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LUISA BERLATO SILVA

AVALIAÇÃO CLÍNICA E HISTOLÓGICA DE OSTEONECROSE MANDIBULAR INDUZIDA POR AGENTES MODIFICADORES ÓSSEOS EM RATOS WISTAR

Santa Maria, RS
2021

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Orientadora: Profª Drª Cristiane Cademartori Danesi
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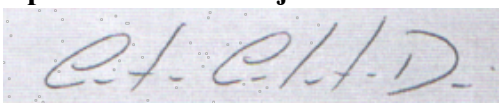

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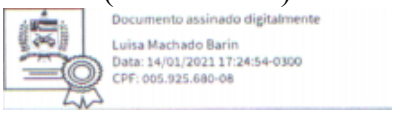

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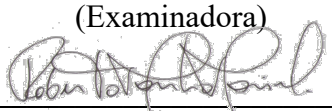

Roberto Marinho (UFSM-RS)
(Examinador)

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2021

Ao meu irmão, que não está mais presente fisicamente, mas presente de alma, o qual sempre me incentivou a ser uma pessoa melhor a cada dia. Dedico também a minha mãe, o qual está ao meu lado sempre na busca dos meus sonhos. E finalmente, ao meu pai, o qual sempre me incentivou a buscar meu melhor.

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- Aos animais, por contribuírem ainda mais para a ciência, pois o desfecho deste estudo não poderia ser concretizado sem eles.

'' É impossível conceber a distância para cima, exceto pelas limitações da sua própria mente''

(W. Clement Stone)

RESUMO

AVALIAÇÃO CLÍNICA E HISTOLÓGICA DE OSTEONECROSE MANDIBULAR INDUZIDA POR AGENTES MODIFICADORES ÓSSEOS EM RATOS WISTAR

AUTORA: Luisa Berlato Silva
ORIENTADORA: Profª Drª Cristiane Cademartori Danesi
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A osteonecrose é uma patologia óssea, com etiopatogenia ainda desconhecida, que foi relatada pela primeira vez em 2003 por Marx. Em 2014 a *Association of Oral and Maxillofacial Surgeons* (AAOMS) descreveu o conceito da osteonecrose, como sendo uma área de exposição óssea na maxila ou mandíbula que não repara em oito semanas devido a uma perda temporária ou permanente de suprimento sanguíneo no local. Acomete, na maioria dos casos, pacientes que utilizam, cronicamente, medicamentos antirreabsortivos e alguns fatores associados como corticoterapia, diabetes mellitus, exodontia e demais procedimentos odontológicos invasivos. Trabalhos recentes investigam em ratos a osteonecrose induzida por esses medicamentos como o alendronato (AL), ácido zoledrônico (Z) e o denosumab (Dmab), a fim de, esclarecer a etiopatogenia, assim como, estudar meios de prevenção e tratamento da doença. o objetivo deste estudo foi comparar as drogas antirreabsortivas em um modelo animal em ratos de MRONJ, utilizando três diferentes fármacos antirreabsortivos: alendronato, ácido zoledrônico e denosumab, uma para cada grupo experimental, baseado nos parâmetros clínicos e histológicos da região submetida à exodontia. Os parâmetros clínicos avaliados foram: área de exposição óssea, fístula, má cicatrização de tecido mole e inflamação no alvéolo. Já os parâmetros histológicos compreenderam a análise de necrose óssea, infiltrado inflamatório (análise qualitativa e quantitativa), vasos sanguíneos (análise quantitativa), sequestro ósseo e resto radicular. Foram 35 ratos machos Wistar, randomizados em 6 grupos: Grupo Controle Negativo (CN) terapia com solução salina fisiológica: GNZ (n=6), GNAL (n=6), GNDmab (n=5); Grupo Alendronato (GAL) terapia com AL (n=6); Grupo Zometa (GZ) terapia com Z (n=6); Grupo Denosumab (GDmab) terapia com Dmab (n=6). A dose aplicada em cada animal será de acordo com seu peso semanal, seguindo a relação: GAL 1 mg/Kg – subcutânea, GZ 0,06mg/kg- intraperitoneal e GDmab 0,25mg/kg – intraperitoneal. O grupo GAL foi submetido a 8 aplicações de AL por um período de 8 semanas (1 aplicação semanal), ao completar a oitava semana os ratos foram submetidos a extração dentária. E após 28 dias da extração dentária foram eutanasiados, juntamente com 6 ratos do grupo GNAL. A medicação foi mantida, 1 vez na semana, até a eutanásia (décima terceira semana) para o grupo GAL. O grupo GZ recebeu o Z quatro vezes por semana, por quatro semanas, quando completou a quarta semana foi realizado a extração dentária, posteriormente foi aguardado o período de 28 dias para a realização da eutanásia dos animais do respectivo grupo e mais 6 do grupo GNZ. O grupo GDmab recebeu um total de 8 aplicações de Dmab, por um período de 4 semanas (2 aplicações semanais), quando completou a quarta semana os animais do grupo foram submetidos a extração dentária e depois de 28 dias foram eutanasiados, juntamente com os 6 ratos restantes do grupo GNDmab. A análise estatística para as variáveis qualitativas foi utilizada o teste exato de Fisher, e para a análise das variáveis quantitativas foi utilizado One-way ANOVA com post hoc de Tukey. Considerando um nível de significância de 0,05. Nossos resultados demonstraram uma maior prevalência de osteonecrose histológica no grupo nos BF's quando comparado ao grupo do Denosumab, bem como a diminuição do número de vasos foi mais frequente nos grupos do BFs. Com isso, concluímos que, pela diminuição da angiogênese e pelo aumento da MRONJ no tecido ósseo, os bisfosfonatos possuem uma maior alteração no mecanismo ósseo.

Palavras-chave: Bisfosfonatos. Exodontia. Denosumab.

ABSTRACT

CLINICAL AND HISTOLOGICAL E EVALUATION OF MANDIBULAR OSTEONECROSIS INDUCED BY BONE MODIFYING AGENTS IN WISTAR RATS

AUTHOR: Luisa Berlato Silva
ADVISOR: Profª Drª Cristiane Cademartori Danesi
CO-ADVISER: Profª Drª Kivia Linhares Ferrazzo

Osteonecrosis is a bone pathology, with a still unknown etiopathogenesis, which was first reported in 2003 by Marx. In 2014 the Association of Oral and Maxillofacial Surgeons (AAOMS) described the concept of osteonecrosis, as an area of bone exposure in the jaw or jaw that does not repair in eight weeks due to a temporary or permanent loss of blood supply at the site. It affects, in most cases, patients who chronically use antiresorptive drugs and some associated factors such as corticosteroid therapy, diabetes mellitus, tooth extraction and other invasive dental procedures. Recent work investigates in rats the osteonecrosis induced by these drugs, such as alendronate (AL), zoledronic acid (Z) and denosumab (Dmab), in order to clarify the etiopathogenesis, as well as to study means of prevention and treatment of the disease. the aim of this study was to compare antiresorptive drugs in an animal model in MRONJ rats, using three different antiresorptive drugs: alendronate, zoledronic acid and denosumab, one for each experimental group, based on the clinical and histological parameters of the region undergoing extraction. The clinical parameters evaluated were: area of bone exposure, fistula, poor healing of soft tissue and inflammation in the alveolus. The histological parameters included the analysis of bone necrosis, inflammatory infiltrate (qualitative and quantitative analysis), blood vessels (quantitative analysis), bone sequestration and root rest. There were 35 male Wistar rats, randomized into 6 groups: Negative Control Group (CN) physiological saline therapy: GNZ (n = 6), GNAL (n = 6), GNDmab (n = 5); Alendronate Group (GAL) therapy with AL (n = 6); Zometa Group (GZ) Z therapy (n = 6); Denosumab Group (GDmab) Dmab therapy (n = 6). The dose applied to each animal will be according to its weekly weight, following the ratio: GAL 1 mg / Kg - subcutaneous, GZ 0.06mg / kg-intraperitoneal and GDmab 0.25mg / kg - intraperitoneal. The GAL group was submitted to 8 applications of LA for a period of 8 weeks (1 weekly application), when completing the eighth week the rats were submitted to tooth extraction. And after 28 days of tooth extraction, they were euthanized, along with 6 rats from the GNAL group. The medication was maintained, once a week, until euthanasia (thirteenth week) for the GAL group. The GZ group received Z four times a week, for four weeks, when the fourth week was completed, tooth extraction was performed, after which the 28-day period was awaited for the euthanasia of the animals in the respective group and another 6 in the GNZ group. The GDmab group received a total of 8 applications of Dmab, for a period of 4 weeks (2 weekly applications), when the fourth week was completed, the animals in the group underwent dental extraction and after 28 days were euthanized, together with the 6 remaining rats from the GNDmab group. The statistical analysis for qualitative variables was used Fisher's exact test, and for the analysis of quantitative variables, One-way ANOVA with Tukey's post hoc was used. Considering a significance level of 0.05. Our results demonstrated a higher prevalence of histological osteonecrosis in the group in the BFs when compared to the Denosumab group, as well as the decrease in the number of vessels was more frequent in the groups of the BFs. Thus, we conclude that, due to the decrease in angiogenesis and the increase in MRONJ in bone tissue, bisphosphonates have a greater alteration in the bone mechar

Key-words: Disphosphonates. Tooth Extraction. Denosumab

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LISTA DE ABREVIATURAS

AAOMS - American Association of Oral and Maxillofacial Surgeons; Associação americana de cirurgiões orais e maxilofaciais

AL – Alendronato

ATP - Adenosine triphosphate; Adenosina trifosfato

BFs – Bisfosfonatos

Dmab – Denosumab

E.C.T- Eva Castro Torriani

g – Grama

CN- Grupo Controle Negativo

GZ- Grupo Ácido Zolendrônico

GAL – Grupo Alendronato

GDmab – Grupo Denosumab

GZN- Grupo Ácido Zolendrônico negativo

GNDmab- Grupo Denosumab negativo

GNAL- Grupo Alendronato negativo

H_2O_2 – Peróxido de hidrogênio

IV – intravenosa

IP - intraperitoneal

L.B.S- Luisa Berlato Silva

mg/Kg – miligrama por quilograma

MRONJ – Medication related osteonecrosis of the jaw

pH – potencial hidrogeniônico

RANK-L – receptor activator for nuclear factor-Kappa B ligand

SC- subcutânea

UFSM – Universidade Federal de Santa Maria

VEGF - Vascular Endothelial Growth Factor; Fator de Crescimento Endotelial Vascular

Z – Ácido Zoledrônico

SUMÁRIO

1. INTRODUÇÃO

A osteonecrose dos maxilares tem forte associação a pacientes que utilizam fármacos antirreabsortivos como: bisfosfonatos (BFs), Denosumab (Dmab) e alguns antigiogênicos que são indicados para o tratamento de metástases ósseas, provenientes de tumores de mama, de próstata e mieloma múltiplo, pois seu efeito é sobre o metabolismo ósseo de um sistema suscetível a distúrbios circulatórios, inflamatórios, neoplásicos, metabólicos e congênitos. Além disso, são amplamente empregados no tratamento da osteoporose, em situações que determinam significativa redução de complicações como fraturas patológicas e compressão da medula espinhal
(RUGGIERO *et al.,* 2014)

O primeiro estudo a mostrar lesões maxilares em pacientes tratados com BFs foi publicado por Marx e colaboradores, chamado Biphosphonate-related osteonecrosis of the jaw (BRONJ), em 2003. A American Association of Oral and Maxillofacial Surgeons (AAOMS) definiu pela primeira vez, em 2007, que a osteonecrose dos maxilares estava associada ao uso de BFs. Contudo, na sua última Position Paper, de 2014, recomendou a alteração da designação BRONJ, para Medication-related osteonecrosis of the jaw (MRONJ), devido ao aumento do número de casos de osteonecrose dos maxilares provocados por outros fármacos para além dos BFs, como o Dmab e alguns antiangiogênicos.

