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Alendronate improves bone density and type I collagen accumulation but increases the amount of pentosidine in the healing dental alveolus of ovariectomized rabbits

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ABSTRACT

Background: It has been shown that the oral aminobisphosphonate sodium alendronate (ALN) therapy reduces the risk of main fractures in osteoporotic women, but its effect on the jaw bones is poorly known. Here, we hypothesized that ALN affects the newly formed alveolar bone, particularly the quality of the type I collagen cross-linking.

Methods: Osteoporosis was induced by ovariectomy (OVX) in 6-month old rabbits. Six weeks following surgery, eight animals were treated by oral gavage with ALN (OVX + ALN) and ten received placebo (OVX + Pbo). Another six rabbits which were sham operated also received placebo (SHAM + Pbo). One month following the beginning of treatment, the upper and lower left first premolars were removed. Six weeks later, the upper and the lower right first premolars were also extracted. One month after the second extraction, biopsies were collected from the maxillary extraction sites and collagen crosslinks were analyzed in the newly formed bone tissue by HPLC. Also, at this time, mandibular bone segments were subjected to μCT.

Results: Animals treated with ALN achieved a roughly 2-time greater bone volume fraction value at a late healing period than animals in the other groups ($p < 0.05$). Collagen mean results were 2- to 4-times superior in the OVX + ALN group than in the control groups ($p < 0.05$).

ALN-treated animals presented higher amounts of the non-enzymatic collagen cross-link pentosidine (PEN) than the sham-operated rabbits ($p < 0.05$), whereas the OVX + Pbo group presented the highest amount of PEN ($p < 0.05$).

Conclusion: Alendronate increases bone volume and collagen accumulation, but does not fully rescue the non-osteoporotic alveolar tissue quality as is evident from the increased quantity of pentosidine.

1. Introduction

Osteoporosis is a progressive skeletal disorder characterized by reduction of bone mineral density (BMD) and degeneration of bone microarchitecture [1]. That results in bone fragility and fracture, which adversely impact the quality of life and boost the risk of infection and mortality [2]. Estrogen deficiency due to menopause is the main cause of bone loss in women and bisphosphonates (BPs) are the most commonly prescribed class of drugs for its treatment by suppressing bone resorption [3]. These pharmacological agents can reduce the risk of fractures and the rate of mortality related to hip fracture by up to 30% and 60%, respectively [4,5]. Clinical studies in postmenopausal women showed that long-term use of those drugs resulted in persistent anti-fracture and BMD increasing effects beyond three years of treatment [6]. However, in the last decade there have been reports of a greater than before incidence of atypical femur fractures (AFFs)

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associated with long-term BPs therapy, which might be explained by an impaired bone remodeling [7,8]. The continuing and degrading action of BPs on the skeleton fracture-resistance toughening system has indeed been associated with bone fragility [9,10].

Nitrogen-containing BPs accumulate mainly in areas of high osteogenic activity, such as sites of micro fracture repair and dental alveolus healing [11,12]. This might result in local amplification of BP effects, leading to modifications in the organic matrix molecular features and bone mineralization [13,14]. Molecular alterations in the extracellular matrix may interfere with the mineralization process and lead to a brittle osseous tissue. In fact, a higher material modulus (less compliant bone), which might contribute to tissue embrittlement and fracture risk enhancement, was correlated with accumulation of the non-enzymatic glycation-induced pentosidine in a mouse model of type 2 diabetes [15]. That form of advanced glycation end product (AGE), contrasting to the enzyme lysyl oxidase-derived collagen cross-links, has been proposed as a major cause of bone fragility associated with aging and numerous disease states including osteoporosis [16]. Due to its exceptionally high proportion of approximately 90% of the organic matter, it is generally agreed that collagen plays a critical role in the structure and function of bone tissue [17]. Here, we hypothesized that the anti-resorptive therapy with sodium alendronate, an aminobisphosphonate, would affect the type I collagen cross-linking pattern and the quality of the newly-formed alveolar bone after tooth extraction.

2. Materials and methods

This study was approved by the Ethics Committee for the Use of Animals by the Health Sciences Center of the Federal University of Rio de Janeiro (UFRJ, protocol number 007/15), according to the regulations in effect in Brazil. This scientific paper complies with the EU Directive 2010/63 for animal experiments and it is also in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and follows the ARRIVE guidelines.

2.1. Animal model

Twenty eight, 6-month old, New Zealand white rabbits (*Oryctolagus cuniculus*), skeletally mature, and weighing approximately 4 kg were randomly distributed into the following experimental groups: Sham-operation + placebo (SHAM + Pbo, $n = 8$); Ovariectomy + placebo (OVX + Pbo, $n = 10$); Ovariectomy + alendronate (OVX + ALN, $n = 10$). The identified animals were housed in individual cages at the laboratory animal facility/Experimental Surgery Center of the School of Medicine at the UFRJ and maintained in controlled environment conditions (20 to 25 °C, 30% to 35% humidity and a 12 h light/12 h darkness cycle). Animals were fed a balanced rabbit chow and water ad libitum.

