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The influence of estrogen deficiency on bone around osseointegrated dental implants. An experimental study in the rat jaw model

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Abstract

Purpose—The aim of this study was to evaluate the influence of estrogen deficiency on bone around osseointegrated dental implants in a rat jaw model.

Materials and Methods—This study utilized 16 female rats, which had the first molars bilaterally extracted and allowed to heal for 30 days prior to implant placement. Sixty days after implant placement, the animals were randomly submitted to sham surgery (SHAM) or ovariectomy (OVX). The animals were euthanized 90 days post-ovariectomy. Bone to implant contact, bone area fraction occupancy between implant threads, mineral density, turnover markers and TRAP positive cells were assessed for both groups.

Results—The results showed that OVX group presented a decrease of systemic bone density, alterations on bone turnover markers, and an increase of TRAP positive cells relative to the

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SHAM group. However no difference relative to the bone to implant contact and bone area fraction occupancy was observed between groups.

Conclusions—The findings of this study demonstrate that estrogen deficiency may not be considered as a risk factor for osseointegrated implants failure in jaw bone.

Keywords

dental implants; ovariectomy; osteoporosis; estrogen deficiency; rats

INTRODUCTION

Osteoporosis represents a major public health threat and annually results in thousands of hip, wrist and vertebral fractures [1,2]. Estrogen is known to regulate bone remodeling and the cessation of estrogen production induces a bone remodeling imbalance with bone resorption exceeding bone formation, leading to bone fragility and increasing risk of fracture [3,4].

Ovariectomy is established as the most prevalent and pertinent [5-7] protocol to induce estrogen deficiency related osteopenia, bringing about hormonal deficiency similar to that of menopause [8]. In rats, estrogen deprivation appears to mimic the anatomical and kinetic patterns of loss of bone mass observed in humans [8,9]. Thus, several authors have used ovariectomy in order to study the estrogen deficiency induced bone loss as a potential risk factor during the placement and maintenance of dental implants, as good quality of bone tissue have been related to their success [10-12].

Previous animal studies using ovariectomy model of osteoporosis induction with implants inserted in rats have shown that estrogen deficiency resulted in lower bone turnover rate, bone-to-implant contact (BIC), bone/implant interface biomechanical competence, as well as bone density on the cancellous bone [10-20].

Despite these evidences, from a clinical perspective, the literature findings in the topic are sparse and contradictory as case reports, case-controls, and retrospective clinical studies suggest that osteoporosis should not be considered a risk factor for implant therapy [21-23].

Thus, the present study evaluated the influence of estrogen deficiency on bone around osseointegrated dental implants in a rat jaw model.

MATERIALS & METHODS

Animals

Sixteen female Wistar rats weighing from 70 to 90g were used in this study. The animals were kept in the animal facility with controlled temperature, humidity, and light exposure. They were fed in a standard laboratory diet and water *ad libitum*. The study protocol was approved by the Committee of Ethics for Animal Experimentation of Araraquara Dental School, UNESP (Protocol# 02/2007).

The rats included in the study had both maxillary first molars extracted. For that purpose, the animals were anesthetized receiving an intramuscular injection of ketamine hydrochloride (Agener União Ltda, São Paulo, SP, Brazil) at a concentration of 0.08ml/100g and xylazine hydrochloride (Rompum®, Bayer S.A., São Paulo, SP, Brazil) at 0.04 ml/100g of body weight.

Oral cavity antisepsis was performed with chlorhexidine 0.12% prior to surgical procedures. Then, teeth were extracted using elevators. After a healing period of 30 days, two implants

were placed in the healed sockets. For the placement of the implants, the rats were anesthetized and oral cavity antiseptics was performed, as described above. The transmucosal grade 5 screw shaped titanium implants (1.4 mm in diameter by 2.7 mm in length; SIN Sistema de Implante, São Paulo, SP, Brazil) were inserted in the osteotomy prepared bilaterally at the edentulous sites. Sixty days after implant placement the animals were randomly divided into a control (SHAM) and an ovariectomized (OVX) group. A fictitious surgery was carried out for SHAM while OVX had the ovaries removed, as previously described [24], in order to induce the estrogen deficiency. The euthanization of the animals were performed by deepening anesthesia on 180th day after teeth extractions.