Na maioria dos casos, os efeitos dos MRONJ são consistentes com um defeito na remodelação óssea ou na cicatrização de feridas, pois sendo um supressor da atividade osteoclástica, os BFs reduzem o processo de remodelação, aumentando a densidade mineral óssea, reduzindo o risco de fratura. Suas propriedades antiangiogênicas podem afetar o suprimento sanguíneo no tecido ósseo, levando a uma isquemia nos ossos maxilares, observada em pacientes que fazem uso de BFs. Assim, um trauma como por exemplo exodontias, acarretaria a dificuldade de suprimento sanguíneo local, impedindo o reparo e favorecendo o desenvolvimento de uma osteonecrose nos maxilares associada a medicamento (RUGGIERO, 2014; WOO *et al*, 2006).

Howie *et al.,* (2015) sugerem a necessidade da presença concomitante de três fatores para a formação da MRONJ, que atuariam em conjunto como agentes etiológicos da doença: o uso dos BFs; trauma local (ligado ao grau de invasividade) e a resposta óssea local, relacionada ao tipo de resposta individual (sistema imunológico e genética).

Dessa forma, segundo a AAOMS, a MRONJ é agora definida pela presença cumulativa das três seguintes condições: terapêutica atual ou prévia com agentes antirreabsortivos ou antiangiogênicos; Exposição óssea ou presença de fístula intra ou extra oral, que permita acesso ao osso, na região maxilofacial; com duração superior a oito semanas; ausência de história de radioterapia na região da cabeça e pescoço, assim como de metástases ósseas que atinjam os maxilares (RUGGIERO *et al,* 2014; ZANDI *et al*, 2016).

A fisiopatologia da MRONJ ainda está em desconscenso na literatura, contudo, quatro mecanismos essenciais são identificados: inibição da atividade osteoclástica e consequentemente da reabsorção e remodelação/*turnover* ósseos, inflamação/infecção, disfunção imune inata ou adquirida, e inibição da angiogênese. (RUGGIERO *et al,* 2014; UYANNE *et al,* 2014; YAMASHITA *et al,* 2012; DE CEUIAER *et al*, 2014). Ruggiero (2007); Lesclous e colaboradores (2009), além de Rasmusson e Abtahi (2014) relataram que a microbiota da cavidade bucal, pode potencialmente favorecer ao processo patogênico e inflamatório após a extração dentária, podendo levar a uma osteomielite, sendo, portanto, um fator que aumentaria o risco de osteonecrose nos maxilares.

As características histológicas da MRONJ exibem lacunas de osteócitos vazias com ausência de osteoblastos ao redor da matriz óssea e osteoclastos destacados, onde frequentemente, possuem uma infecção secundária associada (MARX R, et al, 2005). Em detrimento da supressão da reabsorção óssea dos osteoclastos nos ossos gnáticos, há uma inibição da angiogênese, alterando as respostas imunes inatas e adaptativas (BARROS S, et al, 2016; ZHANG Q, et al, 2015; POUBEL et al, 2017). Sua peça histológica apresenta-se como um osso necrótico, lamelar, com presença de infiltrado inflamatório agudo e/ou crônico, com linfócitos, plasma, macrófagos e neutrófilos, bactérias colônias, associado, e sua vascularização é escassa no local de necrose óssea (POUBEL et al, 2017).

Existe a necessidade de um modelo animal fundamentado, para que seja utilizado em estudos de opções terapêuticas e preventivas da osteonecrose, uma vez que, a maioria dos modelos experimentais já existentes apresentam a comparação de apenas uma classe de antireabsortivos, ou apenas bisfosfonatos ou apenas denosumab (HOWIE *et al*., 2015).

Os principais fatores que dificultam a reprodutibilidade dos modelos existentes incluem: a falta de padronização no tipo, dose e regimes de administração das drogas, uso de medicamentos associados, e o uso de diferentes espécies animais.

Nesse contexto, o objetivo deste estudo foi comparar as drogas antirreabsortivas em um modelo animal em ratos de MRONJ, utilizando três diferentes fármacos antirreabsortivos: alendronato, ácido zoledrônico e denosumab, uma para cada grupo experimental, baseado nos parâmetros clínicos e histológicos da região submetida à exodontia.

2. ARTIGO – CLINICAL AND HISTOLOGICAL E EVALUATION OF MANDIBULAR OSTEONECROSIS INDUCED BY BONE MODIFYING AGENTS IN WISTAR RATS

Os resultados inseridos nesta dissertação apresentam-se sob a forma de manuscrito, o qual se encontra aqui estruturado da mesma forma a qual foi submetido a Brazilian Oral Research, Qualis Capes (Odontologia) A2, Impact Factor™ 1,223 (Institute for Scientific Information - ISI). Os itens Materiais e Métodos, Resultados, Discussão e Referências encontram-se no próprio manuscrito.

Manuscrito submetido a Brazilian Oral Research e encontra-se sob revisão.

Clinical and histological and evaluation of mandibular osteonecrosis induced by bone modifying agents in wistar rats

Área de concentração: Patologia Oral

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Abstract

Osteonecrosis is a bone pathology, with a still unknown etiopathogenesis, which was first reported in 2003 by Marx. In 2014 the Association of Oral and Maxillofacial Surgeons (AAOMS) described the concept of osteonecrosis, as an area of bone exposure in the jaw or jaw that does not repair in eight weeks due to a temporary or permanent loss of blood supply at the site. It affects, in most cases, patients who chronically use antiresorptive drugs and some associated factors such as corticosteroid therapy, diabetes mellitus, tooth extraction and other invasive dental procedures. Recent work investigates in rats the osteonecrosis induced by these drugs, such as alendronate (AL), zoledronic acid (Z) and denosumab (Dmab), in order to clarify the etiopathogenesis, as well as to study means of prevention and treatment of the disease. the aim of this study was to compare antiresorptive drugs in an animal model in MRONJ rats, using three different antiresorptive drugs: alendronate, zoledronic acid and denosumab, one for each experimental group, based on the clinical and histological parameters of the region undergoing extraction. The clinical parameters evaluated were: area of bone exposure, fistula, poor healing of soft tissue and inflammation in the alveolus. The histological parameters included the analysis of bone necrosis, inflammatory infiltrate (qualitative and quantitative analysis), blood vessels (quantitative analysis), bone sequestration and root rest. There were 35 male Wistar rats, randomized into 6 groups: Negative Control Group (CN) physiological saline therapy: GNZ (n = 6), GNAL (n = 6), GNDmab (n = 5); Alendronate Group (GAL) therapy with AL (n = 6); Zometa Group (GZ) Z therapy (n = 6); Denosumab Group (GDmab) Dmab therapy (n = 6). The dose applied to each animal will be according to its weekly weight, following the ratio: GAL 1 mg / Kg - subcutaneous, GZ 0.06mg / kg- intraperitoneal and GDmab 0.25mg / kg - intraperitoneal. The GAL group was submitted to 8 applications of LA for a period of 8 weeks (1 weekly application), when completing the eighth week the rats were submitted to tooth extraction. And after 28 days of tooth extraction, they were euthanized, along with 6 rats from the GNAL group. The medication was maintained, once a week, until euthanasia (thirteenth week) for the GAL group. The GZ group received Z four times a week, for four weeks, when the fourth week was completed, tooth extraction was performed, after which the 28-day period was awaited for the euthanasia of the animals in the respective group and another 6 in the GNZ group. The GDmab group received a total of 8 applications of Dmab, for a period of 4 weeks (2 weekly applications), when the fourth week was completed, the animals in the group underwent dental extraction and after 28 days were euthanized, together with the 6 remaining rats from the GNDmab group. The statistical analysis for qualitative variables was used Fisher's exact test, and for the analysis of quantitative variables, One-way ANOVA with Tukey's post hoc was used. Considering a significance level of 0.05. Our results demonstrated a higher prevalence of histological osteonecrosis in the group in the BFs when compared to the Denosumab group, as well as the decrease in the number of vessels was more frequent in the groups of the BFs. Thus, we conclude that, due to the decrease in angiogenesis and the increase in MRONJ in bone tissue, bisphosphonates have a greater alteration in the bone mechanism.

Key-words: Disphosphonates. Tooth Extraction. Denosumab

INTRODUCTION

Osteonecrosis of maxillary bones (ONJ) is an adverse effect of therapy with antiresorptive drugs, and its etiology continues to be debated in the literature and can be attributed to trauma, such as tooth extractions; poor oral hygiene [1,7,12,38,39]. It is characterized by being an avascular necrosis, since the use of antiresorptive drugs ends up causing a permanent or temporary loss of the local bone blood supply, causing bone necrosis. It may manifest clinically as a bone exposed in the maxillofacial region or it may have no visible clinical signs [40,13.]

Marx and colleagues in their 2003 study, entitled Biphosphonate-related osteonecrosis of the jaw (BRONJ) [3], demonstrated for the first time that patients treated with Bisphosphonates (BFs) had bone lesions that had not been reported in the literature[4]. In 2014, the American Association of Oral and Maxillofacial Surgeons[5] (AAOMS) defined lesions that had the following items as medicated mandibular osteonecrosis: current or previous treatment with bisphosphonates or denosumab or antiangiogenic therapy; exposed area of mandibular bone or probe in intra or extra oral fistula that persisted for more than 8 weeks; no history of radiotherapy or metastatic disease in the mandible[1].

Because osteonecrosis of the jaws is an adverse effect to antiresorptive therapies, its name changes to medication-related mandibular osteonecrosis (MRONJ), and these main medications used are bisphosphonates (BFs) and Denosumab (Dmab) that are used for treatment of diseases such as Paget's disease, hypercalcemia, giant bone cell tumor and bone metastasis [2,6]. While BFs, such as Zolendronic acid and Alendronate, act on the binding of hydroxyapatite on bone surfaces, that is, they inhibit resorption and decrease bone turnover[1]. Dmab, as a human monoclonal antibody inhibiting RANKL (kappa B nuclear factor activating receptor ligand), binds to the kB receptor activator, preventing the activation of RANK (kappa nuclear factor activating receptor B) on the surface of osteoclasts and their precursors, thereby preventing the formation, function and survival of these bone cells, thus blocking bone resorption[15,19].

