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MARIA CLÁUDIA PINHEIRO CORÔA

INVESTIGAÇÃO DOS EFEITOS DA MINOCICLINA NA PERIODONTITE APICAL INDUZIDA EM RATOS *Wistar*: AVALIAÇÃO MICROTOMOGRÁFICA, HISTOPATOLÓGICA E HISTOQUÍMICA

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Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Ciências Farmacêuticas da Universidade Federal do Pará, para a obtenção do título de mestre.

Orientador: Professor Dr. Rafael Rodrigues Lima.

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RESUMO

A minociclina (MNC) é um antibiótico semissintético derivado da tetraciclina que apresenta amplo espectro de ação e propriedades anti-inflamatórias e osteoregenerativas. A periodontite apical (PA) é uma lesão infecciosa à polpa dentária com repercussões locais e sistêmica. Para além da desinfeção e modelação radicular convencionais, a periodontite apical frequentemente causada por infeção bacteriana pode ser tratada com antibióticos e medicamentos anti-inflamatórios. Embora os antibióticos costumassem ser prescritos apenas para pacientes com doenças sistêmicas, a abordagem contemporânea tem novas terapias adjuvantes. O objetivo deste estudo foi investigar os efeitos da administração sistêmica de minociclina no osso alveolar de ratos com lesões periapicais induzidas experimentalmente. Trinta ratos *Wistar* foram distribuídos aleatoriamente em três grupos (n=10): C = controle sem lesões periapicais; AP = periodontite apical induzida pela exposição da câmara pulpar de molares inferiores; AP+M = periodontite apical + administração intraperitoneal de minociclina durante sete dias. A posologia da minociclina utilizada foi de 50 mg/kg em 12/12h por 2 dias e depois 25 mg/kg 24/24h durante 5 dias, totalizando 7 dias de administração do medicamento. Após 28 dias, os animais foram eutanasiados e suas hemimandíbulas foram preparadas para tomografia microcomputadorizada e análise histopatológica e histoquímica. O volume da lesão, parâmetros de qualidade do osso alveolar circundante e a histoquímica foram analisados estatisticamente para a verificação da normalidade foi usado o teste Shapiro Wilker e para análise dos dados teste ANOVA de uma via com pós-teste de Tukey ($p<0,05$). Em comparação com o grupo AP, o grupo AP+M exibiu um volume de lesão significativamente menor, maior volume ósseo e número trabecular. A administração de minociclina também preservou o ligamento periodontal e minimizou a perda do conteúdo de colágeno e da dimensão das fibras. Assim, a administração sistémica de minociclina durante 7 dias foi eficaz na atenuação do dano ósseo causado pela periodontite apical em ratos, mesmo sem o tratamento convencional do canal radicular; no entanto, é necessária mais investigação para avaliar a sua segurança e eficácia em humanos.

Palavras-chave: Periodontite periapical, Minociclina, Modelo animal, MicroCT, Terapia adjuvante

ABSTRACT

Minocycline (MNC) is a semi-synthetic antibiotic derived from tetracycline that has a broad spectrum of action and anti-inflammatory and osteoregenerative properties. In addition to conventional root disinfection and shaping, apical periodontitis often caused by bacterial infection can be treated with antibiotics and anti-inflammatory drugs. Although antibiotics used to be prescribed only for patients with systemic conditions, the contemporary approach has novel adjuvant therapies. This study aimed to investigate the effects of systemic administration of minocycline on the alveolar bone in rats with experimentally induced periapical lesions. Thirty Wistar rats were randomly assigned to three groups (n=10): C = control without periapical lesions; AP = apical periodontitis induced by exposing the pulp chamber of mandibular molars; AP+M = apical periodontitis + intraperitoneal administration minocycline for seven days. The dosage of minocycline used was 50 mg/kg at 12/12h for 2 days and then 25 mg/kg 24/24h for 5 days, totaling 7 days of administration of the drug. After 28 days, the animals were euthanized and their hemimandibles were prepared for micro-computed tomography, histopathological and histochemistry analyses. The lesion volume, surrounding alveolar bone quality parameters and histochemistry were statistically analyzed using one-way ANOVA and post hoc Tukey's test ($p<0.05$). In comparison to the AP group, the AP+M group exhibited significantly lower lesion volume, higher bone volume, and trabecular number. Minocycline administration also preserved the periodontal ligament and minimized the loss of collagen content and fiber dimension. Thus, a 7-day systemic administration of minocycline was effective in attenuating the bone damage caused by apical periodontitis in rats, even without conventional root canal treatment; however, further research is required to address its safety and efficacy in humans.

Keywords: Periapical Periodontitis, Minocycline, Animal Model, MicroCT, Adjuvant Therapy

LISTA DE ILUSTRAÇÕES

LISTA DE SIGLAS E ABREVIAÇÕES

PA – Periodontite Apical

MNC – Minociclina

FDA – Do inglês *Food and Drug Administration*

GUNA – Gengivite ulcerativa necrotizante aguda

SUMÁRIO

1. INTRODUÇÃO

1.1. Periapicopatias

Na endodontia existe diversas patologias inflamatórias, inclusive as perioapicopatias, as quais essas são doenças que acometem o periodonto de inserção (AMSHIDI et al., 2015). Essas patologias são desenvolvidas no ápice dentário, devido principalmente como consequência da infecção proveniente do sistema de canais radiculares (ABBOTT, 2002; NAIR, 1997). Além da etiologia microbiana, fatores traumáticos e iatrogênicos também podem desencadear essa doença (NAIR, 2004).

A teoria da doença causada por microrganismos foi introduzida no mundo científico em 1876. Os postulados de Robert Coch em 1882 associando microrganismos com algumas doenças sistêmicas, acabaram levando ao surgimento da “Idade dourada da Microbiologia” e o deposito de bactérias sobre o dente e gengiva foram também considerados como origem de contaminação (DA SILVA, 2018). As doenças mais frequentes dos ossos maxilares são as periapicopatias inflamatórias de origem endodônticas e ocorrem principalmente em decorrência da disseminação bacteriana.

1.2. Periodontite apical

A periodontite apical (PA) é uma lesão infecciosa à polpa dentária originada por agentes físicos como traumas, químicos ou bacterianos cuja manifestação clínica pode envolver dor (GOMES et al., 2013). Trata-se de uma inflamação aguda ou crônica do periodonto localizada no ápice da raiz do dente (BARCELOS et al., 2020). Os processos de PA geralmente trabalham para criar uma segunda barreira para impedir a disseminação de micróbios potencialmente ameaçadores (ØRSTAVIK, 2007).

Diversos são os fatores que causam ou pioram a periodontite apical, entre eles estão: microrganismos presentes na cavidade bucal, que colonizam a sistema de canais radiculares, levando a cárie; tratamentos de canal realizados de forma inadequada e restaurações coronárias insuficientes (TIBÚRCIO-MACHADO et al., 2020). Uma condição que pode ser associada com o desenvolvimento da PA é a existência de alguma restauração inadequada ou cárie profunda que apresente comprometimento pulpar (BLAKE te al., 2023). O entendimento da formação e

compreensão da PA, bem como a identificação de biomarcadores inflamatórios podem dar suporte para as diferentes estratégias terapêuticas (BRAZ-SILVA et al., 2019).

Agentes microbianos e seus produtos como endotoxinas que adentram o ambiente pulpar e periapical desencadeiam uma resposta imunoinflamatória local que pode levar ao desenvolvimento de uma lesão apical causada pela resposta imune à infecção endodôntica (BARCELOS et al., 2020). Em seres humanos, o diagnóstico diferencial se faz de suma importância para definir a terapia mais adequada.

O teste de vitalidade é de suma importância para o diagnóstico diferencial, sendo o resultado negativo considerado como uma necrose pulpar, no entanto existem dentes com mais de uma raiz e a necrose pode existir em apenas um dos canais, o que pode vir a dificultar encontrar a verdadeira origem da lesão. Exame complementar como radiografia, é necessário, pois podem evidenciar uma área radiolucida da região afetada (FERNANDES NETO et al., 2017). A mobilidade não pode ser considerada um diagnóstico diferencial, uma vez que é mais evidente em casos de perda óssea mais acentuada.

A progressão da inflamação pode se dá através de ações individuais como o consumo crônico de álcool e uma dieta rica em gordura, e doenças crônicas, por exemplo, diabetes, osteoporose, também podem contribuir para a perda óssea em cavidade oral (TIBÚRCIO-MACHADO et al., 2020).

Apesar do caráter local da PA, SAMUEL et al. (2019) constatou que parâmetros sistêmicos como produção de infiltrado inflamatório, aumento na concentração sérica de linfócitos, leucócitos e a redução na concentração do óxido nítrico, em estudo experimental em ratos *Wistar* portadores de periodontite apical induzida. Além disso, evidenciou-se que a periodontite apical eleva os níveis de PCR, IL-2 e IL-6 provocando alterações na aorta, no miocárdio, no baço e no fígado de ratos (ZHANG et al., 2016). Logo, se não for realizado um tratamento endodôntico adequado, a periodontite apical pode permanecer por mais tempo e influenciar e/ou receber influência de fatores locais e sistêmicos (DAL-FABBRO et al., 2019).

Quando as bactérias se hospedam nos tecidos, duas respostas imune são realizadas: a resposta imune inata (primeira linha de defesa) e a adaptativa. A resposta imune inata serve como uma defesa inicial, ou seja, atua imediatamente usando como

mecanismo a ativação do sistema complemento, a fagocitose e a resposta inflamatória (BASTONE et al., 2000). A ativação do complemento gera subprodutos envolvidos com a opsonização, formação do complexo de ataque à membrana e a estimulação da resposta inflamatória (LOPES et al., 2020). Já os fagócitos utilizam receptores de superfície para reconhecer bactérias extracelulares, como: os receptores da região do fragmento cristalizável e os receptores do complemento. Além de receptores os fagócitos ativados secretam citocinas, quimiocinas e outros mediadores químicos que induzem a inflamação e a atração de leucócitos (LOPES et al., 2020).

A resposta imune adaptativa subsequente surge após o aumento da inflamação que consequentemente aumenta o fluxo de linfa, que coleta antígenos bacterianos na forma solúvel ou capturados por células dendríticas ou macrófagos e os conduz aos linfonodos regionais. Isso facilita a apresentação dos antígenos bacterianos a linfócitos circulantes específicos contra esses antígenos, que, após reconhecimento, se tornam ativados e dão origem a uma resposta imune adaptativa eficaz e direcionada (LOPES et al., 2020).

O desenvolvimento de lesão perirradicular, ou lesão periapical, está diretamente relacionada as respostas imunes contra uma infecção intrarradicular, na tentativa de conter a propagação da infecção ao osso e outros locais do corpo (GOMES et al., 1996). Sua intensidade depende do número de bactérias, associado a resposta de defesa do indivíduo é possível classificar em uma resposta inflamatória aguda ou a uma resposta crônica. Se a agressão causada por bactérias que saem pelo forame apical for de alta intensidade, haverá o desenvolvimento de uma resposta inflamatória aguda, caracterizando a lesão perirradicular sintomática (ou periodontite apical sintomática) (LOPES et al., 2020).

