Effects of Bisphosphonate on Bone Reaction After Placement of Titanium Implants in Tibiae of Ovariectomized Rats

Yuki Tokugawa, DDS, PhD[1]/Tatsuo Shirota, DDS, PhD[2]/
Kohsuke Ohno, DDS, PhD[3]/Akira Yamaguchi, DDS, PhD[4]

Purpose: *This study was undertaken to investigate the action of bisphosphonate on bone reactions after the placement of titanium screw implants into the tibiae of ovariectomized rats.* ***Materials and Methods:*** *Twelve-week-old female Wistar rats were divided into 4 groups of 18 animals each. The first group (Sham) was sham-operated, the second group (OVX) was ovariectomized only, the third group (Estrogen) was ovariectomized and received continuous estrogen with a 17β-estradiol pellet, and the fourth group (YM-175) was ovariectomized and received bisphosphonate at a dose of 10 μg/kg of body weight. Titanium screw implants were placed in the proximal metaphyses of the tibiae 168 days after surgery. The animals were sacrificed 7, 14, and 56 days after implant placement. Undecalcified sections were prepared and evaluated by light microscopy. Histomorphometric measurements were obtained with a computer-based image analyzer to quantify the unit bone mass around the implant and the rate of implant-bone contact.* ***Results:*** *Ovariectomies significantly reduced implant-bone contact and the bone volume around the implants. However, in the YM-175 group, only slight differences in both bone contact and bone volume were noted compared with the Sham and Estrogen group. The woven structure of new bone in the YM-175 group was also replaced by mature lamellar bone, as in the other groups.* ***Discussion and Conclusion:*** *These results suggested that bisphosphonate preserved the implant-bone contact and bone volume around the implants.* (INT J ORAL MAXILLOFAC IMPLANTS 2003;18:66–74)

Key words: *bisphosphonate, bone, dental implants, estrogen, ovariectomy, rat*

Osteoporosis is a bone disease characterized by low bone mass leading to an increased risk of fractures and is a serious public health problem.[1] Postmenopausal osteoporosis results from a reduction in estrogen levels after natural or surgical menopause-accelerated bone loss. The bone loss of estrogen deficiency is attributable to both increases in bone turnover and an imbalance in favor of resorption.[2]

Several studies have suggested correlations between mandibular bone mineral density around the teeth, advanced residual ridge resorption, and total skeletal bone mass in osteoporotic patients.[3–5] Kribbs[5] reported that patients with osteoporosis ran a higher risk of early tooth loss compared with normal subjects in the same age groups. Von Wowern and coworkers[3] demonstrated that the mandible undergoes a continuous physiologic sex- and age-related decrease in bone density and bone mineral content. Klemetti and associates[4] also described the highest Community Periodontal Index of Treatment Needs values in postmenopausal subjects with the highest systemic bone mineral density. The long-term clinical success of dental implants relies on continued osseointegration

[1]Staff, First Department of Oral and Maxillofacial Surgery, School of Dentistry, Showa University, Tokyo, Japan.
[2]Assistant Professor, First Department of Oral and Maxillofacial Surgery, School of Dentistry, Showa University, Tokyo, Japan.
[3]Associate Professor, First Department of Oral and Maxillofacial Surgery, School of Dentistry, Showa University, Tokyo, Japan.
[4]Professor and Chairman, Department of Oral Pathology, School of Dentistry, Nagasaki University, Nagasaki, Japan.

Reprint requests: Dr Tatsuo Shirota, First Department of Oral and Maxillofacial Surgery, School of Dentistry, Showa University, 2-1-1 Kitasenzoku, Ohta-ku, Tokyo, 145-8515, Japan. Fax: +81-3-3787-7104. E-mail address: tshirota@senzoku.showa-u.ac.jp

in the jawbone. Therefore, suitable control of the bone mass and bone metabolism may be necessary to enable more successful dental implant treatment in patients with osteoporosis.

Bisphosphonates are pyrophosphate analogs containing a P-C-P bond, which is stable to chemical and enzymatic hydrolysis; they strongly bind to hydroxyapatite crystals and inhibit osteoclastic bone resorption.[6,7] The efficacy of their bone mass–preserving action has been elucidated in conditions characterized by increased resorption. In animal models, maintenance of bone mass and strength by bisphosphonate treatment has been extensively evaluated in ovariectomized rats,[8–15] dogs,[16] and monkeys.[17] In addition, bisphosphonates have been used for adjunct therapy for bone diseases involving increased bone resorption, such as metastatic bone disease,[18] Paget's disease,[19] and osteoporosis.[20]

Starck and Epker[21] described a patient who experienced the loss of 5 implants that had successfully osseointegrated while taking etidronate disodium for osteoporosis. They proposed that bisphosphonate therapy is a risk factor for the osseointegration of dental implants. Given that a substantial number of patients need dental implants, it is probable that some would be women who required treatment because of postmenopausal osteoporosis. Although several studies indicate that dental implants placed in healthy patients have been successful, their placement in patients with osteoporosis remains controversial. There have been no controlled studies examining the effects of bisphosphonates on the implant-tissue interface. The purpose of this study was to investigate the action of bisphosphonate on bone reactions adjacent to endosseous implants in ovariectomized rats.

