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# Effect of Alendronate on Healing of Bone Defect and Gingival Tissue in Osteopenic Rats

## Efecto del Alendronato en la Reparación de Defectos Óseos y Tejido Gingival en Ratas con Osteopenia

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**SUMMARY:** The aim was to evaluate bone repair and gingival tissue repair in osteopenic rats. Fifteen female wistar rats were included; in all of them ovariectomy was realized to induce osteopenia; after 45 days, the animals were submitted to 2 surgical techinques 1) dental extraction of the upper central incisor with no socket preservation and 2) 5 mm cranial defect in the calvarium; 5 rats were included in the control group (G1) withput alendronate application; in the group 2 (G2) was used subcutenous alendronate (0.5 mg/kg) once for three weeks and then was realizd the both surgical techniques. In group 3 (G3), after ovariectomy was realized the both dental extraction and the calvarium defect and after that was realized the alendronate protocol. In each group, after six week was realized euthanasia and descriptive histological analysis of the surgical areas involved. In bone formation of the 5 mm cranial defect was observed with good progression in the 3 experimental models and no modification in quality of bone repair was observed. For the gingival tissue in the extraction socket, no differences were observed between G1 and G3. On other hand, in G2 a thinner and reduced gingival epithelium was found. Our results showed that alendronate was not an obstacle for bone repair; deficiencies in re-epithelialization of oral mucosa show the impact of alendronate before dental extraction.

**KEY WORDS: Biphosphonate; Osteonecrosis; Osteoporosis.**

## INTRODUCTION

Bisphosphonates (BPs) are routinely drugs to treat osteoporosis, involving close to 200 million people in the world (Agis *et al*., 2010). BPs are drugs responsible for reducing bone resorption by their metabolized nitrogen compounds be toxic to osteoclasts, affecting their survival and function (Li *et al*., 1997; Landesberg *et al*., 2008; Brozoski *et al*., 2012; Kumar & Sinha, 2013). They may have approximately 10 years -of half life and their continued use may also trigger osteonecrosis of the jaws with incidence on population ranges from 0.7 to 12 % (Cooper *et al*., 2010; Mozzati *et al*., 2013).

Osteonecrosis induced by BPs is characterized for the involvement of the maxillomandibular complex with preference in mandible related to subjects not exposed to radiotherapy on the head and neck region (Ohashi *et al*., 2009). The most common clinical finding is related to ulcerated mucosa area with localized bone necrotic exposure. The signs and symptoms as well as the etiology and treatment of this disease have been not well revised.

On other hand, it is well documented in the literature the effect of BPs on the bone, however, there are few studies evaluating the toxicity of BPs on oral mucosa (Ruggiero & Woo, 2008). The available studies show that these drugs are responsible for reducing the proliferation of epithelial and fibroblastic cells with a limited in healing of soft tissue and more bone exposure to the oral microflora (Lin & Lane, 2004).Therefore the aim of this research was to investigate the effect of alendronate in hard and soft tissue repair in an in vivo osteopenic model developed with ovariectomized rats.

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## MATERIAL AND METHOD

The procedure involving the use of animals was approved by the Ethics Committee on Animal Experimentation (CEEA) of the State University of Maringa (Protocol No 057/2014). Fifteen female Wistar rats (Rattus norvegicus) with 60 days of age, weighing approximately 300 g, were placed in cages containing three animals, covered with sawdust and kept in standard vivarium conditions with clear light / dark cycle of 12 hours at controlled temperatures (25 ° C) with water and food ad libitum (NuvitalL®).

In this methodology, the first surgical step was the ovariectomy to induce the osteopenia. This surgery was realized by vet surgeon with experience in this technique and after forty-five days, the bone mineral density decreased as reported previously (Scheper *et al*., 2009) and at this moment the sample was divided in three differents groups. The surgical model included two surgical defect in each animal, being 1) dental extraction of the upper right central incisor with no socket preservation and 2) the creation of 5 mm cranial defect on the calvarium realized by a 5 mm trephine using low speed (Yano *et al*., 2014).

