FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
DOUTORADO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO EM ENDODONTIA

LARISSA MAGNUS KLASSMANN

COMPARAÇÃO DOS EFEITOS DE DIFERENTES MEDICAÇÕES INTRACANAL NO REPARO DE LESÕES PERIAPICAIS EM RATOS

Porto Alegre

2013

LARISSA MAGNUS KLASSMANN

COMPARAÇÃO DOS EFEITOS DE DIFERENTES MEDICAÇÕES INTRACANAL NO REPARO DE LESÕES PERIAPICAIS EM RATOS

Tese apresentada ao Programa de Pós Graduação em Odontologia como parte dos requisitos para obtenção do Título de Doutor em Odontologia, Área de concentração em Endodontia.

Orientadora: Profª. Drª Maria Martha Campos

Porto Alegre

2013

Catalogação na Publicação

K63c Klassmann, Larissa Magnus

Comparação dos efeitos de diferentes medicações intracanal no reparo de lesões periapicais em ratos / Larissa Magnus Klassmann. – Porto Alegre, 2013.

93 p.

Tese (Doutorado) – Faculdade de Odontologia, PUCRS
Orientadora: Profª. Drª. Maria Martha Campos

1. Odontologia. 2. Periodontite. 3. Medicamentos. 4. Lesões Periapicais. 5. Hidróxidos. 6. Ratos – Experiências. I. Campos, Maria Martha. II. Título.

CDD 617.634

Bibliotecária responsável: Salete Maria Sartori, CRB 10/1363

Dedico esta Tese à minha família,

que com seu amor incondicional e torcida constantes,

são a base para meu crescimento

e amadurecimento profissional.

Agradecimentos Especiais

A **Deus** por iluminar meus caminhos, mostrar-me que tudo ocorre na hora certa e fortalecer minha fé, dando-me a certeza de que na vida tudo é possível, com força e determinação.

A minha mãe, **Regina**, pelo amor incondicional, conselhos e apoio constantes, pela educação, pelo incentivo ao estudo e ao crescimento profissional.

Ao meu irmão, **Kássio**, por participar de cada momento de minha vida, pelo incentivo e pelo ombro amigo, sempre disposto a me ajudar;

A meu padrasto, **Gilberto**, a quem carinhosamente chamo de "super pai" por toda dedicação, por todas "as missões executadas com êxito", pelo carinho e apoio;

A minha **Tia Beth**, por todo carinho e amor, por ser muito mais que minha tia e ser minha preferida, pelo apoio, incentivo e torcida constantes para meu crescimento profissional.

Ao meu namorado **Claudio** por me compreender, incentivar, apoiar, dar suporte na área de informática e por ser meu companheiro, amigo e amante.

Agradecimentos

A minha orientadora **prof. Dra. Maria Martha Campos** pela orientação do trabalho, pelo exemplo de vida e de profissional, pela compreensão, pela solidariedade nos momentos difíceis e pela bondade e dedicação infinitas.

Ao professor **Dr. José Antônio Poli de Figueiredo** pelo exemplo de profissionalismo, aprendizado, compreensão e conhecimento transmitido.

À professora **Adriana Etges** pela orientação, auxílio, acolhida e ensinamentos que foram fundamentais para conclusão deste trabalho.

Ao radiologista **Vinicius Dutra** por toda amizade, aprendizado e profissionalismo.

Ao **Instituto de Toxicologia e Farmacologia da PUCRS** (INTOX) e todos seus integrantes por toda acolhida, receptividade e auxilio.

À técnica de laboratório **Raquel Oliveira** por todo suporte, atenção e auxilio.

À secretária **Camile Dutra Ferreira** por todo auxilio e eficiência.

Aos **docentes** e ao **Programa de Pós Graduação em Odontologia** da Pontifícia Universidade Católica do Rio Grande do Sul por todo aprendizado e crescimento proporcionados.

À professora **Fernanda B. Morrone** pela atenção dispensada.

Aos bolsistas de iniciação científica **Luciana A. Zollmann, João Paulo Gonçalves** e **Patricia Oliboni de Bairros** pela colaboração e amizade.

Ao técnico de Laboratório **Juliano Soares** da Faculdade de Farmácia da PUCRS por todo auxilio e suporte durante os experimentos.

Às técnicas de Laboratório **Silvana e Ivana** da UFPEL por todo auxilio e colaboração na parte histológica.

Aos funcionários da secretaria de Programa de Pós Graduação em Odontologia da PUCRS **Ana Lúcia Silveira Prestes, Davenir Brusch, Marcos Corrêa e Paulo Silva** pela simpatia, cordialidade e atenção dispensadas durante a realização deste curso.

À **CAPES** pela bolsa de estudos que permitiu a conclusão deste doutorado e a realização deste sonho.

E a todos aqueles que deixei de citar mas que, de alguma forma, contribuíram para a realização deste trabalho.

"O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínimo fará coisas admiráveis."

José de Alencar

LISTA DE ABREVIATURAS

#Amoxicilina-AMX

#Ácido etilenodiamino tetra-acético-EDTA

#Espécies reativas a oxigênio-ROS

#Hematoxilina e Eosina-H&E

Hidróxido de Cálcio – $Ca(OH)_2$

#Resveratrol – RSVO

#Tomografia computadorizada Cone Beam – CBCT

#Fator de crescimento endotelial vascular - VEGF

SUMÁRIO

1. RESUMO

Na Endodontia, o preparo químico-mecânico produz uma redução significativa da quantidade de bactérias no interior do sistema de canais radiculares. Este procedimento pode ser complementado pelo uso de medicações intracanal, a fim de aumentar a possibilidade de sucesso da terapia endodôntica. O presente estudo teve como objetivo avaliar a efetividade de diferentes medicações intracanal, em esquemas isolados ou em associação, para o controle de lesões periapicais em ratos. Esta tese foi dividida em capítulos, contemplado as diferentes medicações estudadas e os artigos originados. No capítulo 1, foi enfatizada a metodologia utilizada, com aplicação da Tomografia Cone Beam (CBTC), como ferramenta para avaliar o reparo das lesões periapicais após terapia endodôntica e, curativo de demora, demonstrando a eficácia do exame para finalidade proposta. Testou-se o hidróxido de cálcio (lado direito) versus grupo controle negativo (lado esquerdo), sem medicação. Na avaliação com CBTC, a área da lesão no grupo controle foi de 9.38 ± 0.68 mm^2 e, no grupo tratado de 7.08 ± 0.44 mm^2, sendo esta diferença significativa ($p<0.05$). No capítulo 2, foi avaliado o efeito do antioxidante, resveratrol e, das medicações consideradas padrão-ouro em Endodontia, clorexidina e hidróxido de cálcio, em esquemas isolados ou em associações. Ademais, o resveratrol foi testado em conjunto com a vitamina E ou com o óleo de arroz. Neste artigo, as comparações entre grupos tratados e controle demonstraram diferença significativa ($p<0.05$) nos grupos em que foi utilizado hidróxido de cálcio, resveratrol em propilenoglicol e resveratrol com clorexidina. Estes grupos apresentaram uma redução de aproximadamente 25% em relação ao seu grupo controle, de acordo com avaliação por CBTC. Os efeitos das medicações foram confirmados por análise histológica qualitativa, tendo sido observado maior infiltrado inflamatório, além de áreas de reabsorção e supuração nos grupos controle. Já, no capítulo 3, foi avaliada ação local da amoxicilina na região periapical, quando utilizada de maneira isolada ou, em combinações. Neste estudo, a amoxicilina mostrou-se bastante

eficaz, tanto isoladamente, quanto em conjunto com hidróxido de cálcio ou clorexidina ($p<0.05$). As percentagens de redução foram de 47 ± 5%, para amoxicilina em propilenoglicol; 46 ± 5% para amoxicilina + gluconato de clorexidina e; 65 ± 10 % para amoxicilina + hidróxido de cálcio. Para confirmar os resultados da CBTC, foi realizada análise histológica qualitativa. Na coloração de H&E, o infiltrado inflamatório variou de leve a moderado nos grupos com medicação. No grupo controle, o infiltrado inflamatório foi classificado como intenso, com áreas de reabsorção. Na coloração de Mallory, os grupos teste mostraram grande quantidade de fibroblastos e fibras colágenas íntegras. Em contrapartida, o grupo controle apresentou infiltrado intenso, áreas de abscesso e supuração. Por fim, é apresentada uma discussão geral dos resultados, bem como, uma seção de considerações finais contemplando o desempenho das diversas medicações testadas e o significado do trabalho para a Endodontia. Conclui-se que a CBTC pode ser uma ferramenta importante para estudos pré-clínicos na área da endodontia, mostrando aplicação para determinação de alterações na região apical. Ademais, sugere-se que o agente antioxidante resveratrol ou o antibiótico amoxicilina podem ser alternativas efetivas como medicação intracanal.

