Effects of Alendronate on the Removal Torque of Implants in Rats with Induced Osteoporosis

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Purpose: *In this study, the removal torques of commercially pure titanium implants that had been implanted simultaneously with the start of treatment for osteoporosis were compared to those of a group without treatment and a healthy group.* ***Materials and Methods:*** *Rats treated by ovariectomy or sham surgery at the age of 12 weeks were used. Twenty-eight days after surgery, the rats treated by ovariectomy were divided into an alendronate-treated group and an untreated (ovariectomy-control) group. At the start of administration of alendronate, a titanium implant was placed in the distal metaphysis of the femur. After 1 month of administration of alendronate and a vehicle, removal torque, the percentage of bone-implant contact (BIC), and parameters of treatment using alendronate were measured.* ***Results:*** *The removal torque values were 10.1 ± 1.6 Ncm for the group of osteoporotic rats that had been administered alendronate and 6.4 ± 1.0 Ncm for the group of osteoporotic rats that did not receive alendronate, indicating that the removal torque was significantly higher in the former group than in the latter group. However, there was no significant difference between the alendronate-treated group and the healthy control group (ie, sham surgery) (9.3 ± 1.3 Ncm).* ***Discussion and Conclusion:*** *These results suggested that implant placement together with treatment of osteoporosis is possible in the ovariectomized rat model.* (INT J ORAL MAXILLOFAC IMPLANTS 2003;18:218–223)

Key words: *alendronate, dental implants, osteoporosis, ovariectomy, rat, removal torque, titanium*

Osseointegrated implants have been prescribed for elderly patients.[1–6] However, appropriate management of any underlying illnesses is important. Osteoporosis is increasingly common in an aging society. An increasing number of patients with osteoporosis, in whom dental treatment using implants may be difficult,[7] want to undergo such treatment. Osteoporosis can be detected by preoperative screening of patients who desire treatment using dental implants.

Hormone replacement therapy (HRT)[8,9] and administration of bisphosphonate as a strong suppressor of bone absorption are used for the treatment of osteoporosis. Bisphosphonate has been used for the treatment of postmenopausal osteoporosis as a second-line therapy, and more active analogs with fewer side effects are being developed and draw attention to drugs for the treatment of osteoporosis. The mechanism of action[10,11] of alendronate, which is the third-generation bisphosphonate having 1,000-fold or higher in vivo suppression of bone absorption than the first-generation bisphosphonate, etidronate,[12] is being clarified.

Studies have been performed on the effects on implants of bisphosphonate administered locally to animals without osteoporosis[13,14] and on the effects on polyethylene implants of alendronate administered to animals with osteoporosis.[10] However, no studies have been undertaken on the effects of alendronate on commercially pure titanium implants, which are among the most commonly used dental implants, in animals with osteoporosis.

In this study, to examine the effects of generally administered alendronate on commercially pure titanium implants in osteoporosis, commercially pure titanium implants were placed in rats with osteoporosis induced by ovariectomy. Subsequently, the removal torque for these implants was measured.

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MATERIALS AND METHODS

Implants

The implants used in this study were screw-type with a machined surface and manufactured from commercially pure titanium (Catalog no. CS275; Implant Innovations Japan, Osaka, Japan). The length of the implant was 4 mm, the diameter of the thread was 1.95 mm, and the diameter of the implant head was 2.1 mm with an internal hexagon (Fig 1).

Animals and Anesthesia

The experimental animals were 25 female Sprague-Dawley rats (Clea Japan, Tokyo, Japan) (Fig 2). General anesthesia was induced by intraperitoneal injection of 0.5 mL of a 1:8 mixture of 20 mg/mL Rompun (Bayer AG, Leverkusen, Germany) and 50 mg/mL Ketalar (Sankyo, Tokyo, Japan). In addition, about 0.2 mL xylocaine adrenaline (Astra, Osaka, Japan) was used for local anesthesia.