Control Group (G1) included five animals with no use of alendronate, submitted to dental extraction and 5 mm cranial defect filled with blood clot. Group 2 (G2) included 5 osteopenic animals treated with subcutaneous alendronate (0.5 mg / kg) once a week for 3 weeks; after drug treatment, the animals were subjected to dental extraction and the same 5 mm cranial defect on the calvarium filled with xenogenic biomaterial (Bionnovation®) and collagen membrane (Baummer®) on the grafted defect. Group 3 (G3) included 5 osteopenic animals treated with dental extraction and the calvarium defects grafted with xenogenic biomaterial (Bionnovation®) and collagen membrane (Baummer®) over the defect; 3 week after surgery, was applied subcutaneous alendronate (0.5 mg / kg) once a week for 3 weeks (Fig. 1).

All the surgical procedure was performed at Laboratory of the Department of Pharmacology and Therapeutics at State University of Maringá on the same afternoon, following intraperitoneal anesthesia with anesthetic solution of xylazine (10 mg / kg) and ketamine (100 mg / kg). All fifteen animals were operated with the same protocol.

Euthanasia of the animals was performed with triple anesthetic dose of xylazine and ketamine, six week after the last intervention in each group, using a routine pharmacological protocol. Samples of calvarium and the area involved in the extraction socket were collected and process with 10 % formalina solution for 72 h; after that, samples were decalcified with acid nitric 5 % for 21 days and then embedded in paraffin. For histological analysis, 6 µm slice were made, stained with haematoxylin and eosin. Histology was descriptive for all the samples.

## RESULTS

Calvaria 5 mm Defect: In G1 was observed low incremental growth lines, a low level of osteoclasts, osteoblasts and osteocytes, low level of bone formation and a greater presence of connective tissue, mainly in the center of the defect (Fig. 2 a-c). In the same line, was observed a minor bone formation than G2 and G3 (Fig. 2 d-f).

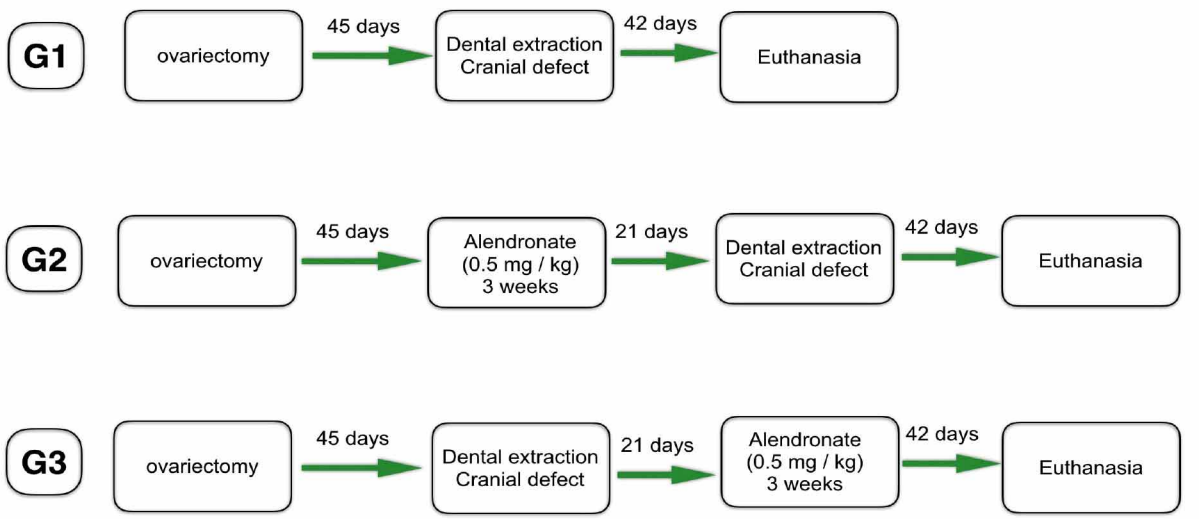


Fig. 1. Sample included in this research; each group, with 5 animals each, included a protocol to participate as control group (G1), and experimental groups (G2 and G3)

In G3 was observed osteocytes surrounded by acidophilic bone matrix close to some osteogenic and fibroblastic cells; some particles of biomaterial was observed, however, a large amount of bone tissue with advanced remodeling was present, showing macrophages in connective tissue, bone channels and a large number abundant blood vessels (Fig. 2 d-f).