2.2. Bone mineral density

Animals had the whole-body BMD measured by dual-energy x-ray absorptiometry (DXA, Lunar Prodigy Advance Plus, GE Lunar, Milwaukee, WI, USA) at three different time points: baseline, six weeks after ovariectomy and 12 weeks following the beginning of medication/placebo administration. Exams were carried out under general anesthesia by IM administration of xylazine 5 $mg \cdot kg^{-1}$ and ketamine 35 $mg \cdot kg^{-1}$, in duplicate, by the same trained examiner who was blinded to the experimental group allocation. Images were captured in the standard scanning mode of 1.8 μGy with the Lunar Prodigy Advance software (GE Healthcare, Chicago, IL, USA) specific for small animals.

2.3. Surgical procedures

One week after the baseline bone densitometry, bilateral ovariectomy was carried out under general anesthesia with xylazine and ketamine. The animals were intubated, and anesthesia was maintained with isoflurane 1.5% and oxygen 0.8–1.5 $L \cdot min^{-1}$, using a non-rebreathing circuit. The surgical procedure was performed as follows: after a longitudinal incision in the lower third of the abdomen, both distal uterine horns were ligated followed by ovaries removal. The abdominal cavity was closed by muscle and skin layers suture. In addition, animals received the antibiotic enrofloxacin 2.5 $mg \cdot kg^{-1}$ (Baytril 10%, Bayer, São Paulo, Brazil) and the anti-inflammatory/analgesic ketoprofen 1.0 $mg \cdot kg^{-1}$ (Ketojet 100 mg, AgenerUnião, Apucarana, PR, Brazil) medication, both given IM once daily for five days. One week following the surgery, ovariectomized animals were fed low calcium (0.14%) and phosphorus (< 1%) diet (Algomix Agroindustrial Ltda, Ouro Verde do Oeste, PR, Brazil) for six weeks according to an established osteoporosis model for rabbits [18]. Sham-operated animals received a regular diet. After that six week period, animals started receiving either ALN or placebo according to the experimental group allocation.

2.4. Interspecies extrapolation of drug dose and dosing interval

The dose conversion of ALN 70 mg once-weekly [19] from a 70 kg human to a 4 kg rabbit was based on the metabolic size, measured by the minimum energy cost (MEC), and the metabolic rate, measured by the specific minimum energy cost per unit weight (SMEC), according to the equations: $MEC = K.W_{kg}^{0.75}$, and $SMEC = K.W_{kg}^{0.75}/W_{kg}$, respectively. In those equations, "K" means a theoretical constant of proportionality that, in line with the Hainsworth's energy group to which placental mammals belongs, equals to 70; "W_{kg}" is the weight of the animal species; and the exponent "0.75" refers to the slope of the metabolic regression line [20]. Firstly, the MEC dose was calculated by using the equation: MEC dose = treatment dose in humans / MEC in humans; the treatment dose in rabbits was, then, computed by using the equation: treatment dose in rabbits = MEC dose x MEC in rabbits. After the calculation, one ended up with a dose of ALN 2.03 $mg \cdot kg^{-1}$. Secondly, the dosing interval was determined from the SMEC. To accomplish that, one has to calculate the following equation: SMEC interval in humans = SMEC in humans × dosing interval in humans. Afterwards, to estimate the treatment interval in rabbits, the SMEC interval in humans was divided by the SMEC in rabbits. The result was an 82.14 h interval. To avoid overstressing the animals, it was established an adjusted drug regimen of 16 mg dissolved in distilled water to a final volume of 16 mL, administered by oral gavage once weekly for twelve weeks. Placebo groups received 16 mL of distilled water. The oral gavage procedure was executed under gas sedation (isoflurane 1.5% and oxygen 0.8–1.5 $L \cdot min^{-1}$), using a flexible cannula attached to a syringe.

2.5. Tooth extraction

One month following the beginning of drug/placebo treatment, the upper and the lower left first premolars were removed for late healing analyses. Six weeks later, the upper and the lower right first premolars were extracted for early healing studies. Tooth extraction was carried out under general anesthesia with xylazine and ketamine. Access to the extraction site was facilitated by an external approach, which consisted of 1 cm extension incision from the labial commissure to the last molar, as previously described [21]. Mucoperiosteal flap was raised to expose the tooth cervical area and the surrounded alveolar bone. Periotome and pediatric dental forceps were used for tooth luxation and extraction, respectively. Tissue wound was closed in two layers with absorbable polyglycolic acid 4-0 suture and skin was closed with nylon 4-0 suture. One month after the second extraction procedure, animals were sacrificed with a 100 $mg \cdot kg^{-1}$ thiopental sodium intracardiac injection, under general anesthesia.