Experimental Procedures

Twelve-week-old rats were divided into 2 groups. Twenty-five rats were treated under general anesthesia by bilateral ovariectomy (OVX group), and the remaining 15 rats were treated by sham operation (sham-CNTL group) (Fig 2). Four weeks after the surgery, blood was collected from 5 rats in each group by puncturing the heart, and the level of serum bone-specific alkaline phosphatase (BAP) was measured, by which induction of osteoporosis in the OVX group was confirmed.[15,16]

A titanium screw was implanted in the distal metaphysis of the right femur of the 30 remaining rats (Figs 3a and 3b). This region was chosen because it is abundant in spongy bone, which is susceptible to bisphosphonate.[17,18] When making screw implantation holes, a low rotation speed of drilling (≤ 800 rpm) was maintained while physiologic saline was profusely injected. Bicortical stabilization of the titanium implants was achieved. The rats could walk immediately after awaking from the anesthesia.

The OVX group was further divided into 2 groups. The OVX-ALN group received administration of alendronate (10 rats), and the rats of the OVX-CNTL group were administered a saline vehicle (10 rats). In the OVX-ALN group, 70 μg/kg alendronate sodium (Teiroc; Teijin, Osaka, Japan) was injected subcutaneously twice per week.[19] In the OVX-CNTL and sham-CNTL groups, saline alone was subcutaneously injected. Four weeks after the placement of titanium screws, blood was collected by puncturing the heart under general anesthesia, and the level of serum BAP was measured. The rats were sacrificed by injection of an excessive amount of anesthetic.

Fig 1 Titanium implant used in this study. The scale shown on the side is 1 mm.

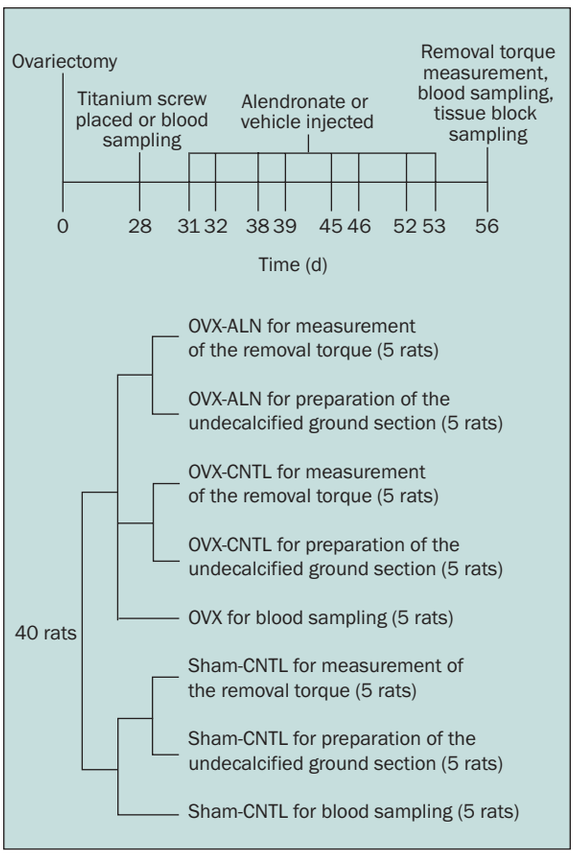

Fig 2 Experimental protocol.

The serum BAP activity was measured using Bessery-Lowry as a substrate.[20] Each group was divided in half. In the first (n = 5 rats per group), the femur in which a titanium implant had been placed was removed and fixed on a Swivel vise (Sumflex, Osaka, Japan) (Fig 4). The removal torque was measured using a torque-gauged wrench (1200ATG-N-S; Tohnichi, Tokyo, Japan).[21] In the other animals,

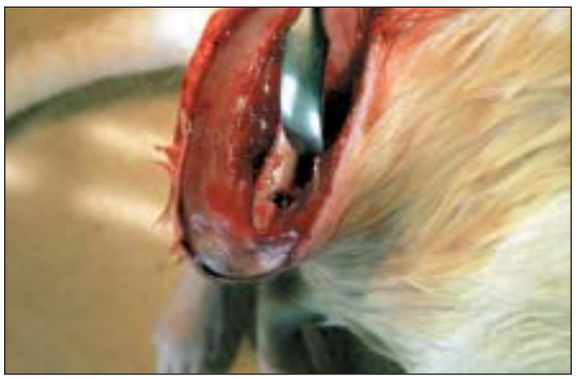

Fig 3a Implant placed into a rat femur.