O aumento da permeabilidade vascular associado à inflamação produz edema, que causa elevação da pressão hidrostática tecidual. Como resultado, as fibras nervosas são comprimidas, produzindo dor. A bradicinina, as prostaglandinas e a histamina também podem causar dor, agindo sobre as fibras nervosas. Contudo, a compressão das fibras é mais significativa nesse aspecto (KOTTOOR et al., 2010).

Quando instalada a lesão crônica, no ligamento periodontal adjacente ao forame apical ou às ramificações, observa-se a presença de um infiltrado inflamatório,

composto basicamente por linfócitos, plasmócitos e macrófagos, fibroblastos, fibras nervosas e vasos sanguíneos. O não tratamento nesse momento pode levar a uma evolução do quadro clínico, levando a formação de um granuloma. Este é caracterizado por reabsorção óssea e substituição do osso reabsorvido por um tecido mole com inflamação crônica. A partir do momento que há reabsorção óssea detectada na radiografia ou na tomografia computadorizada de feixe cônico, e na ausência de sintomas, uma lesão perirradicular assintomática está estabelecida (LOPES et al., 2020).

1.3. Uso dos antibióticos na endodontia

É importante ressaltar que a primeira escolha de tratamento para tratamento da PA é a remoção do agente casual e o procedimento clínico endodôntico como o tratamento de canal convencional (NG, MANN et al., 2008). No entanto, conforme a literatura odontológica, substâncias podem ser utilizadas durante o tratamento convencional. Entre essas substâncias, existe possibilidade de aplicação terapêutica da minociclina em especialidades como dentística, endodontia, periodontia e estomatologia. Verificou-se que uma mistura de drogas antibacterianas, isto é, ciprofloxacino, metronidazol e minociclina pode esterilizar lesões cariosas, polpas necróticas e dentina radicular infectada de dentes decíduos (HOSHINO et al., 1996). O tratamento através de processo cirúrgico somente está indicado caso o convencional não seja possível ou falhar (PEREIRA et al., 2017).

A tripla pasta antibiótica tem demonstrado ser uma combinação de drogas de sucesso em desinfecção do canal radicular e protocolo de regeneração e revascularização pulpar, esterilizando o sistema de canais, para que o novo tecido possa se infiltrar na área radicular. Além disso, relatos de casos mostraram que o desenvolvimento radicular de dentes imaturos necróticos com periodontite apical continua após a aplicação do antibiótico curativo, ou seja, não há prejuízos no processo de fechamento apical (AKGUN et al, 2009).

Na odontologia, especificamente na periodontia, o uso da MNC é bastante investigado. De acordo com PEDRON et al. (2007), “a utilização tópica, no interior das bolsas periodontais em comparação com a sistêmica, apresentou resultados mais significativos, oferecendo vantagens como a manutenção constante da concentração

do fármaco no sítio, permitindo que a dosagem seja mantida em baixo nível, reduzindo os riscos de efeitos adversos e a possível resistência microbiana". Para KHATRI et al. (2012), "a administração sistêmica de antibióticos tem certas vantagens sobre administração tópica, como penetração do medicamento, locais múltiplos da atividade da doença e efeito sobre os dentes locais, por exemplo, língua e áreas tonsilares. Isso melhora a eliminação de microrganismos de toda a boca e diminui o risco de reinfecção dos outros sítios". E para CIANCIO et al (1982), a rota sistêmica poderia permitir o antibiótico que entre na bolsa periodontal com o fluido gengival crevicular para afetar micro-organismos, o que seria difícil de alcançar com a administração tópica. Além disso, uma dose oral eficaz na inibição o crescimento da maioria das espécies bacterianas periodontais é prontamente alcançado em todas as bolsas periodontais durante todo o período de tratamento. Um estudo in vivo comparou os efeitos da administração sistêmica e tópica. A administração tópica reduziu os efeitos da infamação gengival, e a administração sistêmica apontou resultados interessantes na manutenção do tecido ósseo alveolar (Xu & Wei, 2006).

Atualmente, os antibióticos mais indicados para as infecções odontogênicas são as penicilinas, e seu uso sistêmico em endodontia é recomendado para situações nas quais haja a propagação da infecção. Ainda não há evidências dos benefícios de antibioticoterapia no tratamento contra pulpite irreversível, polpa necrótica, retratamento ou redução da dor pós-operatória (Segura-Egea et al., 2017). A terapia antibiótica coadjuvante está indicada para pacientes com doenças sistêmicas e com imunidade comprometida, como pacientes com endocardite infecciosa, próteses de válvulas cardíacas ou com recente prótese articular (SEGURA-EGEA et al., 2016). Dessa forma, os tratamentos farmacológicos adjuvantes em endodontia são importantes para contornar problemas que podem surgir na sequência do tratamento cirúrgico, por reação inflamatória, dor e infecção, e para prevenir eventuais complicações em pacientes com predisposição sistêmica, proporcionando maior conforto e segurança a estes.

Muitas alternativas têm sido estudadas com adjuvantes nos tratamentos endodônticos. Um exemplo disso são os tratamentos com antioxidantes, que se mostraram com grande eficácia para a melhora da doença periodontal (CASTRO et al., 2019). Na endodontia, há também antioxidantes e anti-inflamatórios, como o ácido

alfa-lipóico, que apresentaram grande eficácia no que diz respeito a não progressão da lesão de periodontite apical em ratos Wistar (SEHIRLI et al., 2019) e a MNC, que possui efeitos bactericidas, anti-inflamatórios, antioxidantes, antiapoptóticas e imunomoduladoras (Nazarian & Akhondi, 2023). Suas propriedades antiinflamatória, imunomoduladora e neuroprotetora pode ser associada com diversos mecanismos como efeitos que inibitórios da atividade de enzimas importantes, como iNOS (Amin et al., 1997), metaloproteinases (Golub et al., 1991), inibição de capases 1 e 3, respectivamente (Chen te al., 2000), o aumento dos efeitos derivados de Bcl-2, protegendo as células da morte celular (Jordan et al., 2007), diminuição da fosforilação da p38 MAPK (Corbacella et al., 2004). As tetraciclinas incluindo a MNC, são capazes de ligar-se a cátions como Ca 2+ e Mg 2+, seja por meio da quelação desses íons ou o transporte deles para o meio intracelular, logo essa capacidade pode ser associada a essas atividades biológicas (White e Pearce, 1982).

1.4. Histórico da classe das tetraciclinas

Em 1945, houve a descoberta do primeiro membro da família das tetraciclinas por Benjamin Duggar, sendo um produto da fermentação de uma bactéria do solo (*Streptomyces aureofaciens*) que provocou uma corrida na pesquisa e obtenção de novas tetraciclinas no mercado. Sendo, a partir de 1950 até 1970 diversos membros da família das tetraciclinas haviam sido desenvolvidos, sendo a minociclina descoberta em 1972 (PEREIRA-MAIA et al., 2010)

Tetraciclinas possuem várias propriedades favoráveis, tais com um aspectro bem amplo de ação, baixo custo, baixa toxicidade e podem ser na maioria dos casos administrados por via oral. Estas constituem uma família de produtos naturais (oxitetraciclina, clorotracíclina, tetraciclina, demeclociclina) e semisintética (metaciclina, doxiciclina, minociclina, limeciclina, tigeciclina, rolitetraciclina) (VICENTE et al., 2010 e PÉREZ-TRALLERO et al., 2003).

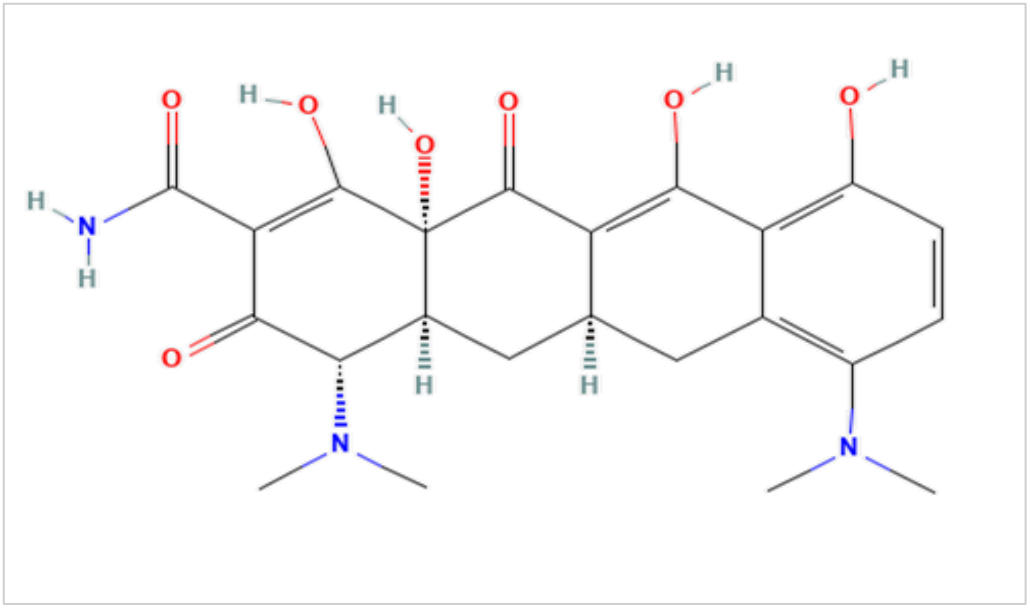
Tetraciclina, minociclina e doxiciclina são consideradas as mais precitas para o tratamento de quadros infecciosos em humanos. A doxiciclina e minociclina, são as drogas que obtiveram os menores efeitos colaterais em relação número de doses reduzidas por dia (PEREIRA-MAIA et al., 2010).

1.4.1. Minociclina

A minociclina (MNC) é um derivado semissintético da tetraciclina de amplo espectro antibiótico aprovado pela Food and Drug Administration (FDA), possuindo atividade contra diversas bactérias gram-positivas, gram-negativas, anaeróbias, aeróbias, riquetsias, clamídias e micoplasmas. Por isso, ela apresenta uma extensa aplicação na medicina, como no tratamento de acne vulgar; gonorreia; meningococo; sífilis; gengivite ulcerativa necrotizante aguda (GUNA) e periodontite. Sua ação é bacteriostática, agindo através da inibição da síntese proteica por ligação reversível com a subunidade 30S do ribossomo bacteriano, impedindo a adição de aminoácidos ao peptídeo em crescimento (SINGH et al., 2021). Quanto a posologia, ainda não está bem elucidada e difere os protocolos de acordo com a patologia a ser tratada. No entanto, a dose usada neste estudo condiz com as usada para atenuar modelos de acidente vascular cerebral e doença de Parkinson (FONTES-JÚNIOR et al., 2016; DU et al., 2001). É importante destacar que essa dose ainda não extrapola para humanos, porém é adaptada considerando a massa corporal e metabolismo dos roedores. Evidências mostram que ao se administrar 120 mg/ Kg por gavagem intraoral, os níveis séricos plasmáticos após 8h da administração foi de 0,32 mg/g (DU et al., 2001).