The pathophysiology of MRONJ is not yet fully understood, however, four essential mechanisms are identified: inhibition of osteoclastic activity and consequently bone resorption and remodeling / turnover, inflammation / infection, innate or acquired immune dysfunction, and inhibition of angiogenesis. Thus, the patient who is submitted

to a dental extraction, can potentially develop osteomyelitis due to the association of the microbiota of the oral cavity with the inflammatory process resulting from tooth extraction, and thus may develop osteonecrosis [8,10].

Studies in animal models have gained space in the scientific literature to discover the possible etiology of this complication resulting from the use of antiresorptive drugs, MRONJ. Animal models available in the literature compare different BFs and Dmab, exposing animals to possible causes of osteonecrosis, such as: tooth extraction, over dosage of antiresorptive medication, presence of dental biofilm and periodontal diseases[14]. However, it is already reported in the literature that yes, osteonecrosis present in patients using BFs has different conditions than patients using Dmab, this is taken into account due to its completely different aspects of imaging [32].

In this context, the objective of this study was to compare antiresorptive drugs in an animal model in MRONJ rats, using three different antiresorptive drugs: alendronate, zoledronic acid and denosumab, one for each experimental group, based on the clinical and histological parameters of the region submitted to the tooth extraction.

MATERIALS AND METHODS

Study design and ethical aspects

The research methodology is based on the animal model, with in vivo experiments using Wistar rats, being a controlled, blind and randomized study. The animals were kept and handled in accordance with the Ethical Principles in Animal Experimentation, so the animals were subjected to an adaptation period at the UFSM vivarium, so as not to obtain animal stress bias. After the drug application process started.

The procedures were performed according to the rules established by the Guidelines for Ethical Care of Experimental Animals, approved by the International Animal Care and Use Committee and this study was approved by the Ethics Committee of the University of Santa Maria (CEUA) with the protocol 0967260318 (Annex A) .

Animal Model

In the present study, 36 heterogeneous rats (Rattus norvegicus) from the Wistar colony, male, with three months of age, 200g in weight, from the Central Vivarium of the Federal University of Santa Maria (UFSM) were used.

They were initially housed in 9 polypropylene cages (49cmx34cmx16cm) lined with wood shavings (Pinus Eliottii), with 4 animals per cage, located in the Experimental Vivarium of the Federal University of Santa Maria, Building 20. The cages were kept in a ventilated shelf with a filter Hepa, with microenvironment of 22 ± 2 ° C, air humidity at 60%, 100 air changes / hour, 12h light-dark cycle (6h-18h). The animals had commercial rodent feed, presented in pellets and drinking water, both ad libitum. For the environmental enrichment of the species, cardboard paper tubes were made available. After the 7-day adaptation period, the animals were randomly assigned to four experimental groups, which occupied 12 cages with 3 animals each.

Experimental Groups

They were divided into three positive control groups, Zolendronic Acid Group (GZ) (n = 6), Alendronate Group (GAL) (n = 6) and Denosumab Group (GDmab) (n = 6) and 3 negative control groups corresponding to each drug used in positive controls, Negative Zolendronic Acid Group (GNZ) (n = 6), Negative Alendronate Group (GNAL) (n = 6) and Denosumab Negative Group (GNDmab) (n = 6), totaling n = 18.

In the animals belonging to the negative control group (CN), physiological saline solution (0.9% NaCl - IP) was administered in the same period as their counterparts in the other groups, who received antiresorptive drugs (Figure 1).

As a result, due to the unequal completion of the dosage of the listed drugs, each sample group and corresponding control, had different moments to be euthanized, due to the changes related to the development of the animal itself, over time, the control groups must be rigorously similar in body development.

Randomization

Initially, the animals were randomly numbered between 1 and 36. For the formation of treatment groups, the initial sequence was subjected to randomization, generated by the computer program (Random Allocation Software, version 1.0, May 2004), from the new sequences , 6 experimental groups were set up: GNZ, GNAL, GNDmab (n = 18), (GAL) (n = 6), (GZ) (n = 6) and (GDmab) (n = 6).

Analgesia Protocol

The analgesia protocol was performed, before extraction procedures, and in cases where signs consistent with pain in the species were observed. Analgesia was provided with administration of tramadol hydrochloride (Tramal, 2mL ampoule, União Química Farmacêutica Nacional S / A, Embu Guaçu, SP, Brazil) (DCB 08806), dose of 20 mg / Kg - IP [15.41]

Anesthesia Protocol

Two anesthetic protocols were applied in the study. The first protocol was previously applied to surgical procedures. The animals were anesthetized with the association of Ketamine 10% (Cetamin, Syntec do Brasil Ltda, Cotia, SP, Brazil) (DCB 01936), at a dosage of 70mg / Kg and Xylazine Hydrochloride 2% (Xilazin, Syntec do Brasil Ltda, Cotia, SP, Brazil) (DCB 09208), at a dosage of 6mg / kg, both IP 16 (with changes regarding the administration of the drugs). The second anesthesia protocol used isoflurane (Isofluorane Biochimico, Itatiaia, RJ. Brazil) (DCB 05082) as an inhalation agent[17], made available to the patient both in cotton swabs present in a glass hood and in a face mask, at ambient pressure. There was no direct contact between isoflurane and the animal. This protocol preceded the collection of intracardiac blood.

Administration protocol for Alendronate (GAL)

Alendronate (AL) solutions (Galena Química e Farmacêutica, Campinas, SP, Brazil) (DCB 00097) were prepared by dissolving the monosodium salt of alendronic acid (ALCON, São Paulo, SP, Brazil) in physiological saline solution (0.9% NaCl) at the appropriate concentrations to obtain the corresponding doses 1mg / kg SC[18]. After checking the body weight of the rats, 12 doses of alendronate (1 mg / kg - SC) were administered, with an interval of 7 days between them. The first dose was applied at the beginning of the experiment and the twelfth, at the end of 12 weeks. After the application of the eighth dose, an interval of 7 days was followed, which resulted in extraction. Thereafter, the treatment continued for another 28 days, until euthanasia. The ninth dose was applied 7 days after extraction[18].

Administration protocol for Zoledronic Acid (GZ)

After weighing the animals in the zoledronic acid group (Zometa, Novartis Pharma, Basel, Switzerland) (DCB 00379), zolendronic acid was administered at a dose of 0.06mg / kg - IP with insulin syringe once a week[19]. The first application occurred at the beginning of the experiment, with an interval of 7 days between doses. The extraction was performed at 21 days of the experiment. Thereafter, the treatment continued for another 28 days, until euthanasia[19].

Denosumab administration protocol (GDmab)

Animals in the Denosumab group (GlaxoSmithKline, London) (DCB 09825) were administered 0.25mg / kg - IP of the drug, with an insulin syringe totaling 8 doses, with an interval of 4 days between them [20]. The extraction of teeth occurred 4 days after administration of the eighth dose. From then on, a period of 28 days was observed, until euthanasia[20]. The animals were weighed to calculate dosages before the drug was administered. In (ANNEX B) the outline of the experimental protocol is present.

Exodontia

The extraction of the first right lower molars was performed by the same operator who was blinded to the experimental groups, using the same surgical technique in all animals. Initially, the animals were stabilized in a dorsal position for extraction of the lower first molars, using dissociative anesthesia injectable (xylazine - 6 mg / kg associated with ketamine - 70 mg / kg-IP). Then, with the aid of an explorer probe, the gingival

tissue syndesmotomy was performed, running the probe tip around the tooth in question. After this detachment, the teeth were dislocated in mesio-distal and cervical-apical directions, being then divided into two segments with the aid of a Hollemback spatula positioned in the furcation region. The segments were then extracted using a hemostat18. After the surgical procedure, all animals received an intramuscular dose of antibiotic (Pentabiótico®, Wyeth-Whitehall Ltda, São Paulo, Brazil, 0.1 mg / Kg) and was also materialized with the preventive use of tramadol hydrochloride[41] (20 mg / kg), an opioid drug effective in the treatment of moderate to severe pain, that is, analgesia was preventive and not just responsive.

Operator blinding

A single blind operator (E.C.T) performed the extraction, the same received the animals for the surgical procedure.

Euthanasia

After 28 days of extraction in the respective treatments and after the collection of the intracardiac blood aliquot, all animals were euthanized by total exsanguination [21], which was used for other evaluations relevant to the study. Euthanasia resulted from the complete extraction of blood from the cardiac chambers (total exsanguination). For that, the animal was anesthetized in a glass bell, which had cotton pads soaked in isoflurane (inhalation anesthesia), which were separated from direct contact with the animal, using a screened plastic screen. After verifying the anesthetic plan (foot reflex test), the animal was placed in the supine position and had the support of inhalation anesthesia maintained by means of a face mask. Subcutaneous lidocaine hydrochloride (local anesthesia - 7 mg / kg) (DCB 05314) was applied to the incision line of the laparotomy procedure (line alba), distributed in subcutaneous buttons from the beginning of the scalpel blade insertion point (region abdominal), up to; the region of the sternal manubrium (chest region), the limit of the incision enlargement to access the heart.

After the procedure was finished, the carcasses were collected in specific bags for biological remains, and kept frozen until collected by a specialized firm (Stericycle).

Drug application

Alendronate resulted in a total of 12 applications, one dose per week, while zolendronic acid resulted in 7 doses, one per week and lastly, denosumab totaled 8 doses, 2 doses per week. Regarding their negative controls, they followed the dosages corresponding to each drug (Figure 2).

Macroscopic Analysis

The oral cavity was examined and the presence of lesion and exposed bone was observed in the entire cavity and in the region of the lower right first molar, which was a dental element listed for extraction. The criteria for clinical evaluation were: the presence or absence of soft tissue healing, bone exposure, purulent secretion and intra and / or extraoral fistula [18].

Laboratory Procedures

The mandibles were first fixed and later demineralized in a 10% EDTA solution (Química Moderna Ind. E Com. LTDA, São Paulo, SP, Brazil) buffered with sodium hydroxide (pH 7.4) (Química Moderna Ind. and Com. LTDA, São Paulo, SP, Brazil) for 8 weeks 16, until they are considered suitable for histological processing by the needle penetration test.

The samples were fixed, diaphanized, clarified and paraffinized in the tissue processor (PT-05, Lupetec® applied technology, Lupe Indústria Tecnológica de Equipamentos para Laboratório LTDA, São Carlos, SP, Brazil) and, afterwards, included in paraffin in a standardized way, by the Laboratory of Pathology and Oral Biology (LAPBIO) in the pathology department of UFSM. With the aid of a microtome (MH-455Automatic Microtome®, Leipzig Instruments Co. Ltd., DMI Científica do Brasil LTDA, São Paulo), two slides were obtained for each animal, sections of 4 μm were obtained in the vestibule-lingual direction. After assembling the slides, they were placed in an oven at 60ºC for 24 hours to remove excess paraffin and subjected to histological staining.