Fig 3b Softex (Nippon Softex, Tokyo, Japan) radiograph of an implant placed into a rat femur. The image was taken at 60 kVp, 4 mA, and 60 cm for 30 seconds.

Fig 4 Setup for removal torque testing.

Table 1 Serum Bone-Specific Alkaline Phosphate

Experimental day	BAP (IU/L)		
	OVX		
	ALN	CNTL	Sham-CNTL
28	111.2 ± 10.2*		69.3 ± 11.0
56	67.2 ± 11.6	94.7 ± 9.4*	65.8 ± 12.8

*Significant difference ($P < .05$) versus sham-CNTL.

the femur in which a titanium implant had been placed was removed and fixed in 70% ethanol. The tissue blocks were dehydrated in gradients of ethanol and embedded in methylmethacrylate resin. Undecalcified ground sections at a final thickness of 30 µm were obtained using an Exakt sawing machine and grinding equipment (Exakt Apparatebau, Norderstedt, Germany). The sections were stained with Villanueva-Goldner stain prior to light microscopic investigation. Histomorphometry was performed with a semiautomatic image analyzing system (Osteoplan II; Carl Zeiss, Thornwood, NY) linked to a light microscope. Histometric analysis determined the percentage of bone-implant contact (BIC), including the entire perimeter of the implant.[22–24] Figure 2 provides information concerning the timing of these procedures.

Statistical Analysis

The removal torque, body weight, BAP, and BIC in the groups were examined by 1-way analysis of variance. The Bonferroni post hoc test was used to determine the difference at each time point. A value of $P < .05$ was considered to indicate a significant difference.

RESULTS

Serum BAP

At the time of screw placement (28 days after OVX, before the treatment by administration of alendronate), the serum BAP level was significantly higher in the OVX rats than in the sham-CNTL rats. The BAP level measured immediately before death (day 56) was significantly higher in the OVX-CNTL group than in the OVX-ALN and sham-CNTL groups, and there was no significant difference between the OVX-ALN and sham-CNTL groups (Table 1).

Body Weight

At the time of screw placement, the mean body weight was 326.6 ± 21.1 g in the OVX-ALN group,

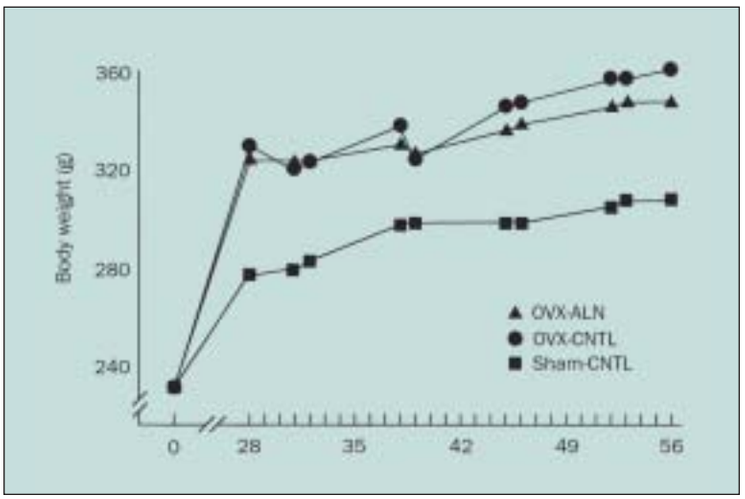

Fig 5 Changes in body weight over the course of the experiment. The day of ovariectomy (or sham ovariectomy) was regarded as day 0, and the day of implant placement was day 28. There were significant differences between the sham-CNTL and OVX-ALN groups and between the sham-CNTL and OVX-CNTL groups in all time intervals over the entire period ($P < .05$).

331 ± 12.5 g in the OVX-CNTL group, and 278 ± 9.3 g in the sham-CNTL group. Immediately before death, it was 348.2 ± 32.3 g in the OVX-ALN group, 360.2 ± 25.1 g in the OVX-CNTL group, and 308.2 ± 17.8 g in the sham-CNTL group. There were no significant differences in the body weight between the OVX-ALN and OVX-CNTL groups at all time points over the test period from the time of screw placement to death, and body weight was significantly higher in these 2 groups than in the sham-CNTL group ($P < .05$) (Fig 5).

