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Bone Healing
Research Paper

Raloxifene enhances peri-implant bone healing in osteoporotic rats

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Abstract. The aim of this study was to evaluate bone healing at the bone–implant interface in rats with induced osteoporosis. The rats underwent a bilateral ovariectomy (OVX) and were fed a low calcium and phosphate diet. The OVX rats were divided into three groups: one was treated with raloxifene (OVX-RAL), one with alendronate (OVX-ALE), and one received no medication (OVX-NT). The control group rats (SHAM-DN) underwent sham surgery and were fed a normal diet. Each animal received one implant in each tibia: a machined surface implant in the right tibia and an implant with surface etching in the left tibia. All animals were euthanized after 42 days. Analysis of variance (ANOVA) and Tukey post hoc tests were applied to the biomechanics (reverse torque) and bone–implant contact (BIC) data ($P < 0.05$). The RAL and ALE groups showed improved peri-implant bone healing. However, the ALE group showed no significant difference from the OVX-NT group. Surface treatment promoted higher corticalization at the bone–implant interface, but showed the same characteristics of mature bone and bone neoformation in concentric laminations as the machined implant. There were no statistically significant differences in reverse torque ($P = 0.861$) or BIC ($P = 0.745$) between the OVX-RAL and SHAM-DN groups. Therefore, the use of raloxifene resulted in good biomechanical, BIC, and histological findings in the treatment of induced osteoporosis in rats.

Key words: alendronate; raloxifene; osteoporosis.

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One of the determining factors for the proper osseointegration of dental implants is the quality of bone tissue, since the characteristics of the bone microarchitecture influence the ability of the bone to transmit and distribute physiological forces.[1] Therefore, when the cortical or trabecular structure of the bone has low density, the bone–implant interface is compromised.[2]

Decreased bone density is observed in two-thirds of women because of oestrogen deficiency after the menopause.[3] Thus, oestrogen deficiency associated with ageing can cause osteoporosis.[3,4] The age-related bone loss is due to decreased intestinal absorption of dietary calcium, which results mainly in cortical bone loss.[5]

There are numerous published reports on rehabilitative treatment using dental implants. Yamazaki et al.[6], Ozawa et al.[7], and Dvorak et al.[8] analyzed the contact between bone tissue and implants placed in the tibia of ovariectomized (OVX) female rats. These authors observed that the reduction in bone mass of osteoporotic rats led to a smaller contact area between the bone and the implant, which compromised the ability of the bone to support the prosthesis.

Among the treatments for osteoporosis, hormone replacement is the most used, but this therapy has several contraindications and side effects.[9] Several other drugs, including bisphosphonates[10,11] and selective oestrogen receptor modulators (SERMs),[12,13] are promising alternative therapies for the treatment of postmenopausal osteoporosis.

Changes in the surface topography of dental implants through the addition and subtraction techniques have improved biological responses in peri-implant osteogenesis, especially in areas of lower density, as seen in osteoporosis.[14–17] The surface treatment of dental implants increases porosity and surface roughness, which causes osteoblastic lineage cells to reach the peri-implant region more quickly and more efficiently.[18,19]

The aim of this study was to evaluate the biomechanical behaviour of implants, with and without changes to their surfaces, in OVX rats fed on a low calcium and phosphate diet to induce osteoporosis. In addition, we sought to evaluate the healing at the bone–implant interface in the tibias of the same rats. Furthermore, we aimed to determine whether treatment with the SERM alendronate (ALE) or raloxifene (RAL) improves peri-implant bone healing and could, therefore, be beneficial in rehabilitation with dental implants.

It was hypothesized that drug treatment with RAL or ALE would improve the peri-implant healing process as well as increase the reverse torque and the bone–implant contact (BIC) values in OVX rats, compared with an OVX group that received no drug. Furthermore, it was hypothesized that surface treatment would promote better biomechanical, BIC, and histological results.

Materials and methods

Animals

This research project was approved by the Ethics Committee on the Use of Animals (CEUA). Female Wistar rats (*Rattus norvegicus albinus*, $n = 72$) weighing approximately 200 g were divided into three groups according to the proposed analysis: group I, histological study; group II, histometric study; and group III, reverse torque biomechanical analysis. Within these groups, the rats were further divided into four subgroups ($n = 6$) as follows: 'SHAM' – rats subjected to sham surgery only, with exposure of the ovaries, and fed a balanced diet; OVX-NT – OVX rats fed a low calcium (Ca^{2+}) and phosphate (PO_{4-}) diet, without drug treatment; OVX-ALE – rats fed a low Ca^{2+} and PO_{4-} diet and treated with sodium alendronate; and OVX-RAL – rats fed a low Ca^{2+} and PO_{4-} diet and treated with raloxifene. Euthanasia was performed 42 days after implant placement.