In G2 showed bone matrix regions with different degrees of acidofilias, featuring a woven bone, presence of blood vessels, osteogenic cells (osteoblasts, osteoclasts, osteocytes) and a connective tissue with abundant collagen fibers and fibroblasts (Fig. 2 g-i).

Dental extraction with no socket preservation: As a summary, in all the samples was observed gingival epithelium, connective tissue, collagen fibers, blood vessels and bone septum. In G1 was observed a stratified squamous epithelium gum, keratinized, thicker with granular layer and epithelial ridges deep and well defined (Fig. 3 a-c) and in G3, a regular description as G1 was observed (Fig. 2 d-f). G2 showed differences when compared to G1 and G3, presenting a gingival epithelium fragile, reduced cell and areas with poor definition and areas without epithelium (Fig. 2 g-i).

## DISCUSSION

For gingival tissue analysis, in G3, using three-week time for tissue healing before receiving treatment with alendronate, was observed similar condition to the control group (G1). In clinical evaluation, Marx *et al*. (2005) recommend a dental assessment before the patients starting treatment with BFs, to eliminate sources of infection; if surgical procedure is needed, it is recommended wait healing for 30 days, and only after this period start treatment with

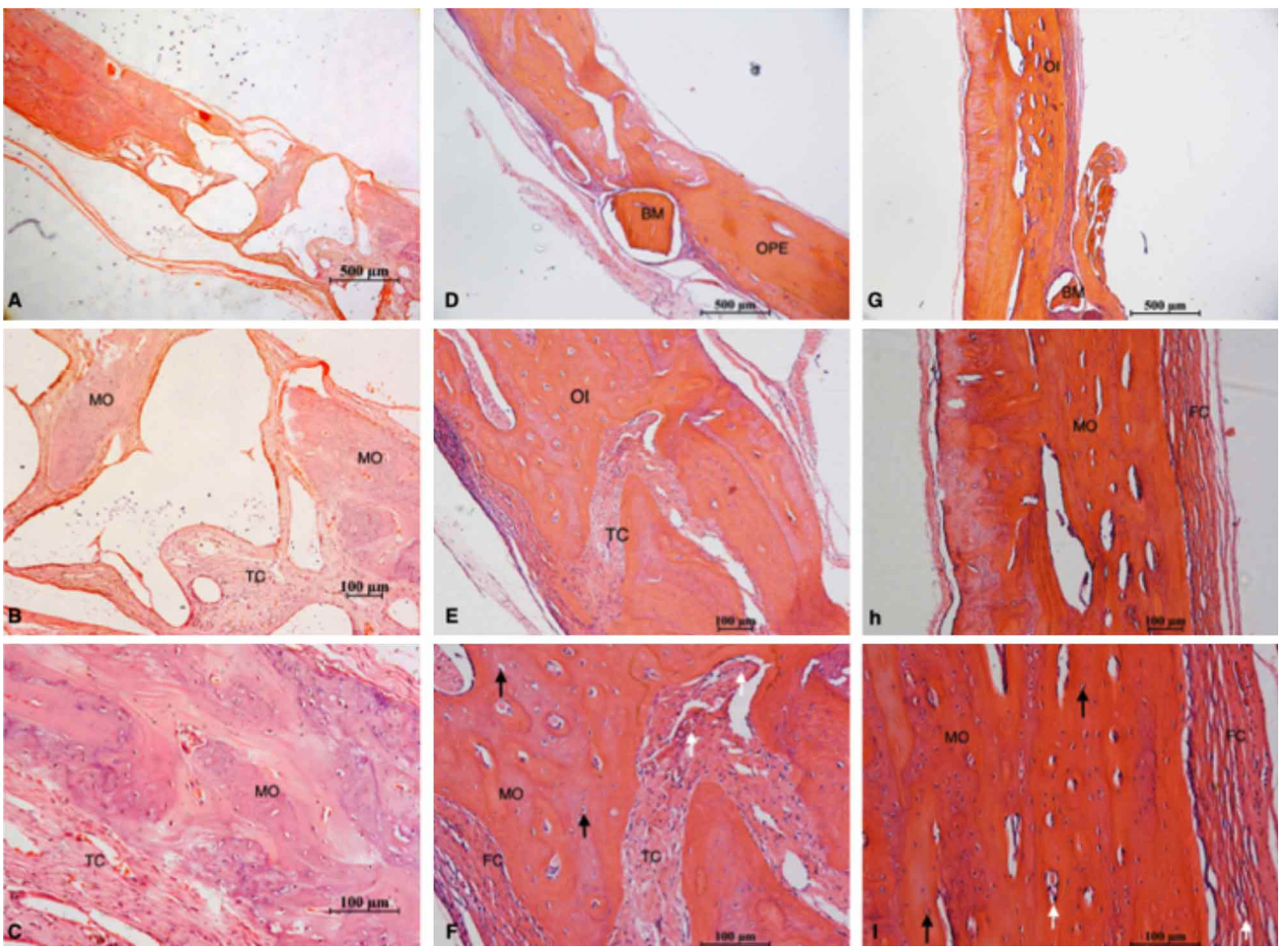


Fig. 2. Histological sections obtained 6 weeks after the creation of 5 mm cranial defects. G1 (A, B, C), G2 (D, E, F) and G3 (G, H, I). Histological analysis was observed shiwong bone matrix (MO), connective tissue (TC), preexisting bone (OPE), immature bone (OI), bundles of collagen fibers (FC), bio-materials (BM), osteocytes (black arrows) and blood vessels (white arrows) (hematoxylin and eosin, 4X, 10X and 20X).