2. ABSTRACT

In endodontics, the chemomechanical preparation produces a significant reduction in the amount of bacteria in the root canal system. It has been demonstrated that the adjuvant use of intracanal medications, might increase the possibility of success of endodontic therapy. The present study aimed to evaluate the effectiveness of different intracanal medications, when used alone or in combination, in a rat model of periapical lesions. This thesis was divided into three chapters, according to the scientific articles. The Chapter 1 emphasizes the methodology used, which employed Cone Beam Tomography to measure the repair of periapical lesions after endodontic therapy and root canal dressing, demonstrating the effectiveness of this tool. Calcium hydroxide was tested, versus control group, which had received no medication. The lesion area was 9.38 ± 0.68 mm^2 in the control group, and 7.08 ± 0.44 mm^2 in the treated group ($p<0.05$), according to CBTC evaluation**.** This data was confirmed by histological evaluation, indicating the applicability of CBTC in the rat model of apical periodontitis. The Chapter 2 evaluated the natural compound resveratrol, and the medications considered as gold standards in Endodontics, chlorhexidine and calcium hydroxide, in isolated schemes or in associations. The effects of resveratrol were also assessed in combination to vitamin E or rice oil, which also have known antioxidant effects. In this paper, the comparison between treated and control groups showed significant differences in the groups that received calcium hydroxide, resveratrol in propyleneglycol or resveratrol with chlorhexidine with inhibition percentages of about 25%. The effects of medications were confirmed by qualitative histological analysis, which showed higher inflammatory infiltrate, areas of resorption and suppuration in the control groups. The Chapter 3 shows data on the use of amoxicillin as intracanal medication, when used alone or in combined protocols. In this study, amoxicillin was highly effective, presenting significant differences when tested alone or in combination with calcium hydroxide or chlorhexidine.

Amoxicillin vehicled in propyleneglycol produced an inhibition of 47 ± 5% of periapical lesion areas, and a similar degree of reduction was observed in the group that received amoxicillin plus chlorhexidine gluconate (46 ± 5%). Notably, the combination of amoxicillin and calcium hydroxide paste resulted in a marked inhibition of periapical lesions (65 ± 10 %). Qualitative H&E histological analysis showed that inflammatory infiltrate ranged from mild to moderate in the test groups, and the control samples presented intense inflammatory infiltrate, with visible resorption areas. Regarding the Mallory staining, it was possible to observe a massive presence of fibroblasts, surrounded by intact and well-bonded collagen fibers. Otherwise, the control groups showed intense inflammatory infiltrate and areas of abscess. There is a final chapter with a general discussion and some specific considerations on the various strategies tested herein and the meaning of our study for Endodontics. In conclusion, the CBTC might represent an important tool for studies in pre-clinical endodontics, showing applicability for measuring changes in the apical region. Furthermore, either the antioxidant resveratrol, or the antibiotic amoxicillin, appear to be useful strategies as alternative intracanal dressings.

Introdução

O tratamento endodôntico tem a finalidade de reduzir os microorganismos e seus produtos do sistema de canais radiculares(1-3). As bactérias e as reações inflamatórias induzidas por seus produtos metabólicos, enzimas e, toxinas estão entre os fatores causadores das doenças endodônticas(3-7). Na Endodontia, o preparo químico-mecânico, através da limpeza, modelagem e irrigação, tem mostrado redução significativa da quantidade de bactérias no interior do sistema de canais radiculares; porém, estes procedimentos podem ser complementados pelo uso de medicações intracanal, para aumentarem a possibilidade de sucesso da terapia endodôntica(2-3, 5-6, 8). As razões para utilização das medicações intracanal são: a) reduzir ao máximo as bactérias no sistema de canais radiculares; b) prevenir a proliferação de microorganismos entre as consultas; e c) criar uma barreira físico-química, prevenindo a reinfecção do canal radicular e o suprimento nutritivo para os microorganismos remanescentes (3, 8-9).

Dentre as medicações intracanal existentes, o hidróxido de cálcio e a clorexidina são as mais utilizadas na Endodontia(3-7, 10-12). O hidróxido de cálcio possui ação antimicrobiana relacionada ao seu alto pH, o que resulta na inativação da membrana enzimática bacteriana, (1, 3, 5, 9, 12-14), embora esta medicação não seja totalmente eficaz na eliminação de microorganismos facultativos anaeróbios e leveduras(1, 3, 13). O gluconato de clorexidina tem sido recomendado tanto como solução irrigadora, como medicação intracanal, devido a sua forte atividade antibacteriana contra microorganismos gram-positivos e gram-negativos, assim como leveduras, anaeróbios facultativos e aeróbios (15-17). Entretanto, até o momento, a clorexidina não preenche os requisitos necessários para um "curativo de demora" satisfatório, particularmente quanto à inativação do LPS bacteriano (18), pelo fato de não possuir a habilidade de dissolução tecidual (3). Além disso, a concentração de 2% tem sido considerada tóxica aos tecidos vivos (18).

Deve-se considerar que, até o momento, não existe uma medicação intracanal ideal. Assim, pesquisas sobre novas medicações são necessárias e a utilização de substâncias naturais isoladas ou em combinação com medicações já consagradas pode ser uma alternativa para o aprimoramento dos curativos de demora em endodontia (1, 3-4, 7, 19-20). O resveratrol é um agente antioxidante, que possui diversas propriedades biológicas descritas na literatura e, já é utilizado em algumas áreas da Medicina. Entretanto, a ação desta substância na Odontologia e, mais especificamente na Endodontia, ainda não foi estudada. Além disso apesar de ser utilizada sistemicamente na Odontologia, não há estudos que tenham investigado a eficácia da amoxicilina como medicação intracanal. Assim, justifica-se este trabalho pelo fato de não terem sido encontradas na literatura, evidências que comprovem que em endodontia há uma medicação intracanal considerada totalmente satisfatória.

Revisão de Literatura

4. Revisão de Literatura

4.1 CONSIDERAÇÕES GERAIS

O tratamento endodôntico é direcionado ao controle das infecções pulpares e perirradiculares. O envolvimento dos microrganismos nas patogêneses da polpa, bem como nas doenças perirradiculares, já foi demonstrado por KAKEHASHI (1965), MOLLER (1981) e SUNDQVIST (1992), sendo que a eliminação dos microrganismos do sistema de canais radiculares envolve o uso de várias técnicas de instrumentação, irrigação e medicação intracanal(21-24). Dada a relevância dos microrganismos para patogênese das periodontites apicais, torna-se claro que o sucesso do tratamento endodôntico, depende da redução ou eliminação destes agentes agressores (14). A instrumentação mecânica sozinha, porém, não resulta em eliminação completa dos microrganismos do sistema de canais radiculares, fato também justificado pela complexidade anatômica do referido sistema(22). Neste contexto, o uso da medicação intracanal tem sido indicado para complementar a redução do número de microrganismos do sistema de canais radiculares (25).

Na terapia endodôntica, o preparo químico-mecânico é essencial para a desinfecção radicular; contudo, a medicação intracanal é utilizada para complementar a redução de bactérias remanescentes (14, 22). A medicação intracanal tem vantagens e limitações, sendo estas últimas relacionadas com a presença de microrganismos residuais, os quais poderão interferir na cicatrização ou no desenvolvimento de periodontite apical, levando, assim, à falha do tratamento Endodôntico (20). A medicação intracanal, portanto, é utilizada como auxiliar na desinfecção endodôntica, buscando a redução ou eliminação das bactérias localizadas no interior do sistema de canais radiculares, criando um ambiente favorável ao reparo dos tecidos periapicais.

A periodontite apical pode ser definida como um processo inflamatório dos tecidos perirradiculares, provavelmente causada pela presença de microorganismos no interior do

sistema de canais radiculares, onde os anaeróbios são considerados os agentes etiológicos primários (11). No tratamento endodôntico, diversas substâncias químicas têm sido utilizadas para controlar os microrganismos encontrados no interior do sistema de canais radiculares, a fim alcançar a resolução do processo (26).

Dentre as substâncias químicas utilizadas como medicação intracanal, destacam-se como mais efetivas e melhor caracterizadas, o hidróxido de cálcio e a clorexidina (7, 11, 13-14, 25, 27-28).