MATERIALS AND METHODS

Experimental Animals

Seventy-two female Wistar rats were used in this study. These animals were 12 weeks old and weighed 200 g at the beginning of the experiment. For all experiments, the rats were housed at room temperature (24°C to 25°C) and 55% humidity with a circadian light rhythm of 12 hours and fed a pelleted commercial standard diet (Oriental Yeast, Tokyo, Japan) containing 1.1% calcium and 0.83% phosphorus. Water was available ad libitum.

Medications

For estrogen treatment, slow-release pellets containing 0.5 mg of 17β-estradiol were purchased from Innovative Research of America (Rockville, MD). YM-175 (disodium cycloheptylaminomethylenedisphosphonate monohydrate) was provided by Yamanouchi Pharmaceutical (Tokyo, Japan) and was prepared in a vehicle of normal saline.

Experimental Design

Briefly, the animals were anesthetized by abdominal administration of a 1:1 mixture of ketamine chloride (Ketarat, Sankyo, Tokyo, Japan) and xylazine (Ceractal, Bayer, Leverkusen, Germany). Bilateral ovariectomies were performed from a dorsal approach in 72 animals. The remaining rats were subjected to sham surgery that exposed but did not remove the ovaries. The rats were then randomly divided into 4 groups of 18 animals each based on the following treatments (Fig 1).

1. Sham group, sham-operated
2. OVX group, bilaterally ovariectomized
3. Estrogen group, bilaterally ovariectomized and treated with a 17β-estradiol pellet that was implanted subcutaneously in the back of the neck every 3 weeks starting 4 weeks after surgery throughout the experiment
4. YM-175 group, bilaterally ovariectomized and treated with bisphosphonate (YM-175) at the dose of 10 μg/kg of body weight injected subcutaneously 3 times per week from 4 weeks after surgery throughout the experiment

Implantation

Implants were made of commercially pure titanium and fabricated as screw-type by machining. They were 2.0 mm in diameter and 5.0 mm long, with a pitch height of 1.0 mm (Taguchi, Tokyo, Japan). They were cleaned in trichloroethylene, rinsed in absolute ethanol in an ultrasonic bath, and sterilized in an autoclave. As a postmenopausal osteoporosis model, rats in which bone mass is sufficiently reduced are considered appropriate. Therefore, the implants were placed in the proximal metaphyseal region of both sides of the tibiae in 18 rats of each group 168 days after ovariectomy or sham surgery. Under general anesthesia, the skin of the bilateral tibial region was shaved and disinfected with 70% ethanol. A 15-mm incision was made on the anteromedial aspect of the proximal end of the tibia, and the bone surface was exposed. Then, an implantation hole 1.0 mm in diameter was drilled with a rotatory speed not exceeding 2,000 rpm, accompanied by profuse saline irrigation. The implants were then placed via self-tapping in this site.

Histologic Preparation

Six rats in each group were sacrificed at 7, 14, and 56 days after implantation. The tibiae were removed, fixed in 4% buffered formalin, and dehydrated by an

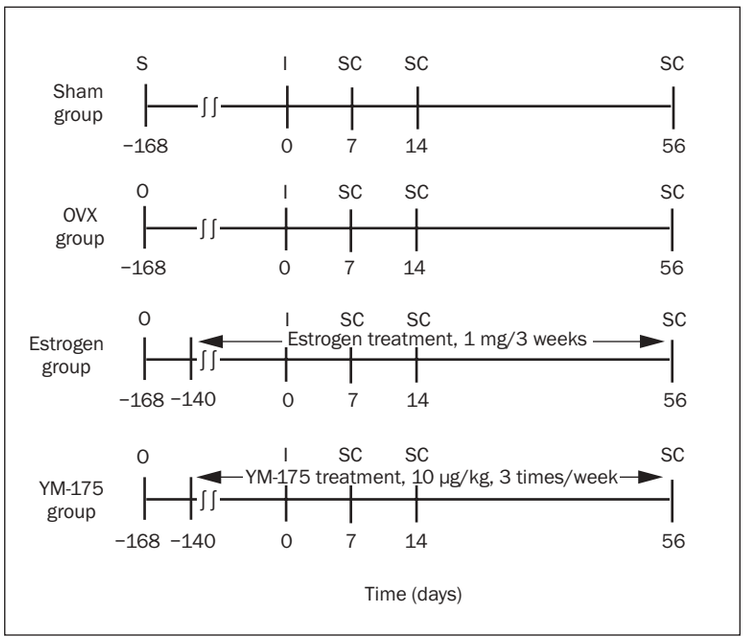

Fig 1 Experimental protocol. S = sham operation; O = ovariectomy; I = implantation; SC = sacrifice.

ascending (percentage) series of alcohol. Simultaneously, the body and uterus weights were measured. The specimens were embedded in methylmethacrylate resin (Technovit, Kulzer, Wehrheim, Germany), and approximately 50-μm-thick undecalcified sections of the tibia were prepared along the long axis of the implant with an EXAKT Cutting-Grinding System (Exakt-Apparatebau, Norderstedt, Germany). Each section was reground to a thickness of 15 μm and stained with 1% toluidine blue for general histologic observation.

Histomorphometric Analysis

To quantify the changes in bone tissue around the implants, the parameters below were measured morphometrically. Primary parameters were assessed via an automatic image analyzing system (SPECTRUM, Mitani, Tokyo, Japan). Secondary parameters were calculated from primary parameters.