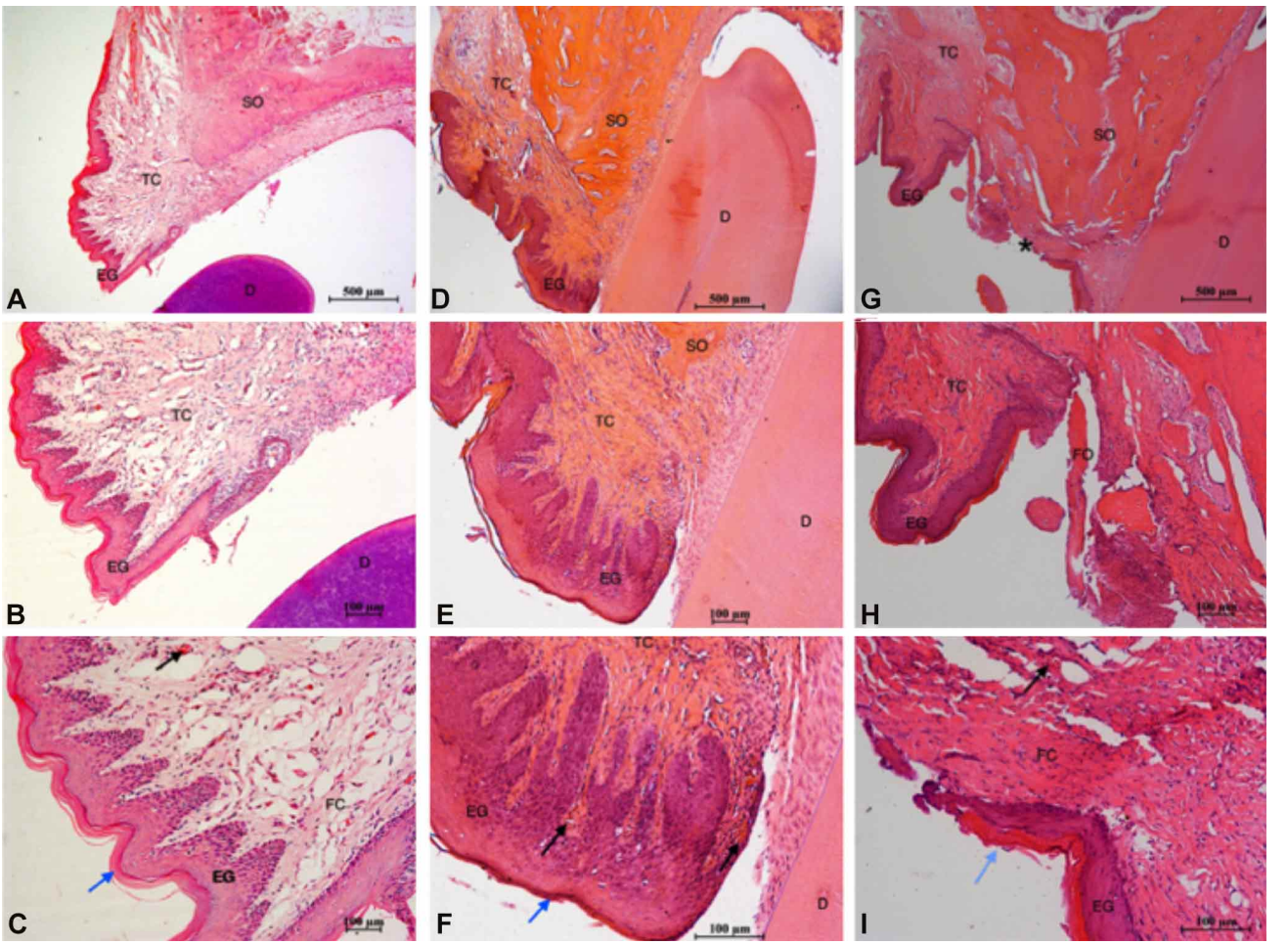


Fig. 3. Histological sections obtained 6 weeks after extraction surgery of the upper right central incisor stained with hematoxylin and eosin (4X, 10X and 20X). G1 (a, b, c), G2 (d, e, f) and G3 (g, h, i) show sept bone (OS), tissue conjuntive (TC), gingival epithelium (EG), tooth (D), keratin (blue arrow), blood vessels (black arrow), bone fragment (FO) asence epithelial (*).

BPs (Ruggiero *et al*., 2014). The validation of this clinical practice can be better evaluated in our experiment where the results showed a satisfactory wound healing in animals involved in procedures before the use of alendronate. Recently the American Association of Oral and Maxillofacial Surgeons (2014), published a clinical follow-up recommending the suspension of the drug three months before and three months after the surgical procedure, for better wound healing (Ruggiero *et al*.).

G2 had the gingival epithelium reduced, thinner, even with areas of epithelial absence, suggesting a toxic action of BFs to the oral epithelium. There are few studies in the literature discussing the potential toxicity of bisphosphonates to the oral epithelium (Dimitrakopoulos *et al*., 2006; Mozzati *et al*.). An animal model was suggested that BPs suppress cell proliferation and impair oral wound healing, showing osteonecrosis by the bone exposure to oral micro flora (Ruggiero *et al*.). In a survery, rats subjected to subcutaneous treatment with alendronate (Miglioratir *et al*., 2005), also showed deficiency in epithelial wound healing, and in some cases a necrotic bone were exposed. A similar result was found in our study where the G2 had oral epithelium delayed, rendering them more fragile and susceptible to ulcerations, allowing the bone exposed and contributes to osteonecrosis pathogenesis.

BFs in the oral mucosa induce bone exposure to oral microflora and contributing to osteonecrosis pathogenesis (Miglioratir *et al*., 2005; McLeod *et al*. 2014); therefore, trauma on the mucosa of patients undergoing treatment with BFs must be avoided (Brozoski *et al*.). Our results suggest that the treatment of alendronate for a short time, could not cause osteonecrosis after invasive dental procedures, and in such cases a previous dental evaluation at the beginning of drug treatment is required. Therefore, an explain to do not detect alteration on mineral content in our study can be related on the experiment time, dose and time of

administration of alendronate (0.5 mg / kg) for 3 weeks were low and in human could be infrequently. The literature shows that patients in treatment with oral BPs for less than three years may be undergoing to surgery (Dimitrakopoulos *et al*.; Reid *et al*., 2007).