4.2 HIDRÓXIDO DE CÁLCIO

O hidróxido de cálcio é uma das medicações mais versáteis em Odontologia, especialmente por seu uso como medicação intracanal, tanto em dentes vitais, quanto não vitais, sendo a medicação de escolha para casos que incluem procedimentos de apexificação, reabsorção dentária interna e reparo de perfurações (27). O hidróxido de cálcio é uma substância alcalina, com pH aproximado de 12,5. Em solução aquosa, se dissocia em íons hidroxila, apresentando diversas propriedades biológicas, tais como: atividade antimicrobiana, capacidade de dissolução tecidual, inibição da reabsorção dentária e indução do reparo pela formação tecidual (14). Tem sido demonstrado que os patógenos presentes nos canais radiculares não são capazes de sobreviver no ambiente alcalino criado pelo hidróxido de cálcio. A atividade antimicrobiana desta substância estaria relacionada à liberação de íons hidroxila, levando à morte das células bacterianas por pelo menos três mecanismos: (a) dano à membrana citoplasmática bacteriana, (b) dano ao DNA e/ou, (c) desnaturação proteica (14). Entretanto, para que fosse totalmente efetiva, esta medicação intracanal deveria ocupar todo espaço do sistema de canais radiculares, o que não acontece de fato (27). Assim, a ação antimicrobiana do hidróxido de cálcio depende diretamente da disponibilidade, difusão e velocidade de dissociação dos íons cálcio e hidroxila dentro do canal radicular (2).

Deve-se ressaltar que a pasta de hidróxido de cálcio tem-se mostrado biocompatível e efetiva em dentes com periodontite apical. Esta pasta pode ser preparada com diferentes veículos, tais como água destilada, propilenoglicol, Otosporin®, entre outros, Entretanto, não há evidências definitivas acerca da existência de um sinergismo antimicrobiano entre o veículo e o hidróxido de cálcio (14).

O hidróxido de cálcio, como medicação intracanal, é indicado para tratamento de dentes com necrose pulpar, por possuir ação antisséptica, capacidade de estimular ou criar condições favoráveis para o reparo tecidual, além de possuir elevada ação higroscópica, fato que o torna indicado para tratamento de lesões periapicais de longa duração, possivelmente por apresentar atividade anti-inflamatória (29). Salienta-se também que alguns estudos têm investigado as associações de hidróxido de cálcio com substâncias antimicrobianas, como clorexidina, eritromicina, clindamicina e tetraciclina (30-31) sobre bactérias do gênero Enterococcus, obtendo resultados satisfatórios. Entretanto, até o momento, não foram encontradas evidências concretas que justifiquem o uso do hidróxido de cálcio associado à (a) outra (s) substância (s) (27). Assim, são necessários novos estudos dentro dessa linha de pesquisa.

4.3. CLOREXIDINA

A clorexidina é uma biguanida catiônica, largamente utilizada na periodontia, que age sobre um amplo número de microrganismos, como bactérias gram-positivas e gram-negativas. Tem sido demonstrado que esta substância parece agir adsorvendo a parede celular dos microrganismos, o que resulta em extravasamento dos componentes intracelulares (2, 11, 16, 27). A eficácia do gluconato de clorexidina pode ser explicada pela interação entre suas cargas positivas e, as cargas negativas dos grupos fosfato da parede celular bacteriana (27).

O gluconato de clorexidina foi introduzido na terapia endodôntica com o objetivo de aumentar a ação antibacteriana das medicações intracanais consideradas tradicionais,

buscando a eliminação dos microrganismos associados às infecções persistentes e aos casos de insucesso (7). Quando utilizada como medicação intracanal, a clorexidina tem-se mostrado mais efetiva que o hidróxido de cálcio contra a infecção causada por *Enterococcus faecallis* nos túbulos dentinários (3, 10-11, 16, 32). Finalmente, é importante ressaltar que a clorexidina possui atividade reduzida em presença de matéria orgânica e, assim como o hidróxido de cálcio, apresenta certa dificuldade de remoção do sistema de canais radiculares(1, 3, 6, 10).

4.4. AGENTES ANTIMICROBIANOS

As bactérias estão diretamente envolvidas nas patologias endodônticas, sendo cabível considerar que os antibióticos podem ser úteis no manejo dos sintomas dessas afecções (33). A utilização de medicação intracanal contendo antibióticos pode ser importante para o tratamento de infecções persistentes, já que antibióticos como a clindamicina, quando utilizados sistemicamente, são eficazes no tratamento de infecções agudas, abcessos e *flare-ups* (34). Ressalta-se também que dentre os antibióticos, a amoxicilina tem sido utilizada sistemicamente, como tratamento coadjuvante em infecções endodônticas agudas, por ser este um antibiótico eficaz contra *F. nucleatum e F .necrophorum*, microrganismos frequentemente relacionados com infecções endodônticas primárias (35).

Na periodontite apical, por não haver polpa e, consequentemente, suprimento sanguíneo, as concentrações dos antibióticos sistêmicos que alcançam o canal radicular são mínimas. A ação local de antibióticos como, amoxicilina, clindamicina e doxiciclina sobre os microrganismos tem sido demonstrada apenas *in vitro* (20, 36-39). Estudos utilizando técnicas de biologia molecular demonstraram que a aplicação de amoxicilina (25 mg/ml), clindamicina (15 mg/ml), tetraciclina (25 mg/ml) ou doxiciclina (10 mg/ml) produziu culturas negativas de *E. faecalis*, quando estes antibióticos foram incubados *in vitro* (40). Contudo, não há evidências conclusivas acerca dos efeitos da aplicação local destes agentes *in vivo*.

4.5. RESVERATROL

O resveratrol é conhecido desde a década de 40, época em que foi isolado de diversas plantas, apresentando vários efeitos benéficos (41). É um polifenol encontrado nas uvas, no vinho tinto e em alguns grãos, possuindo propriedades anti-inflamatórias, efeito cardioprotetor, baixa toxicidade e uma série de ações biológicas, incluindo propriedades quimiopreventivas. (42-45). Destaca-se ainda que o resveratrol mostrou-se efetivo em modelos de câncer, além de apresentar ações positivas sobre o metabolismo(41).

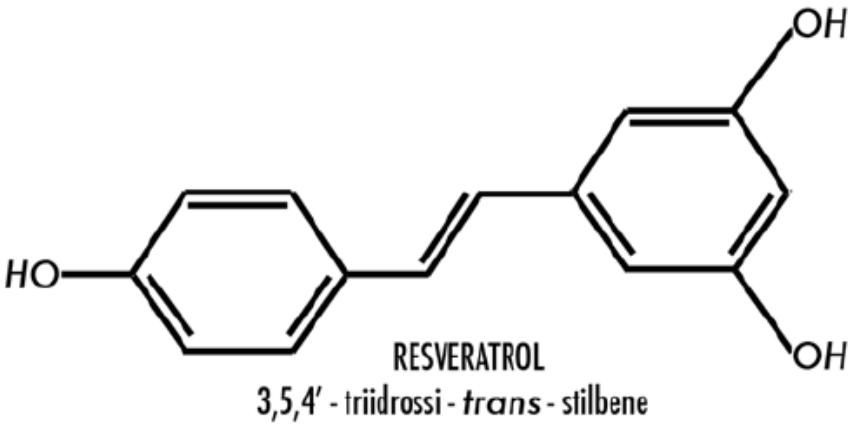

FIGURA 1: Estrutura química do Resveratrol.
Fone: Cucciolla et all (2007)[45]

Ressalta-se que a interferência positiva do resveratrol já foi relacionada a eventos importantes, tais como: (a) radioproteção, (b) radiosensibilidade, (c) supressão da mutagênese, (d) imunomodulação, (e) angiogênese, entre outros (45).

Salienta-se que o resveratrol é conhecido há muito tempo na medicina oriental, sendo utilizado pelos japoneses e chineses para o tratamento de doenças inflamatórias, alérgicas e da aterosclerose (46).

