Effect of Estrogen Replacement and Calcitonin Therapies on Bone Around Titanium Implants Placed in Ovariectomized Rats: A Histometric Study

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Purpose: *The aim of the present study was to evaluate whether hormone replacement therapy (HRT) and calcitonin (CT) administration could influence bone healing around implants placed in ovariectomized (OVX) rats.* ***Materials and Methods:*** *One screw-type titanium implant was placed bilaterally in OVX rats. The animals were assigned to one of the following groups: group 1 (n = 15), sham surgeries; group 2 (n = 15), OVX rats; group 3 (n = 14), OVX rats administered CT 4 days/week (16 IU/kg); group 4 (n = 14), OVX rats administered 17β estradiol daily (20 μg/kg). After 60 days, the animals were sacrificed and undecalcified sections obtained. Bone-to-implant contact (BIC) and bone area (BA) around the implants were determined separately for the cortical (zone A) and cancellous (zone B) bone areas.* ***Results:*** *In zone A, intergroup analysis did not reveal a significant difference regarding BIC. In contrast, the HRT group (group 4) presented greater BA than groups 2 and 3 ($P < .05$). Data from zone B revealed that HRT eliminated the negative effect of the ovariectomy on BIC and BA ($P < .05$), while CT had no effect ($P > .05$).* ***Discussion:*** *It was the first study to evaluate and demonstrate the impact of HRT and CT on bone around titanium implants in an estrogen-deficient model.* ***Conclusion:*** *Within the limits of the present study, it may be concluded that HRT may prevent the influence that estrogen deficiency exerts on bone healing around titanium implants.* (Int J Oral Maxillofac Implants 2002;17:786–792)

Key words: *calcitonin, dental implants, estrogen deficiency, hormone replacement therapy, titanium*

Titanium endosseous implants have been used increasingly often in various edentulous situations.[1–4] However, because the success of osseointegration depends in part on the state of the host bone bed and its healing capacity, concerns have been raised regarding conditions affecting bone quality.[5]

Since osteoporosis is a skeletal disorder in which bone density and bone mass decrease as a function of a high rate of bone turnover,[6] some studies have investigated the impact of osteoporosis on osseointegrated implant outcomes.[5,7] Recently, the reactions of bone tissues following placement of implants under estrogen-deficient conditions have been studied in experimental animals.[8–10] Generally speaking, estrogen deficiency seems to negatively influence bone quality around titanium implants.

To prevent or "treat" bone loss in postmenopausal women, ie, estrogen-deficient individuals, hormone replacement therapy (HRT) and calcitonin (CT) have been used. Increased trabecular bone volume, unchanged mechanical properties, and

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depressed bone turnover have been reported as benefits of HRT.[11–13] Similarly, normal bone volume and decreased bone turnover have been reported for estrogen-deficient animals (ovariectomized [OVX]) treated by osteoclast inhibitors such as CT.[14]

To date, no information is available regarding the effect of HRT and CT administration on bone healing around osseointegrated implants placed in estrogen-deficient animals. Therefore, the present study was designed to evaluate, by histologic analysis, whether HRT and CT administration could prevent the negative influence of estrogen deficiency around titanium implants placed in OVX rats.

MATERIALS AND METHODS

Animals

The experimental animals were 58 female Wistar rats, which were 90 days old and weighed 250 to 300 g at the beginning of the study. The animals were kept in plastic cages with access to water ad libitum. The food consumption of the OVX rats was restricted to that of control rats (pair feeding) to minimize the increase in body weight associated with ovariectomy.[15] The protocol was approved by the University of Campinas Institutional Animal Care and Use Committee (School of Dentistry at Piracicaba, University of Campinas, Piracicaba, SP, Brazil).

Ovariectomy Procedure

General anesthesia was obtained by intramuscular administration of ketamine (0.5 mL/kg). Bilateral ovariectomies were performed in 43 rats from a dorsal approach. The remaining 15 rats were subjected to sham surgeries in which the ovaries were exteriorized and replaced intact. For the sham ovariectomy, the ovaries were lifted up and returned to their original position.