Primary parameters were:

- Total bone tissue area (mm^2), ie, the total area of cancellous bone and marrow tissue;
- Total bone area (mm^2), ie, the cancellous bone area;
- Implant circumference (mm), the boundary length of a single longitudinal profile of the implant; and
- Total contact surface (mm), the total boundary length bone/implant contact.

Secondary parameters were:

- Bone density (%), ie, the ratio of cancellous bone area in the marrow cavity (total bone area/total bone tissue area) × 100; and
- Percentage of bone-implant contact, ie, the ratio of the implant surface covered with bone for the total length of the implant (total contact surface/implant circumference) × 100.

Statistical Analysis

Values were calculated as means ± SEM. The Sham, OVX, and other groups were compared using the Mann-Whitney *U* test. Differences were considered statistically significant at $P < .05$.

RESULTS

Body Weights

The rats in the OVX and the YM-175 groups weighed significantly more than those in the Sham group throughout the observation period. The rats in the Estrogen group displayed significantly lower weights than those in the OVX group at all times (Table 1).

Uterus Weights

The uterus weights of the rats in the OVX and the YM-175 groups were significantly lower than in the corresponding Sham rats throughout the observation period. The rats in the Estrogen group displayed significantly higher weights than those in the

OVX group up to 14 days after implantation. However, there were no statistical differences between the Estrogen and the OVX groups at the 56-day time point (Table 2).

Histologic Findings

Seven Days After Implantation. In the Sham group, the cancellous bone interconnected around the implant. Woven bone was formed from the stumps of original cortical bone and/or the cancellous bone surfaces toward the implant and was in direct contact with its surface in some places (Fig 2a).

In the OVX group, the cancellous bone around the implants was thin and short in comparison with that seen in the Sham group. However, woven bone was formed from the stumps of cortical bone and/or cancellous bone surfaces toward the implant, and some of this was in direct contact with the implant surface (Fig 2b).

In the Estrogen group, abundant cancellous bone was observed around the implants, as in the Sham group. The amount of newly formed bone around the implants, with some in direct contact with the implant surface, appeared equivalent to that seen in the Sham group (Fig 2c).

In the YM-175 group, the distribution of the cancellous bone around the implants appeared equivalent to that of the Sham group. Newly formed trabecular bone was in direct contact with the surface of the implant in parts (Fig 2d).

Fourteen Days After Implantation. In the Sham group, newly formed bone trabeculae around the implants from the stumps of cortical bone and/or cancellous bone surfaces consisted mainly of lamellar bone. The majority of the implant surface was covered with thin lamellar bone (Fig 3a).

In the OVX group, newly formed bone trabeculae consisted mainly of lamellar bone. This new bone was in direct contact with the surface of the implant, but such new ossification appeared smaller, and the distribution of bone surrounding the implants appeared thinner than the corresponding bone noted in the Sham group (Fig 3b).

In the Estrogen group, new bone trabeculae around the implant were also almost the same as in the Sham group (Fig 3c).

In the YM-175 group, new trabecular bone around the implant consisted mainly of lamellar bone. Although part of the implant surface was covered with new bone similar to that of the Sham group, newly formed bone that made contact with the implant surface was thinner than in the Sham group (Fig 3d).

Fifty-six Days After Implantation. In the Sham group, most of the implant surface was in direct contact with the new bone that had formed around the remnants of compact bone in the cortical bone area. Almost the entire implant surface was covered with new lamellar bone that was connected to the cancellous bone in several places (Fig 4a).

In the OVX group, the new bone in contact with the implant surface appeared thinner than that seen in the other groups. In the areas of cortical bone, the surface of the implant was in contact with thick, new, lamellar bone that resembled that of the Sham group (Fig 4b).

In the Estrogen group, the surface of the implant in the cortical bone area was in contact with thick, new, lamellar bone, as seen in the Sham group. Almost the entire implant surface was covered with new lamellar bone that was connected to the cancellous bone in several places (Fig 4c).

In the YM-175 group, the surface of the implant in the cortical bone area was in contact with thick, new, lamellar bone that was similar to the bone noted in the Sham group. Nonetheless, the new bone that contacted the implant surface appeared thinner than in the Sham group (Fig 4d).

Bone Histomorphometric Analyses

Bone Density. In the Sham group, the unit bone mass around the implants increased actively from 7 to 14 days after implantation and reached approximately 46% at 56 days after implantation. In the OVX group, the bone density around the implants reached a maximum of approximately 22% at 14 days and decreased slightly thereafter. It remained significantly lower in the OVX group than in the other groups throughout the experiment. The bone density in the Estrogen group was similar to that in the Sham group and was significantly higher than that in the OVX group. The bone density in the YM-175 group also remained significantly higher than that in the OVX group throughout the experiment. However, the bone density in the YM-175 group was lower than that in the Sham and Estrogen groups, and by 56 days, these differences were statistically significant (Table 3).

Percentage of Bone-Implant Contact. In the Sham group, the rate of bone contact between the implant and new bone in areas of cortical bone began to increase at 7 days after implantation and reached approximately 83% at 56 days after implantation. The rate of bone-implant contact in the OVX, Estrogen, and YM-175 groups was similar to that in the Sham group (Table 4).