Estudos *in vivo* com o resveratrol demonstraram que esta substância possui efeito quimiopreventivo e antitumoral em animais(45) sendo foi mostrado também que o resveratrol diminui os níveis de lipídeos no soro sanguíneo, diminui a agregação plaquetária e aumenta os níveis de HDL, o qual ajuda a remover o colesterol LDL do sangue e a prevenir a obstrução das artérias (46). Ressalta-se que os efeitos do resveratrol já foram demonstrados em modelos animais de doenças inflamatórias crônicas, tais como artrite e pancreatite (43) tendo sido igualmente evidenciado que este composto atua sobre o sistema celular, interferindo no crescimento, diferenciação e morte celular (45), além de apresentar efeitos anti-virais, anti-fúngicos e anti-bacterianos (42-46). De forma interessante, na Odontologia, o resveratrol se apresentou efetivo contra o crescimento de *Porphyromonas gingivalis,* um patógeno envolvido no inicio e na progressão da doença periodontal (44). Entretanto, não há evidências diretas acerca dos efeitos do resveratrol sobre infecções endodônticas.

Assim, diante de todos estes efeitos promissores do Resveratrol constados na literatura, pensou-se em testar o seu efeito como medicação intracanal utilizando o mesmo localmente, no tratamento da periodontite apical. Já que a Endodontia carece de um curativo de demora ideal, a união de substâncias naturais com medicações já consagradas pode representar uma alternativa promissora, como já discutido anteriormente.

Objetivos

5. OBJETIVOS

5.1. OBJETIVO GERAL

O presente estudo teve por objetivo avaliar a efetividade de diferentes medicações intracanais, em esquemas isolados ou em associação, para o controle de lesões periapicais em ratos.

5.2. OBJETIVOS ESPECÍFICOS

a) Avaliar a aplicação da tomografia cone beam (CBTC) como ferramenta para a determinação de lesões periapicais em ratos e da eficácia de medicações intracanal;

b) Comparar os efeitos do hidróxido de cálcio e da clorexidina em gel, isolados ou em associação com diferentes substâncias no reparo de lesões periapicais em ratos;

c) Avaliar os efeitos do composto natural, resveratrol, como medicação intracanal para o controle de lesões periapicais em ratos, em comparação com os outros agentes avaliados no estudo;

d) Avaliar os efeitos do antibiótico amoxicilina, como medicação intracanal de uso local para o controle de lesões periapicais em ratos, em comparação com os outros agentes avaliados no estudo.

Capítulo 1

Use of cone bean tomography to evaluate intracanal medications in a rat model of apical periodontitis

Aceito para publicação na Revista Odontociência em 06 novembro de 2012.

Use of cone bean tomography to evaluate intracanal medications in a rat model of apical periodontitis

Uso da Tomografia Cone Beam para avaliação de Medicação Intracanal em Modelo de Periodontite Apical em Ratos

[1]Larissa Magnus, [2]Vinicius Dutra, [3]Lisiane Bernardi,[1,4]Maria Martha Campos

[1]Postgraduate Program in Dentistry, PUCRS; [2]Radiology Service, Mãe de Deus Hospital; [3] Postgraduate Program in Dentistry,UFRGS; [4]Institute of Toxicology and Pharmacology, PUCRS.

Corresponding Author: Larissa Magnus Klassmann, [1]Postgraduate Program in Dentistry, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, Partenon, 90619-900, Porto Alegre, Brazil. Phone number: 55 51 3320 3562; Fax number: 55 51 3320 3626.

E-mail: larissamagnus@hotmail.com; Larissa.klassmann@acad.pucrs.br

Summary

The aim of this study was to describe a methodology for evaluating the efficacy of intracanal medications in rats, by using cone beam computed tomography (CBCT) to follow-up periapical lesions. Six male Wistar rats were used and periapical lesions were induced in 12 upper molars. The endodontic treatment was performed in all teeth and calcium hydroxide paste was applied in the right molars (treated group, n=6), whereas no medication was used in left molars (control group, n=6). CBCT was performed twenty one days after endodontic treatment, and the lesion area was determined in mm^2. Unpaired Student *t* test was employed to verify the differences between groups. The lesion area was 9.38 ± 0.68 mm^2 in the control group, and 7.08 ± 0.44 mm^2 in the treated group ($p<0.05$), according to CBTC evaluation**.** This data was confirmed by histological evaluation of maxillas, indicating the applicability of CBTC in the rat model of apical periodontitis. The rat model and CBCT was found to be a useful tool to study *in vivo* the effects intracanal medications and their influence in periapical lesions healing.

Key words: Apical Periodontitis- Intracanal Medication – Cone Beam – Rats

RESUMO

O objetivo deste estudo foi descrever uma metodologia para avaliar a eficácia das medicações intracanal em ratos, utilizando a Tomografia Cone Beam (CBCT) para acompanhar as lesões periapicais. Seis ratos Wistar machos foram utilizados e lesões apicais induzidas em 12 molares superiores. O tratamento Endodôntico foi realizado em todos os dentes e pasta de Hidróxido de Cálcio foi aplicada nos molares do lado direito (grupo teste, n=6) enquanto que nenhuma medicação foi utilizada nos molares esquerdos (grupo controle, n=6). CBCT foi realizada vinte e um dias após tratamento endodôntico, e a área da lesão foi determinada em mm^2. Teste t Student não pareado foi empregado para verificar diferenças entre os grupos. A área da lesão era de 9.38 ± 0.68 mm^2 no grupo controle e 7.08 ± 0.44 mm^2 no grupo tratado ($p<0.05$) de acordo com a avaliação da CBTC. Estes dados foram confirmados pela avaliação histológica das maxilas, indicando a aplicabilidade da CBTC no modelo de periodontite apical em ratos. O modelo de rato e a CBCT mostraram-se uma ferramenta útil para estudo in vivo dos efeitos das medicações intracanal e suas influências na cicatrização das lesões periapicais.

Palavras chave:Periodontite Apical- Medicação Intracanal-Cone Beam-Ratos

Introduction

Apical periodontitis is an inflammatory process affecting the periradicular tissues, caused by microbial presence into the root canal system (1-2). The elimination of microorganisms from infected canals involves the use of mechanical and chemical approaches(3-5). In this regard, intracanal medications are currently employed to disinfect the root canal system, in order to prevent bacterial proliferation between endodontic therapy appointments (4-6). Calcium hydroxide has been widely used as a routine intracanal medication(7). In the clinical practice, this chemical agent is pointed out as one of the most effective antimicrobial dressings during the endodontic therapy(7-8).

It has been demonstrated that cone beam computed tomography (CBCT) produces three-dimensional images of individual tooth and surrounding tissues (9-12). Another favorable aspect is the great volume of data that can be collected and processed, by using CBCT (10, 12). Other advantage, is the possibility of performing the exams *in vivo* using its 3-dimensional capabilities, including follow-up and longitudinal studies (9, 12).The potential applications in endodontics include diagnosis of endodontic pathologies and canal morphology, assessment of alterations of non-endodontic origin, evaluation of root fractures and trauma, analysis of external and internal root resorption and invasive cervical resorption, as well as pre-surgical planning (9).

Considering the abovementioned evidence, the present study describes a methodology for evaluating the efficacy of intracanal medications in rats, by using CBCT technology to follow-up the periapical lesions. We have also attempted to correlate the results obtained in vivo by CBCT, with those observed post-mortem by histological assessment.

Material and methods

Animals

Six male Wistar rats (220-250g) were used in this study. The animals were maintained in controlled temperature (22 ± 2 ºC) and humidity (60 - 70 %), under a 12 h light-dark cycle. Food and water were available *ad libitum*. All animal procedures were performed according to the "Principles of Laboratory Animal Care" from NIH publication No. 85-23 and Ethical Guidelines for Investigation of Experimental Pain in Conscious Animals. The Institutional Animal Ethics Committee approved all the experimental protocols. The number of animals was the minimum necessary to demonstrate the consistent effects of the drug treatments. The sample size calculation was based on previous studies (13-14) considering a statistic power of 95% and a level of significance of 5%.

Induction of periapical lesions

Periapical lesions were induced in 12 upper molars, as described previously (13, 15). Briefly, the animals were anesthetized by a mixture of ketamine (100mg/kg) and xylazine (10 mg/kg), given by i.p. route. Pulp exposure was performed at the distal fossa of the right and the left maxillary first molars, by using a 1011 diamond round bur (VortexIndústria e Comércio de Ferramentas Diamantadas Ltda – São Paulo, Brazil). The exposed pulps were left open to the oral environment for twenty one days. The Figure 1 provides a representative image of periapical lesion, in a non-treated tooth.