A farmacocinética da minociclina é caracterizada por uma biodisponibilidade oral alta (95 a 100%) e lipofilicidade de até cinco vezes maior que seu protótipo. Em comparação com outras tetraciclinas, a minociclina é a que possui a maior biodisponibilidade, durando de 16-18 horas, assim como possui propriedade de transpassar a barreira hematoencefálica. Sua meia-vida sérica é longa e possui menor taxa de excreção urinária, permitindo assim o uso de suas doses menores e menos frequente (CIANCIO et al., 1982).

Figura 1 da monografia. Fórmula molecular da minociclina.



Fonte: Centro Nacional de Informações de Biotecnologia (PubChem), 2022.

1.4.2. Minociclina e ação anti-inflamatória

Ademais, a minociclina apresenta características de interesse para o tratamento odontológico, diferenciando-se de outras tetraciclinas por apresentar propriedades tanto antibióticas quanto anti-inflamatórias (KHATRI et al., 2012). Entretanto, enquanto os anti-inflamatórios esteroidais e não esteroidais atuam bloqueando a cascata da inflamação, a ação anti-inflamatória da minociclina caracteriza-se por atuar inibindo a proliferação dos leucócitos e monócitos circulantes, reduzindo citocinas pró-inflamatórias e elevando de citocinas anti-inflamatórias, como TGF-β e IL-10. (YANG et al., 2015). Em consequência, a minociclina diminui a perda de tecido ósseo, por impedir a diferenciação dos macrófagos em osteoclastos, promovendo a formação de tecido ósseo mais proliferativo e menos diferenciado (COELHO et al., 2008). No entanto, o desenvolvimento de cepas bacterianas resistentes, descoloração dos dentes e hiperpigmentação da mucosa, são algumas de suas desvantagens (PARHIZKAR et al., 2018). De acordo com OLIVEIRA et al. (2006), a minociclina é um dos antibióticos mais usados no combate à maioria das bactérias responsáveis pela periodontite como as *Actinomyces*, pois apresenta como ação a inibição de reabsorção óssea e a promoção da formação óssea.

Além disso, estudos prévios relatam a minociclina pode modular a inibição da transmigração e ativação de linfócitos T, metaloproteinase da matriz 9 (MMP-9), inibição da fosfolipase A_2, aumento da regulação da interleucina 10 (IL-10, inibição da expressão de NOS (óxido Nítrico Sintase). Pesquisas recentes estão cada vez mais investigando não somente os efeitos antimicrobianos da MNC, como também o seu potencial anti-inflamatório (Sapadin & Fleischmaje, 2006).

A minociclina pode ser considerada uma alternativa no tratamento da periodontite apical por possuir propriedades antibióticas, anti-inflamatórias e osteoregenerativas. Além disso, a minociclina tem sido empregada nos tratamentos endodônticos regenerativos, como a revascularização endodôntica, por meio da utilizada da pasta tripla antibiótica como medicação intracanal (BECERRA et al., 2014; MARTIN et al., 2012). Considerando-se a escassez de informação disponível sobre os efeitos da minociclina na perda óssea circunscrita na periodontite apical, o objetivo deste estudo foi avaliar os efeitos da minociclina sobre o tecido ósseo alveolar com lesão de periodontite apical em ratos.

2. OBJETIVOS

2.1. Geral

Investigar os efeitos da administração sistêmica da minociclina sob o tecido ósseo alveolar em periodontite apical induzida em ratos

2.2. Específicos

- Verificar se houve diferença no volume da lesão periapical dos animais que receberam MNC
- Analisar microtomograficamente se houve alteração na qualidade do osso alveolar do grupo somente com PA quando comparado ao grupo PA+ MNC
- Avaliar os aspectos histopatológicos do tecido ósseo alveolar com e sem o uso da administração da MNC
- Verificar se a MNC modula o conteúdo de colágeno no tecido ósseo alveolar remanescente à lesão

3. CORPO DO ARTIGO

Minocycline Restrains Lesion Volume and Preserves Alveolar Bone in Rats with Experimentally Induced Apical Periodontitis

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Abstract

In addition to conventional root disinfection and shaping, apical periodontitis often caused by bacterial infection can be treated with antibiotics and anti-inflammatory drugs. Although antibiotics used to be prescribed only for patients with systemic conditions, the contemporary approach has novel adjuvant therapies. This study aimed to investigate the effects of systemic administration of minocycline on the alveolar bone in rats with experimentally induced periapical lesions. Thirty Wistar rats were randomly assigned to three groups (n=10):

C = control without periapical lesions; AP = apical periodontitis induced by exposing the pulp chamber of mandibular molars; AP+M = apical periodontitis + intraperitoneal administration minocycline for seven days. After 28 days, the animals were euthanized and their hemimandibles were prepared for micro-computed tomography and histopathological analyses. The lesion volume and surrounding alveolar bone quality parameters were statistically analyzed using ANOVA and Tukey's test ($p<0.05$). In comparison to the AP group, the AP+M group exhibited significantly lower lesion volume, higher bone volume, and trabecular number. Minocycline administration also preserved the periodontal ligament and minimized the loss of collagen content and fiber dimension. Thus, a 7-day systemic administration of minocycline was effective in attenuating the bone damage caused by apical periodontitis in rats, even without conventional root canal treatment; however, further research is required to address its safety and efficacy in humans.

1. Introduction

Apical periodontitis results from root canal infection that leads to pulp chamber inflammation and damage. Untreated apical periodontitis can progress to inflammatory and potentially necrotic stages (Sehirli et al., 2019; Siqueira Jr and Rôças, 2022). Apical foramen of root canals facilitates intracanal infection that triggers a protective response involving bone resorption and subsequent replacement of granulomatous tissue. This process ultimately leads to periapical lesion development (Sehirli et al., 2019; Karamifar et al., 2020).

The main cause of endodontic diseases is a bacterial infection within the root canal system (Bronzato et al., 2021; Siqueira Jr and Rôças, 2022). The virulence of gram-negative bacteria commonly found in necrotic root canals is associated with several factors such as lipopolysaccharides (LPS)/endotoxins (Martinho et al., 2011; Gomes et al., 2015), which interact with host immunological factors and lead to inflammatory reactions, degradation of periodontal ligament extracellular matrix, and mineralized tissue resorption (Sorsa et al., 2006; Martinho et al., 2011). In addition, the prevalence of gram-positive bacteria such as Enterococcus faecalis in chronic periapical lesions is 77% due to their great resistance to endodontic treatment (Signoretto et al., 2000; Dai et al., 2022).

The use of antibiotics to treat endodontic infections has been traditionally restricted to systemically compromised patients or at risk of endocarditis (Segura-Egea et al., 2017). However, the contemporary approach takes into account the overall health status of patients to customize prescriptions of systemic, topical, and prophylactic antibiotics (Segura-Egea et al., 2018). The first-choice prescription of amoxicillin is substituted by clindamycin in case of penicillin allergy (Segura-Egea et al., 2017).

Tetracyclines have a broad spectrum of activity against a wide range of gram-positive and gram-negative bacteria, spirochetes, obligate intracellular bacteria, and protozoan parasites (Grossman, 2016). These bacteriostatic antibiotics act through ribosomal interaction that interrupts translation by steric interference on aminoacyl transporter RNA (tRNA) binding during elongation (Brodersen et al., 2000). However, the effectiveness of tetracyclines has been undermined by the increasing prevalence of several resistance mechanisms (ribosomal protection, drug degradation, mutation), of which efflux figures as the primary mode of resistance (Nguyen et al., 2014). Since these resistance mechanisms are commonly found in microorganisms such as Enterococcus faecalis, tetracycline is not the first-choice drug to treat endodontic infections (Al-Ahmad et al., 2014); however, it has been recently added to the CTZ paste (500mg chloramphenicol 500mg, 500mg tetracycline, 1g zinc oxide 1g, and 1 eugenol drop), which is suggested as intracanal medication when conventional root canal treatment is not indicated or feasible. The CTZ paste has demonstrated favorable outcomes against periapical lesions and pathogens with great antibiotic resistance such as Enterecoccus faecalis; in addition, it stimulates bone deposition in radiolucent areas and reduces bone loss damage caused by periapical lesions. Nevertheless, further research is needed to address the biocompatibility of CTZ paste (Garrocho-Rangel et al., 2021).

Minocycline is a second-generation tetracycline that has emerged as a promising alternative against both gram-positive and gram-negative bacteria. The enhanced

ribosomal affinity of minocycline decreases the rate of bacterial resistance in comparison to tetracycline, in addition to potent anti-inflammatory properties (Nguyen et al., 2014; Fontes-Júnior et al., 2016; Möller et al., 2016). This new generation of tetracyclines has been added to the triple antibiotic paste (TAP), which effectively disinfects root canals and thus is recommended as part of the conventional disinfection protocol (Segura-Egea et al., 2017; Arruda et al., 2018). Moreover, minocycline exhibits significant anti-inflammatory effects in several tissues and systems. For instance, minocycline reduced the ischemic injuries in rats' brains through the modulation of pro-inflammatory factors expression associated with microglial activity that also detrimentally affects structures in the neural microenvironment (Oliveira et al., 2014; Yang et al., 2015). In addition, such immunomodulatory effects were associated with a reduction of oxidative stress markers such as lipid peroxidation and nitric oxide metabolites in rats (Fontes-Júnior et al., 2016). Moreover, a pre-clinical study showed that minocycline reduced the occurrence of experimentally induced alveolar osteitis in rats and alveolar bone damage (Bosco et al., 2008). Since alveolar bone loss is also induced by the inflammation induced by a periapical lesion, the use of minocycline may attenuate the M1 macrophage pro-inflammatory expression, which involves the up-regulation of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and COX-2/prostaglandins (Song et al., 2022).

While minocycline is commonly used as an intracanal medication, its effects when administered systemically on rats with experimentally induced periapical lesions have not yet been studied. Animal studies are needed to evaluate the effects of minocycline administration on bone health and inflammation and validate potential application in humans (Barré-Sinoussi and Montagutelli, 2015). Therefore, this study aimed to investigate the effects of systemic administration of minocycline on the alveolar bone in rats with experimentally induced periapical lesions.

2. Material and methods

2.1. Animals

Thirty 90-day-old male rats (*Rattus novergicus*, Wistar) were randomly housed in polypropylene cages with unrestricted access to food and water, a 12-hour light/dark cycle (lights on at 7 a.m.), and 25°C. The study was approved by the Ethics Committee on Experimental Animals of the Federal University of Pará (protocol #7599261120) and followed the Guide for the Care and Use of Laboratory Animals (Care and Animals, 1986), ARRIVE guidelines (Du Sert et al., 2020), and the Preferred Reporting Items for Animal Studies in Endodontology (Nagendrababu et al., 2021).

