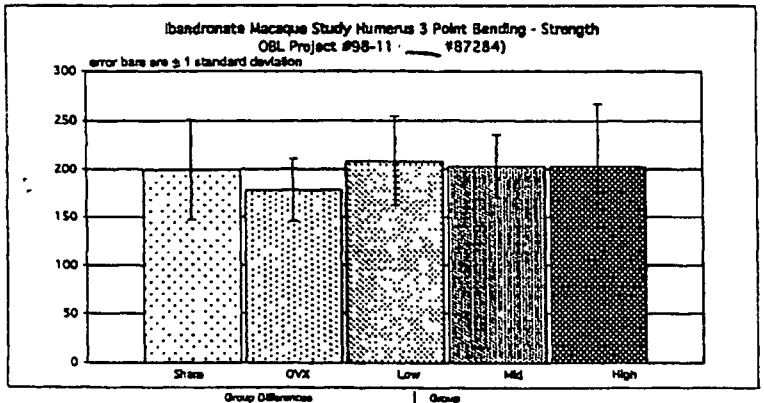
Figure 21: L1 Vertebrae Compression Testing - Apparent Strength

Humerus (beam specimens processed by hand)



Group Differences In percent	from Sham	from OVX	Group COV
Sham	-	+ 30%	34%
OVX	- 23%	-	28%
Low Band	- 38%	- 7%	34%
Mid Band	- 27%	- 5%	28%
High Band	- 1%	+ 30%	37%

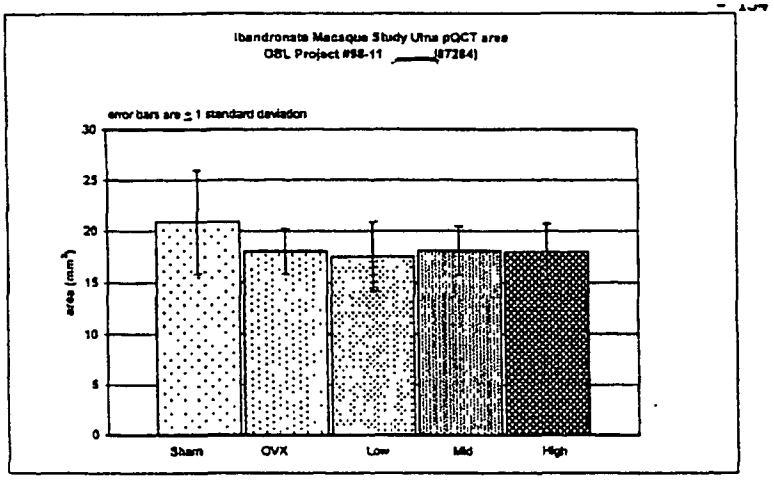
Figure 27: Humerus Cortical Beams 3 Point Bending - Ultimate Load



Group Differences In percent	from Sham	from OVX	Group COV
Sham	-	+ 12%	25%
OVX	- 11%	-	19%
Low Band	- 9%	- 17%	22%
Mid Band	+ 2%	- 14%	16%
High Band	+ 8%	- 14%	31%

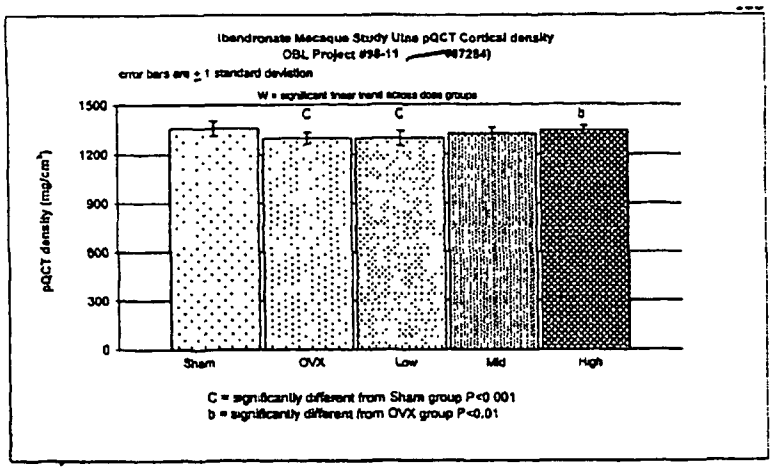
Figure 28: Humerus Cortical Beams 3 Point Bending - Strength