The histological analyzes were performed by a blinded and calibrated observer, which was measured three times by each examiner on different days, in order to reduce the variation of the data, the intra and inter-examiner Kappa coefficient (0.75) was calculated. All images were obtained through an image analysis system (Axiovision, Carl Zeiss MicroImagnig, Jena, Germany), captured with a digital camera coupled to the light

microscope (AxioStar PluSS, Carl Zeiss) and viewed with the aid of a computer with processor (Pentium 4, with 3.00 GHz, 512Mb of RAM - Microsoft Windows XP Operating System - LG monitor FLATRONezT710SH, 64M, 17-inch color), associated with a binocular optical microscope (OLYMPUS, model BX51 / BX52), with camera video (OLYMPUS, model OLY-200) attached.

To capture the images, 4 fields of each slide were chosen, in a standardized way, which covered the area of tooth extraction, with two upper and two lower fields and all images were saved in TIFF format (True Image Format File) [22.18].

Histological analysis

Analysis of Inflammatory Infiltrate

The quantitative analysis of the inflammatory infiltrate was performed based on scores, being 0 (absent; 0%), 1 (mild; ≤10%), 2 (moderate;> 10 and ≤50%) and 3 (increased;> 50%) in a 100% increase [18], in a 400% increase the quality of the inflammatory process (acute, chronic or mixed) [18] was analyzed.

Blood Vessel Count

Quantitative assessment of blood vessels was performed on each slide connective tissue by counting individual capillaries in three distinct fields (60x60µm2) at 400x 23 magnification, the tissue of this analysis was the soft tissue surrounding the alveolus of the first right lower molar, which was placed in an eppendorf for further analysis. The selection of fields for analysis was standardized in an order from left to right, and from top (limit of connective tissue with epithelium) to bottom.

All capillaries with visible lumen and endothelium were included, with or without red blood cells [24,25]. For counting, the software ZEN 2012 (Blue Edition) was used, installed on a computer with an Intel® Core i5 processor model OPTIPLEX 9010, with 3.40 GHz, 8.00 GB of RAM - Microsoft Windows Pro 8 Operating System - DELL Monitor model U2312HM, 23 " LED LCD, associated with a ZEISS binocular optical microscope, model Axio Lab.A1, with video camera AxioCam, model ERc 5S, coupled for the acquisition of microscopic images. All vessel analyzes were performed by two trained examiners (L.B.S and E.C.T), calibrated and blinded to the experimental groups.

Measurement of histological variables

Regarding the quantitative analysis of bone necrosis, it was considered by the presence of eight contiguous empty gaps (without osteocyte) in the bone adjacent to the alveolus, which was performed by tooth extraction, in a 200x increase, and the results were expressed in percentage [19]. The variables, root debris and bone sequestration were evaluated for their absence or presence in the studied cuts and the data expressed as a percentage.

Statistical analysis

Data analysis was performed using STATA 14 (StataCorp. 2014. Stata Statistical Software: version 14.1. College Station, TX: StataCorp LP). Six outcomes were considered: 1) presence of necrosis (no or yes); 2) inflammatory infiltrate - quantitative (absent / mild / moderate / increased); 3) inflammatory infiltrate - qualitative (absent / acute / chronic); 4) bone sequestration (no or yes); 5) presence of root debris (no or yes); and 6) mean blood vessels. For qualitative results, the comparison between groups was performed using Fisher's exact test. For quantitative variables, the One-way ANOVA test with Tukey's post hoc was performed. A significance level of 0.05 was considered

RESULTS

Clinical analysis

According to our clinical analysis, we did not obtain samples showing exposed bone, nor any clinical signs.

Histological analysis

Bone necrosis.

Regarding bone necrosis (Figure 3), when comparing the group Zolendronic Acid (GZ) with Alendronate (GAL), all samples presented bone necrosis, where there was no statistically significant difference ($p < 0.99$). The same occurred when comparing the Zolendronic Acid (GZ) group with Denosumab (GDmab), there was also no statistically significant difference ($p < 0.09$). In the GDmab group, only half of the sample presented necrosis (Table 1).

When comparing the GZ group with GNZ, there was a statistically significant difference ($p < 0.01$), since all samples in the GZ group had bone necrosis, whereas in their negative control group (GNZ) the samples did not present bone necrosis. The same occurs when the comparison is made between the groups of the drug Alendronate, GAL and GNAL, where there was a statistically significant difference between them ($p < 0.01$). The comparison of the GDmab and GNDmab groups did not show any statistically significant difference between their groups, as only half of the sample in the GDmab group obtained bone necrosis, whereas in the GNDmab group, no animal presented bone necrosis ($p < 0.06$).

Inflammatory infiltrate

Quantitative analysis

In the quantitative analysis of the inflammatory infiltrate (Figure 4) where the scores are classified as absent, mild, moderate and increased, the comparison between the groups did not result in a statistically significant difference (Table 2). The comparison of the GAL and GDmab groups did not show any statistically significant difference ($p > 0.2$), however, in the GAL, its slide showed mild inflammatory infiltrate, whereas in the GDmab group, most samples did not present inflammatory infiltrate. The comparison between the GAL and GNAL groups was not statistically significant ($p > 0.2$), where the

most samples obtained mild inflammatory infiltrate compared to GNAL samples, which did not obtain inflammatory infiltrate.

Qualitative analysis

According to the qualitative analysis, which consists of absent, acute or chronic inflammatory infiltrate, the comparison of the groups in this study did not show a statistically significant difference (Table 3). The comparison of the GZ and GDmab groups ($p > 0.2$) where, respectively, presented 3 samples with quality of acute inflammatory infiltrate while the other group resulted in the absence of inflammatory infiltrate. In the comparison between the GAL and GDmab groups ($p > 0.1$), GAL presented 2 samples in each quality of inflammatory infiltrate, while GDmab obtained absence of infiltrate in most samples.

Bone sequestration

The comparison of groups (Table 4), GZ with GAL ($p > 0.2$), GZ with GDmab ($p > 0.2$) and GZ with GNZ ($p > 0.2$), where GZ obtained bone sequestration (Figure 5) in his histological slides, GAL, GDmab and GNZ did not obtain bone sequestration in their samples, however, no analysis with statistically significant results.

Root Rest

According to the measurement of this variable, we did not obtain a statistically significant difference (Table 5). Groups GZ and GAL ($p > 0.2$), where the first contained total absence of root rest on the slide and the second obtained two samples with the presence of this category. GAL with GDmab ($p > 0.2$) where the first group presented 4 samples with the presence of root rest, while the other obtained total absence. And finally, comparison of the GAL with GNAL ($p > 0.2$), where the first presented 4 positive samples to the remaining root present in the slide while its comparative group, resulted in total absence of the variant. Thus, this variable also did not present statistically significant results.

Blood vessels

In the blood vessel count (Table 6), there was a statistically significant difference between the GZ and GDmab groups ($p < 0.05$), with the GZ group having a smaller

number of vessels than the GDmab. In the other groups, there was no statistically significant difference.

DISCUSSION

The results of the study suggest that the bisphosphonate group was able to induce histological MRONJ, as well as a reduction in the amount of blood vessels, demonstrating that MRONJ may be present, even subclinically, depending on the stage 0 presented by Ruggiero and collaborators in 2014 [1.]

This finding can be justified by the mechanism of action of the BFs being more aggressive when compared to Denosumab. Because the BFs increase the affinity with hydroxyapatite and consequently they adhere to bone tissue, resulting in apoptosis of mature osteoclasts and angiogenic alteration, thereby preventing bone resorption. The Denosumab, on the other hand, act on the bone cascade, not severely altering resorption, resulting in minimal angiogenic alteration [31].

The inhibitory effects of BFs on angiogenesis and endothelial cell activity have been frequently reported in the literature. The increase in gene expression of vascular endothelial growth factor (VEGF) is directly influenced by inflammation mediators present mainly after extractions, where there is a delay in healing, thus developing MRONJ [29,26.] The cause of this increase in VEGF can be justified by the presence of cellular hypoxia in bone tissue, which becomes an important regulator in angiogenesis, so in bone tissue with less vascularization, we have a stimulus to increase VEGF 30. While the Dmab, act on the bone mechanism, not significantly altering the resorption 31. In this study, there was a smaller amount of blood vessels in the BF group, while the Dmab group did not show any significant change. Thus, the number of vessels present in the lamina expresses the irrigation of that tissue, the smaller the number of vessels, the greater the risk of necrosis at the site[27].

The decrease in the amount of blood vessels is directly related to inflammatory mediators, as is the case of neutrophils, which are the main cells found in the acute inflammatory infiltrate and responsible for the first defense of the organism. In bone tissues submitted to Dmab, we did not obtain a statistically significant difference regarding the quality and quantity of this infiltrate, which is in line with the results presented in the literature[20]. This can be justified by the fact that by its mechanism of action, the suppression of the RANKL molecule is stopped by altering function and differentiation of osteoclasts. The group of BFs, on the other hand, obtained a prevalence

of acute inflammatory infiltrate, but in another study there was no statistically significant difference in the inflammatory infiltrate[18], which can be explained by the blockage of the signaling pathway that BFs perform in the signaling pathway of marrow cells[28].

Regarding the clinical appearance of these necrotic bone lesions, in our study it was not present, as well as other studies that compare only BFs [22,33], which can be justified by the presence of subclinical jaw osteonecrosis, as reported[1], where there is no bone necrosis exposure, with symptoms such as ulcerations, extra-oral fistula and radiographic changes[9]. Clinically visible MRONJ is reported in the literature in studies that compare both BFs associated with corticosteroids, as well as the comparison of 2 BFs or even BFs with Dmab 18,27,34, and the presence of clinical signs can be justified by the established dosage, trauma list and time of submission of samples to treatment.

Among the risk factors that may favor the appearance of MRONJ, they are summarized in three questions: local risk, pathology and the type of medication used [35].Therefore, it is already known that both BFs and Dmab are potential drugs for inducing MRONJ , in relation to local risk, it is already known that both dental infections and periodontal diseases are collaborative for the development of osteonecrosis of the jaws, as they end up increasing acidity in the area of infection leading to the suppression of healing mechanisms, which may result in necrosis[36], as well as tooth extraction, which is the most common factor for MRONJ development [37].

Variants such as: bone sequestration and root debris, we obtained positive results in the groups that contained BFs, although not statistically significant. However, these are extremely important outcomes for the occurrence of MRONJ, as they are potential local risks that cause secondary infections, which can be further addressed in future scientific research covering this topic.

The results of this study demonstrated that groups submitted to BFs obtained a smaller amount of blood vessels and a higher prevalence of MRONJ. However, visible clinical signs of necrotic bone lesions were not positive in the samples. Therefore, the etiopathogenesis of this necrotic bone lesion has not yet been defined, there is a need for further studies with animal models to remedy this problem in order to assist in clinical practice in a satisfactory manner.