2.2. Apical periodontitis induction

The apical periodontitis induction was adapted from Frazão et al. (2023). The rats were intraperitoneally anesthetized with 2% xylazine (8mg/kg) and 10% ketamine (90 mg/kg). A #1/4 carbide burr was used to expose the pulps of the left and right mandibular first molars, which remained exposed to the oral environment to induce apical lesions. The animals received a daily subcutaneous dose of 100 mg/kg dipyrone for three days to attenuate pain and discomfort (Figure 1).

Insert figure 1 here

2.3. Experimental procedures

The animals were randomly allocated in three groups (n=10): Control; apical periodontitis (AP); and apical periodontitis + minocycline (AP+M). After 21 days from AP induction, the animals from the AP+M group received an intraperitoneal administration of 50 mg/kg of minocycline (Sigma Aldrich, St. Louis, IL, USA) every 12 hours for two days followed by 25 mg/kg every 24 hours for five days. This therapeutic regimen was suggested by Yranick et al., (1997) and studies by our group have also used the same therapeutic regimen, which proved to be efficient and without mortality (Oliveira et al., 2014; Fontes-Júnior et al., 2016). The animals from both the C and AP groups received intraperitoneal administration of sterile 0.9% saline solution for seven days. The body weight of the rats was recorded every week. To verify possible changes in the animals' body weight, they were weighed once a week until the end of the experimental period. At 28 days, the animals were anesthetized with ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (9 mg/kg) until the absence of corneal reflexes. Then, the left ventricles were perfused with 0.9% heparinized saline followed by 4% formaldehyde. The hemimandibles of both sides were dissected by using a scalpel and surgical scissors for micro-computed tomography (micro-CT) and histopathological analyses (Figure 2).

Inserte figure 2 here

2.4. Micro-CT analysis

The right hemimandibles were fixed in a 30 times larger volume of 4% formaldehyde and submitted to micro-CT scanning (MicroCT.SMX-90 CT, Shimadzu, Kyoto, Japan) with 360o rotation, 70kV intensity, and 100 mA. A total of 541 images per specimen were reconstructed with 10 μm voxel size, 1024x1024 resolution, and exported as DICOM files (inspeXio SMX-90CT, Shimadzu, Kyoto, Japan).

The area of alveolar bone resorption was reconstructed using surface rendering software (CTAn v.1.15.4.0, Bruker, Kontich, Belgium). The hemimandibles were standardly positioned to visualize the periodontal ligament through the coronal section and both periodontal ligament space and root destruction were included in the volume of interest (VOI) as described by Chen et al. (Chen et al., 2019). The lesion volume was manually delineated from the mesial root throughout the distal root. The VOI initiated from the first mesial root coronal slice surrounded by the bone crest and extended towards the distal region up to the mandibular second molar.

A 220-image set from the mandibular first molar surrounding bone was also analyzed by the rendering software to determine the alveolar bone quality. The bone was manually delineated in each coronal plane from the closest point to the mesial root up to the farthest point from the distal root. The manufacturer's recommended threshold adjustment procedure was used to differentiate cortical bone, trabecular bone, and bone marrow. The threshold range of 31-71 was used to segment multiple gray color scores. The reminiscent bone volume (BV), bone volume percentage (BV/TV), trabecular spacing (Tb.Sp),

trabecular number (Tb.N), and trabecular thickness (Tb.Th) were measured in the bone area not affected by the lesion.

2.5. Histopathological and morphometric evaluation

The left hemimandibles were fixed for 24 hours in a 4% formaldehyde solution and demineralized for 90 days with a 10% ethylenediaminetetraacetic acid (EDTA) solution. Then, the hemimandibles were dehydrated with alcohol, diaphanized in xylene, embedded in paraplast, and sliced by using a microtome (RM 2045, Leica Microsystems, Nussloch, Germany). The 5-µm thick slices with mesiodistal orientation were mounted on individual slides and stained with hematoxylin and eosin (H&E). The slices were photographed with a digital color camera (Eclipse E200, Nikon, Tokyo, Japan) coupled to an optical microscope (QWin Plus, Leica Microsystems, Nussloch, Germany). The inflammatory characteristics of the periapical lesions were determined in semi-serial sections along the entire mandibular length and the severity of lesions was based on extension, intensity, and characteristics of the inflammatory infiltration, cementum preservation, and bone integrity.

In addition, alveolar bone sections were stained with picrosirius red and examined under a polarized light microscope at 40x magnification to determine the collagen content. An image processing software (Image J, National Institutes of Health, Bethesda, MD, USA) was used to measure the collagen total area (µm²) and perimeter of collagen fibers (µm), while the collagen content was calculated by averaging the threshold percentages from three fields/sections.

2.6. Statistics

The sample size was determined in accordance with Prieto et al. (Prieto et al., 2017) with aid of a dedicated software (G*Power v3.1.9.2, Universität Düsseldorf). The effect size was set at 1.863 with 0.05 error probability and 0.95 power. The normal distribution of data was verified by the Shapiro-Wilk test and the experimental groups were compared by using one-way ANOVA followed by post-hoc Tukey multiple comparisons at a significance level of $p<0.05$. Two-way ANOVA for repeated measures was used to analyze the body weight over time (GraphPad Prism 9.0, GraphPad, San Diego, CA, USA).

3. Results

3.1. Body weight evaluation

The overall bodyweight variation was not significantly different among groups during the four weeks of experimental procedures ($p = 0.99$) (Figure 3).

Insert figure 3 here

3.2. Micro-CT analysis

3.2.1. Minocycline attenuates alveolar bone resorption induced by apical periodontitis

The AP+M group (6.80±0.26 mm^3) exhibited a significantly lower volume of bone resorption than the AP group (9.26±1.10 mm^3) ($p=0.026$), which in turn was significantly higher than the Control group (3.84±0.23 mm^3) ($p=0.0035$) (Figures 4 and 5).

Insert figure 4 here

Insert figure 5 here

3.2.2. Minocycline modulates bone microstructure in rats with apical periodontitis

Both the Control group (46.65±1.97%) and the AP+M group (45.23±3.45%) exhibited a significantly higher BV/TV than the AP group (30.36±3.81%) ($p<0.01$). Interestingly, no significant difference was observed between Control and AP+M groups ($p=0.24$) (Figure 6A). The AP group (0.27±0.02 mm) showed significantly higher Tb.Sp in comparison to both the Control group (0.11±0.01 mm) and the AP+M group (0.19±0.11 mm) ($p<0.001$) (Figure 6B). The AP group (2.56±0.27 /mm) demonstrated significantly lower Tb.N than both the Control group (3.64±0.11 /mm) and the AP+M group (3.65±0.16 /mm) ($p<0.001$) (Figure 6C). The Control group (0.14±0.0012 mm) exhibited a significantly higher Tb.Th when compared to both the AP+M group (0.13±0.008 mm) and Control group (0.11±0.0063 mm) ($p<0.001$) (Figure 6D).

Insert figure 6 here

3.3. Minocycline restored the histological aspects of periodontium

The periapical region of the Control group exhibited no signs of inflammation, while the AP group exhibited an apparent inflammatory infiltrate and osteoclasts in selected areas close to the periapex (Figure 7C and 7D). In addition, specific regions exhibited pronounced inflammatory infiltration within the interradicular alveolar bone (Figure 8). The AP+M group exhibited a mild presence of mononuclear infiltration in the periapical alveolar bone and a reduction of osteoclasts in some areas (Figure 7E and 7F).

Insert figure 7 here

Insert figure 8 here

3.4. Minocycline modulated the quantity and size of collagen fibers within the alveolar bone remaining in the region of the periapical lesion.

Both the Control group (855192±151204 μm²) and the AP+M group (684552±156576 μm²) exhibited significantly higher bone collagen total area in the remaining alveolar bone than the AP group (333760±66136 μm²) ($p<0.0001$) (Figure 9D). The analysis of collagen fiber perimeter showed The Control group (44.63±4.080 μm) showed significantly thicker collagen fibers than the AP+M group (38.10±1.028 μm), which in turn exhibited significantly thicker collagen fibers than the AP group (24.82±3.617 μm) ($p<0.0001$) (Figure 9E).

Insert figure 9 here

4. Discussion

This study provides evidence regarding the systemic use of minocycline to modulate the inflammation and alveolar bone structure induced by apical periodontitis. The micro-CT analysis showed that minocycline effectively mitigated the reduction in trabecular number and thickness, as well as decreased trabecular spacing and alveolar bone resorption. Moreover, minocycline remarkably attenuated the local inflammatory response and thus minimized the loss of collagen content and fiber dimensions in this experimental context. Thus, a systemic therapeutic action of minocycline on alveolar bone structure damage in humans is expected.

When considering pathogens, the importance of their permanence for the onset and progression of periapical lesions is notorious. Scientific evidence shows that the main pathogens that may be involved are Actinomyces, commonly found in lesions of the periapical region (Figdor and Gulabivala, 2008), *Fusobacterium*, which is a Gram-negative anaerobic bacterium (Bronzato et al., 2020). Gram-negative bacteria, *Prevotella and Porphyromonas*, are mainly present when there are abscesses with purulent secretions, and Staphylococcus when there is fistula formation in the oral cavity (Bronzato et al., 2021).

The minocycline dose in this study was described by Cristine Ekdahl et al. (Ekdahl et al., 2003) and has been administered by our research group in other studies. Oliveira et al. (Oliveira et al., 2014) showed that this dose of minocycline reversed microglial activation in ischemic rats exposed to ethanol and suggested its potential effect to mitigate neuroinflammation. Furthermore, Fontes-Júnior et al. (Fontes-Júnior et al., 2016) highlighted the role of minocycline in suppressing oxidative stress and reducing nitrite and MDA levels, which consequently provided additional evidence to support the anti-inflammatory effects of this drug.

Minocycline, a second-generation tetracycline antibiotic, demonstrates anti-inflammatory and immunomodulatory effects that have been reported for several inflammatory conditions such as periodontitis (Garrido-Mesa et al., 2013). A clinical trial showed that the combination of essential clinical procedures such as scaling and root planning with minocycline administration remarkably improved clinical parameters such as pocket reduction and clinical attachment (Arnett et al., 2023). In this context, this study clearly showed, by means of micro-CT and histopathological evidence, that systemic administration of minocycline effectively attenuated the damage caused by AP in the remaining alveolar bone tissue. Thus, minocycline seems to play a key role in reducing apical lesion volume and inflammation, as well as preserving alveolar bone quality and

collagen fiber dimensions. Moreover, these beneficial effects are surprisingly observed even without conventional root canal disinfection and shaping.