Endodontic treatment

After 21 days of pulp exposure, the animals were anesthetized as described before, and the endodontic treatment was performed in the right and left maxillary first molars. The exploration of the root canals was done with a 6-type K file (Dentsply Maillefer, São Paulo, Brazil). The working length was established at 3 mm, according to the anatomy of the rat upper first molars. The technique consisted of manual instrumentation from the 6-type to the

20-type K file (all 21-mm long). The change of each instrument was performed under 0.2 ml sodium hypochlorite irrigation. At the end of instrumentation, the smear layer was removed by the application of 0.2 ml of EDTA (ASFER Indústria Química,São Caetano do Sul, São Paulo, Brazil) followed by irrigation with 0.2 ml of sodium hypochlorite 1% (ASFER Indústria Química,São Caetano do Sul, São Paulo, Brazil). Calcium hydroxide (Pasta Calen, S.S White Duflex, Rio de Janiero, Brazil) was used as intracanal medication into the right molars, whereas the left molar received no medication, and was used as the negative control group. Teeth were restored with glass ionomer (Vidrion R, S.S White Duflex, Rio de Janiero, Brazil). The rats were maintained for an additional period of 21 days, in order to determine the effectiveness of the intracanal dressing.

Cone Beam Tomography

For the tomography procedures, the animals were re-anesthetized and positioned in a modified cage equipped with an adaptor for the head, to permit the standardization of image takings. A 3D i-CAT tomography apparatus (Imaging Sciences, Hatfield, PA, USA) was used, and the images (0.2 mm voxel size) were analyzed by using the equipment's software. A radiologist who was blinded to the groups performed the measurements. The area (length x height) of periapical lesions was provided in mm^2.

Histological analysis

After tomography procedures, euthanasia was performed by deep inhalation of isofluorane. Immediately after, the maxillas were removed and placed in plastic bottles containing 10% formaldehyde in 0.1 M phosphate buffer, for subsequent decalcification and histological processing. The histological slides were stained with (a) Hematoxylin & Eosin (HE) to verify the inflammatory infiltrate or with (b) Mallory to observe collagen fibers.

Histological analysis was performed to complement and reinforce the results of CBTC for checking intracanal medication effects.

Statistical analysis

The results are presented as the mean ± standard error mean of 6 animals (left and right superior molars). Histological results were described in a qualitative manner. The statistical comparison of the data was performed by using the unpaired Student *t* test. *P*-values smaller than 0.05 ($P < 0.05$) were considered significant.

Results

All the specimens developed periapical lesions, with areas ranging from 5.8 to 8.4 mm^2 in the calcium hydroxide-treated teeth, and from 7.9 to 12.1 mm^2, in the non-treated control side, according to assessment by CBCT. The Figure 2 shows a representative image, as captured by the CBCT apparatus. The mean (± SEM) lesion area was 9.38 ± 0.68 mm^2 in the control group, and 7.08 ± 0.44 mm^2 in the calcium-hydroxide-treated group. Most samples in the control group showed a bursting of the cortical layer, an event that was not verified in the treated group (Figure 3). The comparison between the lesion areas of control and treated groups revealed a significant difference ($p<0.05$; unpaired Student *t* test). On the basis of CBCT analysis, the calcium hydroxide dressing produced a reduction of about 25% in relation to the non-treated control group (Figure 4). Histological analysis reinforced the CBTC findings, confirming the effectiveness of calcium hydroxide in our experimental protocol. From the histological analysis, it was possible to observe: 1) fewer inflammatory cells and well-organized tissue in the treated group, in comparison to the controls; 2) the presence of collagen fibers present, offering support and organized connection between periapical tissues

and endodontium in teeth that had receive medication; and 3) control teeth, without dressing, presented visible destruction and lack of continuity of collagen fibers (Figure 5).

Discussion

Several advantages of CBCT have been pointed out in recent literature, especially the rapidity of measurements (9-12). In this regard, depending on the number and the size of samples, CBCT data acquisition and evaluation can be accomplished in a short period of time. Additionally, data collected is readily available for further evaluation and analysis in different planes. We wondered whether research involving endodontic treatment in rats and CBCT technology might be useful for this area of dentistry. Literature search revealed forty five articles regarding the use of CBCT in rodent models, but none of them was related to endodontics. The present study was aimed at describing the use of CBCT technology to follow-up the extension of periapical lesions and the efficacy of intracanal medications *in vivo*.

The induction of periapical lesions in rats has been widely described in the literature (13-14, 16-19). In this experimental model, the lesion areas are commonly estimated by radiographic analysis, providing two-dimensional images (13). In our study, a three-dimensional image of tooth and surrounding tissues was produced, what indicates an obvious advantage in the use of CBCT technology to evaluate periapical lesions, by providing more accurate results. Of note, the use of CBCT apparatus permits taking the images when the animal is alive, allowing multiple observations. On the other hand, in studies employing conventional radiographs (13), taking good quality images depends on rat euthanasia and maxillary dissection.

The limitations of conventional radiographic examination to evaluate the presence of apical periodontitis are related to the amount of bone loss caused by lesion, the spread of bone

resorption into the cortical bone, and operator variability in radiographic interpretation (20). For these reasons, the use of CBCT for assessment of periapical healing might be satisfactory.

One inconvenience of using animals for *in vivo* studies in endodontics is that the anatomy of the apices is different from apical structures in humans (13, 21). Nevertheless, research using animal models is rather relevant to improve the current knowledge on endodontics. In our paper, we have demonstrated that it is possible to analyze the effects of intracanal medications, in a rat model of periapical lesions, by using CBCT. This model allowed determining the effectiveness of calcium hydroxide in the resolution of apical periodontitis. Evaluation by CBCT demonstrated a reduction of about 25% in the lesion area in the treated groups. This might be considered a satisfactory outcome as most apical lesions are still radiographically evident 1 year after treatment (20, 22-24), and 3 to 4 years might be required to truly evaluate healing (20, 25).

In the clinical practice of endodontics, histological analysis cannot be performed, but this methodology provides important information about tissue healing in animal models. In this work, the histological evaluation was mainly employed to certify the validity of CBTC for analysis of endodontic lesions. The histology assessment confirmed tomography results showing less inflammatory infiltrate and better cellular organization in the test group, according to H&E staining. The microscopy also showed that the test group presented better organized collagen fibers, indicating a healthier endodontium, with integrity of the supporting tissues, as observed form Mallory-colored sections.

In conclusion, the methodology described for endodontic treatment in rats with the use of CBCT technology sounds rather appropriate and showed efficacy to be used in further studies for analyzing innovative intracanal medications, and to the *in vivo* follow-up of periapical lesions

References

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The Effects of Surgical Exposures of Dental Pulps in Germ-Free and Conventional Laboratory Rats. Oral Surg Oral Med Oral Pathol. 1965 Sep;20:340-9.

2. Moller AJ, Fabricius L, Dahlen G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res. 1981 Dec;89(6):475-84.

3. Gondim JO, Avaca-Crusca JS, Valentini SR, Zanelli CF, Spolidorio DM, Giro EM. Effect of a calcium hydroxide/chlorhexidine paste as intracanal dressing in human primary teeth with necrotic pulp against Porphyromonas gingivalis and Enterococcus faecalis. Int J Paediatr Dent. 2012 Mar;22(2):116-24.

4. Panzarini SR, Trevisan CL, Brandini DA, Poi WR, Sonoda CK, Luvizuto ER, et al. Intracanal dressing and root canal filling materials in tooth replantation: a literature review. Dent Traumatol. 2012 Feb;28(1):42-8.

5. Taneja S, Kumari M. Use of triple antibiotic paste in the treatment of large periradicular lesions. J Investig Clin Dent. 2012 Feb;3(1):72-6.

6. Riccitiello F, Stabile P, Amato M, Rengo S, D'Ambrosio C. The treatment of the large periradicular endodontic injury. Minerva Stomatol. 2011 Sep;60(9):417-26.

7. Sathorn C, Parashos P, Messer H. Antibacterial efficacy of calcium hydroxide intracanal dressing: a systematic review and meta-analysis. Int Endod J. 2007 Jan;40(1):2-10.

8. Basrani B, Tjaderhane L, Santos JM, Pascon E, Grad H, Lawrence HP, et al. Efficacy of chlorhexidine- and calcium hydroxide-containing medicaments against Enterococcus faecalis in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003 Nov;96(5):618-24.

9. Cotton TP, Geisler TM, Holden DT, Schwartz SA, Schindler WG. Endodontic applications of cone-beam volumetric tomography. J Endod. 2007 Sep;33(9):1121-32.

10. Kim TS, Caruso JM, Christensen H, Torabinejad M. A comparison of cone-beam computed tomography and direct measurement in the examination of the mandibular canal and adjacent structures. J Endod. 2010 Jul;36(7):1191-4.

11. Michetti J, Maret D, Mallet JP, Diemer F. Validation of cone beam computed tomography as a tool to explore root canal anatomy. J Endod. 2010 Jul;36(7):1187-90.