Experimental Design

On the day after the ovariectomies, the animals were assigned to 1 of 4 treatment groups:

- Group 1 (n = 15): sham surgeries (negative control)
- Group 2 (n = 15): OVX rats (positive control)
- Group 3 (n = 14): OVX rats injected subcutaneously with CT (Miacalcic, Sandoz, Ravenburg, Germany) 4 days/week at a dose of 16 IU/kg body weight
- Group 4 (n = 14): OVX rats injected subcutaneously daily with 17β estradiol (Sigma Chemical, St Louis, MO), dissolved in 100% ethanol and diluted in mineral oil at a dose of 20 μg/kg body weight

Implant Surgery

Twenty-one days after the ovariectomies, all animals were anesthetized (ketamine, 0.5 mL/kg). The skin was cleansed with iodine surgical soap, an incision of approximately 1 cm in length was made, and the bone surface of the tibia was surgically exposed by blunt dissection. Under profuse saline irrigation, bicortical implant beds were drilled at a rotary speed not exceeding 1,500 rpm, and 1 screw-type commercially available pure titanium implant (AS Technology, São José dos Campos, SP, Brazil), 4.0 mm in length and 2.2 mm in diameter, was placed bilaterally. The soft tissues were replaced and sutured. Postoperatively, the animals received antibiotic (Pentabiótico, Wyeth-Whitehall, São Paulo, SP, Brazil) given as a single intramuscular injection.

Serum Analyses

At the time of sacrifice (60 days after implant placement), blood was drawn to measure the serum level of alkaline phosphatase and calcium. Alkaline phosphatase activity was determined colorimetrically (Gold Analisa Diagnóstica, Belo Horiaonte, MG, Brazil) and calcium by the ion-selective electrode method (Eletrolyte Analyzer, AVL Scientific Corporation, Roswell, GA).

Clinical Analyses

To confirm the success of the ovariectomy, 2 weeks after the ovariectomy and sham surgeries, the estrus cycle of the rats was monitored daily for 1 week by collecting vaginal smears. In addition, the success of the ovariectomy was also confirmed at necropsy by marked atrophy of the uterine horns and no histologic evidence of ovarian tissue at the surgical site.

Histometric Procedure

After 60 days, the animals were sacrificed; the tibiae were removed and fixed in 4% neutral formalin for 48 hours. Undecalcified sections were prepared as previously described,[16] ie, the blocks were dehydrated in an ascending series of ethanols (60% to 100%) and embedded in glycolmethacrylate resin (Technovit 7200; Heraeus Kulzer, Wehrheim, Germany). Subsequently, sections (20 to 30 μm) were obtained and stained using 1% toluidine blue. The percentages of bone-to-implant contact (BIC) and bone area (BA) within the threads of the implants were determined bilaterally (Image-Pro; Media Cybernetics, Silver Spring, MD) and arranged separately in cortical (zone A) and cancellous bone (zone B) areas.

Statistical Analysis

Data from zones A and B (cortical and cancellous bone, respectively) were averaged separately. The

hypothesis that HRT and CT administration had no influence on the bone healing around the implants placed in the OVX rats was tested using an intergroup analysis (Kruskal-Wallis test, alpha = .05). If a statistical difference was detected, Dunn's method was used to isolate the groups that differed from the others. In addition, to test the hypothesis that HRT and CT administration did not influence serum levels of alkaline phosphatase and calcium, a 1-way analysis of variance (ANOVA) (alpha = .05) was used. If a statistical difference was detected, a pairwise multiple comparison procedure was used (Bonferroni *t* test).

Finally, an intergroup analysis was used to test the hypothesis that there was no difference between the groups with respect to the animals' body weights at the end of the experimental period (1-way ANOVA, alpha = .05).