This study not only underscores the anti-inflammatory properties of minocycline but also highlights its critical role as a member of the tetracycline class, known for its broad bacteriostatic activity(Khaje Roshanaee et al.). As a tetracycline, minocycline exerts a potent effect against a spectrum of microorganisms that are typically present in periapical lesions(Siqueira Jr and Rôças, 2022). Notably, the effectiveness of minocycline in minimizing periapical lesions can be partially attributed to its ability to target these pathogenic bacteria, thereby controlling infection and reducing microbial-induced inflammation. The dual mechanism of action of minocycline—its anti-inflammatory properties alongside its bacteriostatic capabilities—plays a pivotal role in the observed reduction of lesion severity. Minocycline not only mitigates the direct inflammatory response but also alters the microbial landscape within the periapical region, which is instrumental in reducing tissue destruction and promoting recovery. These findings suggest that the clinical benefits of minocycline extend beyond its anti-inflammatory impact, encompassing a substantial antibacterial effect that warrants further exploration to fully understand its therapeutic potential in endodontic applications.

In addition to antibiotic effects, the therapeutic scope of minocycline covers anti-inflammatory, anti-apoptotic, and neuroprotective actions and thus can be used to treat dermatitis, atherosclerosis, autoimmune disorders, Parkinson's disease, spinal cord injury, malignant cell growth, HIV replication, and bone resorption (Garrido-Mesa et al., 2013). The anti-inflammatory effects of minocycline have been related to its ability to inhibit iNOS enzymes, metalloproteinases, and caspases, as well as to modulate the expression of pro-apoptotic Bcl-2 proteins that have an effect on the outer mitochondrial membrane (Jordan et al., 2007).

In endodontics, minocycline can also be used for pulp revascularization, which is characterized by blood clot stimulation and is more conservative than apicification. The literature shows that minocycline contributes to re-establish pulp vitality and vital connective tissue with reactive dentin layers after two months of revascularization; in addition to infection reduction, tetracycline and its derivatives such as minocycline have inhibited metalloproteinases that degrade the extracellular matrix (Ritter et al., 2004). These drugs also interact with fibroblasts to regenerate the periodontium (Terranova et al., 1986) and inhibit osteoclast action that causes bone resorption (Rifkin et al., 1993).

Since minocycline benefits diverse aspects of bone physiology, its effects have been investigated to treat several bone-related conditions (Garrido-Mesa et al., 2013). A study on guided bone augmentation in rats showed that the systemic administration of minocycline hydrochloride has an accelerating and enhancing effect on vertical bone augmentation; in addition, the combination of minocycline and calvaria perforation resulted in the strongest tissue augmentation and increased mineralization (Biewer et al., 2023). These findings further support the potential protective effect of minocycline on alveolar bone structure and highlight its significant role in bone tissue formation.

The micro-CT analysis conducted in this study is widely used to evaluate bone and periapical lesions (Von Stechow et al., 2003) since the bone parameters can be accurately measured on the 2D and 3D images obtained (Kang et al., 2013; Yang et al., 2014). Moreover, this non-invasive and non-destructive method is widely used in vivo to evaluate the area and volume of periapical lesions (Balto et al., 2000; Schambach et al., 2010). In this study, Micro-CT findings showed that minocycline attenuated the damage by reducing the volume of the periapical lesion and improving BV/TV, Tb.Sp, and Tb.N

parameters. Other studies also reported that minocycline stimulates the proliferation and differentiation of osteoblastic cells (Ma et al., 2020) and inhibits the differentiation of bone marrow-derived macrophages (Kinugawa et al., 2012). Evidence suggests minocycline modulates the main mechanism of bone remodeling by downregulating RANKL and upregulating OPG expressions (Ma et al., 2020). The histopathological analysis conducted in this study also showed that minocycline administration modulated the number of osteoblastic cells in the alveolar bone. Studies show that doses of between 1 and 10 μg/ml of MNC can positively modulate osteoblast proliferation (Gomes and Fernandes, 2007). Furthermore, although the mechanism of how MNC is able to modulate bone cells is not well understood, evidence indicates that MNC is able to bind to the calcium receptor present on the cell membrane of osteoclasts, interfering in the possible induction of differentiation of these cells.

The alveolar bone exhibits a complex structure with diverse components and several types of collagen fibers of different sizes and densities, which are arranged in bundles and provide resilience and support to the tissue (Jiang et al., 2016). In this study, the collagen content and thickness of remaining alveolar bone were observed under polarized light and indicated that minocycline significantly increased the quantity and thickness of collagen fibers in the areas affected by the periapical lesion and can be attributed to its anti-inflammatory and antimicrobial properties. Since minocycline reduces inflammation and host response, fibroblasts and osteoblasts are stimulated to proliferate and produce new collagen fibers (Gomes and Fernandes, 2007; Zhu et al., 2021); in addition, minocycline has an innate ability to inhibit collagenases (Sorsa et al., 2006; Vandenbroucke and Libert, 2014). The increase of newly formed collagen fibers in terms of quantity and thickness supports alveolar bone tissue repair in the affected region, mitigates alveolar bone tissue degradation, and increases the efficiency of bone regeneration (Jain et al., 2020; Zhu et al., 2021). In summary, collagen fiber augmentation after minocycline administration can be attributed to an enhanced inflammatory response, infection control, and bone tissue destruction, thereby facilitating tissue remodeling around the periapical lesion.

Minocycline is a broad-spectrum antibiotic that can act on gram-positive and gram-negative bacteria, anaerobic and facultative bacteria (Segura-Egea et al., 2017). Satisfactory levels of minocycline were found in the crevicular fluid after 14 days of administration (Williams et al., 2001). The slow release of minocycline inhibits bacterial proliferation for an adequate period for tissue regeneration such as pulp revascularization (Ritter et al., 2004). Another benefit reported for the topical administration of minocycline is the reduction of alveolar bone loss (Williams et al., 2001). Nevertheless, this antibiotic may lead to dentin color change and should be used with caution by pregnant women (Muanda et al., 2017).

Minocycline demonstrates a high affinity for mineralized tissues, making it an exceptionally favorable drug for the preservation of bone tissue, as evidenced by the results of micro-computed tomography in this study (Biewer et al., 2023). Its antibiotic capacity appears to have positively influenced the preservation of bone tissue, potentially altering the virulence or microbiota of the periapical lesion. This action might contribute to reducing the extent of bone loss and supporting the maintenance of bone structure, ultimately highlighting minocycline's beneficial impact on periapical lesions. Further research is needed to fully elucidate the mechanisms through which minocycline achieves these effects and to explore its potential application in clinical practice.

Given this study’s promising findings, further studies are encouraged to address the pharmacokinetic implications of minocycline doses and to compare them to other

antibiotics commonly used in endodontics, as well as to investigate the potential effects of different administration modes such as topical use. Similarly, the effects of the addition of minocycline administration to conventional root canal treatment must also be determined. Finally, further research is needed to address the potential systemic effects of minocycline administration on other macrophage populations.

5. Conclusion

In our study, a 7-day systemic administration of minocycline was effective in reducing bone damage caused by apical periodontitis in rats, even without conventional root canal treatment. However, these findings are not intended to suggest routine antibiotic use for standard root canal treatment, and further research is needed to determine its safety and efficacy in humans.

6 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7 Author Contributions

DRF, JDMM, RRL: study concept and design. MCPC, DRF, RMFN, FON, VSC, JDMM, FMC: data analysis and interpretation. MCPC, DRF, VSC, JDMM: manuscript preparation. LOB, GSB, FMC, JMB, RRL: critical revision. All authors contributed to the article and approved the submitted version.

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Figure Captions

Figure 1. (A) A carbide bur was used to expose the pulp of the mandibular first molar, which remained exposed to the oral environment to induce apical lesion. (B, C) Clinical photographs of a Wistar rat's molar before coronal access and with bleeding exposed pulp.

Figure 2. Experimental groups and procedures.

Figure 3. Bodyweight means of different groups at baseline and four weeks of experimental procedures. The whiskers represent the standard error of the mean.

Figure 4. The volume of alveolar bone resorption in different groups. The top lines of the boxes represent the mean and the whisker indicates the standard error of the mean. Groups with the same letter are not significantly different ($p>0.05$).

Figure 5. Representative micro-CT images. Sagittal, transversal, and coronal sections of Control group (A, B, and C), AP group (D, E, and F), and AP+M (G, H, and I). The periapical lesions are indicated with asterisks (*).

Figure 6. Alveolar bone parameters (BV/TV, Tb.Sp, Tb.N, and Tb.Th) measured for each experimental group. The top lines of the boxes represent the mean, and the whisker indicates the standard error of the mean. Groups with the same capital letter are not significantly different ($p>0.05$).

Figure 7. Representative 50µm- and 10µm-scale photomicrographs of the periapical area in the Control group (A and B), AP group (C and D), and AP+M group (E and F). Dashed lines indicate the preservation of the periodontal ligament space/apex-alveolus distance. Yellow asterisks indicate the alveolar bone. Black asterisks indicate the periapex.

Figure 8. Representative 10µm-scale photomicrographs of the interradicular area in the Control group (A), AP group (B), and AP+M group (C). Yellow asterisks indicate the alveolar bone.

Figure 9. Representative images of histochemical analysis of collagen content for the Control group (A), AP group (B), and AP+M group (C). The collagen total area and collagen fiber perimeter for each group are shown in D and E. The top lines of the boxes represent the mean, and the whisker indicates the standard error of the mean. Groups with the same capital letter are not significantly different ($p>0.05$).