12. Patel S, Dawood A, Ford TP, Whaites E. The potential applications of cone beam computed tomography in the management of endodontic problems. Int Endod J. 2007 Oct;40(10):818-30.

13. Lee YL, Hong CY, Kok SH, Hou KL, Lin YT, Chen MH, et al. An extract of green tea, epigallocatechin-3-gallate, reduces periapical lesions by inhibiting cysteine-rich 61 expression in osteoblasts. J Endod. 2009 Feb;35(2):206-11.

14. Sobrinho AP, Barros MH, Nicoli JR, Carvalho MA, Farias LM, Bambirra EA, et al. Experimental root canal infections in conventional and germ-free mice. J Endod. 1998 Jun;24(6):405-8.

15. Metzger Z, Klein H, Klein A, Tagger M. Periapical lesion development in rats inhibited by dexamethasone. J Endod. 2002 Sep;28(9):643-5.

16. Alshwaimi E, Purcell P, Kawai T, Sasaki H, Oukka M, Campos-Neto A, et al. Regulatory T cells in mouse periapical lesions. J Endod. 2009 Sep;35(9):1229-33.

17. Fouad A, Barry J, Russo J, Radolf J, Zhu Q. Periapical lesion progression with controlled microbial inoculation in a type I diabetic mouse model. J Endod. 2002 Jan;28(1):8-16.

18. Garlet TP, Fukada SY, Saconato IF, Avila-Campos MJ, da Silva TA, Garlet GP, et al. CCR2 deficiency results in increased osteolysis in experimental periapical lesions in mice. J Endod. 2010 Feb;36(2):244-50.

19. Wagner C, Barth VC, Jr., de Oliveira SD, Campos MM. Effectiveness of the proton pump inhibitor omeprazole associated with calcium hydroxide as intracanal medication: an in vivo study. J Endod. 2011 Sep;37(9):1253-7.

20. Burgener B, Ford AR, Situ H, Fayad MI, Hao JJ, Wenckus CS, et al. Biologic markers for odontogenic periradicular periodontitis. J Endod. 2010 Aug;36(8):1307-10.

21. Holland R, Mazuqueli L, de Souza V, Murata SS, Dezan Junior E, Suzuki P. Influence of the type of vehicle and limit of obturation on apical and periapical tissue response in dogs' teeth after root canal filling with mineral trioxide aggregate. J Endod. 2007 Jun;33(6):693-7.

22. Penesis VA, Fitzgerald PI, Fayad MI, Wenckus CS, BeGole EA, Johnson BR. Outcome of one-visit and two-visit endodontic treatment of necrotic teeth with apical periodontitis: a randomized controlled trial with one-year evaluation. J Endod. 2008 Mar;34(3):251-7.

23. Trope M, Delano EO, Orstavik D. Endodontic treatment of teeth with apical periodontitis: single vs. multivisit treatment. J Endod. 1999 May;25(5):345-50.

24. Waltimo T, Trope M, Haapasalo M, Orstavik D. Clinical efficacy of treatment procedures in endodontic infection control and one year follow-up of periapical healing. J Endod. 2005 Dec;31(12):863-6.

25. Peters LB, Wesselink PR. Periapical healing of endodontically treated teeth in one and two visits obturated in the presence or absence of detectable microorganisms. Int Endod J. 2002 Aug;35(8):660-7.

Figure 1

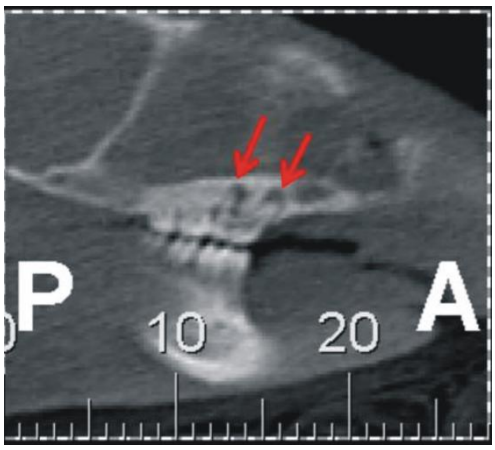

Figure 1. CBCT showing apical periodontitis in rat superior untreated molar (arrow).

Figure 2

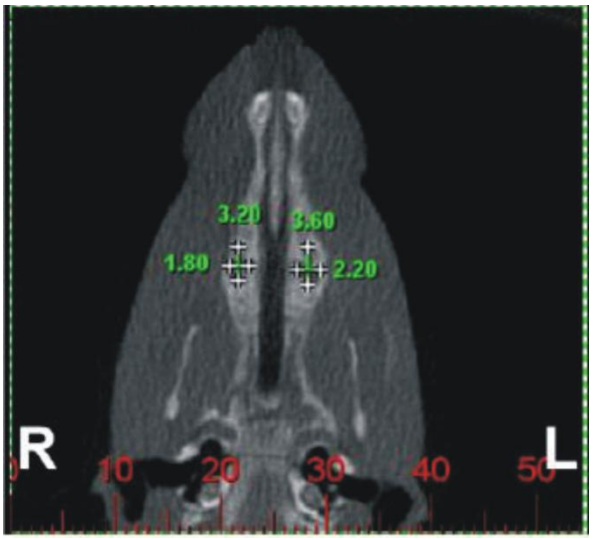

Figure 2. Representative CBCT showing the apical lesion area in the control (left side) and the treated group (right side).

Figure 3

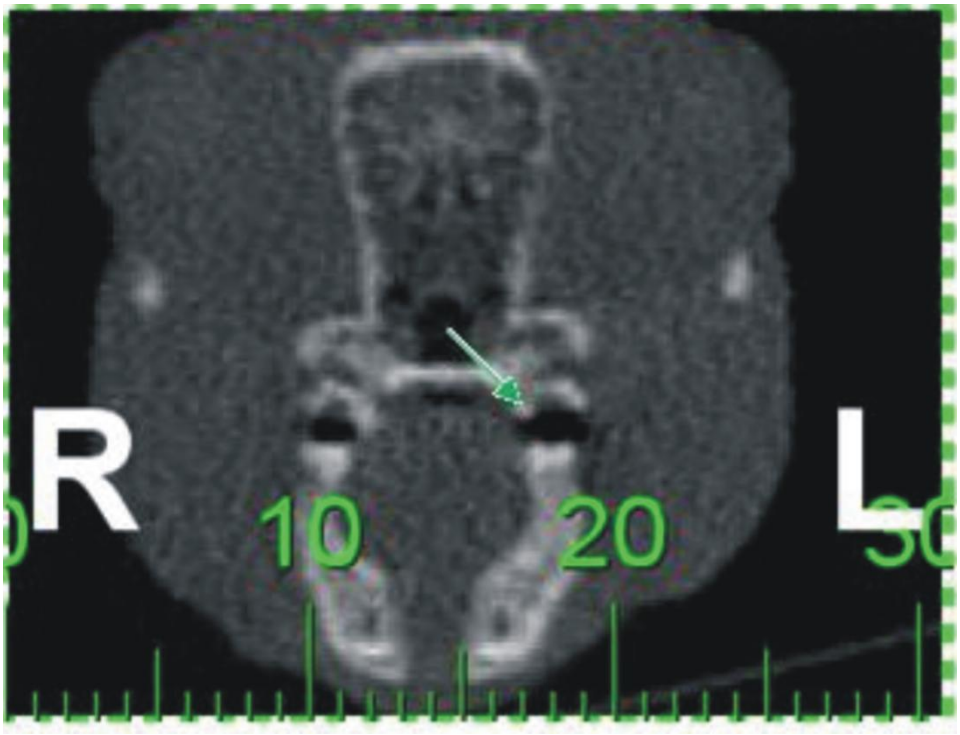

Figure 3. Representative CBCT showing bursting of cortical in the control (left side, as indicated by an arrow), but not in the treated group (right group).