Ulna (cortical bone)



Group Differences in percent from Sham			from OVX			Group COV
Sham	-		+	16%		24%
OVX	-	14%		-		12%
Low Iband	-	16%	-	3%		19%
Mid Iband	-	13%	+	1%		13%
High Ibanc	-	14%	-	1%		15%

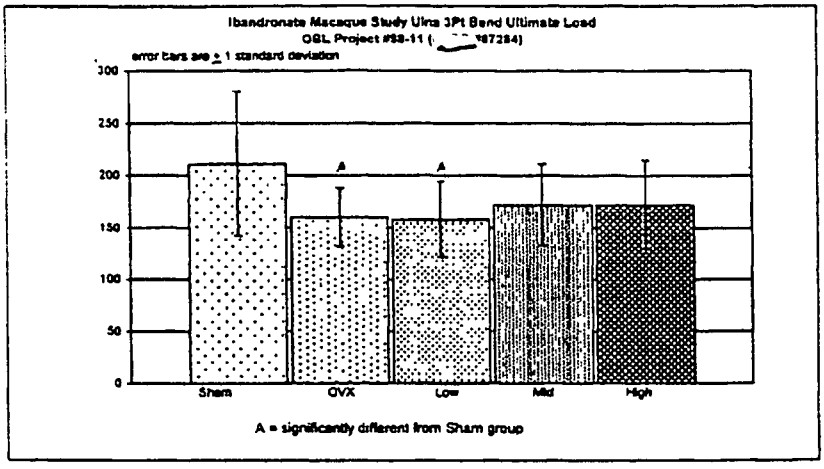
Figure 32: Ulna pQCT area



Group Differences in percent		from Sham		from OVX	Group COV
Sham	-		*+	3%	3%
OVX	*-	4%	-		3%
Low Iband	*-	4%		0%	3%
Mid Iband	-	2%	+	2%	3%
High Ibanc	-	1%	*+	4%	2%

* = significant difference

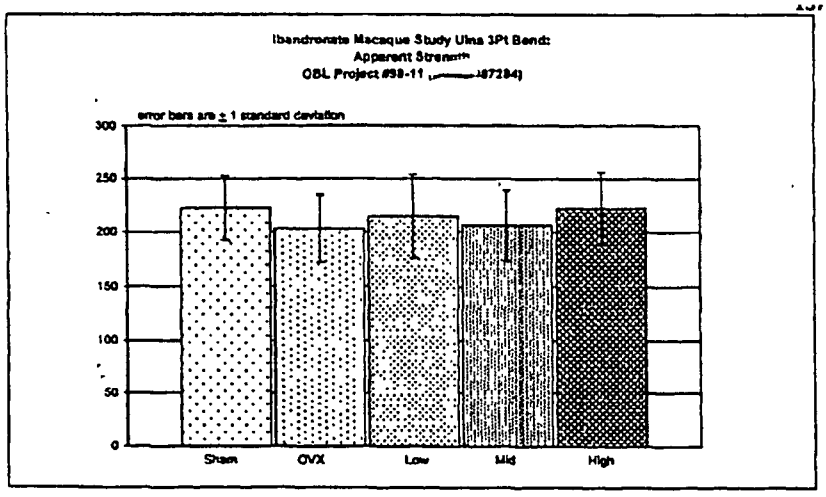
Figure 34: Ulna pQCT Cortical Density



Group Differences in percent	from Sham	from OVX	Group COV
Sham	-	+ 32%	33%
OVX	- 24%	-	18%
Low Ibandr	- 25%	- 1%	23%
Mid Ibandr	- 18%	+ 8%	23%
High Ibandr	- 16%	+ 9%	25%

