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The effect of ovariectomy and 2 antiresorptive therapeutic agents on bone response in rats: A 3-dimensional imaging analysis

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Objective. The aim of this study was to evaluate bone mineral density (BMD) and microarchitecture in femurs and maxillary bones of ovariectomized (OVX) rats treated or not treated with alendronate (ALD) or odanacatib (ODN).

Study Design. Twenty rats were divided into groups: SHAM, OVX, OVX/ALD, and OVX/ODN. After 12 weeks, the femurs and maxillae were removed and subjected to 3-dimensional analysis by micro–computed tomography. Results were analyzed with 1-way analysis of variance and Tukey's post hoc test ($\alpha = 0.05$).

Results. OVX decreased maxillary and femoral BMD and altered femoral microarchitecture ($P < .05$). The drugs increased BMD of both types of bones, but only ALD maintained the phenotype similar to the SHAM group. The action of ALD was limited to the femoral trabecular separation (Tb.Sp). OVX and the drugs had no effect on the microarchitecture of the maxilla ($P > .05$).

Conclusions. ALD and ODN therapy increased BMD in both bones after ovariectomy. ALD was more successful than ODN in preserving the morphology of bone similar to the SHAM group. ALD maintained the phenotype for Tb.Sp in the femur, but ODN did not. In the maxillae, neither ovariectomy nor the 2 antiresorptive drugs had significant effects on microarchitecture. (Oral Surg Oral Med Oral Pathol Oral Radiol 2018;■■:■■–■■)

Osteoporosis is a metabolic bone disease characterized by decrease of bone mineral density (BMD), leading to skeletal fragility and increased risk of fractures.[1,2] Studies evaluating osteoporosis and the therapeutic agents for the prevention and treatment of this disease often use ovariectomized rats as an experimental model, approved by the U.S. Food and Drug Administration, to mimic postmenopausal conditions, such as reduced BMD.[3] Micro–computed tomography (micro-CT) is considered the gold standard to evaluate bone morphology and microarchitecture in rodents and other animal models, presenting excellent reproducibility and accuracy.[4,5]

The effect of osteoporosis on BMD and the microarchitecture of maxillary bones has been studied, but the results are still controversial.[6,7] There have been reports of a decrease in BMD after ovariectomy (OVX) in rats, with differences of results depending on the type of bone. Long bones and vertebrae are more sensitive than maxillary bones to the estrogen deficiency that occurs after OVX.[8] Some studies have shown that maxillary bones are minimally affected by OVX,[8-10] whereas some others have demonstrated changes in alveolar bone porosity, with a resulting decrease in BMD in the mandibles of ovariectomized rats.[7,11] In addition, there are large discrepancies among the studies using ovariectomized rats[8] with regard to animal age at the time of surgery, type of bone, and duration of the experimental period.

The prevention and treatment of bone loss and osteoporosis after menopause is of utmost importance in maintaining bone integrity and preventing possible fractures.[12] Bisphosphonates are the first-choice drugs for the pharmacologic treatment of osteoporosis.[13] Alendronate (ALD), a bisphosphonate containing nitrogen, is considered a drug capable of preventing bone loss induced by estrogen deficiency[12,14] and is associated with increased BMD and reduced risk of fracture.[15] However, an association between the systemic use of alendronate and a high incidence of osteonecrosis of the jaw has been suggested.[16] Therefore, there is an urgent need to develop new potential alternative antiresorptive agents,[17] with the mechanism of action based on the inhibition of bone resorption.

Odanacatib (ODN) is an antiresorptive drug that acts specifically on the inhibition of cathepsin K, an enzyme secreted by osteoclasts that degrades type I collagen. ODN has been shown to increase BMD of the spine and hips,[18] as well as the radius and tibia,[19] and has the ability to reduce bone resorption without decreasing the number of osteoclasts and preserving bone formation.[20] ODN leaves the osteoclasts alive and unaffected but inhibits bone resorption by inhibiting cathepsin K activity.[21] The mechanisms by which cathepsin K inhibition increases

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bone modeling, mainly at the periosteal surface, remain unclear.[22] Currently, ODN is being clinically tested (phase III) in the treatment of postmenopausal osteoporosis. Although cathepsin K inhibitors have the ability to suppress ovariectomy-induced bone loss in rats,[23] there are no studies evaluating the effect of ODN on maxillary bones. Therefore, the objective of the present study was to evaluate BMD and microarchitecture in femurs and maxillary bones of ovariectomized (estrogen-deficient) rats, subjected or not subjected to treatment with bisphosphonate (alendronate) or a cathepsin K inhibitor (odanacatib) for prevention of osteoporosis.