In the cancellous bone area, the contact percentage in the Sham group rapidly increased to approximately 77% during the period between 7 and 14 days after implantation. This percentage was significantly higher than that in the OVX

Table 1 Comparison of Body Weight (g) Between Groups

Group	Time (days)		
	7	14	56
Sham	249.8 ± 4.8	274.8 ± 6.7	282.8 ± 14.6
OVX	318.3 ± 11.5[b]	332.0 ± 25.0[b]	352.8 ± 5.0[b]
Estrogen	272.7 ± 4.1[b,d]	282.8 ± 16.8[c]	270.6 ± 5.6[d]
YM-175	331.4 ± 7.8[b,f]	353.4 ± 19.6[b]	372.2 ± 18.2[b,f]

Each value is mean ± SE. [a]$P < .05$ versus Sham group; [b]$P < .01$ versus Sham group; [c]$P < .05$ versus OVX group; [d]$P < .01$ versus OVX group; [f]$P < .01$ versus Estrogen group.

Table 2 Comparison of Uterus Weight (mg) Between Groups

Group	Time (days)		
	7	14	56
Sham	602.4 ± 34.9	920.0 ± 391.4	675.0 ± 130.7
OVX	65.2 + 6.4[a]	95.0 + 11.1[a]	59.8 ± 38.8[a]
Estrogen	290.0 ± 40.6[d]	307.0 ± 14.6[d]	507.0 ± 15.6[b,d]
YM-175	59.6 ± 26.6[b,f]	45.6 ± 11.5[b,c,f]	52.4 ± 18.1[b,f]

Each value is mean ± SE. [a]$P < .05$ versus Sham group; [b]$P < .01$ versus Sham group; [c]$P < .05$ versus OVX group; [d]$P < .01$ versus OVX group; [f]$P < .01$ versus Estrogen group.

Figs 2a to 2d *Specimens at 7 days after implantation (toluidine blue; original magnification ×50).*

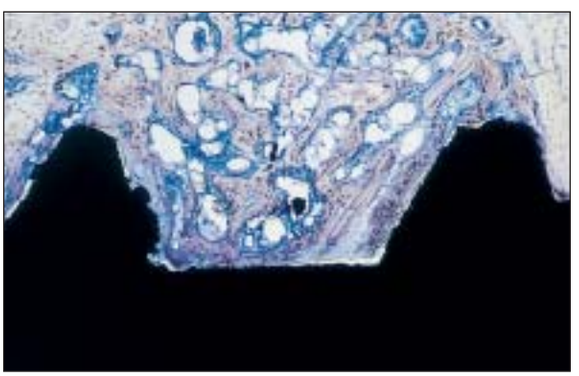

Fig 2a Sham-operated rat. Abundant pre-existing cancellous bone is observed around the implant, and newly formed woven bone has been formed around the implant.

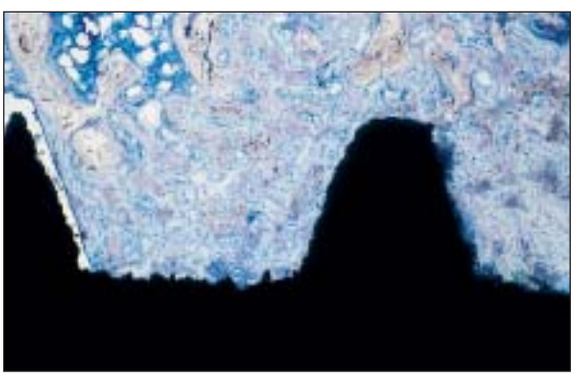

Fig 2b OVX rat. Pre-existing cancellous bone around the implant is thin in comparison with that seen in Sham rats.

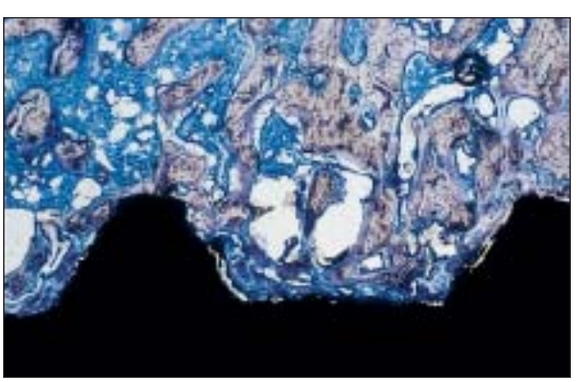

Fig 2c Estrogen-treated rat. Pre-existing cancellous bone is observed around the implant as in Sham rat.

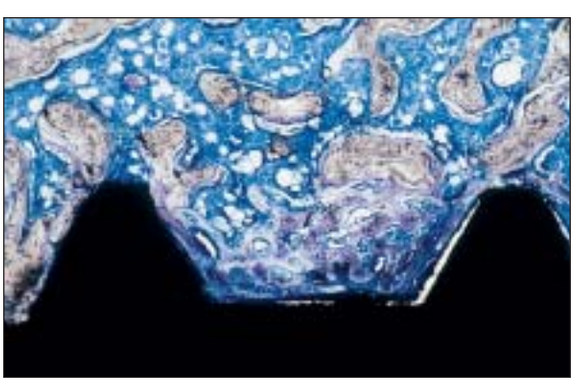

Fig 2d YM-175-treated rat. Pre-existing cancellous bone is also seen around implant as in Sham rat.

groups at all time points after implantation ($P < .05$ or $P < .01$; Table 5). In the Estrogen group, no significant difference was noted in bone contact compared to that in the Sham group at 7 and 14 days after implantation. In the YM-175 group, the rate of implant-bone contact reached approximately 23% at 7 days after implantation, and was significantly higher than that in the other groups. Thereafter, there were no significant differences in the bone contact between the Sham and the YM-175 groups up to 56 days after implantation (Table 5).