Removal Torque

The mean removal torque after sacrifice was 10.1 ± 1.6 Ncm in the OVX-ALN group, 6.4 ± 1.0 Ncm in the OVX-CNTL group, and 9.3 ± 1.3 Ncm in the sham-CNTL group. There was no significant difference between the OVX-ALN and sham-CNTL groups, but there were significant differences between the OVX-CNTL and OVX-ALN groups and between the OVX-CNTL and sham-CNTL groups ($P < .05$) (Fig 6).

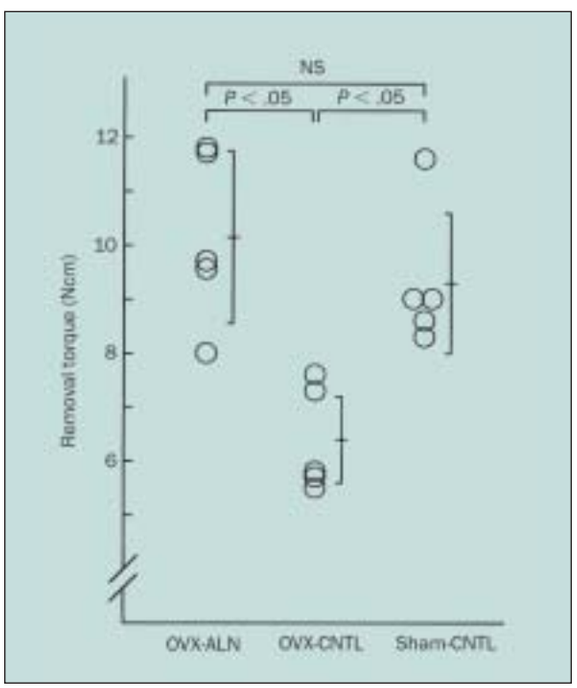

Fig 6 Removal torque of the titanium implants after treatment with alendronate or saline vehicle for 28 days.

Histologic Observations

The implants in the OVX-ALN group were osseointegrated, with direct bone-implant contact visible at the light microscopic level (Fig 7a). Osseointegration was determined by the apparent direct attachment or connection of vital osseous tissue to the surface of an implant without intervening connective tissue. The implants were in contact with one of the cortical plates, and a continuous strut of bone encircled the implant.

Although the implants in the OVX-CNTL group were osseointegrated, the bony architecture was located a distance away from the implant surface and appeared to be more immature (Fig 7b).

The implants in the sham-CNTL groups exhibited similar trends as found in the OVX-ALN group. The implants in the sham-CNTL groups were osseointegrated, and showed more mature and extensive bone formation (Fig 7c).

Histometric Observations

BIC percentages were as follows: 55.9 ± 2.0% for the OVX-ALN group, 46.0 ± 3.0% for the OVX-CNTL group, and 79.1 ± 1.2% for the sham-CNTL group.

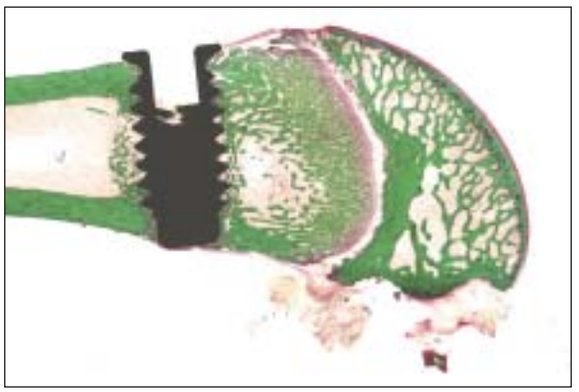

Fig 7a Ground section representing a titanium implant 28 days after placement in the OVX-ALN group. Red = osteoid; yellowish-green = calcified bone (original magnification ×5).