Figure 4

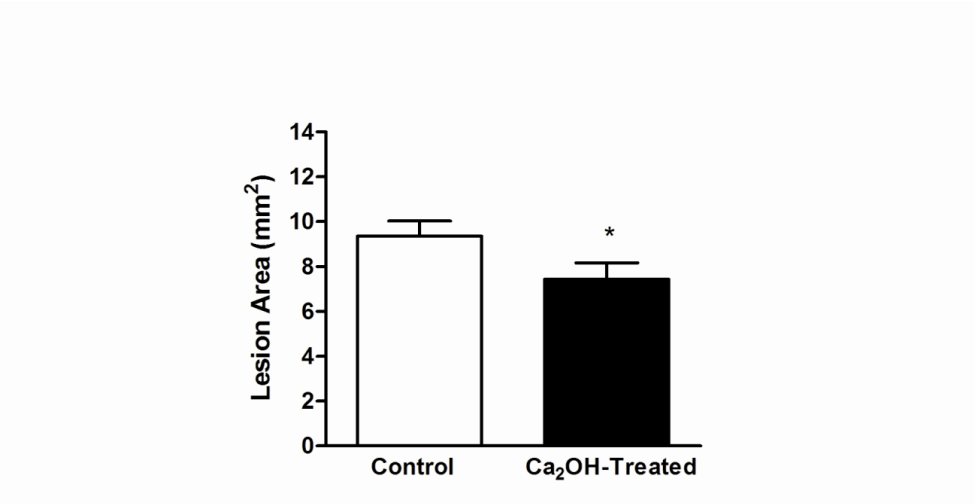

Figure 4. Lesion areas (in mm^2) as determined by CBCT, in control (left upper molars) and calcium hydroxide-treated (right upper molars) groups. Each column represents the mean of 6 samples and the vertical lines show the SEM. *P<0.05 denotes the significance levels in comparison to control values.

Figure 5

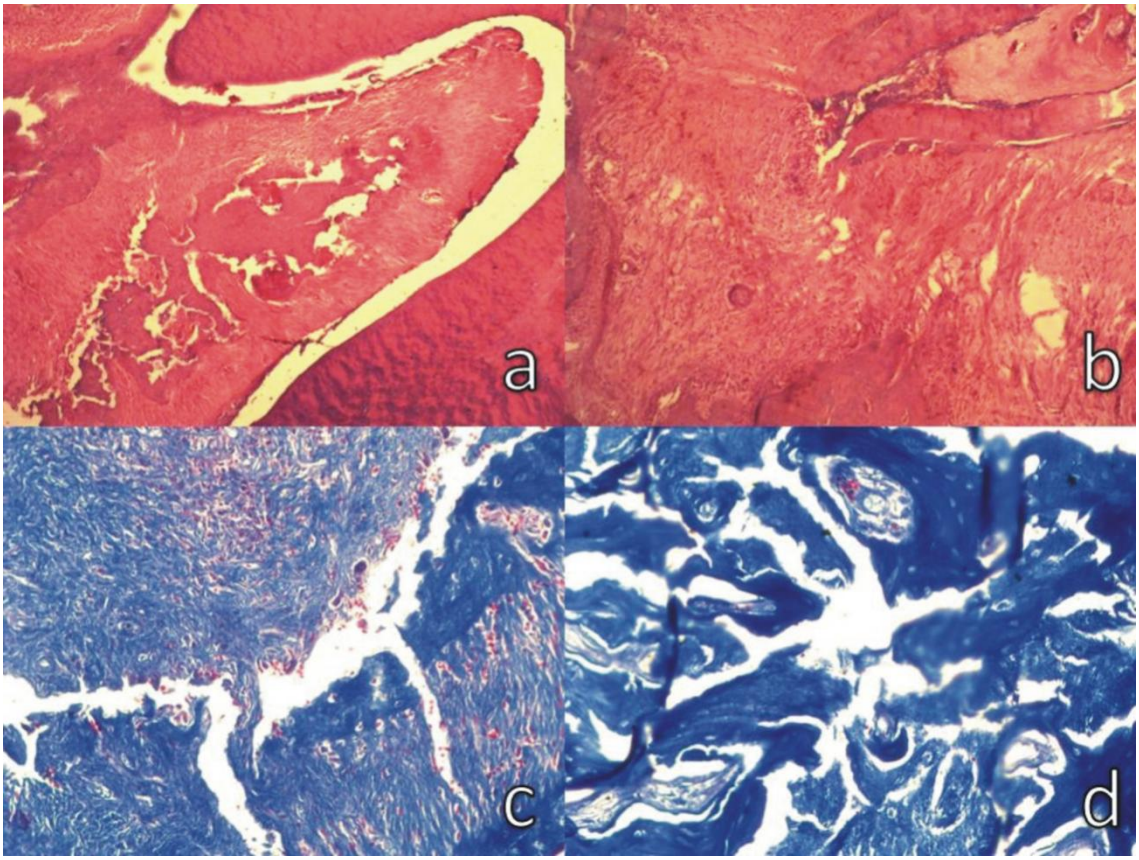

Figure 5. Optical microscopy showing fewer and well-organized inflammatory infiltrate in the treated group (a), when compared to the control teeth (b). Teeth that received intracanal medication displayed more collagen fibers, offering support and organized connection between periapical tissues and endodontium (c); control teeth, without dressing, presented destruction and lack of continuity of collagen fibers (d).He (a and b)-10x ; Mallory (d and c) - 40x.

Capítulo 2

Efficacy of different formulations containing the antioxidant compound resveratrol as intracanal medication in a rat model of periapical lesions

Submetido ao Periódico Journal of Endodontics

Efficacy of different formulations containing the antioxidant compound resveratrol as intracanal medication in a rat model of periapical lesions

[1]Larissa Magnus, [2]Fabiana Moresco , [3]João Paulo Gonçalves, [4]Vinicius Dutra, [5]André Arigony Souto, [1,3]Maria Martha Campos

[1]Postgraduate Program of Dental College, Pontifical Catholic University of Rio Grande do Sul, PUCRS, Porto Alegre, RS, Brazil; [2]Stomatology Municipal Service, Imbé, RS, Brazil; [3]Institute of Toxicology and Pharmacology, PUCRS. [4]Radiology Service, Mãe de Deus Hospital, Porto Alegre, RS, Brazil. [5]Laboratory of Polyphenols, Faculty of Chemistry, PUCRS, Porto Alegre, RS, Brazil.

Corresponding Author: Maria Martha Campos, School of Dentistry, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, Partenon, 90619-900, Porto Alegre, Brazil. Phone Number: 55 51 3320 3562; Fax Number: 55 51 3320 3626; E-mail: camposmmartha@yahoo.com; maria.campos@pucrs.br

Acknowledgments

J.P.G. held undergraduate grants from the Institutional BPA Program (PUCRS). L.M. is a PhD student in Dentistry supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). We would like to thank Mr. Juliano Soares for his excellent technical assistance. This study was financially supported by CNPq, PUCRS, and the FINEP research grant “Implantação, Modernização e Qualificação de Estrutura de Pesquisa da PUCRS” (PUCRSINFRA) # 01.11.0014-00. The authors deny any conflicts of interest related to this study.

Abstract

Objectives*:** The purpose of this study was to evaluate the profile of different formulations containing the antioxidant agent resveratrol, as intracanal medications in a rat model of periapical lesions. ***Methods: Forty two male Wistar rats were used. Periapical lesions were induced in the first upper molars of both sides. After 21 days, the endodontic instrumentation was performed in the right and left maxillary first molars. Intracanal medication was applied into the right molars, whereas the left molar received no medication, and it was used as the negative control group. The medications were: Group 1: calcium hydroxide paste; Group 2: 2% chlorhexidine gluconate; Group 3: resveratrol + propyleneglycol; Group 4: calcium hydroxide paste + resveratrol; Group 5: 2% chlorhexidine gluconate + resveratrol; Group 6: resveratrol + rice oil; Group 7: resveratrol + vitamin E + propyleneglycol. After an additional period of 21 days, both tomography and histological analysis were performed. ***Results:*** The comparison between the lesion areas of control and treated groups revealed a significant difference in the Groups (1) calcium hydroxide, (3) resveratrol + propyleneglycol and (5) 2% chlorhexidine gluconate + resveratrol. The Groups 1, 3 and 5 showed a reduction of about 25% in relation to the respective non-treated control group, according to tomography evaluation. The effects of these medications were confirmed by qualitative histological analysis. ***Conclusions:*** It is tempting to suggest that resveratrol could be introduced to the endodontics therapeutic arsenal, especially as an alternative to the currently available options. Our study demonstrates, for the first time, that antioxidant agents can be effective when used locally, as components of intracanal medications.

Keywords: resveratrol, calcium hydroxide paste, 2% chlorhexidine gluconate, periapical lesions, rats.