Figs 3a to 3d *Specimens at 14 days after implantation (toluidine blue; original magnification ×50).*

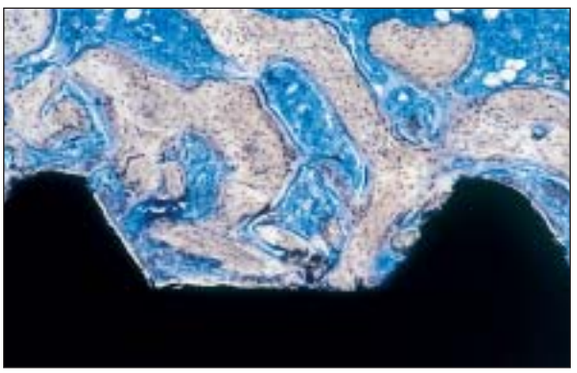

Fig 3a Sham-operated rat. Newly formed trabeculae have matured, and the surface of the implant is extensively covered with new bone.

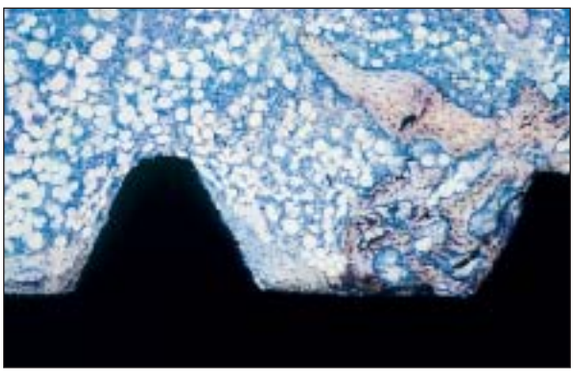

Fig 3b OVX rat. Newly formed bone trabeculae contact the implant surface, but the amount of bone around the implant is less than that in the Sham rat.

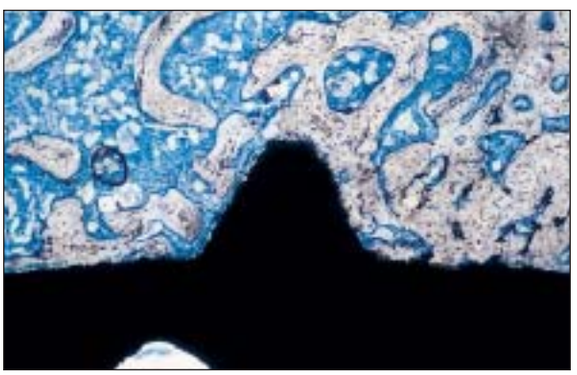

Fig 3c Estrogen-treated rat. Entire implant surface is in contact with mature new bone, as in Sham rat.

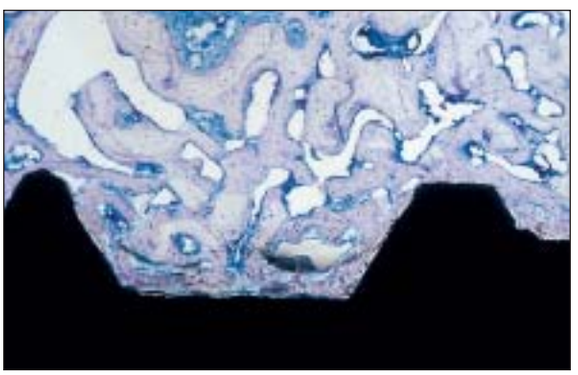

Fig 3d YM-175-treated rat. The surface of the implant is extensively in contact with new bone, as in Sham rat.

DISCUSSION

YM-175 is a new-generation bisphosphonate that has been shown to be effective in preventing bone resorption in various experimental models, including in experimental hypercalcemia induced by Walker-256 tumors and by parathyroid hormone (PTH) administration,[22] in animals immobilized by neurectomies,[23] and in OVX rats after cessation of PTH treatment.[24] Motoie and coworkers[25] also found that YM-175 preserved bone mass and strength without impairment of mineralization in OVX, calcium-restricted beagle dogs. In the animal model of PTH-induced hypercalcemia, Takeuchi and associates[26] reported that YM-175 was 10- and 1,000-fold more potent than pamidronate and etidronate, respectively. Based on this background understanding, YM-175 can be expected to be useful in the treatment of humoral hypercalcemia of malignancy and osteoporosis. The results of this study may provide important information regarding dental implants in patients who have received YM-175 for the treatment of systemic bone loss.

The dose of YM-175 used in this study (10 μg/kg 3 times per week as a subcutaneous injection) might be suitable for the treatment of OVX rats because of certain effects related to prevention of bone loss.[24]

There are different opinions of bone formation activity in fracture healing under bisphosphonate treatment. Nyman and colleagues[27] found that clodronate inhibited fracture healing by delaying osteoblast recruitment and differentiation. Li and associates[28] also reported inactive bone formation in the fracture healing process of rat tibiae under YM-175 treatment. However, Peter and coworkers[29] demonstrated that etidronate had no adverse effects on bone fracture healing, bone strength, or mineralization. In the present study, there was only a slight difference in new bone formation around implants in the cortical bone area among the study groups.