RESULTS

Clinical Observations

All animals gained weight during the course of the study. The animals from groups 1, 2, and 3 weighed significantly more than the animals from group 4 ($P < .05$). The final mean body weights were 256.27 ± 15.70 g, 261.67 ± 14.98 g, 255.47 ± 20.25 g, and 228.57 ± 13.68 g for the animals from groups 1, 2, 3, and 4, respectively.

In the present study, macro- and microscopic analyses of the uterine horns and assessment of the estrus cycle of the rats confirmed the success of the ovariectomy surgery. All OVX (group 2) and OVX/CT (group 3) animals were in the diestrus stage and displayed atrophied reproductive organs, therefore confirming the reduction of serum estrogen levels in these 2 groups.[17] In contrast, the sham group presented the 4 stages of the estrus cycle (estrus, metestrus, diestrus, and proestrus), while the OVX/HRT animals (group 4) remained in the estrus stage. Furthermore, macro- and microscopic analyses showed that the reproductive organs of sham-operated and HRT groups were intact, confirming that the serum estrogen levels were maintained in both groups.

Serum Analyses

The serum levels of alkaline phosphatase varied between the experimental groups. They were similar for the animals from groups 1 and 4 (27.60 ± 10.93 IU/L and 33.20 ± 14.91 IU/L, respectively; $P > .05$) but statistically significantly higher in the animals from groups 2 and 3 (80.47 ± 20.16 IU/L and 98.20 ± 14.27 IU/L, respectively; $P < .05$).

Regarding the serum levels of calcium, the OVX animals that did not receive either HRT or CT (group 2) presented higher values than the animals from groups 1, 3, and 4 ($P < .05$). The mean serum calcium levels were 1.10 ± 0.07 mmol/L, 1.24 ± 0.08 mmol/L, 1.10 ± 0.14 mmol/L, and 1.07 ± 0.13 mmol/L for groups 1, 2, 3, and 4, respectively. Figures 1a and 1b illustrate the results observed for serum levels of alkaline phosphatase and calcium, respectively.

Histometry

Intergroup analysis did not reveal significant differences regarding BIC in zone A ($P = .64$). Therefore, in the cortical bone area, estrogen deficiency did not appear to influence the osseointegration of the titanium implants. On the other hand, in zone B, data analysis showed that estrogen deficiency could result in a lower percentage of BIC than in the animals not submitted to ovariectomy ($P < .05$). However, this negative effect was reversed in the OVX/HRT animals (which received 17β estradiol) to a similar percentage as that of the sham surgery animals. In addition, data analysis revealed a slight positive effect of CT administration on the percentage of BIC in the group 3 animals, but this difference was not statistically significant ($P > .05$) (Table 1).

Data analysis also showed that in the cortical and cancellous bone regions (zones A and B), a slight difference in the BA was noted between groups 1, 2, and 3, but this was not statistically significant ($P > .05$). In contrast, the OVX/HRT animals (group 4) presented a statistically higher percentage of BA than did the animals from groups 2 and 3 ($P < .05$) (Table 1). Figures 2 to 5 illustrate the histologic findings.

DISCUSSION

Risk factors for osteoporosis have been studied extensively.[18,19] Estrogen levels present prior to menopause are protective against bone loss resulting from osteoporosis. Therefore, early menopause, whether naturally occurring, drug-induced, or surgically induced, predisposes individuals to osteoporosis.[18]

The influence that osteoporosis exerts on implant outcome has been investigated. Some authors have suggested, on the basis of clinical observations, that osteoporosis is not always a risk factor in osseointegration.[7,20] On the other hand, studies based on histologic observations have reported some negative effects of estrogen deficiency on the bone healing around titanium implants (induced osteoporosis).[8–10]

Fig 1a Means and standard deviations (IU/L) of serum levels of alkaline phosphatase.