CONCLUSION

Osteonecrosis of the jaws (MRONJ) has an unknown etiopathogenesis, requiring animal models to help define this problem. In order to collaborate with this problem, in this study it was evidenced that anti-resorptives of the bisphosphonate class (Alendronate Sodium and Zolendronic Acid) result in a greater number of samples with MRONJ and with a smaller amount of blood vessels, when compared to another class of antiresorptive agents, Denosumab (Prolia). Thus, we conclude that, due to the decrease in angiogenesis and the increase in MRONJ in bone tissue, bisphosphonates have a greater alteration in the bone mechanism.

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REFERENCES

[1] S. Ruggiero, T. Dodson, J. Fantasia, R. Goodday, et al. Medication-Related Osteonecrosis of the Jaw - 2014 Update. American Association of Oral and Maxillofacial Surgeons. (2014). Doi: 10.1016/j.joms.2014.04.031.

[2] G. Ficarra, F. Beninati. Bisphosphonate-related Osteonecrosis of the Jaws: An Update on Clinical, Pathological and Management Aspects. Head Neck Pathology. 2 (2007). Doi: 10.1007/s12105-007-0033-2

[3] R. Marx. Pamidronate (Aredia®) and zoledronate (Zometa®) induced avascular necrosis of the jaws: a growing epidemic. Journal of Oral and Maxillofacial Surgery. 61 (2003). Doi: 10.1016 / s0278-2391

[4] S. Kuhl, C. Walter, S.Acham, R. Pfeffer, J.T. Lambrecht. Bisphosphonate-related osteonecrosis of the jaws – A review. Oral Oncology. 48 (2012) 938-947. Doi: 10.1016/j.oraloncology.2012.03.028

[5] AAOMS, American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaws. Journal Oral Maxillofacial Surgery. 65 (2007) 369-376. Doi: 10.1016/j.joms.2006.11.003

[6] O.N. Galatis, M. Schiedt, R.A. Mendes, L.D. Coates, *et al*. Medication-related osteonecrosis of the jaw: definition and best practice for prevention, diagnosis, and treatment. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 127 (2019). Doi: 10.1016/j.oooo.2018.09.008.

[7] S. Ruggiero, S Drew. Osteonecrosis of the jaws and bisphosphonate therapy. Journal Dental Research. 86 (2007) 1013-1021. Doi: 10.1177/154405910708601101

[8] J. Uyanne, C. C. Calhoun, A. D. Le Antiresorptive drug-related osteonecrosis of the jaw. Dental Clinica North American. 58 (2014) 369-384. Doi: 10.1016/j.cden.2013.12.006.

[9] N. A AlDhalaan, A. BaQais, A. Al-Omar. Medication-related Osteonecrosis of the Jaw: A Review. Cureus. 12 (2020). Doi: 10.7759/cureus.6944.

[10] J. De Ceulaer, E. Tacconelli, S.J Vandecasteele. Actinomyces osteomyelitis in bisphosphonate- related osteonecrosis os the jaw (BRONJ): the missing link? Europe Journal Clinical Microbiology Infect Dis. 33 (2014) 1873-1880. Doi: 10.100^{7}/s10096-014-2160-5

[11] P. Lesclous, S. Abi Najm, J. Carrel, B Baroukh,. et al. Bisphosphonate-associated osteonecrosis of the jaw: a key role of inflammation? Bone. 45 (2009) 843-852. Doi: 10.1016/j.bone.2009.07.011

[12] L. Rasmusson, J. Abtahi, Bisphosphonate Associated Osteonecrosis of the Jaw: An Update on Pathophysiology, Risk Factors, and Treatment. International Journal of Dentistry. (2014). Doi: 10.1155/2014/471035

[13] S. Fedele, G. Bedogni, M. Scoletta, G. Favia, G. Colella, A. Agrillo, A. Bedogni. Up to a quarter of patients with osteonecrosis of the jaw associated with antiresorptive agents remain undiagnosed. Brit. Journal Oral Maxillo Surgery. 53(2015). Doi: 10.1016/j.bjoms.2014.09.001x

[14] R. Howie, J. Borke, Z. Kurago, A. Daoudi, et al. A Model for Osteonecrosis of the Jaw with Zoledronate Treatment following Repeated Major Trauma. PLoS ONE. 10 (2015). Doi: 10.1371/journal.pone.0132520

[15] M. Rashidpour et al, Effect of Tramadol (μ-opioid receptor agonist) on orthodontic tooth movements in a rat model. Journal of Dentistry, Tehran University of Medical Sciences. 9 (2012). PMID: 23066472

[16] V. G. Garcia, et al. Treatment of experimental periodontitis in rats using repeated adjunctive antimicrobial photodynamic therapy. Lasers Medical Science. (2012). Doi: 10.1007/s10103-012-1099-y

[17] N. E. Taylor, J.J. Chemali, E.N. Brown, K. Solt, Activation of D1 dopamine receptors induces emergence from isoflurane general anesthesia. Anesthesiology. 118 (2013) 30-39. Doi: 10.1097/ALN.0b013e318278c896

[18] N.C. Neto et al. Experimental osteonecrosis: development of a model rodents administered alendronate. Brazilian Oral Research. 30 (2016). Doi: 10.1590/1807-3107-2016.vol30.0099

[19] M. Zandi, et al. Introducing a protocol to create bisphosphonate-related osteonecrosis of the jaw in rat animal model. Journal of Cranio-Maxillo-Facial Surgery. 44 (2016) 271 3 278. Doi: 10.1016/j.jcms.2015.12.010

[20] V.LD.N. Poubel, D.L Capella, A.R.S Santos, M. Correa, L.R Correa, E.R.C Rivero, Evaluation of mandibular bone after dental extraction in rats treated with antiresorptive drugs. Journal Oral Maxillofacial Surgery (2017). Doi: 10.1016/j.joms.2017.07.172

[21] A. B. Carregaro, M. B. Castro, F. S Martins, Estudo da ação inflamatória aguda do tiopental intraperitoneal em ratos. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 57 (2005) 191-195. Doi: 10.1590/S0102-09352005000200009.

[22] M.A.P. Maahs, V.P.Nora, A.A Azambuja, K. Cherubini. Bisfosfonatos e osteonecrose dos maxilares. Revista OdontoCiência. 24 (2009) 337-344. Doi:

[23] B. O. Cetinkaya, *et al*. The expression of vascular endothelial growth factor in a rat model at destruction and healing stages of periodontal disease. Journal of Periodontology. 78 (2007) 1129-1135. Doi: 10.1902/jop.2007.060397.

[24] M.A.N. Diniz. Avaliação da angiogênese, inflamação e crescimento tumoral em camundongos com deleção gênica dos receptores para o PAF (PAFR-KO). Tese (Doutorado em Fisiologia e Farmacologia) - Universidade Federal de Minas Gerais, Belo Horizonte, 2006.

[25] F. H. G. D. Silva, Avaliação da angiogênese inflamatória em camundongos induzida por antígenos da cepa Y do Trypanosoma cruzi. 75 f. Dissertação (Mestrado em Ciências Biológicas) - Universidade Federal de Ouro Preto, Minas Gerais, 2012.

[26] ADA, American Dental Association Council on Scientific Affairs. Dental management of patients receiving oral bisphosphonate therapy: expert panel recommendations. Journal American Dental Association. 137 (2006) 1144-1150. Doi: 10.14219/jada.archive.2006.0355

[27] S. Sonis, B. Watkins, G. Lyng, M. Lerman, K. Anderson, Bony changes in the jaws of rats treated with zoledronic acid and dexamethasone before dental extractions mimic bisphosphonate-related osteonecrosis in cancer patients. Oral Oncol. 45 (2009) 164-172. Doi: 10.1016/j.oraloncology.2008.04.013.

[28] S. Khosla, D. Burr, J. Cauley, D.W. Dempster, P.R. Ebeling, F. Felsenberg *et al*. Bisphosphonate-Associated Osteonecrosis of the Jaw: Report of a Task Force of the American Society for Bone and Mineral Research. Journal Bone Miner Research. 22 (2007) 1479-1491. Doi: 10.1359/jbmr.0707onj.

[29] M.Q. S. Soares. Effect os long-term zolendronic acid treatment on cancellous bone in the mandible and femur of Wistar rats. Tese Doutorado USP. Acesso em: 2019. Doi: 10.11606/T.25.2018.tde-23042018-215507

[30] G.Z. Ferreira, E.V.Z. Filho, I.R.F. Rubira-Bullen, G.P. Garlet, C.F. Santos, P. S. S. Santos. Delayed alveolar bone repair and osteonecrosis associated with Zoledronic Acid therapy in rats: macroscopic, microscopic and molecular analysis. Journal Appl Oral Science. 25 (2020) Doi: 10.1590/1678-7757-2020-0204

[31] M.V.G. Viana, M.M.M. Carvalho, P.V.Fialho *et al*. Clinical considerations on the use of do L-PRF in the medicinal therapeutics: case report. Brazilian Jounal of Health Review. 2 (2019). Doi: 10.34119/bjhrv2n4-095

[32] R. Querrer, N. Ferrare, N. Melo, C.M. Stefani *et al*. Differences between bisphosphonate- related and denosumab-related osteonecrosis of the jaws: a systematic review. Support Care Cancer. (2020). Doi: 10.1007/s00520-020-05855-6

[33] R.D. G Caminha, G. M. Chicrala, L.A.V Soares, P.S.S. Santos, Perfil de risco para osteonecrose dos maxilares associada a agentes antiangiogênicos. Einstein Journal. 17 (2019). Doi: 10.31744.

[34] S. A. Berti-Couto, A.C.U. Vasconcelos, J.E. Iglesias, M.A.Z. Figueiredo *et al*. Diabetes Melitus and corticotherapy as risk factors for alendronate – related osteonecrosis of the jaws: a study in Wistar rats. Head Neck. 36 (2014). Doi: 10.1002/hed.23260

[35] K. McGowan, T. McGowan, S. Ivanovski. Risk factors for medication-related osteonecrosis of the jaws: a systematic review. Oral Diseases. 24 (2018). Doi: 10.1111 / odi.12708.

[36] T. Shibahara, T. Tohoku. Antiresorptive Agent-Related Osteonecrosis of the Jaw (ARONJ): A Twist of Fate in the Bone. The Tohoku Journal of Experimental Medicine. 247 (2019). Doi: 10.1620/tjem.247.75.

[37] S. Kuroshima, M. Sasaki, H. Murata, T. Sawase. Medication-related osteonecrosis of the jaw-like lesions in rodents: a comprehensive systematic review and meta-analysis. Gerodontology. 36 (2019). Doi: 10.1111 / ger.12416.