* = significant difference

Figure 37: Ulna 3 Point Bend - Ultimate Load



Group Differences in percent	from Sham	from OVX	Group COV
Sham	-	+ 9%	13%
OVX	- 9%	-	15%
Low Ibandr	- 3%	+ 6%	16%
Mid Ibandr	- 7%	+ 2%	16%
High Ibandr	0%	+ 9%	15%

Figure 38: Ulna 3 Point Bend Apparent Strength

Femur

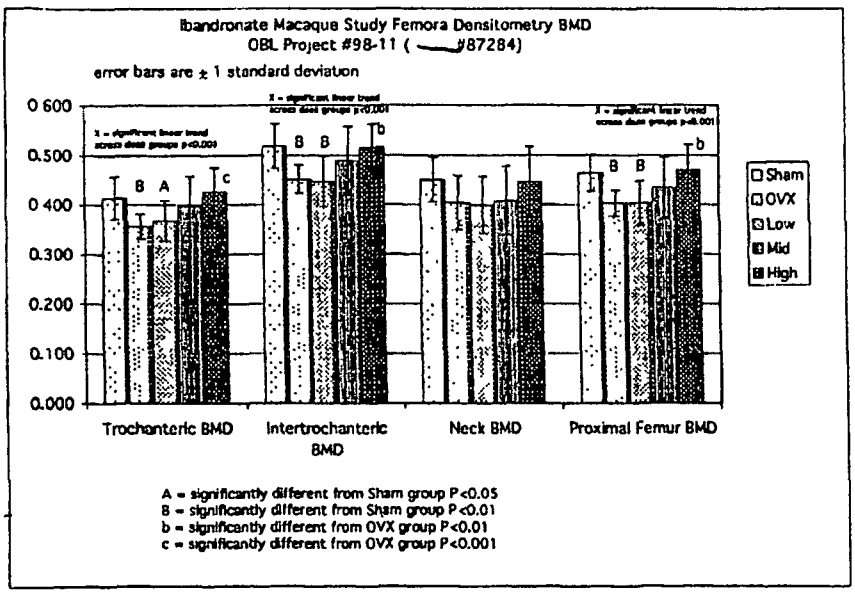
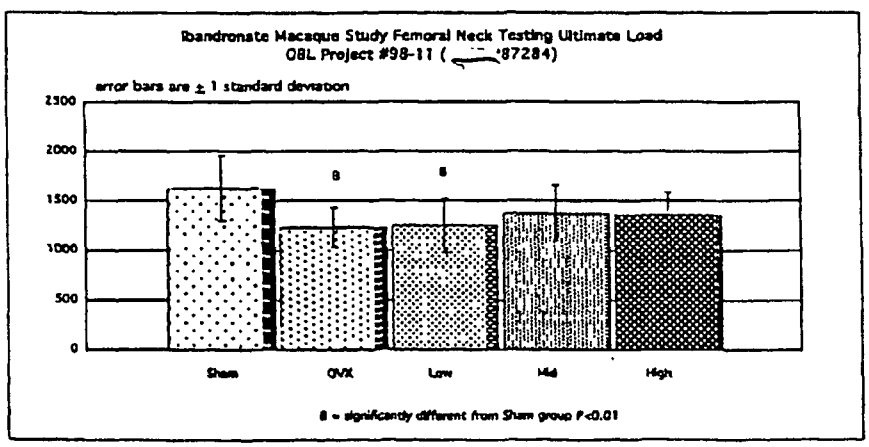


Figure 41: Combined Proximal Femur BMD



Group Differences (in percent)	from Sham	from OVX	Group COV
Sham	-	↑ 33%	20%
OVX	↓ 24%	-	17%
Low ibandr	↓ 23%	↑ 1%	22%
Mid ibandr	↓ 18%	↑ 12%	21%
High ibandr	↓ 18%	↑ 11%	18%

* = significant difference

Figure 43: Femoral Neck Testing - Ultimate Load

TABLE 3 (from Smith et al, Bone Vol. 32, 2003)

Table 3
Biomechanical testing of lumbar vertebrae in compression, whole ulnae in three-point bending and femoral neck strength, after 16 months treatment with ibandronate