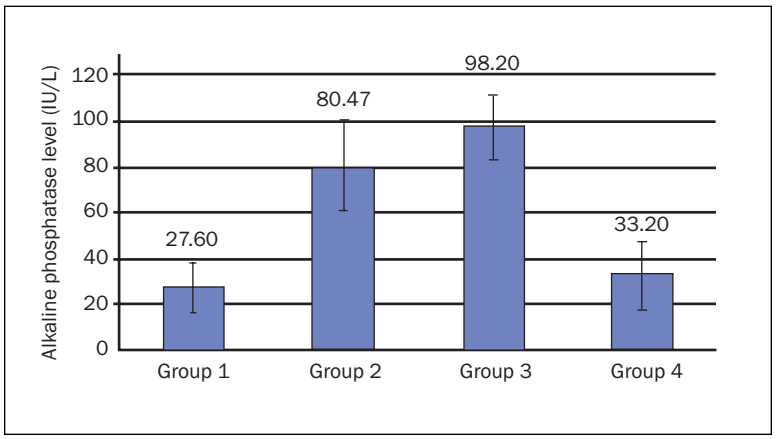

Fig 1b Means and standard deviations (mmol/L) of serum calcium levels.

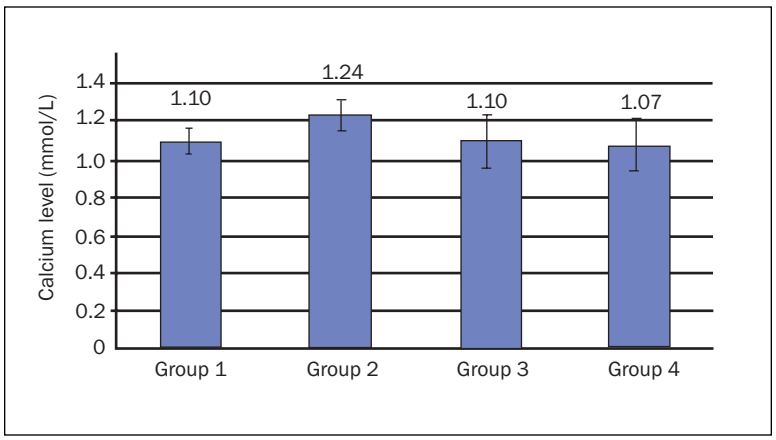

Table 1 Means and Standard Deviations (%) of Bone-to-Implant Contact (BIC) and Bone Area (BA) Within the Limits of the Implant Threads for Groups 1 to 4 at Zones A and B

	Zone A		Zone B	
Group	BIC	BA	BIC	BA
1	50.98 ± 15.19[a]	84.76 ± 3.74[ab]	51.75 ± 12.53[a]	49.26 ± 5.60[ab]
2	46.00 ± 7.94[a]	81.35 ± 3.81[a]	40.28 ± 9.51[b]	41.38 ± 10.82[a]
3	49.79 ± 14.81[a]	82.65 ± 5.15[a]	48.60 ± 10.16[ab]	42.64 ± 5.49[a]
4	50.45 ± 12.76[a]	88.36 ± 3.39[b]	52.99 ± 10.27[a]	58.56 ± 12.22[b]

Superscript letters should be considered in columns.

Although the present study did not aim to investigate the effect of ovariectomy on the bone around titanium implants, the present results showed that estrogen deficiency may slightly influence bone healing around titanium implants in the cortical area (zone A) and significantly decrease BIC in the cancellous bone region (zone B). Extrapolation of the observations found in animal models to a clinical situation is not possible. However, the indication provided by the experimental studies that estrogen deficiency may influence BIC and BA around titanium implants in humans should be investigated on a long-term basis.

Most bone diseases, including osteoporosis, are caused by increased bone resorption, rendering its inhibition a primary therapeutic objective. Indeed, most bone therapies currently available belong to this category. Inhibition of bone resorption can be accomplished by reducing either osteoclast generation (for example, with estrogens) or osteoclast activity (with CT). Estrogen replacement therapy has long been considered the first line of therapy

Fig 2 *(Left)* Ground section showing the histologic findings for the animals submitted to sham surgery (group 1). Note that after 60 days in the cortical zones, the bone is in a close contact with the implant surface, *(arrows)* and in the cancellous bone area *(asterisks),* some bone proliferation on the implant surface is evident (toluidine blue; original magnification ×6.25).