Figs 4a to 4d *Fifty-six days after implantation (toluidine blue; original magnification ×50).*

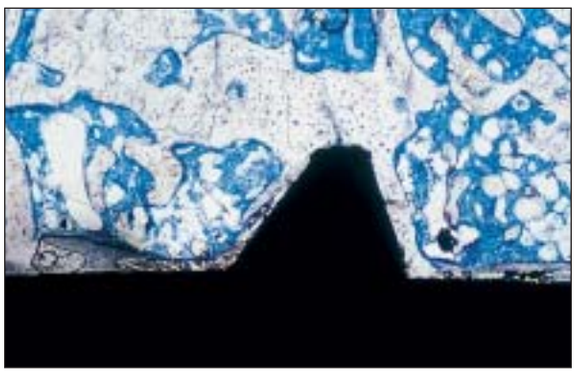

Fig 4a Sham-operated rat. The implant surface remains in contact with mature new bone.

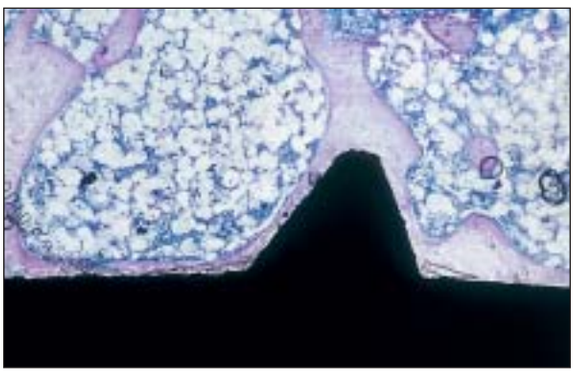

Fig 4b OVX rat. The greater part of the implant surface is covered with thin and uniform new bone.

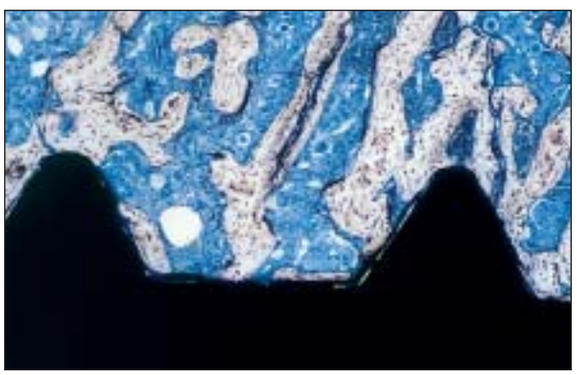

Fig 4c Estrogen-treated rat. Most of the implant surface is in contact with mature new bone, as in Sham rat.

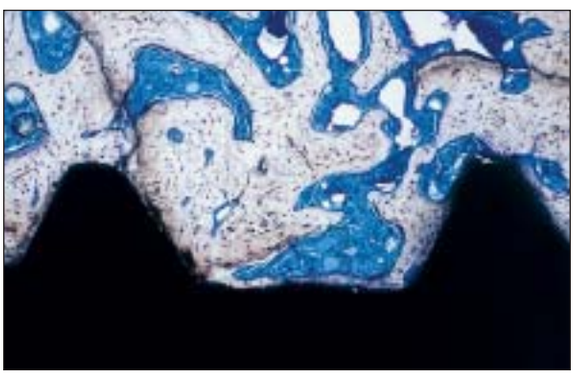

Fig 4d YM-175-treated rat. Most of the implant surface remains in contact with thin and uniform new bone.

Table 3 Bone Density (%) Around the Implant at Each Time Point

	Time (days)		
Group	**7**	**14**	**56**
Sham	32.8 ± 3.2	37.9 ± 7.7	45.7 ± 4.5
OVX	21.2 ± 5.3[b]	22.1 ± 7.9[a]	16.4 ± 2.3[b]
Estrogen	40.8 ± 6.6[a,b]	39.1 ± 5.4[c]	39.6 ± 5.6[d]
YM-175	25.1 ± 8.5[e]	33.3 ± 5.2[c]	37.9 ± 5.6[a,d]

Each value is mean ± SE. [a]$P < .05$ versus Sham group; [b]$P < .01$ versus Sham group; [c]$P < .05$ versus OVX group; [d]$P < .01$ versus OVX group; [f]$P < .01$ versus Estrogen group; [e]$P < .05$ versus Estrogen group.

Table 4 Percentage of Bone/Implant Contact (%) in Cortical Bone Area at Each Time Point

	Time (days)		
Group	**7**	**14**	**56**
Sham	30.1 ± 15.3	50.5 + 12.9	83.0 ± 17.4
OVX	27.4 ± 18.8	50.7 ± 11.1	80.3 ± 5.2
Estrogen	20.3 ± 4.4	46.4 ± 8.6	87.2 ± 5.0
YM-175	35.4 ± 12.9[e]	31.4 ± 14.3	88.3 ± 5.6

Each value is mean ± SE. [e]$P < .05$ versus Estrogen group.

This indicated that not only OVX, but also YM-175, had minimal influence on bone formation after placement of implants into the rat tibia. In the cancellous bone area of OVX rats, in which bone mass decreased by estrogen depletion, newly formed bone trabeculae around the implants were less numerous than in Sham rats. It is possible that such bone formation inferiority might be attributed to the loss of pre-existing cancellous bone for osteogenesis. In contrast, treatment of OVX rats with YM-175 maintained pre-existing bone mass and structure, as in the estrogen-treated rats, and new bone formation around the implants in the cancellous bone area was almost the same as in both Sham and estrogen-treated rats. Thus, in this model, YM-175 treatment had no negative effects on new bone formation around the implants, as estrogen treatment.