Figures

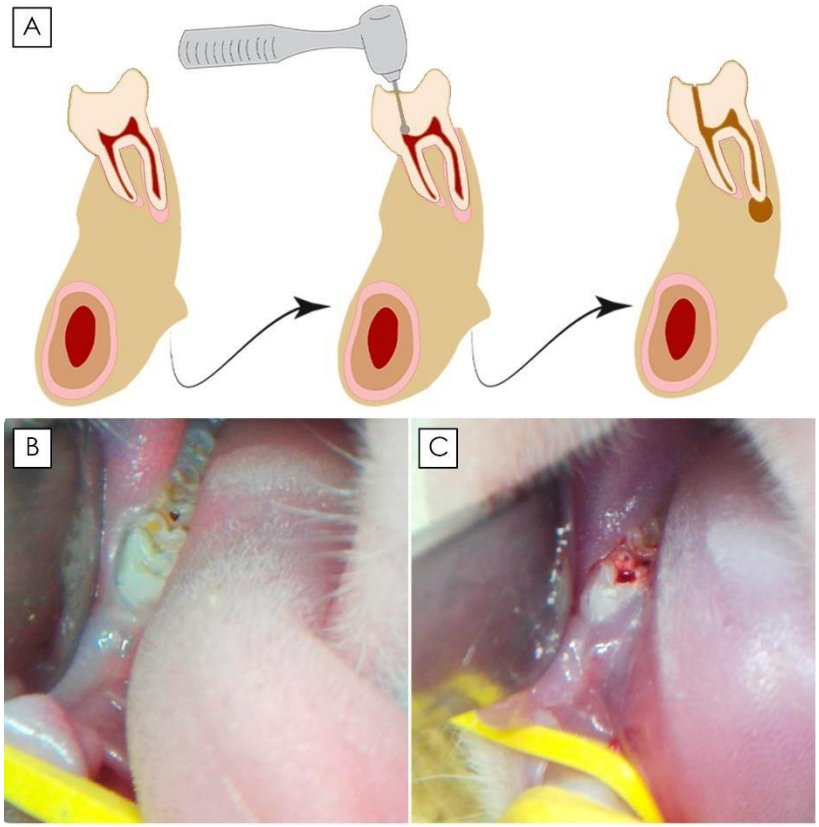

Figure 1

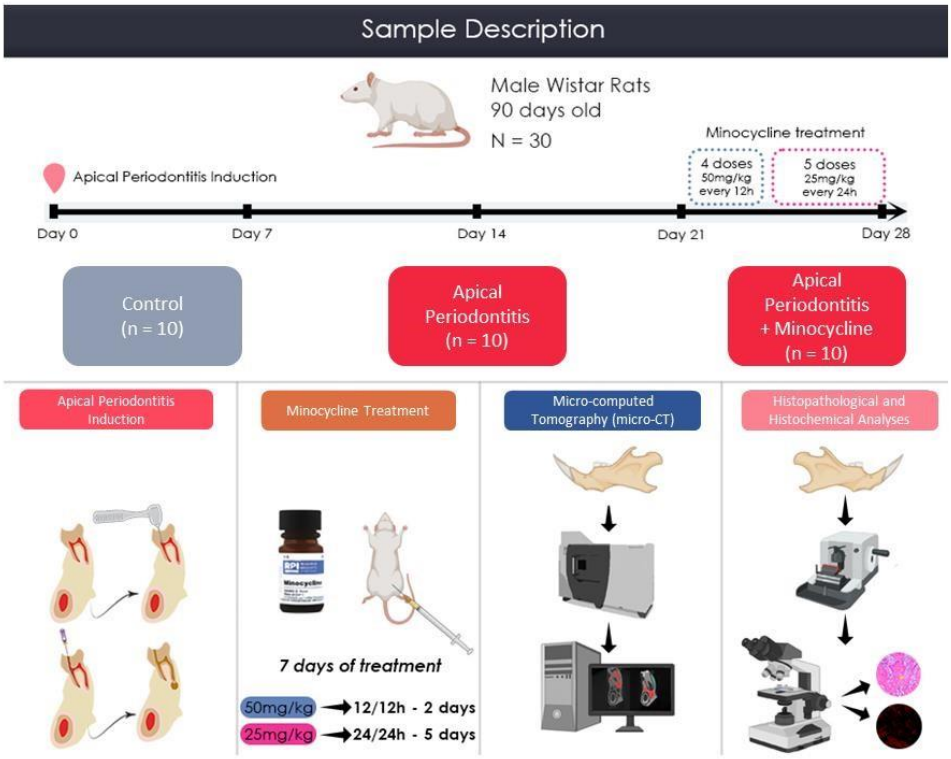

Figure 2

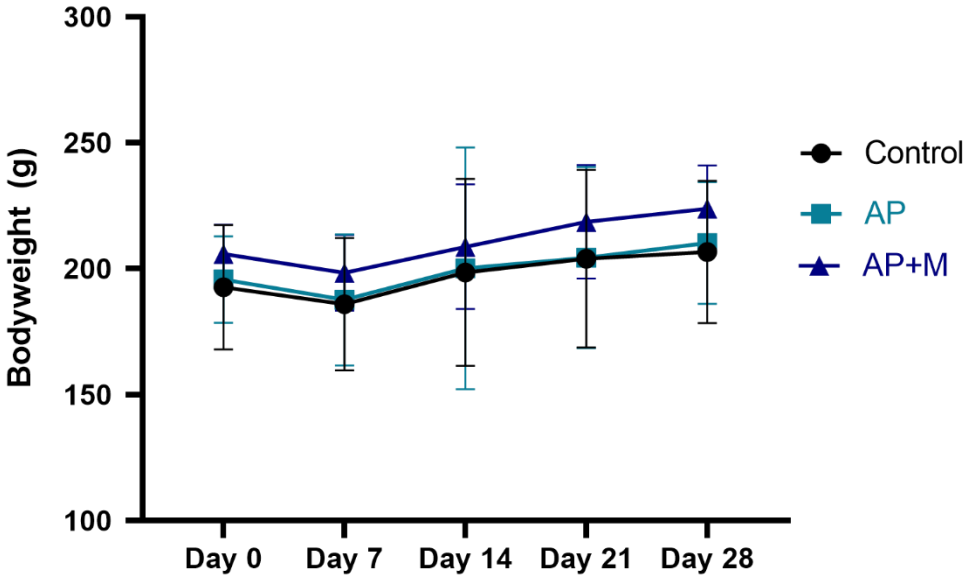

Figure 3

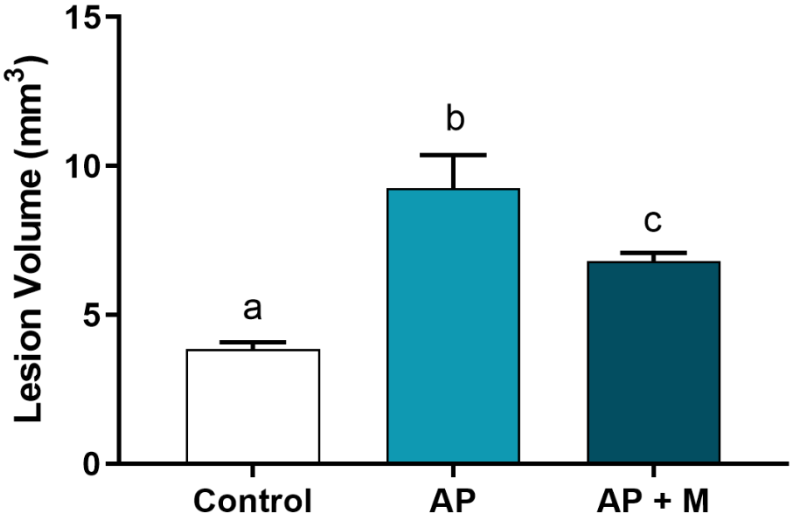

Figure 4

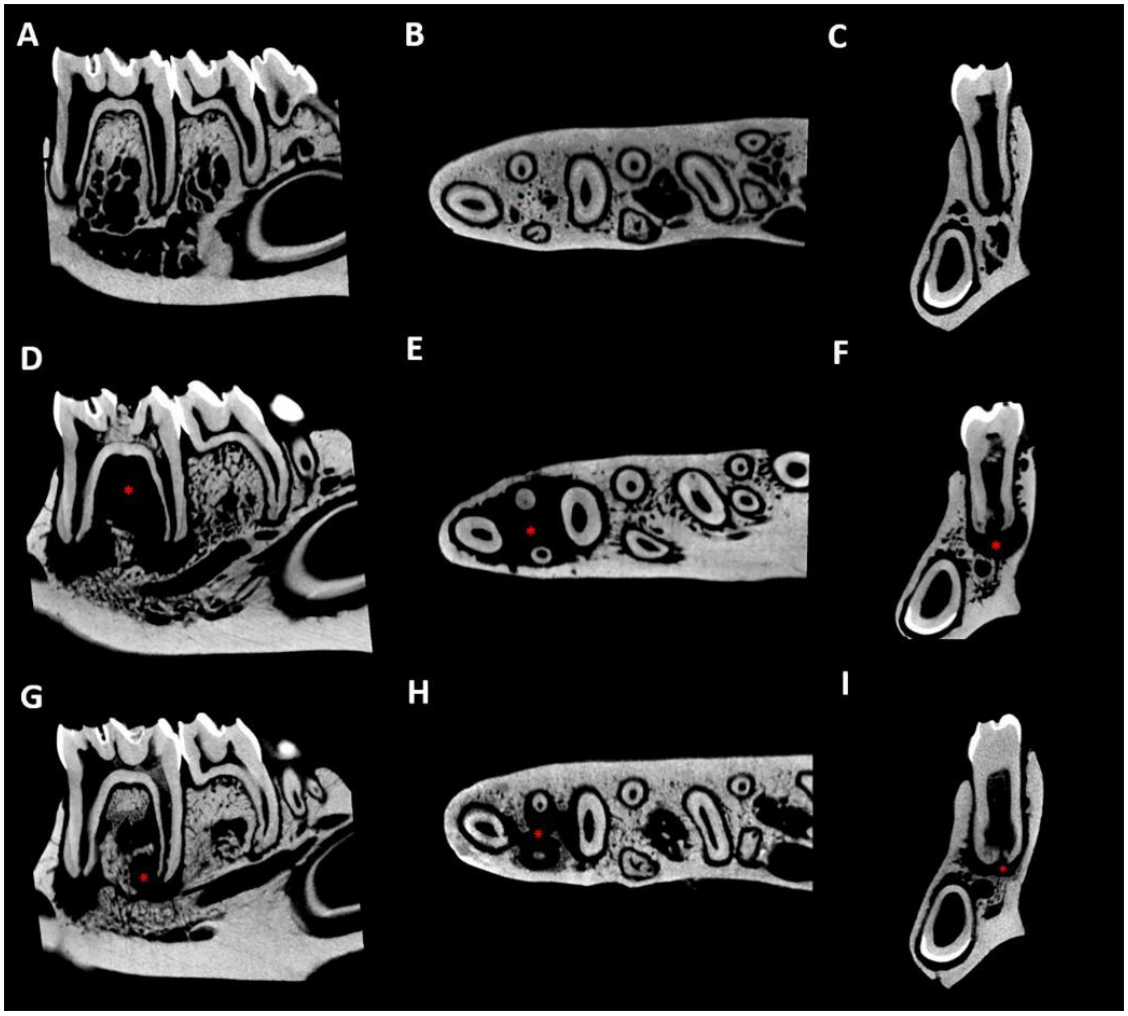

Figure 5

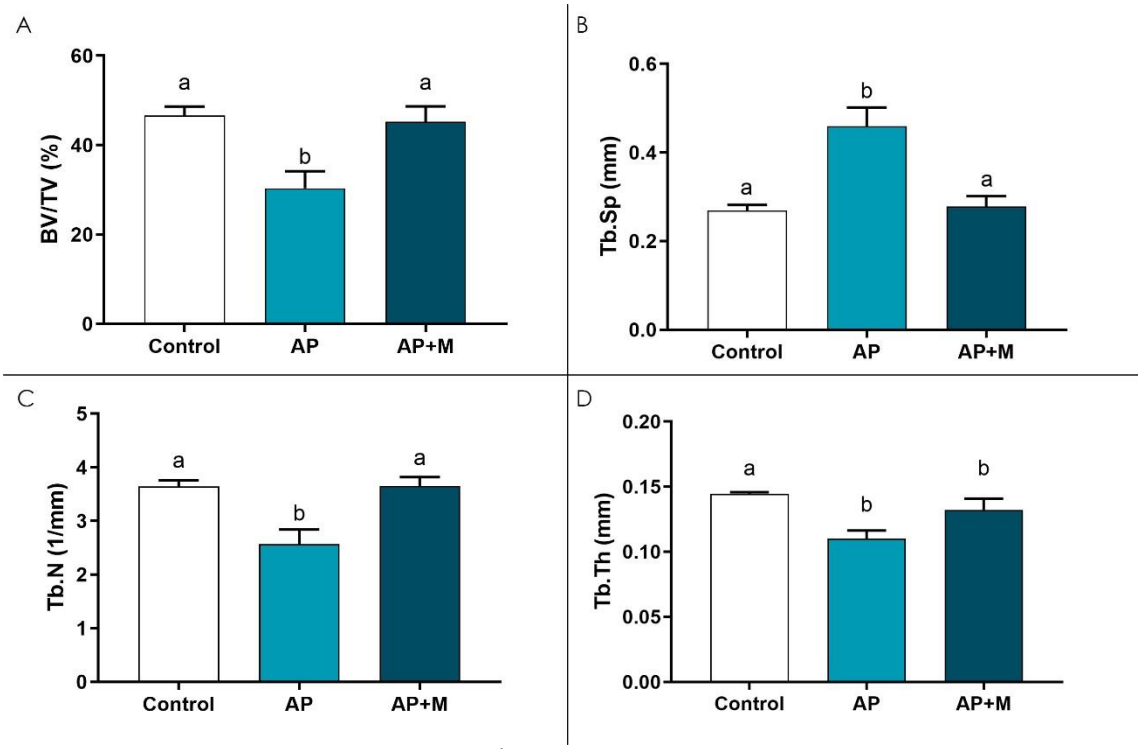

Figure 6

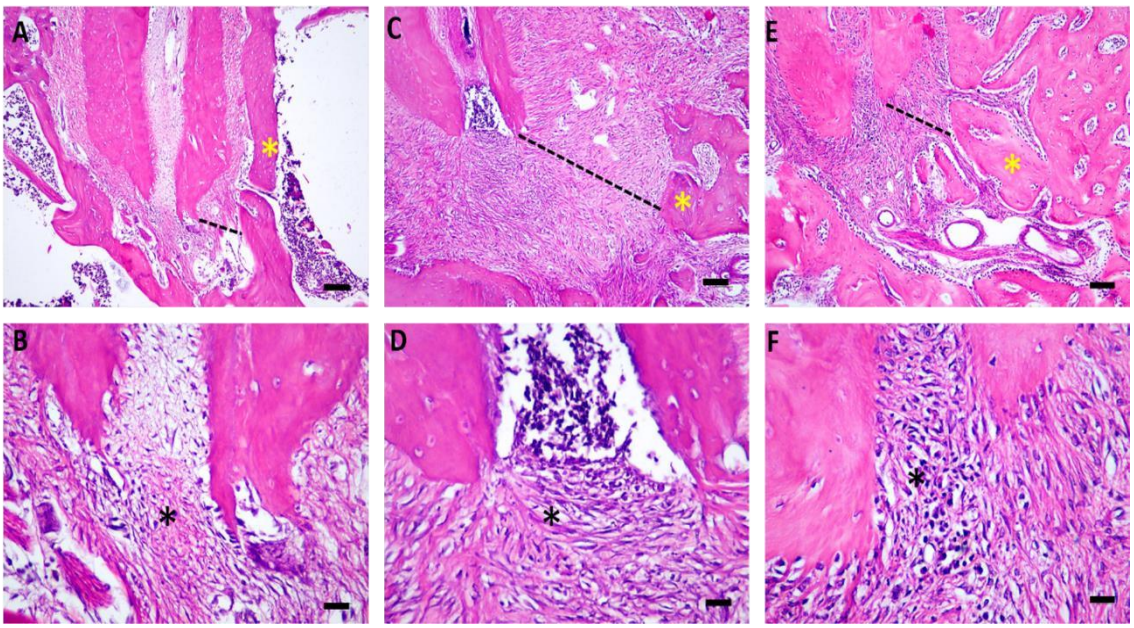

Figure 7

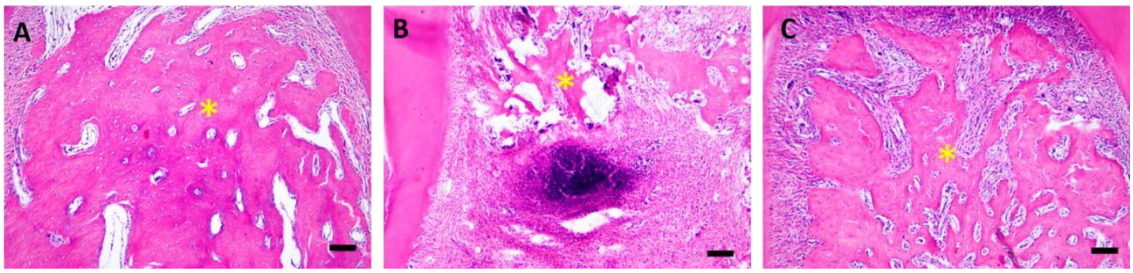

Figure 8

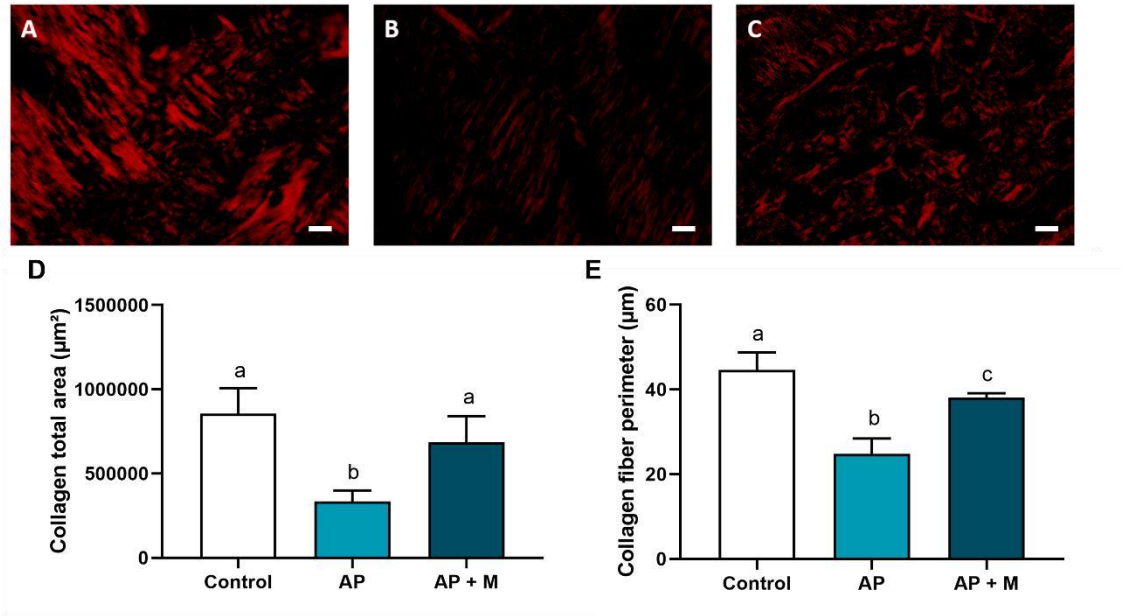

Figure 9

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ANEXO 1

UFPA
Universidade Federal do Pará

Comissão de Ética no
Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "AVALIAÇÃO DOS EFEITOS DA MINOCICLINA SOBRE A ESTRUTURA DO TECIDO ÓSSEO ALVEOLAR SOB A INDUÇÃO DE PERIODONTITE POR LIGADURA E PERIODONTITE APICAL EXPERIMENTAL EM RATOS", protocolada sob o CEUA nº 6545250320 (ID 0034111), sob a responsabilidade de **Rafael Rodrigues Lima** *e equipe; RAILSON DE OLIVEIRA FERREIRA* - 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 do Pará (CEUA/UFPA) na reunião de 30/04/2020.

We certify that the proposal "EVALUATION OF THE EFFECTS OF THE MINOCYCLINE ON THE STRUCTURE OF THE ALVEOLAR BONE TISSUE UNDER INDUCTION OF LIGATURE PERIODONTITIS AND EXPERIMENTAL APICAL PERIODONTITIS IN RATS", utilizing 40 Heterogenics rats (40 males), protocol number CEUA 6545250320 (ID 0034111), under the responsibility of **Rafael Rodrigues Lima** *and team; RAILSON DE OLIVEIRA FERREIRA* - 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 Para (CEUA/UFPA) in the meeting of 04/30/2020.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 06/2020 a 05/2021 Área: Instituto de Ciências Biológicas

Origem:	Biotério Central ICB/UFPA				
Espécie:	Ratos heterogênicos	sexo: Machos	Idade:	90 a 120 dias	N: 40
Linhagem:	Rattus novergicus/wistar		Peso:	150 a 250 g	