Fig 3 *(Right)* Histologic aspect around the implants placed in animals that were submitted to ovariectomy (group 2). Note that estrogen deficiency negatively influenced the bone tissue around the implant, especially in the cancellous bone compartment *(asterisks)* (toluidine blue; original magnification ×6.25).

Fig 4 *(Left)* Histologic illustration of the bone tissue around the implants placed in the animals that were submitted to ovariectomy and received daily injections of CT (group 3). Observe that CT did not prevent the negative effect of estrogen deficiency on bone around the implant, especially in the cancellous bone area *(asterisks)* in comparison to the control group (see Fig 2) (toluidine blue; original magnification ×6.25).

Fig 5 *(Right)* Histologic findings for animals that were submitted to ovariectomy and treated by HRT. A significantly positive effect of the treatment was observed for either the cortical or cancellous bone as compared to groups 2 and 3 (toluidine blue; original magnification ×6.25).

for preventing osteoporosis. Treatment with estrogens clearly inhibits bone loss and bone turnover and increases bone mineral density.[21] The molecular mechanism of estrogen action on bone, as well as on other tissues, is under investigation.[22]

CT is a polypeptide hormone that has been used to treat or prevent bone metabolism disorders, because of its capacity to inhibit bone resorption[23] and because of its analgesic properties.[24] Because no information is available regarding the effect of HRT and CT administration on bone healing around osseointegrated implants placed in estrogen-deficient animals, the present study aimed to evaluate whether HRT or CT administration could prevent the negative influence of estrogen deficiency on bone around titanium implants placed in OVX rats. The results of the present study showed that administration of estrogen immediately after ovariectomy may neutralize the negative effects of estrogen deficiency on both parameters analyzed, ie, BIC and BA around implants placed in the tibiae of the rats. The OVX rats that daily received 17β

estradiol (group 4) presented levels of BIC and BA similar to those of the sham-operated animals (group 1). On the other hand, in the present study, although data analysis revealed a slight positive effect of CT administration after ovariectomy, this was not statistically significant.

The biochemical serum analysis supported the histologic results of this study. HRT in OVX rats resulted in levels of alkaline phosphatase and calcium that were similar to those in the control group (sham), indicating that estrogen administration might have controlled the high bone turnover promoted by the ovariectomy (increased alkaline phosphatase levels) and blocked its influence on the bone tissue around the implants. In contrast, although CT may have presented its biologic properties, decreasing the level of serum calcium, it did not decrease bone turnover in the OVX animals. CT-induced loss of CT receptors resulting in hormone-induced resistance has been reported previously.[25,26] Whether this was the case in the present investigation remains to be investigated. Thus, the bone tissue around the implants in the CT-treated animals was affected negatively by estrogen deficiency.

The results of the present study are in agreement with previous studies regarding the benefits of HRT on bone metabolism in estrogen-deficient animals.[11–13,27] Moreover, regarding the effect of CT on bone metabolism in OVX rats, the results of the present study are closely related to the study by Shen and coworkers,[28] who showed that CT partially prevented bone loss in OVX rats. Results, though, are not in agreement with the report by Wronski and coworkers,[14] who observed that CT treatment depressed bone turnover and prevented the development of osteopenia in OVX rats.

SUMMARY

Within the limits of the present investigation, it can be suggested that HRT immediately after ovariectomy may be important to prevent the influence that estrogen deficiency may exert on bone healing around titanium implants. Nevertheless, further studies should be considered to clinically confirm the present data and to investigate whether other inhibitors of osteoclast activity, such as diphosphonates, may influence bone around titanium implants in estrogen-deficient animals. In addition, the question of whether HRT is able to reverse, rather than merely prevent, the effect of estrogen deficiency on bone healing around titanium implants should also be investigated.

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