Bone densitometry

Femur and lumbar vertebral bone mineral density (BMD) evaluations were performed by Dual energy X-ray Absorptiometry (DXA) in order to assess the systemic impairment of the animals due to ovariectomy. DXA was achieved using a densitometer (QDR Discovery, Hologic, Bedford, MA, USA) operating in the high-resolution mode and using specific software for small animals supplied by the equipment's manufacturer.

Histologic preparation of the specimens

Two implants were inserted in each animal, one of the implants was prepared for hard tissue histology and the other was subjected to decalcification and embedded in paraffin. After euthanization, the maxillary implants were fixed in 4% buffered formaline saline for 48h. Non-decalcified ground sections were prepared after dehydration by a series of ethanol solutions and embedded in methacrylate-based resin (Technovit 9100, Heraeus Kulzer, Wehrheim, Hesse, Germany). The blocks were cut into 100µm thickness sections aiming the center of the implant diameter along its long axis using a precision diamond saw (Isomet 2000, Buehler Ltd., Lake Bluff, IL, USA). The sections were reduced to a final thickness of approximately 30µm by grinding and polishing (Metaserv 3000, Buehler Ltd., Lake Bluff, IL, USA) with a sequence of SiC abrasive papers (400, 600, 800, 1200 and 2400) (Buehler Ltd., Lake Bluff, IL, USA). The sections were stained in 1% toluidine blue (Sigma-Aldrich Co, St. Louis, MO, USA) and referred to light microscopy evaluation.

Measurements of the percentages of bone to implant contact (BIC) and the bone area fraction occupancy (BAFO) were performed at 100× magnification (Leica DM1200M, Leica Microsystems, Wetzlar, Hesse, Germany) by using the National Institutes of Health image analyzer software (ImageJ 1.41o, National Institutes of Health, USA).

The decalcified processed specimens were treated for 28 days in 10% ethylenediamine tetraacetic acid (EDTA) solution containing 0.1M Tris base (pH 6.9), dehydrated, diafanized and embedded in paraffin. Sections of 5µm were obtained and prepared for hematoxylin eosin staining, histochemically reacted for tartrate-resistant acid phosphatase (TRAP) to demonstrate the presence of osteoclasts, and also reacted to immunolabeling of osteocalcin (OCN) and alkaline phosphatase (ALP) by using ABC system (DAB Substrate Kit, Vector Laboratories Inc. Burlingame, CA, USA).

Measurement of biochemical markers of bone turnover

Blood samples were collected from the rat caudal artery at the euthanization day. Serum concentrations of osteocalcin (OCN – Biomedical Technologies Inc, Stoughton, MA, USA), the cross-linked C-terminal telopeptides (CTX, RatLaps EIA, Immuno Diagnostic System, IDS, Fountain Hills, AZ, USA), osteoprotegerin (OPG - Peprotech, Rocky Hill, NJ, USA) and the receptor activator of nuclear factor-κB ligand (RANKL - Peprotech, Rocky Hill, NJ, USA) were measured by Enzyme-Linked Immunosorbent Assay (ELISA) following the manufacturer's instructions. Tartrate-resistant acid phosphate (TRAP) and alkaline

phosphatase (ALP) activities were also measured in serum by biochemical assay using the α -naphthyl- phosphate (Sigma Aldrich, Saint Louis, MO, EUA) as a substrate.

Data Analysis

After using D'Agostino test for evaluation of data normality, both analysis of Mann Whitney (used for OCN, CTX, OPG, RANKL, TRAP and ALP activity measurements) and Student's t-test (for BMD of femur and lumbar vertebrae, BIC and BAFO analyses) were used for comparison of the groups. Statistical significance was set to 95%.

RESULTS

BMD evaluation revealed the establishment of bone mass loss by estrogen deficiency as OVX presented significantly lower BMD values than SHAM for femur (Figure 1a) and lumbar vertebrae (Figure 1b).

Statistically significant differences in TRAP ($p=0.0247$) and ALP activity ($p=0.0411$) was detected between groups (Figure 2a and 2b, respectively). While the OVX group showed increased TRAP activity, its ALP activity was decreased in comparison to SHAM. These enzymes ratio also presented significantly higher values for OVX ($p=0.0087$) (Figure. 2c). Although both RANKL and OPG did not present statistically significant difference (Figure 2d and 2e), the ratio between these parameters was slightly increased for the OVX group relative to SHAM ($p= 0.0863$).

The OVX group also presented increased OCN ($p=0.0317$) and CTX ($p=0.0083$) serum concentrations (Figures 2g and 2h, respectively).