MATERIAL AND METHODS

Animals

After approval of the research project by the Institutional Animal Research Ethics Committee (process number #2013.1.1404.58.4), 20 female Wistar rats (*Rattus norvegicus albinus*), approximately 12 weeks old, were selected from the University's animal facility and kept under standard laboratory conditions. The rats were fed standard laboratory animal chow and were offered filtered water ad libitum.

The animals were randomly assigned to 4 groups: (1) SHAM: animals subjected to sham surgery, (2) OVX: animals subjected to OVX, (3) OVX/ALD: animals subjected to OVX and treatment with ALD, and (4) OVX/ODN: animals subjected to OVX and treatment with ODN.

The rats were anesthetized by an intramuscular injection of 10% ketamine (55 mg/kg weight) and 2% xylazine (10 mg/kg), and the ovaries were excised, as a whole, bilaterally in the experimental groups. In the SHAM control group, surgery was simulated, and the ovaries were gently moved and immediately returned to their original position, only to simulate surgical stress, as described by Orrico et al.[24] The success of OVX was confirmed by monitoring the animal's weight and the weight of the uterus at the day of euthanasia, considering that OVX is associated with weight gain and uterine atrophy.[14]

Administration of drugs

Administration of the study drugs started 1 day after OVX. The OVX/ALD group received 2 doses per week (1 mg/kg) of chemically pure sodium alendronate (Alendronate Monosodium Trihydrate; Galena Química e Farmacêutica Ltda., Campinas, Brazil), diluted in distilled water and administered by inserting a gavage needle into the oral cavity, totaling 24 doses during the experimental period of 12 weeks.[14] The OVX/ODN group received, by gavage, a total of 7 doses (2.12 mg/kg) of ODN (Santa Cruz Biotechnology Inc., Santa Cruz, CA) diluted in polyethylene glycol (PEG 400) (2.5 mg/mL) every 12 days, totaling also 12 weeks. The dose and frequency of administration were calculated by interspecies extrapolation by allometric rules from mice to rats, based on previous data presented by Hao et al.[25]

For administration of the drugs via gavage, the animals were fasted for 2 hours. A curved stainless steel oral gavage needle specific for rats (Ciencor Scientific Ltda., São Paulo, SP, Brazil) with a cannula measuring 1.2 mm in diameter, 38 mm long, and 2.25 mm ball tip, was used to inject the solution directly into the gastrointestinal tract, as described by Nelson-Filho et al.[26] After gavage, the animals were maintained for a few minutes in an upright position to avoid the occurrence of gastroesophageal reflux and/or irritation and to ensure complete ingestion of the volume administered in the procedure. The animals were weighed weekly to adjust the concentration of the ALD and ODN solutions according to the changes occurring in weight over time.

After the experimental period, the animals were euthanized by using ketamine hydrochloride (Ketamina Agener, União Química Farmaêutica Nacional S/A, Embu-Guaçu, Brazil) for anesthesia, xylazine hydrochloride (Dopaser; Caleir S.A., Barcelona, Spain), and carbon dioxide inhalation. The bones were surgically removed with the aid of sterilized scissors and a scalpel blade and stored in 70% ethyl alcohol for fixation until scanning by micro-CT.

Micro-CT to analyze BMD and bone microarchitecture

A high-resolution micro-CT device (SkyScan 1174 v2; Bruker-microCT, Kontich, Belgium) was used to quantify the BMD and 3-dimensional (3-D) microarchitecture parameters in the femur and maxillae. Cross-sectional images of each specimen were reconstructed with specific software (NRecon version 1.6.3; Bruker-microCT), with ring artifact correction of value 10.

The regions of interest (ROI) were limited to the cortical-free trabecular bone, which is more delicate and comparable with osteoporotic human bone.[27] In each bone, the ROIs were selected according to Liu et al.[8]: the trabecular alveolar bone in the interradicular septum of the maxillary first molar and the axial trabecular alveolar bone located 3 mm under the growth plate.

For accurate calculation of BMD, appropriate calibration of the CTAn software (v. 1.14.4.1 + , Bruker-microCT, Kontich, Belgium) was performed by using calcium hydroxyapatite phantoms of known density (0.25 and 0.75 g/cm^3). Once the BMDs of the phantoms were calibrated in the CTAn software, the following structural parameters of the ROI were calculated: BMD; bone volume fraction (bone volume/total volume = BV/TV), defined as the fraction of bone in the total ROI; trabecular thickness (Tb.Th); trabecular number (Tb.N), defined as the number of times that a trabecular is crossed by others per unit of length; and trabecular separation (Tb.Sp).[7,8]