During the acquisition of bone contact with implants placed into the metaphyses of tibiae in normal rats, it has been previously indicated that bone-implant contact was almost complete by 56 days after implantation.[30,31] Histomorphometric examination in the present study demonstrated a similar level of bone-implant contact in the cortical bone area among the study groups throughout the experiment. In the cancellous bone area, a significant reduction in the amount of bone-implant contact in the OVX group was seen relative to that of the Sham group. In contrast, the bone contact ratios in both the YM-175 and Estrogen groups were higher than that in the OVX group and were almost the same as that noted in the Sham group. It appears that the pharmaceutical treatment in this experiment produced favorable results in achieving bone-implant contact in the cancellous bone area. It is important to note that the administration of YM-175 for the treatment of osteoporosis might not inhibit the osseointegration of dental implants.

The present study showed lower amounts of bone mass in the OVX group compared to the Sham group at each time point after implantation. In contrast, the bone mass in the YM-175 group was equivalent to that in both Sham and Estrogen groups throughout study. Therefore, YM-175 treatment appeared to be effective in the prevention of bone loss around the implants in the OVX group, as was the case with estrogen treatment.

Osteoclasts play an important role in the remodeling of woven bone into lamellar bone in the process of bone repair.[32,33] Bisphosphonates inhibit the activity of mature osteoclasts and the production of the osteoclast progenitor cells.[34] The influence of bisphosphonates on fracture healing has also been reported previously, as these agents have been known to disturb the remodeling process during fracture healing.[27,28] Bone repair after the placement of implants into the tibiae of normal rats was studied previously, and it was reported that the woven structure of new bone first appeared around the implant and was replaced by mature lamellar bone by 56 days after implantation.[30] The present study revealed that the woven structure of new bone in the YM-175 group was also replaced by mature lamellar bone as in the other groups. These results suggest that with clinical usage, YM-175 might slightly hinder bone remodeling in the healing processes after implantation.

Table 5 Percentage of Bone/Implant Contact (%) in Cancellous Bone Area at Each Time Point

	Time (days)		
Group	7	14	56
Sham	16.4 ± 1.9	77.1 ± 5.2	90.8 ± 5.4
OVX	12.2 ±2.8[a]	29.3 ± 8.0[b]	76.7 ± 5.8[a]
Estrogen	12.4 ± 8.1	51.7 + 18.6	92.2 ± 3.4[d]
YM-175	23.3 ± 5.4[a,c]	56.3 ± 9.2[d]	86.9 ± 7.5[d]

Each value is mean ± SE. [a]$P < .05$ versus Sham group; [b]$P < .01$ versus Sham group; [c]$P < .05$ versus OVX group; [d]$P < .01$ versus OVX group.

CONCLUSION

In the present study, the effects of YM-175 on bone reactions after placement of titanium screw implants into the tibiae of OVX rats were investigated. The results showed only a slight difference in both bone contact and bone area in the YM-175 group compared with the Sham and Estrogen groups. The woven structure of new bone in the YM-175 group was also replaced by mature lamellar bone, as in the other groups. These results suggested that YM-175 preserved implant-bone contact and that the bone area around the implants might also be slightly compromised in the healing processes after implantation in the tibial metaphyses of ovariectomized rats.

ACKNOWLEDGMENTS

This study was supported in part by the Ministry of Education, Science, and Culture of Japan (Great-in Aid for Science Research 11672006). The authors would like to acknowledge Dr Ken-ichi Michi, Professor Emeritus, Showa University, Tokyo, Japan, for helpful suggestions of this work.