Regarding the TRAP staining of decalcified sections, at the OVX group multinucleated TRAP⁺ cell was observed to be more frequently found than in sections of SHAM group on bone tissue in proximity of the implant threads. (Figure 3).

Evaluation of the bone tissue around the implants showed no significant differences in BIC (Figure 4a) and BAFO (figure 4b), between groups ($p=0.9254$ and $p=0.7445$, respectively). From a morphologic standpoint, HE and toluidine blue staining of histologic sections showed that both groups of the study presented no difference among the groups (Figure 5).

OCN and ALP immunoreactivity presented agreement with their serum concentration assessment, where both bone formation markers were strongly expressed on OVX group. At the implantation site, OCN was expressed at the osteocytes and distributed all along the bone matrix lamellae, while ALP was detected mainly in endosteal cells for both groups (Figure 6).

DISCUSSION

From a public health standpoint, the effects of osteoporosis account for a large number of medical and medical-related procedures per year [1,2]. Concerning potential effects of osteoporosis on dental implant therapy, the lack of relationship between laboratory in vivo and clinical studies have prevented a clear definition of practice guidelines for affected patients whether these have developed the condition prior or after implant placement.

Confirming previous reports, the results of the present study showed that ovariectomy was an efficient method to establish the estrogen deficiency related bone loss [9,25,26]. The systemic bone density was decreased relative to the SHAM group, as well as serum parameters of bone remodeling were altered. These results are in agreement with Rico and Villa [27], who demonstrated that serum TRAP concentration was increased after

menopause and that such observation was inversely related to bone mass. Also in agreement with previous studies, our results also revealed an increase of OCN and CTX along with a decrease in ALP serum for the OVX group [28-31].

The loss of bone mass due to estrogen deficiency during the healing period of dental implants or its implication on implants that are already osseointegrated has been previously addressed. These investigations have shown that estrogen deficiency alters bone density around implants, decreases the overall biomechanical competence of the bone-implant system, as well as decreases the degree of osseointegration [10,15,16,32,33].

In a similar animal model but at a different implantation site (tibiae), Giro et al. [19] had shown significant bone mass loss along the implant threads, and decreased BIC and BAFO values in ovariectomized animals relative to the SHAM group. On the contrary, such observations did not occur in the present study when jawbone was considered. Our results support that although estrogen suppression resulted in a systemic impairment of the animals, the morphologic and morphometric parameters were relatively unaffected. Such observation could be explained by the fact that bone remodeling rate may differ on axial and appendicular skeleton [34]. Another plausible explanation is related to the fact that adult rats might have an activation of osteonal remodeling due to ovariectomy [8] and different turnover is expected for different bones in the skeletal system. Moreover a special attention should be given to the mechanical loading of the implants in this study. The physiological masticatory forces would stimulate periosteal mineralization [35] and, consequently, enabling the dynamics of bone turnover to reach equilibrium, thereby attenuating the loss in bone incurred by estrogen deficiency.

The results of this study are in direct agreement with a human retrieval study which did not observe differences in bone morphology between osteoporotic and non osteoporotic subjects [22]. Other authors have also shown that systemic osteoporosis was not considered an important indicator of dental implant outcome under masticatory function [21,36,37].

Within the limitations of the experimental model, the results obtained in the present study further suggests that estrogen deficiency is not a risk factor for the maintenance of jaw bone around osseointegrated implants.

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Figure 1.
Graphical representation of the (a) femur and (b) lumbar vertebral BMD (g/cm²).

Figure 2. Comparison of (a) TRAP and (b) ALP activity, (d) RANKL, (e) OPG, (g) OCN and (h) CTX serum concentrations between the groups. Ratio between TRAP/ALP and RANK/OPG are represented in Figure (c) and (f), respectively.

Figure 3.

TRAP staining representing SHAM and OVX group are indicated in Figures (a) and (b), respectively. 200× magnification. (c) demonstrates the presence of osteoclast in OVX group in a higher magnification (400×).

Figure 4.
Graphical representation of histometrical parameters measured on non-decalcified section.
No statistically significant difference was shown for BIC and BAFO between different groups.

Figure 5. Photomicrographs of the bone/implant interface in decalcified HE stained section (a and b) and non-decalcified toluidine blue stained ones (c and d), for SHAM and OVX respectively. No difference was found in the histological morphology comparing both groups.

Figure 6. Representative sections of the expression and distribution of OCN (A and B) and ALP immunolabelling (C and D) for the different groups.