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Effect of alendronate sodium on tooth movement in ovariectomized rats

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ABSTRACT

Objectives: The aim of the present study was to evaluate the effect of two different dosages of alendronate on induced orthodontic movement in an experimental model involving rats with osteoporosis following ovariectomy.

Design: Female Wistar rats (*Rattus norvegicus*) eight weeks of age were divided into four groups ($n = 12$/group): ovariectomized (OVX group); ovariectomized and treated with alendronate sodium at 1 mg/kg (Group OVX + ALN1); ovariectomized and treated with alendronate sodium at 2 mg/kg (Group OVX + ALN2); and sham operated (control). Three months after ovariectomy, the maxillary right first molar was submitted to movement for five and seven days. After the death of the animals, the maxilla were removed and processed for microscopic evaluation. The maxillary left first molar (without movement) was used for comparison purposes in all groups. The samples were processed for the quantification of alveolar bone and tooth movement.

Results: Intragroup comparisons showed significant movement after five and seven days ($p < 0.05$) for all groups. Comparison among groups revealed greater tooth movement in the OVX group ($p < 0.05$), on day 7.

Conclusions: Both alendronate sodium doses similarly decreased tooth movement in ovariectomized rats ($p > 0.05$). Movement in ovariectomized + alendronate groups were also smaller than non-ovariectomized rats, however without statistical difference.

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1. Introduction

Orthodontic treatment has become more accessible to the population and a greater number of adults currently seek such treatment. As a consequence, orthodontists are more likely to have patients with systemic diseases stemming from the ageing process, such as osteoporosis. The correction of malocclusions in such patients requires knowledge on the influence of orthodontic movement on bone tissue and teeth.

Osteoporosis causes the loss of bone mass in long bones and vertebrae. This condition can also alter the bone tissue of the maxilla and mandible involved in tooth movement during orthodontic treatment.[1,2] Systemic drugs used to treat osteoporosis can have an effect on orthodontic treatment by acting on osteogenesis and structures of the periodontium.[3,4]

Bisphosphonates are among the indicated medications for the treatment of osteopenic and osteoporotic states.[5] The amino-bisphosphonate alendronate sodium (ALN) is one of the most widely used medications for this purpose due to its high potential for inhibiting bone resorption.[6,7] ALN has a strong affinity for circulating calcium and to mineral at bone surfaces[8] and inhibits enzyme activity in osteoclasts, thereby impeding the dissolution of bone tissue and the degradation of collagen.[8] Upon being transported with isolated components of bone tissue through the cytoplasm of osteoclasts, ALN induces biochemical events capable of initiating apoptosis in these cells.[9–11] Thus, bisphosphonates contribute to controlling bone turnover and preventing osteopenia/osteoporosis.

Case reports involving patients submitted to prolonged bisphosphonate treatment describe lower rates of tooth movement, which hampers orthodontic treatment.[12,13] However, further evidence is needed to determine the extent to which altered bone metabolism in patients under systemic treatment for osteopenia and osteoporosis affects tooth movement.[3,4,14]

The experimental ovariectomy is an important study model that evaluates, in animals, the consequences of loss of bone mass in several situações,[14] since it is increasingly frequent presence of individuals with frames of osteopenia and osteoporosis under treatment with alendronate sodium in dental offices.

In humans, the recommended dose of alendronate is 1 mg/kg body weight, once a week, however there is no consensus regarding the appropriate dosage for animal testing. Therefore, the aim of this work was evaluate the effect of two different dosages of alendronate on induced orthodontic movement in an experimental model involving rats with osteoporosis following ovariectomy.

2. Materials and methods

The procedures employed in this study were approved by the Ethics Committee on Animal Experimentation under process number 2011-03696.

Forty-eight female Wistar rats (*Rattus norvegicus*) aged eight weeks were used. The animals were anesthetized with an intramuscular injection of xylazine 8 mg/kg (Anasedan Agribrands do Brasil Ltda, Paulínia, São Paulo, Brazil) and ketamine 70 mg/kg (Dopalen Sespo Industria e Comercio Ltda, Jacareí, São Paulo, Brazil) prior to bilateral laparotomy for the removal of the ovaries. The animals were divided into four groups (n = 12/group): ovariectomized (OVX group); ovariectomized and treated with 1 mg/kg or 2 mg/kg alendronate sodium salt (DEG ''Importation of Chemical Products'', India) diluted in saline solution (Group OVX + ALN1 and Group OVX + ALN2); and a sham operated group used as the control. Throughout the experiment, the animals were kept in an animal lodging facility with a 12-h light/dark cycle at a temperature of 20 °C and free access to ration and water.

For 90 days following ovariectomy, the OVX + ALN1 and ALN2 groups received a subcutaneous injection of ALN at respective doses, twice a week on non-consecutive days.[15] After three months, the animals were anesthetized with an intramuscular injection of 8 mg/kg xylazine and 70 mg/kg ketamine prior to the placement of a mechanical device for the induction of tooth movement. For such, the appliance designed by Heller and Nanda (1979) [16] was modified with the replacement of the steel spring with a nickel-titanium spring (Sentalloy, GAC, New York, USA) and a light-curing resin in the cervical region of the incisor to enhance the retention of the wire. The appliance was anchored on the upper incisors and right first molar to produce the mesialization of the molar.[14] The upper right first molar was wrapped with steel wire measuring 0.20 mm in diameter (Morelli, Sorocaba, São Paulo, Brazil) and attached to the spring measuring 3 mm in length for the application of a continuous force of 50 cN.[17–19] The spring was tied in a cervical position to the resin (Z100, 3M, Saint Paul, MN, USA) of the upper right incisor with steel wire measuring 0.25 mm in diameter (Morelli, Sorocaba, São Paulo, Brazil) (Fig. 1).

The animals in all four groups were euthanized with an overdose of ketamine five and seven days (n = 6 animals/group/evaluation time) following the placement of the orthodontic appliance.[20,21] The upper left first molar, which was not submitted to movement, was used for comparison purposes in each group. On induced tooth movement in rats, the initial resorptive phenomena are the first three days, with its peak at 5 days and its consequences markedly present at 7 days.[22] From the viewpoint of optical microscopy, the analysis of the effects of the forces three days before or after seven days do not bring significant results because they do not cover the main tissue and cellular phenomena of induced tooth movement.[15]

All animals were weighed at the beginning and end of the experiment (Table 1). The maxillae were removed, dissected, immersed in a 10% formalin solution for 48 h, washed in running water for 24 h and decalcified in 18% EDTA solution for six weeks. The specimens were then dehydrated, cleared and embedded in paraffin. Semi-serial cuts measuring 7 μm were made in the longitudinal direction along the larger axis of the molars and stained with haematoxylin and eosin.

For the histomorphometric analysis, the histological cuts were examined under an optical microscope (BX41, Olympus, Tokyo, Japan) coupled to a digital camera (QColor3, Olympus, Tokyo, Japan) and photographed. Measures were performed with the aid of the Image Pro Plus® program, version 4.5 (Media Cybernetics, USA).