[38] M. Scheper, A. Badros, R. Chaisuparat, K. Cullen, *et al*. Effect of zoledronic acid on oral fibroblasts and epithelial cells: a potential mechanism of bisphphonate-associated osteonecrosis. British Journal Haematology**.** 144 (2009). Doi: 10.1111 / j.1365-2141.2008.07504.x

[39] R. Weinstein, P. Roberson, S. Manolagas. Giant osteoclast formation and long-term oral bisphosphonate therapy. The New England Jounal of Medicine. 360 (2009). Doi: 10.1056 / NEJMoa0802633

[40] A. Fassio, F. Bertoldo, L. Idolazzi, O. Viapiana, M. Rossini, D. Gatti. Drug-induced osteonecrosis of the jaw: the state of the art. Reumatismo. 69 (2017). Doi: 10.4081/reumatismo.2017.983

[41] R.S. Fecchio; M.S. Gomes. Analgesia em roedores e lagomorfos. Tratamento da dor na Clinica de Pequenos Animais. Elselvier. 34 (2012). Doi:

Figure 1. Experimental study groups.

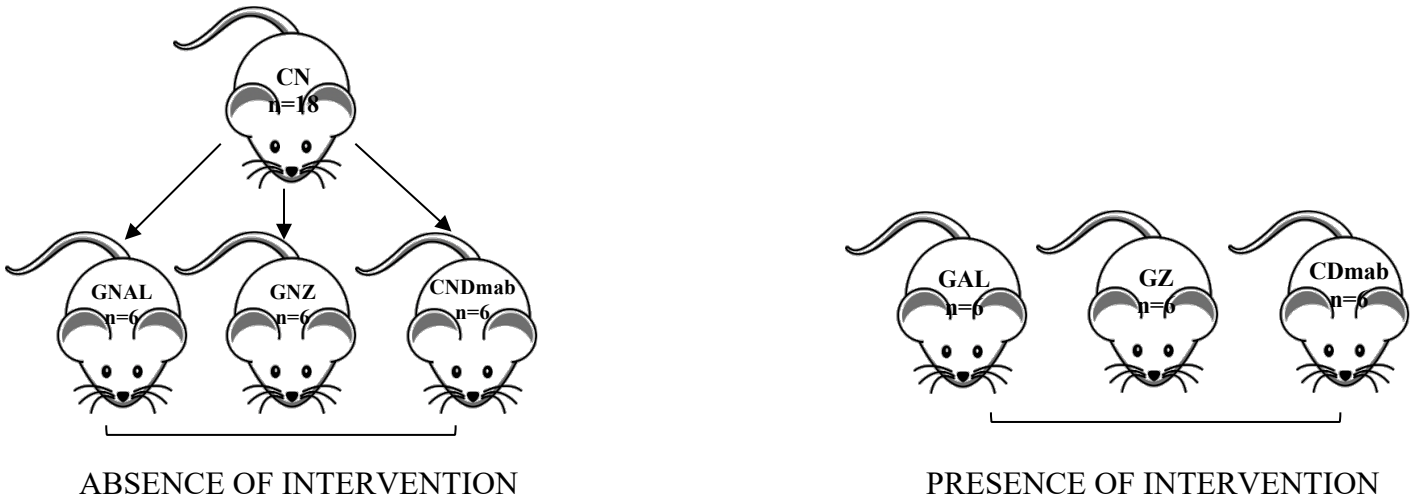


Figure 2. Experimental study design.

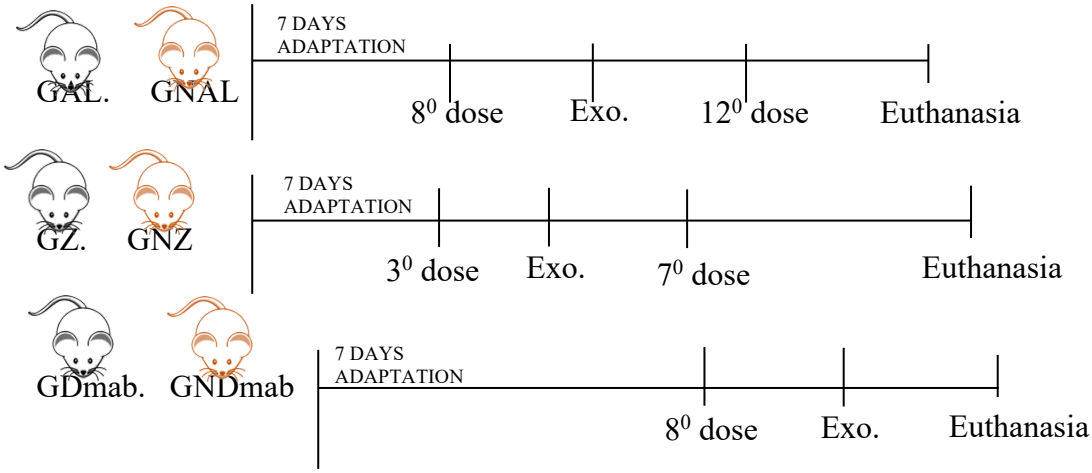
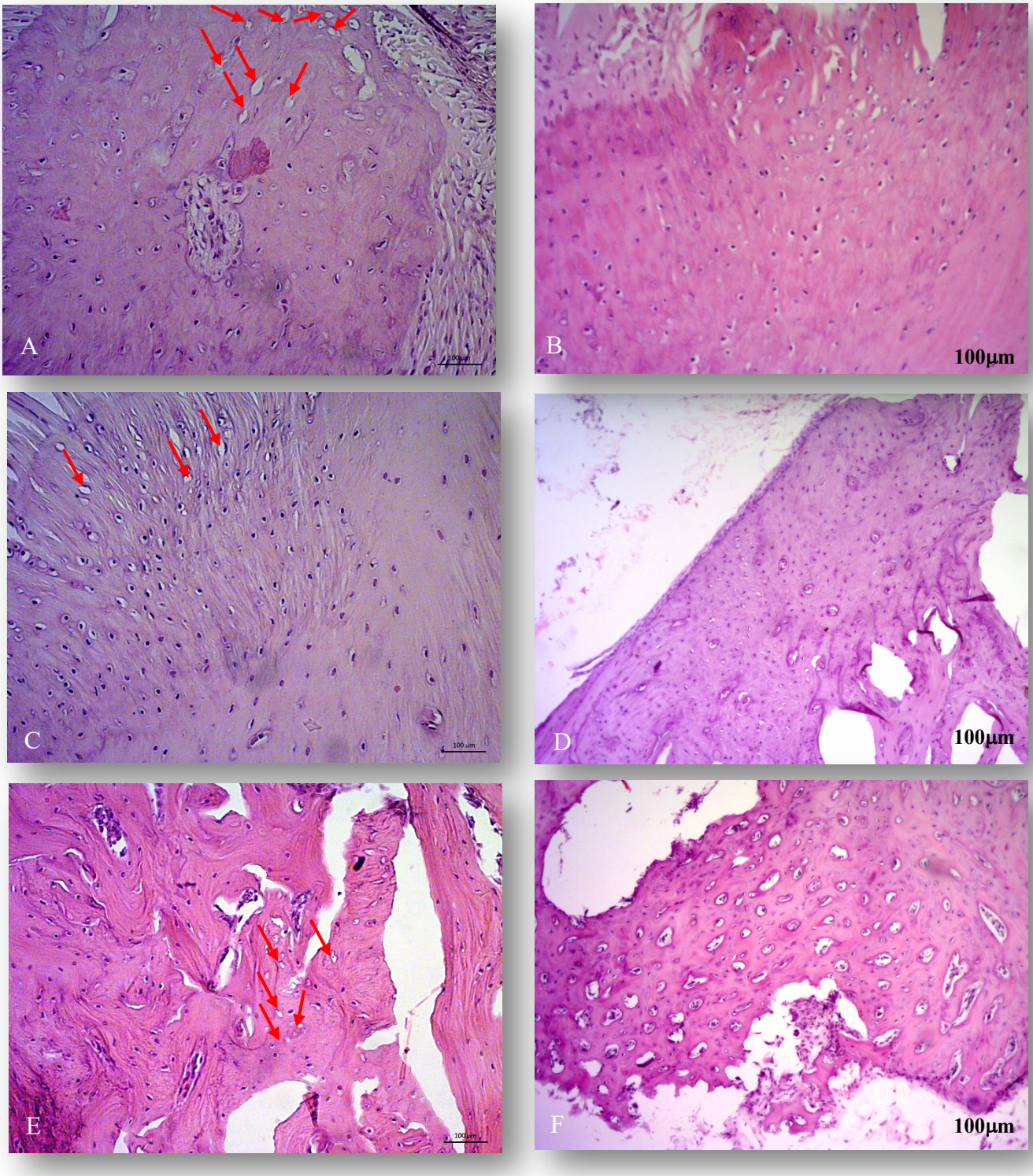
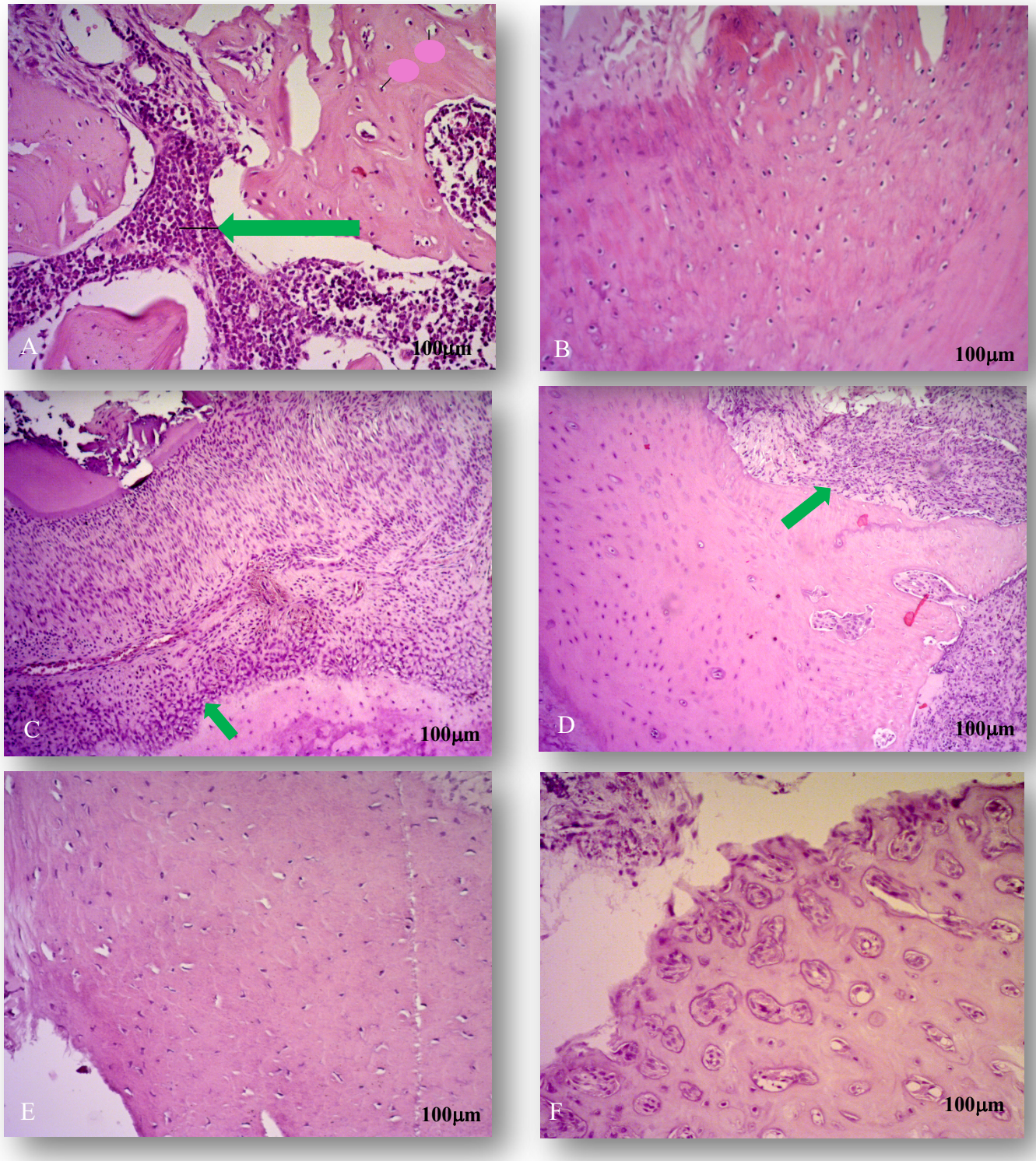