In the literature (Ravosa *et al*., 2011; Paiva-Fonseca *et al*., 2014) it is well documented toxicity of the BPs in the gastrointestinal tract cells and had been related to esophagitis, gastritis and ulcers. Histological studies conducted in animals that received alendronate orally, show the stomachs with impair anti-oxidant system of the gastric mucosa and decrease the cell differentiation, causing exfoliation of the mucosa and damage in the epithelium. It is reasonable to assume that in oral cavity, high concentrations of BFs in the underlying bone can produce a similar effect. In vitro studyindicate that risedronate, another type of BPs administered orally, inhibited cell proliferation, and consequently decreased the oral wound healing (Lin & Lane). Our results showed similar results with use of alendronate and we observed that after dental surgery, the epitelial cells prolifaration is low, and this may contribuit to weaken physical barrier created by the mucosa that protect the adjacent bone and if the drug concentration is high enough in the local, a secondary bone infection may occur.

Recently, a in vitro assessment of three BPs (Zoledronate, Alendronate and Clodronate) using keratinocytes and fibroblasts of the oral mucosa, showed that high doses of these drugs would be responsible to inhibit cell proliferation, reducing viable cells, however subtoxic doses of this drug didn't show specific effect on the epithelium; the BPs are quickly attached to the bone tissue due to the drug's affinity to hydroxyapatite, so their time in the bloodstream is low (Papapetrou *et al*., 2009). Thus, our results suggest that the treatment of alendronate for a short time, could not cause osteonecrosis after invasive dental procedures, and in such cases a previous dental evaluation at the beginning of drug treatment is required. The gingival epithelium of the group treated with alendronate and then operated (G2), presented soft tissue abnormalities, suggesting toxicity by alendronate to epithelial cells.

Treatment for osteoporosis aims to improve bone density. In animal studies of Oliveira *et al*. (2017), results showed improvement in bone repair around implants installed in tibia of osteoporotic rats treated with Alendronate. Authors have concluded that cases with short-term treatment with Alendronate this advantage could occur (Oliveira *et al*.). But in other study with Canettieri *et al*. (2009) bone defects created in femur of rats were filled with alendronate, and another group with alendronate and hydroxyapatite; The authors concluded that in cases where alendronate was present, bone repair was impaired compared to the control group and the hydroxyapatite groups only. Based on results like these, more studies are necessary to evaluate the quality of bone in cases of sodium alendronate administration.

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**RESUMEN:** El objetivo fue evaluar la reparación ósea y gingival en ratas con osteopenia. Quince ratas wistar hembras fueron incluidas; en todas ellas se realizo ovarectomia y fue realizada la inducción de osteopenia; después de 45 días, los animales fueron sometidos a dos técnicas quirúrgicas 1) extracciones dentales del incisivo central superior sin preservación alveolar y 2) creación de un defecto craneano de 5 mm en la calota; 5 animales fueron incluidos como grupo control (G1) sin la aplicación de alendronato; en el grupo 2 (G2) se utilizó alendronato subcutáneo (0,5 mg/kg) una vez a la semana durante 3 semanas. En el grupo 3 (G3), después de la ovarectomia se realizó la exodoncia y el defecto en el cráneo y después de ello se inicio el protocolo con alendronato. En cada grupo, después de seis semanas se realizó la eutanasia con descripción histológica de los hallazgos. En el hueso formado en el defecto craneano de 5 mm se observó una adecuada progresión de reparación en los 3 modelos experimentales y no se observó cambios importantes en el modelo de reparación. Para el tejido gingival en el sitio de extracción, no se observaron diferencias entre el grupo G1 y G3. Por otra parte, el G2 presentó un tejido mas delgado con reducción del epitelio gingival; nuestros resultados demuestran que el alendronato no fue un obstáculo en la reparación ósea; deficiencias en la re epitelización de la mucosa oral muestran el impacto del alendronato después de la exodoncia.

**PALABRAS CLAVE: Bifosfonatos; Osteonecrosis; Osteoporosis.**

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