Initially, all animals were kept in cages, fed a normal laboratory diet (NUVILAB, Curitiba PR, Brazil) containing 1.4% Ca^{2+} and 0.8% PO_{4-}, and allowed access to water *ad libitum*. The animals were separated into treatment groups (SHAM-DN, OVX-NT, OVX-ALE, or OVX-RAL) prior to the induction of osteoporosis and drug treatment. After surgery, the animals in the SHAM group continued on the normal diet with water provided *ad libitum*, while all the OVX rats were fed a diet containing 0.1% Ca^{2+} and 0.5% PO_{4-} (Rhoster Ind., Vargem Grande Paulista, SP, Brazil) and had access to water *ad libitum*.

Experimental design

Determination of the oestrous cycle

The rats were placed in individual cages for the oestrous cycle evaluation, which was conducted daily according to the method of Evans and Long.[18] After two or three regular cycles, the animals were used for the experiments.

Induction of osteoporosis

The induction of osteoporosis was performed according to the model described by Teófilo et al.[5] Briefly, this involved combining a bilateral OVX with the administration of a low Ca^{2+} and PO_{4-} diet for a period of at least 4 weeks. To confirm the development of osteoporosis, the tibias of the SHAM-DN and osteoporotic animals (OVX-NT) were processed through quantitative computed microtomography (SkyScan 1176; Bruker MicroCT, Aartselaar, Belgium) and the bone mineral density (BMD) values of the cortical bones were obtained. Data from our laboratory showed that the BMD in the OVX-NT animals was 0.12525 g/cm^3 compared with 0.35255 g/cm^3 in the SHAM-DN animals. This result confirmed the presence of the osteopenia that is characteristically observed in the osteoporosis model in rats.[20] The OVX-ALE and OVX-RAL groups showed BMD values of 0.33302 and 0.51231 g/cm^3, respectively.

Bilateral ovariectomy (OVX)

The rats in the OVX-NT, OVX-ALE, and OVX-RAL groups were anaesthetized with xylazine (Coopazine; Coopers Brasil Ltda, Campinas, São Paulo, Brazil) and ketamine hydrochloride injection (Vetaset; Fort Dodge Saúde Animal Ltda, Campinas, São Paulo, Brazil), and incisions were made in both flanks to remove the ovaries. The rats in the SHAM-DN group underwent the same procedure, but without removal of the ovaries.

Drug treatment – sodium alendronate (ALE) and raloxifene (RAL)

Eight days after the OVX, rats in the OVX-ALE and OVX-RAL groups were treated with sodium alendronate (0.1 mg/kg/day) and raloxifene (1.0 mg/kg/day), respectively, for 30 days; the drugs were administered by gavage. Both drugs were dissolved in an aqueous solution.[13,19] The drugs were administered for a total of 72 days of dosing, up to the end of the experiment (euthanasia).

Surgery for tibia implants

The animals were fasted for 8 h prior to surgery and anaesthetized with a combination of 50 mg/kg intramuscular ketamine and 5 mg/kg xylazine. The rats were also administered mepivacaine hydrochloride (0.3 ml/kg 2% Scandicaine, 1:100,000 epinephrine; Septodont, Saint-Maur-des-Fossés, France) for local anaesthesia and to provide haemostasis in the operative field.

Following the induction of anaesthesia, the surgical site on the medial portion of the right and left tibia of the animal was shaved, after disinfecting with topical polyvinylpyrrolidone iodine and degerming (10% PVP, riodeine degermante; Rioquímica, São José do Rio Preto, SP, Brazil). A 2.0-cm incision was made, followed by the separation of the soft tissue to the right and left of the exposed tibial metaphysis.

A grade 4 titanium implant with a machined surface (IMPLALIFE Biotechnology, Jales, São Paulo, Brazil) was installed in the right tibia of each rat. A grade 4 titanium double acid-etched surface implant (IMPLALIFE Biotechnology, Jales,