Figure 3. Influence of the use of antiresorptives on bone tissue



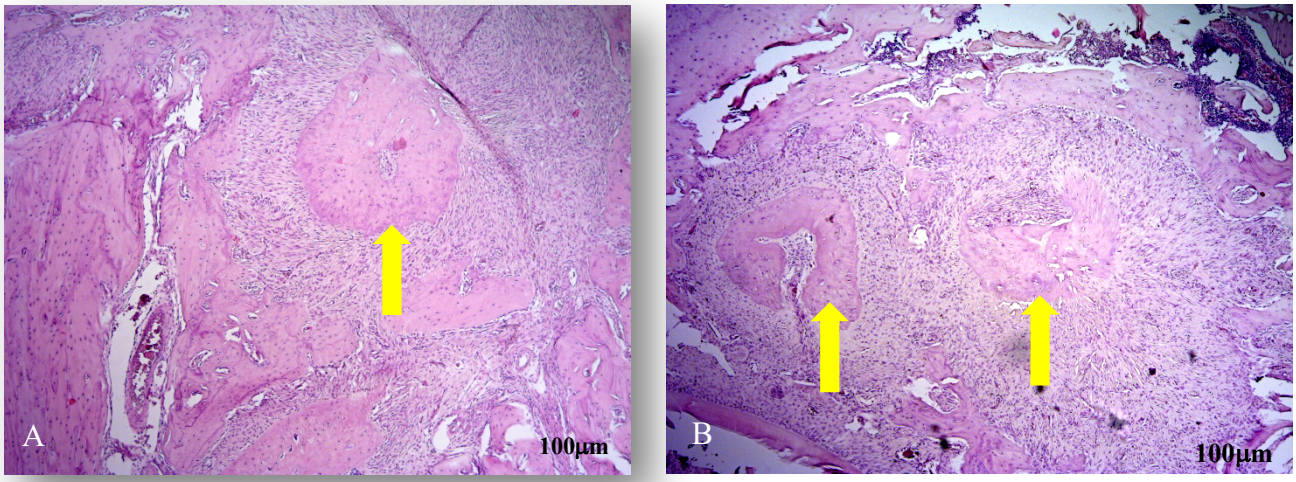
Photos of histological slides from the regions adjacent to the extraction of the first molar after euthanasia of animals. Demonstrates, at the tip of the red arrows, GZ (A) with 8 continuous gaps without the presence of osteocytes, confirming bone necrosis. GNZ (B) in a listed section, presence of osteocytes in the gaps, as well as the GNAL (D) and GNDmab (F) group, characterizing normal bone tissue. In relation to the GAL (C) it presented empty gaps, however in a reduced number, as well as GDmab (E). Hematoxylin / Eosin staining technique, 400x increase.

Figure 4. Influence of antiresorptives on inflammatory mediators



Photos of histological slides from the regions adjacent to the extraction of the first molar after euthanasia of animals. Demonstrates, at the tip of the green arrows, GZ (A) an inflammatory infiltrate of acute quality. GNZ (B) with no inflammatory infiltrate, as well as GDmab (E) and GNDmab (F). GAL (C) shows an inflammatory infiltrate in the mild quantity and in the acute quality, indicated by the green arrow; its negative control (GNAL) (D) also has an inflammatory infiltrate, but in chronic quality, indicated by the green arrow. Hematoxylin / Eosin staining technique, 400x increase.

Figure 5. Result of the use of bisphosphonates in bone tissue.



Photos of histological slides from the regions adjacent to the extraction of the first molar after euthanasia of animals. Demonstrates, at the tip of the yellow arrows, in GZ (A) and GAL (B) bone sequestration. Hematoxylin / Eosin staining technique, 200x increase.

Table 1. Comparison of the different medication groups in the occurrence of necrosis

Variables	Necrosis [n (%)]		p-value*
	No	Yes	
Groups			0.99
Zoledronic acid	0 (0.0)	6 (100.0)	
Alendronate	0 (0.0)	6 (100.0)	
Groups			0.09
Zoledronic acid	0 (0.0)	6 (100.0)	
Denosumab	3 (50.0)	3 (50.0)	
Groups			0.09
Alendronate	0 (0.0)	6 (100.0)	
Denosumab	3 (50.0)	3 (50.0)	
Groups			<0.01
Zoledronic acid	0 (0.0)	6 (100.0)	
GNZ	6 (100.0)	0 (0.0)	
Groups			<0.01
Alendronate	0 (0.0)	6 (100.0)	
GNAL	6 (100.0)	0 (0.0)	
Groups			0.06
Denosumab	3 (50.0)	3 (50.0)	
GNDmab	5 (100.0)	0 (0.0)	

*Fisher's exact test.

Table 2. Comparison of the different medication groups in the infiltrate inflammatory (quantitative evaluation)

Variables	Infiltrate inflammatory [n (%)]				p-value*
	Absent	**Mild**	**Moderate**	**Increased**	
Groups					0.75
Zoledronic acid	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)	
Alendronate	2 (33.3)	3 (50.0)	0 (0.0)	1 (16.7)	
Groups					0.31
Zoledronic acid	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)	
Denosumab	5 (83.3)	1 (16.7)	0 (0.0)	0 (0.0)	
Groups					0.24
Alendronate	2 (33.3)	3 (50.0)	0 (0.0)	1 (16.7)	
Denosumab	5 (83.3)	1 (16.7)	0 (0.0)	0 (0.0)	
Groups					0.74
Zoledronic acid	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)	
GNZ	4 (66.6)	1 (16.7)	1 (16.7)	0 (0.0)	
Groups					0.24
Alendronate	2 (33.3)	3 (50.0)	0 (0.0)	1 (16.7)	
GNAL	5 (83.3)	1 (16.7)	0 (0.0)	0 (0.0)	
Groups					0.72
Denosumab	5 (83.3)	1 (16.7)	0 (0.0)	0 (0.0)	
GNDmab	4 (80.0)	1 (20.0)	0 (0.0)	0 (0.0)	

*Fisher's exact test.

Table 3. Comparison of the different medication groups in the infiltrate inflammatory (qualitative evaluation)

Variables	Infiltrate inflammatory [n (%)]			p-value*
	Absent	Acute	Chronic	
Groups				0.76
Zoledronic acid	2 (33.3)	3 (50.3)	1 (16.7)	
Alendronate	2 (33.3)	2 (33.3)	2 (33.3)	
Groups				0.24
Zoledronic acid	2 (33.3)	3 (50.3)	1 (16.7)	
Denosumab	5 (83.3)	1 (16.7)	0 (0.0)	
Groups				0.19
Alendronate	2 (33.3)	2 (33.3)	2 (33.3)	
Denosumab	5 (83.3)	1 (16.7)	0 (0.0)	
Groups				0.56
Zoledronic acid	2 (33.3)	3 (50.3)	1 (16.7)	
GNZ	4 (66.7)	2 (33.3)	0 (0.0)	
Groups				0.31
Alendronate	2 (33.3)	2 (33.3)	2 (33.3)	
GNAL	5 (83.3)	0 (0.0)	1 (16.7)	
Groups				0.36
Denosumab	5 (83.3)	1 (16.7)	0 (0.0)	
GNDmab	4 (80.0)	0 (0.0)	1 (20.0)	

*Fisher's exact test.

Table 4. Comparison of the different medication groups in the occurrence of bone sequestrum

Variables	Bone sequestrum [n (%)]		p-value*
	No	Yes	
Groups			0.22
Zoledronic acid	4 (66.7)	2 (33.3)	
Alendronate	6 (100.0)	0 (0.0)	
Groups			0.22
Zoledronic acid	4 (66.7)	2 (33.3)	
Denosumab	6 (100.0)	0 (0.0)	
Groups			0.99
Alendronate	0 (0.0)	6 (100.0)	
Denosumab	6 (100.0)	0 (0.0)	
Groups			0.22
Zoledronic acid	4 (66.7)	2 (33.3)	
GNZ	6 (100.0)	0 (0.0)	
Groups			0.99
Alendronate	0 (0.0)	6 (100.0)	
GNAL	6 (100.0)	0 (0.0)	
Groups			0.99
Denosumab	3 (50.0)	3 (50.0)	
GNDmab	5 (100.0)	0 (0.0)	

*Chi-squared test.

Table 5. Comparison of the different medication groups in the presence of root rest

Variables	Root rest[n (%)]		p-value*
	No	Yes	
Groups			0.22
Zoledronic acid	6 (100.0)	0 (0.0)	
Alendronate	4 (66.7)	2 (33.3)	
Groups			0.99
Zoledronic acid	6 (100.0)	0 (0.0)	
Denosumab	6 (100.0)	0 (0.0)	
Groups			0.22
Alendronate	4 (66.7)	2 (33.3)	
Denosumab	6 (100.0)	0 (0.0)	
Groups			0.99
Zoledronic acid	6 (100.0)	0 (0.0)	
GNZ	6 (100.0)	0 (0.0)	
Groups			0.22
Alendronate	4 (66.7)	2 (33.3)	
GNAL	6 (100.0)	0 (0.0)	
Groups			0.99
Denosumab	6 (100.0)	0 (0.0)	
GNDmab	5 (100.0)	0 (0.0)	

*Chi-squared test.

Table 6. Comparison of the different medication groups in the mean of blood vessels

Variables	**Blood vessels [mean (SD)]**	**p-value***
Groups		0.99
Zoledronic acid	6.0 (4.9)	
Alendronate	5.6 (3.2)	
Groups		<0.05
Zoledronic acid	6.0 (4.9)	
Denosumab	7.8 (5.6)	
Groups		0.98
Alendronate	5.6 (3.2)	
Denosumab	7.8 (5.6)	
Groups		0.98
Zoledronic acid	6.0 (4.9)	
GNZ	8.1 (6.5)	
Groups		0.50
Alendronate	5.6 (3.2)	
GNAL	11.1 (6.4)	
Groups		0.71
Denosumab	7.8 (5.6)	
GNDmab	3.2 (4.6)	

SD, standard deviation; *Anova one-way, post hoc Tukey.