Local do experimento: Laboratório de Biologia Estrutural e Funcional - ICB/UFPA

Belém, 15 de maio de 2020

Mª Vivina B. Monteiro
Profa. Dra. Maria Vivina Barros Monteiro
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal do Pará

Profa. Dra. Vanessa Joia de Mello
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal do Pará

ANEXO 2

Comprovante de submissão

Gmail **maria claudia pinheiro <mcp.coroa@gmail.com>**

Your manuscript submission - 1450671

1 mensagem

17 de junho de 2024

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Responder a: Frontiers in Pharmacology Editorial Office <pharmacology.editorial.office@frontiersin.org>
Para: Maria Coroa <mcp.coroa@gmail.com>

Dear Dr Coroa

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----------MANUSCRIPT DETAILS----------
Manuscript title: Minocycline Restrains Lesion Volume and Preserves Alveolar Bone in Rats with Experimentally Induced Apical Periodontitis
Manuscript ID: 1450671
Submitted By: Rafael Rodrigues Lima
Authors: Maria Cláudia Coroa, Deborah Frazão, Leonardo Oliveira Bittencourt, João Daniel Mendonça De Moura,
Felipe Oliveira Nunes, Rayssa Nazario, Victoria Chemelo, Juliana Brandão, Douglas Guimarães, GABRIELA
BALBINOT, Walace Gomes-Leal, Fabricio Mezzomo Collares and Rafael Rodrigues Lima
Journal: Frontiers in Pharmacology, section Inflammation Pharmacology

Article type: Original Research
Submitted on: 17 Jun 2024

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ANEXO 3

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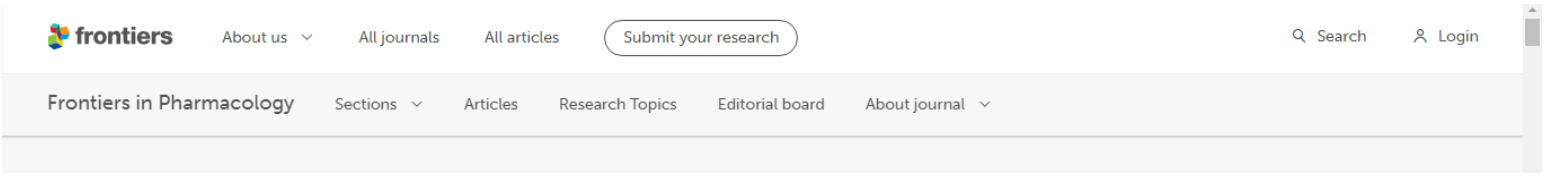

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Acknowledgments

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Figures should be prepared with the PDF layout in mind. Individual figures should not be longer than one page and with a width that corresponds to 1 column (85 mm) or 2 columns (180 mm).