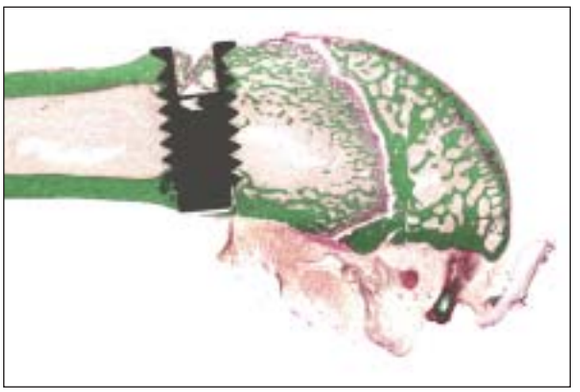

Fig 7b Ground section representing a titanium implant 28 days after placement in the OVX-CNTL group. The extent of calcified bone is markedly reduced versus the OVX-ALN group. Red = osteoid; yellowish-green = calcified bone (original magnification ×5).

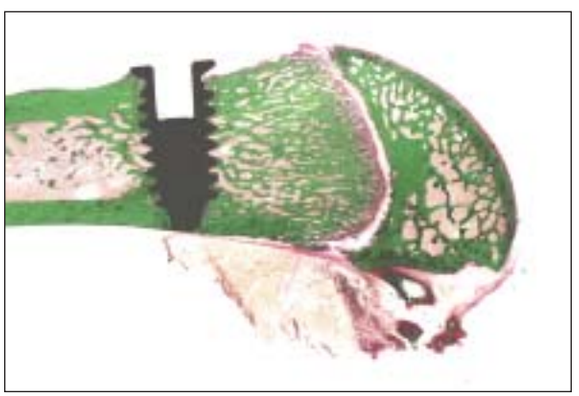

Fig 7c Ground section representing a titanium implant 28 days after placement in the sham-CNTL group. Findings are similar to those in the OVX-ALN group. Red = osteoid; yellowish-green = calcified bone (original magnification ×5).

There was no significant difference in BIC between the OVX-ALN and OVX-CNTL groups, but there were significant differences between the OVX-ALN and sham-CNTL groups and between the OVX-CNTL and sham-CNTL groups ($P < .05$).

DISCUSSION

The results of this study demonstrated the possibility of implant placement performed simultaneously with the treatment of osteoporosis in untreated postmenopausal osteoporosis using experimental animals. The criteria for treatment using implants in patients with osteoporosis has been established to some extent by prolongation of the healing time.[25] The present study suggested that the healing time of treatment using implants in patients with osteoporosis can be reduced. The femoral metaphysic, with its thick cortical bone and abundant spongy bone, may be considered structurally similar to the mandible. The duration of implantation in this study was short (1 month), but it has been reported that bone volume and structure in OVX rats administered bisphosphonate 3 times per week for 1 month were maintained for more than 12 months after discontinuation of the drug.[26]

To evaluate the relationship between osseointegration, the level of serum BAP (which is a simple parameter of clinical treatment of osteoporosis), and body weight, measurement of these parameters was performed. Serum BAP is a marker of bone formation and is considered to be a useful biochemical marker of osteoporosis.[27] Alendronate, which is a bisphosphonate, increases bone volume by suppressing osteoclasts. Measurement of serum BAP is used for the evaluation of treatment of osteoporosis using alendronate.[28,29]

In the present study, the level of serum BAP was correlated with the implant removal torque. Serum BAP may be a biochemical parameter of loss of integration during long-term follow-up observation of implants. Body weight is considered to be increased by postmenopausal osteoporosis and has been reported to be reduced by the treatment of osteoporosis using alendronate.[30] However, body weight proved to be an unsatisfactory parameter of treatment in short-term examinations as in the present study.

In the OVX group, the removal torque was significantly increased by administration of alendronate, but the increase in BIC was not significant. This may be because fixation in the early stage after implantation was achieved mainly by the cortical bone, and because of the results in body weight, the observation period was short compared to that in the previous study.[26] In other words, ossification of the entire circumference of the implants

was not completed within the short healing time, but integration was established in the region of the cortical bone.

CONCLUSIONS

This study indicated that the removal torque of commercially pure titanium screws implanted in the femurs of rats with osteoporosis induced by OVX at the start of alendronate administration was significantly improved compared to those animals not given alendronate. However, another useful parameter, BIC, could not be detected as improved in this short-term experiment.

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