REFERENCES

1. Riggs BL. Overview of osteoporosis. West J Med 1991;154: 63–77.
2. Lindsay R. Prevention of postmenopausal osteoporosis. Obstet Gynecol Clin North Am 1987;14:63–76.
3. Von Wowern N, Klausen B, Kollerup G. Osteoporosis: A risk factor in periodontal disease. J Periodontol 1994;65: 1134–1138.
4. Klemetti E, Collin HL, Forss H, Markkanen H, Lassila V. Mineral status of skeleton and advanced periodontal disease. J Clin Periodontol 1994;21:184–188.
5. Kribbs PJ. Comparison of mandibular bone in normal and osteoporotic women. J Prosthet Dent 1990;63:218–222.
6. Fleisch H. Bisphosphonate: Pharmacology and use in the treatment of tumor-induced hypercalcemia and metastatic bone disease. Drugs 1991;42:919–944.
7. Rodan GA, Fleisch H. Bisphosphonate: Mechanisms of action. J Clin Invest 1996;97:2692–2696.
8. Wronski TJ, Dann LM, Scott KS, Crooke LR. Endocrine and pharmacological suppressors of bone turnover protect against osteopenia in ovariectomized rats. Endocrinology 1989;125:810–816.
9. Toolan BC, Shea M, Myers RE, et al. Effects of 4-amino-1-hydroxybutylidene bisphosphonate on bone biomechanics in rats. J Bone Miner Res 1992;7:1399–1406.
10. Lauritzen DB, Balena R, Shea M, et al. Effects of combined prostaglandin and alendronate treatment on the histomorphometry and biomechanical properties of bone in ovariectomized rats. J Bone Miner Res 1993;8:871–879.
11. Ammann P, Rizzoli R, Caverzasio J, Shigematsu T, Slosman D, Bonjour JP. Effects of the bisphosphonate tiludronate on bone reaction. J Bone Miner Res 1993;8:1491–1498.
12. Mosekilde L, Sogaard CH, McOsker JE, Wronski TJ. PTH has more pronounced effect on vertebral bone mass and biomechanical competence than antiresorptive agents (estrogen and bisphosphonate) -assessed in sexually mature, ovariectomized rats. Bone 1994;15:401–408.
13. Søgaard CH, Wronski TJ, McOsker JE, Mosekilde L. The positive effect of parathyroid hormone on femoral neck bone strength in ovariectomized rats in more pronounced than that of estrogen or bisphosphonates. Endocrinology 1994; 134:650–657.
14. Qi H, Li M, Wronski TJ. A comparison of the anabolic effects of parathyroid hormone at skeletal site with moderate and severe osteopenia in aged ovariectomized rats. J Bone Mineral Res 1995;10:948–955.
15. Ammann P, Rizzoli R, Meyer JM, Bonjour JP. Bone density and shape as determinants of bone strength in IGF-I and/or pamidronate-treated ovariectomized rats. Osteoporosis Int 1996;6:219–227.
16. Faugere MC, Friedler RM, Bauss F, Malluche HH. A new bisphosphonate, BM 21.0955, prevents bone loss associated with cessation of ovarian function in experimental dogs. J Bone Miner Res 1993;8:1345–1355.
17. Balena R, Toolan BC, Shea M, et al. The effects of 2-year treatment with the aminobisphosphonate alendronate on bone metabolism, bone histomorphometry, and bone strength in ovariectomized nonhuman primates. J Clin Invest 1993;92:2577–2586.
18. Fitton A, McTravish D. Pamidronate. A review of its pharmacological properties and therapeutic efficacy in resorptive bone disease. Drugs 1991;41:289–318.
19. Cantrill JA, Anderson DC. Treatment of Paget's disease of bone. Clin Endocrinol 1990;32:507–518.
20. Watts NB, Harris ST, Genant HK, et al. Intermittent cyclical etidronate treatment of postmenopausal osteoporosis. N Engl J Med 1990;323:73–79.
21. Starck WJ, Epker BN. Failure of osseointegrated dental implants after diphosphonate therapy for Osteoporosis: A case report. Int J Oral Maxillofac Implants 1995;10:74–78.
22. Kudo M, Abe T, Kawamuki K, et al. Effects of YM-175 on experimental hypercalcemia and tumorinduced osteolysis in rats. J Bone Miner Res 1990;5(Suppl):S166.
23. Kawamuki K, Abe T, Kudo M, et al. Effect of YM-175 on bone positively correlates with its concentration in bone. J Bone Miner Res 1990;5(Suppl):S245.
24. Takano Y, Tanizawa T, Mashiba T, Endo N, Nishida S, Takahashi H. Maintaining bone mass by bisphosphonate incadronate disodium (YM-175) sequential treatment after discontinuation of intermittent human parathyroid hormone (1-34) administration in ovariectomized rats. J Bone Miner Res 1996;11:169–177.
25. Motoie H, Nakamura T, O'uchi N, et al. Effects of the bisphosphonate YM-175 on bone mineral density, strength, structure, and turnover in ovariectomized beagles on concomitant dietary calcium restriction. J Bone Miner Res 1995;10:910–920.
26. Takeuchi M, Sakamoto S, Yoshida M, Abe T, Isomura Y. Studies on novel bone resorption inhibitors. I. Synthesis and pharmacological activitis of aminomethylenebisphosphonate derivatives. Chem Pharmaceut Bull (Tokyo) 1993;41:688–693.
27. Nyman MT, Gao T, Lindholm TC, Lindholm TS. Healing of a tibial double osteotomy is modified by clodronate administration. Arch Orthop Trauma Surg 1996;115:111–114.
28. Li J, Mori S, Kaji Y, Mashiba T, Kawanishi J, Norimastu H. Effects of bisphosphonate (incadronate) on fracture healing of long bones in rats. J Bone Miner Res 1999;14:969–979.
29. Peter CP, Cook WO, Nunamaker DM, Provost MT, Seedor JG, Roda GA. Effect of alendronate on fracture healing and bone remodeling in dogs. J Orthop Res 1996;14:74–79.
30. Motohashi M, Shirota T, Tokugawa Y, Ohno K, Michi K, Yamaguchi A. Bone reactions around hydroxyapatite-coated implants in ovariectomized rats. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;87:145–152.
31. Pan J, Shirota T, Ohno K, Michi K. Effects of ovariectomy on bone remodeling adjacent to hydroxyapatite-coated implants in the tibia of mature rats. J Oral Maxillofac Surg 2000;58:877–882.
32. Aro HT, Wippermann BW, Hodgson SF, Chao KY. Internal remodeling of periosteal new bone during fracture healing. J Orthop Res 1990;8:238–246.
33. Frost HM. The biology of fracture healing. An overview for clinicians. Part I. Clin Orthop Res 1989;248:283–293.
34. Hiraga T, Tanaka S, Yamamoto M, Nakajima T, Ozawa H. Inhibitory effects of bisphosphonate (YM-175) on bone resorption induced by a metastatic bone tumor. Bone 1996; 18:1–7.