	Sham	OVX			
		Vehicle	Ibandronate (μg/kg/dose)		
			10	30	150
L1 whole vertebrae					
Ultimate load (N)	2116 (561)	1586 (286)[a]	1614 (163)[a]	1745 (299)	2089 (479)[b]
Apparent strength (N/mm²)	22.80 (5.13)	17.14 (4.15)[a]	17.61 (3.34)[a]	19.30 (3.26)	22.45 (3.64)[b]
Stiffness (N/mm)	1375 (395)	1131 (257)	1345 (527)	1266 (350)	1489 (417)
Apparent modulus (N/mm²)	246.20 (68.25)	194.84 (49.21)	220.94 (76.19)	233.64 (74.55)	257.50 (51.14)
pQCT BMD (mg/cm³)	548 9 (81.1)	462.0 (98.6)[a]	468.9 (64.7)	477.0 (85.5)	533 7 (93.3)
Ulna					
Ultimate load (N)	211 (70)	160 (28)[a]	158 (37)[a]	172 (39)	172 (43)
Strength (N/mm²)	222.53 (29 85)	203.39 (31.28)	214.89 (38.83)	206.50 (32.83)	222.60 (33.93)
Stiffness (N/mm)	70 (29)	53 (11)	51 (15)	57 (17)	54 (16)
Modulus (N/mm²)	2.45 (0.34)	2.43 (0.40)	2.51 (0.50)	2.38 (0.37)	2.54 (0.41)
Cortical pQCT BMD (mg/cm³)	1357.0 (45.1)	1296.7 (33 6)[a]	1296.1 (43.8)[a]	1324.2 (36.5)	1344 6 (26 8)[b]
Femoral neck					
Ultimate load (N)	1624 (331)	1229 (203)[a]	1246 (278)[a]	1372 (285)	1362 (221)
Stiffness (N/mm)	1027 (245)	862 (184)	991 (242)	946 (279)	881 (151)
Proximal DXA BMD (g/cm²)	0 464 (0.037)	0.403 (0.026)[a]	0.403 (0.044)[a]	0.434 (0.063)	0.470 (0.050)[b]

Data are means (SD).
[a] Significantly different from sham controls.
[b] Significantly different from OVX controls.
$P < 0.05$.

BMD, BMC, size and strength parameters at various bone sites

		Sham	OVX			
			Vehicle	10 ukd	30 ukd	150 ukd
L5 vertebral cores	BMD QCT (mg/cm3)	290	210 (a)	261	316 (b)	352 (b)
	BMC QCT (mg/mm)	3.35	2.16	2.97	3.64	3 85
	Ultimate Load (N)	48	39	40	48	50
L1 whole vertebrae	BMD QCT (mg/cm3)	549	462 (a)	469	477	534
	BMC QCT (mg/mm)	51	43	43	44	51
	BMD DXA/APØ (g/cm3)	0.31	0 25 (a)	0.27 (a)	0.29 (b)	0 31 (b)
	BMC DXA (g)	0.70	0.59	0.57	0.67	0.77 (b)
	Ultimate Load (N)	2116	1586 (a)	1614 (a)	1745	2089 (b)
Humerus	Ultimate Load (N)	198	152	142	145	197
	Width (of specimen)	4.47	4.31	3.83 (a)	4.33	4.85 (b)
	Strength (N/mm2)	199	178	208	203	203
Ulna	BMD QCT (mg/cm3)	1357	1297 (a)	1296 (a)	1324	1345 (b)
	BMC QCT (mg/mm)	28.2	23.4 (a)	22.7 a)	24.0	24.2
	Area (mm2)	20.9	18.0	17.5	18.1	18.0
	AP diameter (mm)	4.96	4.60	4.56	4.73	4 56
	MI (mm4)	47	35	34	39	34
	Ultimate Load (N)	211	160 (a)	158 (a)	172	172
Femoral neck	BMD DXA (g/cm2)	0.45	0.40	0.40	0.41	0.45
	BMC DXA (gr)	0.18	0.16	0.13 (a)	0.17	0 20 (b)
	Ultimate Load (N)	1624	1229 (a)	1246 a)	1372	1362

(a) significantly different from sham
(b) significantly different from OVX