All images must have a resolution of 300 dpi at final size. Check the resolution of your figure by enlarging it to 150%. If the image appears blurry, jagged, or has a stair-stepped effect, the resolution is too low.

The text should be legible and of high quality. The smallest visible text should be no less than eight points in height when viewed at actual size.

Solid lines should not be broken up. Any lines in the graphic should be no smaller than two points wide.

Please note that saving a figure directly as an image file (JPEG, TIF) can greatly affect the resolution of your image. To avoid this, one option is to export the file as PDF, then convert into TIFF or EPS using a graphics software.

Format and color image mode

The following formats are accepted: TIF/TIFF (.tif/.tiff), JPEG (.jpg), and EPS (.eps) (upon acceptance). Images must be submitted in the color mode RGB.

Chemical structures

Chemical structures should be prepared using ChemDraw or a similar program. If working with ChemDraw please use our ChemDraw template. If working with another program please follow the guidelines below.

- Drawing settings: chain angle, 120° bond spacing, 18% width; fixed length, 14.4 pt; bold width, 2.0 pt; line width, 0.6 pt; margin width, 1.6 pt; hash spacing, 2.5 pt. Scale 100% Atom Label settings: font, Arial; size, 8 pt
- Assign all chemical compounds a bold, Arabic numeral in the order in which the compounds are presented in the manuscript text.

Table requirements and style guidelines

Tables should be inserted at the end of the manuscript in an editable format. If you use a word processor, build your table in Word. If you use a LaTeX processor, build your table in LaTeX. An empty line should be left before and after the table.

Table captions must be placed immediately before the table. Captions should be preceded by the appropriate label, for example 'Table 1.' Please use only a single paragraph for the caption.

Kindly ensure that each table is mentioned in the text and in numerical order.

Please note that large tables covering several pages cannot be included in the final PDF for formatting reasons. These tables will be published as supplementary material.

Tables which are not according to the above guidelines will cause substantial delay during the production process.

Accessibility

Frontiers encourages authors to make the figures and visual elements of their articles accessible for the visually impaired. An effective use of color can help people with low visual acuity, or color blindness, understand all the content of an article.

These guidelines are easy to implement and are in accordance with the W3C Web Content Accessibility Guidelines (WCAG 2.1), the standard for web accessibility best practices.

Ensure sufficient contrast between text and its background People who have low visual acuity or color blindness could find it difficult to read text with low contrast background color. Try using colors that provide maximum contrast.

WC3 recommends the following contrast ratio levels:

- Level AA, contrast ratio of at least 4.5:1
- Level AAA, contrast ratio of at least 7:1

You can verify the contrast ratio of your palette with these online ratio checkers:

- WebAIM
- Color Safe

Avoid using red or green indicators More than 99% of color-blind people have a red-green color vision deficiency.

Avoid using only color to communicate information Elements with complex information like charts and graphs can be hard to read when only color is used to distinguish the data. Try to use other visual aspects to communicate information, such as shape, labels, and size. Incorporating patterns into the shape fills also make differences clearer; for an example please see below:

Supplementary material

Data that are not of primary importance to the text, or which cannot be included in the article because they are too large or the current format does not permit it (such as videos, raw data traces, PowerPoint presentations, etc.), can be uploaded as supplementary material during the submission procedure and will be displayed along with the published article. All supplementary files are deposited to Figshare for permanent storage and receive a DOI.

Supplementary material is not typeset, so please ensure that all information is clearly presented without tracked changes/highlighted text/line numbers, and the appropriate caption is included in the file. To avoid discrepancies between the published article and the supplementary material, please do not add the title, author list, affiliations or correspondence in the supplementary files.

The supplementary material can be uploaded as:

- data sheet (Word, Excel, CSV, CDX, FASTA, PDF or Zip files)
- presentation (PowerPoint, PDF or Zip files)
- image (CDX, EPS, JPEG, PDF, PNG or TIF/TIFF),
- table (Word, Excel, CSV or PDF)
- audio (MP3, WAV or WMA)
- video (AVI, DIVX, FLV, MOV, MP4, MPEG, MPG or WMV).

Technical requirements for supplementary images:

- 300 DPIs
- RGB color mode.

For supplementary material templates (LaTeX and Word), see our supplementary material templates.

References

Frontiers' journals use one of two reference styles, either Harvard (author-date) or Vancouver (numbered). Please check our help center to find the correct style for the journal to which you are submitting.

- All citations in the text, figures or tables must be in the reference list and vice-versa
- The names of the first six authors followed by et al. and the DOI (when available) should be provided
- Given names of authors should be abbreviated to initials (e.g., Smith, J., Lewis, C.S., etc.)
- The reference list should only include articles that are published or accepted
- Unpublished data, submitted manuscripts, or personal communications should be cited within the text only, for article types that allow such inclusions
- For accepted but unpublished works use 'in press' instead of page numbers
- Data sets that have been deposited to an online repository should be included in the reference list. Include the version and unique identifier when available

- Personal communications should be documented by a letter of permission
- Website URLs should be included as footnotes
- Any inclusion of verbatim text must be contained in quotation marks and clearly reference the original source
- Preprints can be cited as long as a DOI or archive URL is available, and the citation clearly mentions that the contribution is a preprint. If a peer-reviewed journal publication for the same preprint exists, the official journal publication is the preferred source. See the preprints section for each reference style below for more information.

Harvard reference style (author-date)

Many Frontiers journals use the Harvard referencing system; to find the correct reference style and resources for the journal you are submitting to, please visit our help center. Reference examples are found below, for more examples of citing other documents and general questions regarding the Harvard reference style, please refer to the Chicago Manual of Style.

In-text citations

- For works by a single author, include the surname, followed by the year
- For works by two authors, include both surnames, followed by the year
- For works by more than two authors, include only the surname of the first author followed by et al., followed by the year
- For humanities and social sciences articles, include the page numbers.

Reference examples

Article in a print journal Sondheimer, N., and Lindquist, S. (2000). Rnq1: an epigenetic modifier of protein function in yeast. Mol. Cell. 5, 163-172.

Article in an online journal Tahimic, C.G.T., Wang, Y., Bikle, D.D. (2013). Anabolic effects of IGF-1 signaling on the skeleton. Front. Endocrinol. 4:6. doi: 10.3389/fendo.2013.00006

Article or chapter in a book Sorenson, P. W., and Caprio, J. C. (1998). "Chemoreception," in The Physiology of Fishes, ed. D. H. Evans (Boca Raton, FL: CRC Press), 375-405.

Book Cowan, W. M., Jessell, T. M., and Zipursky, S. L. (1997). Molecular and Cellular Approaches to Neural Development. New York: Oxford University Press.

Abstract Hendricks, J., Applebaum, R., and Kunkel, S. (2010). A world apart? Bridging the gap between theory and applied social gerontology. Gerontologist 50, 284-293. Abstract retrieved from Abstracts in Social Gerontology database. (Accession No. 50360869)

Website World Health Organization. (2018). E. coli. https://www.who.int/news-room/fact-sheets/detail/e-coli [Accessed March 15, 2018].

Patent Marshall, S. P. (2000). Method and apparatus for eye tracking and monitoring pupil dilation to evaluate cognitive activity. U.S. Patent No 6,090,051. Washington, DC: U.S. Patent and Trademark Office.

Data Perdiguero P, Venturas M, Cervera MT, Gil L, Collada C. Data from: Massive sequencing of Ulms minor's transcriptome provides new molecular tools for a genus under the constant threat of Dutch elm disease. Dryad Digital Repository. (2015) http://dx.doi.org/10.5061/dryad.ps837

Theses and dissertations Smith, J. (2008) Post-structuralist discourse relative to phenomological pursuits in the deconstructivist arena. [dissertation/master's thesis]. [Chicago (IL)]: University of Chicago

Preprint Smith, J. (2008). Title of the document. Preprint repository name [Preprint]. Available at: https://persistent-url (Accessed March 15, 2018).

Vancouver reference style (numbered)

Many Frontiers journals use the numbered referencing system; to find the correct reference style and resources for the journal you are submitting to, please visit our help center.

Reference examples are found below, for more examples of citing other documents and general questions regarding the Vancouver reference style, please refer to Citing Medicine.

In-text citations

- Please apply the Vancouver system for in-text citations
- In-text citations should be numbered consecutively in order of appearance in the text – identified by Arabic numerals in the parenthesis (use square brackets for physics and mathematics articles).

Reference examples

Article in a print journal Sondheimer N, Lindquist S. Rnq1: an epigenetic modifier of protein function in yeast. Mol Cell (2000) 5:163-72.

Article in an online journal Tahimic CGT, Wang Y, Bikle DD. Anabolic effects of IGF-1 signaling on the skeleton. Front Endocrinol (2013) 4:6. doi: 10.3389/fendo.2013.00006

Article or chapter in a book Sorenson PW, Caprio JC. "Chemoreception". In: Evans DH, editor. The Physiology of Fishes. Boca Raton, FL: CRC Press (1998). p. 375-405.

Book Cowan WM, Jessell TM, Zipursky SL. Molecular and Cellular Approaches to Neural Development. New York: Oxford University Press (1997). 345 p.

Abstract Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, editor. Genetic Programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3–5; Kinsdale, Ireland. Berlin: Springer (2002). p. 182–91.

Website World Health Organization. E. coli (2018). https://www.who.int/news-room/fact-sheets/detail/e-coli [Accessed March 15, 2018].

Patent Pagedas AC, inventor; Ancel Surgical R&D Inc., assignee. Flexible Endoscopic Grasping and Cutting Device and Positioning Tool Assembly. United States patent US 20020103498 (2002).

Data Perdiguero P, Venturas M, Cervera MT, Gil L, Collada C. Data from: Massive sequencing of Ulms minor's transcriptome provides new molecular tools for a genus under the constant threat of Dutch elm disease. Dryad Digital Repository. (2015) http://dx.doi.org/10.5061/dryad.ps837

Theses and dissertations

Smith, J. (2008) Post-structuralist discourse relative to phenomological pursuits in the deconstructivist arena. [dissertation/master's thesis]. [Chicago (IL)]: University of Chicago

Preprint Smith, J. Title of the document. Preprint repository name [Preprint] (2008). Available at: https://persistent-url (Accessed March 15, 2018).