3. Conclusão

A MRONJ esteve presente neste estudo de forma histológica na lâmina de animais submetidos a BFs, bem como uma diminuição na quantidade de vasos. Assim demonstrando, que pelo seu mecanismo de ação ser mais local e mais duradouro, os BFs apresentam mais MRONJ do que quando comparado aos Dmab. Portanto estudos que possuam uma metodologia padronizada, considerando o estágio clínico da MRONJ zero, necessitam ser feitos para corroborar para a descoberta da etiopatogenia desta lesão.

Referências

AAOMS. American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaws. **Journal Oral Maxillofac. Surgery**, v.65, n.3, p. 369-376, 2007.

ABTAHI, J.; AGHOLME, F.; SANDBERG, O.; ASPENBERG, P. Bisphosphonate-induced osteonecrosis ofthe jaw in a rat model arises first after the bone has become exposed. No primary necrosis inunexposed bone. **J Oral Pathol Med**. 41(6):494-9. 2012.

BARROS, S.P.G.; OLIVEIRA, C.C.; BRIZENO L. *et all*. Perfil celular imune da osteonecrose da mandíbula relacionada ao bifosfonato. Oral Dis., v.22, n.7, p. 649-657, out. 2016

HOWIE, R.; BORKE, J.; KURAGO, Z.; DAOUDI, A. et al. A Model for Osteonecrosis of the Jaw with Zoledronate Treatment following Repeated Major Trauma. ***PLoS ONE***, Volume 10(7). 2015.

HOKUGO, A.; CHRISTENSEN, R.; CHUNG, E.M. et al. Increased prevalence of bisphosphonate-related osteonecrosis of the jaw with vitamin D deficiency in rats. **American Society for Bone and Mineral Research,** Volume 25, Jun, 2010.

LESCLOUS, P.; ABI NAJM, S.; CARREL, J.; BAROUKH, B. et al. Bisphosphonate-associated osteonecrosis of the jaw: a key role of inflammation? ***Bone*****,** Volume 45(5), p. 843-52. 2009.

MARX, R. E.; SAWATARI, Y.; FORTIN, M.; BROUMAND, V. Bisphosphonate-induced exposed bone (osteonecrosis/osteopetrosis) of the jaws: Risk factors, recognition, prevention, andtreatment. **J Oral Maxillofac Surg**,63:1567-75, 2005.

MARX, R. E. Pamidronate (Aredia®) and zoledronate (Zometa®) induced avascular necrosis of the jaws: a growing epidemic. **Journal of Oral and Maxillofacial Surgery**, Philadelphia, v. 61, n. 9, p. 1115-1117, Sept. 2003.

NETO, N.C. et al. Experimental osteonecrosis: development of a model in rodents administered alendronate. **Braz. Oral Res.,** 30(1):e99, 2016.

OHKAWA, H.; OHISHI, N. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction **Anal. Biochem.**,v. 95, p. 351, 1979.

POUBEL, V. L. D. N.; CAPELLA, D. L.; SANTOS, A. R. S.; CORREA, M.; CORREA, L. R.; RIVERO, E. R.C. Evaluation of mandibular bone after dental extraction in rats treated with antiresorptive drugs, ***Journal of Oral and Maxillofacial Surgery***doi: 10.1016/j.joms.2017.07.172. 2017.

RASHIDPOUR, M. et al. Effect of Tramadol (μ-opioid receptor agonist) on orthodontic tooth movements in a rat model. **Journal of Dentistry, Tehran University of Medical Sciences**, v.9, n. 2, p. 83-89, 2012.

RASMUSSON, L. AND ABTAHI, J. Bisphosphonate Associated Osteonecrosis of the Jaw: An Update on Pathophysiology, Risk Factors, and Treatment. ***International Journal of Dentistry***, Volume 2014, p. 1-9. 2014.

RUGGIERO, S. I. et al. Osteonecrosis of the jaws associated with the use of bisphosphonates: a review of 63 cases. **Journal of Oral and Maxillofacial Surgery**, Philadelphia, v. 62, n.5, p. 527-534, May 2004.

RUGGIERO, S.; AND DREW, S. Osteonecrosis of the jaws and bisphosphonate therapy. **J Dent Res**, Volume 86(11), p. 1013-21. 2007.

RUGGIERO, S.; DODSON, T.; FANTASIA, J.; GOODDAY, R. et al. Medication-Related Osteonecrosisof the Jaw - 2014 Update. **American Association of Oral and Maxillofacial Surgeons**, 2014.

UYANNE, J.; CALHOUN, C. C.; LE, A. D. Antiresorptive drug-related osteonecrosis of the jaw. **Dent Clin North Am.** 58(2):369-84, 2014.

WOO, S. B.; HELLSTEIN, J. W.; KALMAR, J. R. Systematic review: bisphosphonates and osteonecrosis of the jaws. **Archives of Internal Medicine**, Chicago, v. 144, n. 10, p. 753-761, May 2006.

YAMASHITA, J.; MCCAULEY, L. K. Antiresorptives and Osteonecrosis of the Jaw. Journal of Evidence Based Dental Practice.,12(3):233-47, 2012.

ZANDI, M. et al. Introducing a protocol to create bisphosphonate-related osteonecrosis of the jaw in rat animal model. **Journal of Cranio-Maxillo-Facial Surgery.,** 44; 271 e 278, 2016.

ZHANG X, HAMADEH I.S, SONG S, LESKO L.J, GONG Y. Osteonecrosis of the Jaw in the United States Food and Drug Administration's Adverse Event Reporting System (FAERS). **Journal Bone Miner Research**, 2015.

ANEXO A- APROVAÇÃO PELA COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "AVALIAÇÃO DE MODELOS DE OSTEONECROSE MANDIBULAR INDUZIDA POR DROGAS ANTIRREABSORTIVAS EM RATOS WISTAR", protocolada sob o CEUA nº 9967260318 (ID 002000), sob a responsabilidade de **Cristiane Cademartori Danesi** *e equipe; Victor de Mello Palma; Eva Aguiar Almeida Campos Castro Torriani; Roberto Marinho Maciel ; Gustavo Nogara Dotto; Kivia Linhares Ferrazzo; Raquel Cristine Silva Barcelos; Luisa Machado Barin ; Luisa Berlato Silva* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 07/06/2018.

We certify that the proposal "EVALUATION OF MANDIBULAR INDUCED OSTEONECROSIS MODELS BY ANTIRREABORESTATIVE DRUGS IN WISTAR RATS", utilizing 36 Heterogenics rats (36 males), protocol number CEUA 9967260318 (ID 002000), under the responsibility of **Cristiane Cademartori Danesi** *and team; Victor de Mello Palma; Eva Aguiar Almeida Campos Castro Torriani; Roberto Marinho Maciel ; Gustavo Nogara Dotto; Kivia Linhares Ferrazzo; Raquel Cristine Silva Barcelos; Luisa Machado Barin ; Luisa Berlato Silva* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 06/07/2018.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 04/2018 a 08/2019 Área: Bioquímica E Biologia Molecular

Origem:	Biotério Central UFSM						
Espécie:	Ratos heterogênicos	sexo:	Machos	idade:	2 a 3 meses	N:	36
Linhagem:	Wistar			Peso:	200 a 300 g		

Local do experimento: Biotério setorial da Parasitologia (prédio 20) LAPBIO Laboratório de Patologia e Biologia Oral (prédio 20)

Santa Maria, 02 de junho de 2020

Profa. Dra. Patrícia Severo do Nascimento
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

ANEXO B. NORMAS PARA PUBLICAÇÃO NO PERIODICO BRAZILIAN ORAL RESEARCH.

ISSN 1807-3107 *online version*

INSTRUCTIONS TO AUTHORS

Mission, scope, and submission policy

Brazilian Oral Research - BOR (online version ISSN 1807-3107) is the official publication of the *Sociedade Brasileira de Pesquisa Odontológica* - SBPqO (the Brazilian division of the International Association for Dental Research - IADR). The is rated A2 Qualis Capes (Dentistry), Impact Factor ™ / 20182019 1,508 (Institute for Scientific Information - ISI), is peer-reviewed (double-blind system), and its mission is to disseminate and promote an information interchange concerning the several fields in dentistry research and/or related areas with gold open access.

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Presentation of the manuscript

The manuscript text should be written in English and provided in a digital file compatible with "Microsoft Word" (in DOC, DOCX, or RTF format).

All figures (including those in layouts/combinations) must be provided in individual and separate files, according to recommendations described under the specific topic.

Photographs, micrographs, and radiographs should be provided in TIFF format, according to the recommendations described under the specific topic.

Charts, drawings, layouts, and other vector illustrations must be provided in a PDF format individually in separate files, according to the recommendations described under the specific topic.
Video files may be submitted as per the specifications, including the author's anonymity (for purposes of evaluation) and respect for the patient's rights.

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- Results
- Discussion
- Conclusion
- Acknowledgments
- References: maximum of 40 references
- Figure legends
- Figures: a maximum of 8 (eight) figures, as described above
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- Methodology
- Results
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- Conclusion
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Layout

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- Results
- Discussion
- Conclusion
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- Critical Evaluation and Data Collection
- Data analysis and presentation
- Improvement
- Review update
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EXAMPLES OF REFERENCES

Journals

Bhutta ZA, Darmstadt GL, Hasan BS, Haws RA. Community-based interventions for improving perinatal and neonatal health outcomes in developing countries: a review of the evidence. Pediatrics. 2005;115(2 Suppl):519-617. https://doi.org/10.1542/peds.2004-1441

Articles with title and text in a language other than English

Li YJ, He X, Liu LN, Lan YY, Wang AM, Wang YL. [Studies on chemical constituents in herb of Polygonum orientale]. Zhongguo Ahong Yao Za Zhi. 2005 Mar;30(6):444-6. Chinese.

Supplements or Special Editions

Pucca Junior GA, Lucena EHG, Cawahisa PT. Financing national policy on oral health in Brazil in the context of the Unified Health System. Braz Oral Res. 2010 Aug;24 Spec Iss 1:26-32.

Books

Stedman TL. Stedman's medical dictionary: a vocabulary of medicine and its allied sciences, with pronunciations and derivations. 20th ed. Baltimore: Williams & Wilkins; 1961.

Online Books

Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available

from: http://www.nap.edu/books/0309074029/html/

Websites

Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000 [cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/

Instituto Brasileiro de Geografia e Estatística [homepage]. Brasília (DF): Instituto Brasileiro de Geografia e Estatística; 2010 [cited 2010 Nov 27]. Available from: http://www.ibge.gov.br/home/default.php

World Health Organization [homepage]. Geneva: World Health Organization; 2011 [cited 2011 Jan 17]. Available from: http://www.who.int/en/