Introduction

The endodontic treatment aims to eradicate or to reduce the amount of microorganisms in the root canal systems and to promote the healing of periapical tissues(2-4). The diseases affecting the pulp and the periapical tissues are mainly caused by bacteria and their metabolic products, enzymes or toxins that induce inflammation in endodontium (2, 5). The preparation of the root canal system through irrigation, shaping and cleaning significantly reduces the amount of bacteria in the root canal, and this process can be successfully improved by the use of intracanal medications (2, 6). The reasons for the use of intracanal medications are (a) to improve the elimination of bacteria in root canals; (b) to prevent bacterial proliferation between appointments, and (c) to provide a physiochemical barrier, preventing the root canal re-infection and nutrient supply to the remaining microorganisms (2, 4, 6).

Among the available intracanal dressings, calcium hydroxide and chlorhexidine gluconate are the most used agents in the endodontic practice (7-8). Calcium hydroxide has antimicrobial actions related to its high pH, which results in the inactivation of bacterial membrane enzymes (2, 4, 9-11), although it is insufficient in the elimination of facultative anaerobes and yeasts (2, 11). Chlorhexidine gluconate has been recommended as both irrigation solution and intracanal medication due to its good antibacterial activity against bacteria and yeast (12). However, chlorhexidine does not have the ability to dissolve tissues, and removing gels containing chlorhexidine from the canal spaces can represent a complex procedure (2).

Therefore, the search for new intracanal medications is patent and the use of natural substances alone or in combination with currently available substances could be a very attractive alternative for endodontic dressings (2-3, 5, 13-14). Resveratrol (3,5,4'-trihydroxystilbene), a well-known antioxidant ingredient found in red wine, is a polyphenolic phytoaxelin produced by grapes, mulberries and peanuts (15-16). This compound has a

variety of biological properties, such as anti-inflammatory, analgesic, immunomodulatory, cardioprotective and chemopreventive activities (15, 17-20). The impact of resveratrol in dentistry has been studied in vascular inflammation and vascular endothelial factor (VEGF) expression, induced by periodontal pathogens (19, 21). Furthermore, it was demonstrated recently that resveratrol is able to reduce the production of inflammatory cytokines in human periodontal ligament cells, stimulated with LPS from *Porphyromonas gingivalis* (22). However, the action of this substance in endodontics has not yet been reported. Following this basis, the purpose of this study was to evaluate the profile of resveratrol as an intracanal medication in periapical lesions in rats. Special attempts have been made in order to assess the effects of resveratrol when combined to the classical intracanal dressings, calcium hydroxide or chlorhexidine gluconate, or even to test it in new formulations, containing other active principles such as vitamin E and rice oil.

Material and Methods

Animals

Forty two male Wistar rats (220-250g) were used in this study. The animals were maintained in controlled temperature (22 ± 2 ºC) and humidity (60 - 70 %), under a 12 h light-dark cycle. Food and water were available *ad libitum*. All animal procedures were performed according to the "Principles of Laboratory Animal Care" from NIH publication No. 85-23 and Ethical Guidelines for Investigation of Experimental Pain in Conscious Animals. The Institutional Animal Ethics Committee approved all the experimental protocols (Approval number Nº 051/09). The number of animals was the minimum necessary to demonstrate the consistent effects of the intracanal treatments. The sample size calculation was based on previous studies (23-24) considering a statistic power of 95% and a level of significance of 5%, what indicated a number of six animals per experimental group.

Induction of periapical lesions

Periapical lesions were induced in the first upper molars, as described previously (23-24). Briefly, the animals were anesthetized by a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg), given by i.p. route. Pulp exposure was performed at the distal fossae of the right and the left maxillary first molars, by using a 1011 diamond round bur. The exposed pulps were left open to the oral cavity for 21 days, to allow the lesion formation.

Endodontic treatment procedures

After 21 days of pulp exposure, the animals were anesthetized as described before, and the endodontic instrumentation was performed in the right and left maxillary first molars, according to the method described previously (25-26). The exploration of the root canals was done with an 8-type K file. The working length was established at 3 mm, according to the anatomy of the rat upper first molars. The technique consisted of manual instrumentation from the 8-type to the 20-type K file (all 21-mm long). The change of each instrument was performed under 200-µl sodium hypochlorite irrigation. At the end of instrumentation, the smear layer was removed by the application of 200 µl of EDTA, followed by irrigation with 200 µl of sodium hypochlorite. Intracanal medication was applied into the right molars, whereas the left molar received no medication, and it was used as the negative control group. The medications were:

Group 1: calcium hydroxide paste;

Group 2: 2% chlorhexidine gluconate;

Group 3: resveratrol + propyleneglycol;

Group 4: calcium hydroxide paste + resveratrol;

Group 5: 2% chlorhexidine gluconate + resveratrol;

Group 6: resveratrol + rice oil;

Group 7: resveratrol + vitamin E + propyleneglycol.

Teeth were restored with glass ionomer (Vidrion R, SS White Duflex, RJ, Brazil). The rats were maintained for an additional period of 21 days, in order to determine the effectiveness of the intracanal dressings.

Cone Beam Tomography

The areas of periapical lesions were determined by means of cone beam tomography, as described before (26). Briefly, the animals were re-anesthetized as described above, and positioned in a modified cage equipped with an adaptor for the head, to permit the standardization of image takings. A 3D i-CAT tomography apparatus (Imaging Sciences, Hatfield, PA, USA) was used, and the images (0.2 mm voxel size) were analyzed by using the equipment's software. A radiologist who was blinded to the experimental groups performed the measurements. The area (length x height) of periapical lesions was provided in mm^2.

Histological analysis

After tomography procedures, euthanasia was performed by deep inhalation of isofluorane. Immediately after, the maxillae were removed and placed in recipients containing 10% formaldehyde in 0.1 M phosphate buffer, for subsequent decalcification and histological processing. The histological slides were stained with Hematoxylin & Eosin (HE) to assess the inflammatory infiltrate. Histological analysis was performed to complement the results of cone beam tomography, in order to confirm the effects of intracanal medications. Two slides of each tooth were analyzed by a pathologist blinded to the experimental groups. Histological data is provided in a qualitative manner.

Statistical analysis

The results from tomography are presented as the mean ± standard error mean of 6 animals (left and right superior molars). Histological results were described in a qualitative manner. The statistical comparison of the lesion areas was performed by using Student's *t* test. *P*-values less than 0.05 ($P < 0.05$) were considered significant.

Results

All the specimens analyzed developed periapical lesions. Figure 1 shows a representative image of the periapical lesion as it is acquired after cone beam computed tomography. The panel A shows a general view of the tomography images, whereas the panel B reveals a lateral prospect of the periapical lesions. In the panel C, it is possible to observe the periapical lesion areas in the left and the right first molars, as calculated by using i-Cat software, from a specimen obtained from the resveratrol group (Group 3).

The comparison between the lesion areas of control and treated groups revealed a significant difference in the Groups (1) calcium hydroxide paste, (3) resveratrol + propyleneglycol, and (5) 2% chlorhexidine gluconate + resveratrol (Figures 2A, 2C and 2E) when compared to the control teeth. On the basis of tomography analysis, the Groups 1, 3 and 5 displayed a reduction of about 25% in relation to the respective non-treated control group. The Group 4, which was treated with calcium hydroxide paste + resveratrol presented a partial, but not significant reduction of the lesion extent (Figure 2D), whereas 2% chlorhexidine gluconate alone (Group 2) failed to significantly modify the periapical lesion area, at the conditions tested in our study (Figure 2B). To gain further insights on the effects of resveratrol when associated with other antioxidant products, we have also evaluated the outcome of periapical lesions, when using resveratrol plus rice oil (Group 6), or resveratrol

plus vitamin E (Group 7). Both associations failed to significantly affect the periapical lesions, in comparison to control teeth.

The histological analysis reinforced the tomography findings, confirming the effectiveness of intracanal medications in the groups that received (1) calcium hydroxide paste, (3) resveratrol + propyleneglycol, or (5) 2% chlorhexidine gluconate + resveratrol as intracanal dressings (Figure 3, Panels A, B and C), when compared to control teeth (Figures 3F, 3G and 3H, respectively). These intracanal medications produced a visible reduction of inflammatory infiltrate, with reduced areas of suppuration in relation to their respective control groups.

Discussion

Intracanal medications have been considered valuable tools to support the preparation of the root canal system, in an attempt to improve the elimination of the microorganisms. Despite the antimicrobial properties of the chemomechanical preparation and intracanal dressings, the elimination of microorganisms may not be uniform due to different reasons, such as (a) the varying vulnerability of the microorganism species; (b) the anatomical complexity of root canals; or (c) the limited access by instruments or irrigants (5, 10, 12, 24).

In the Endodontic practice, calcium hydroxide and chlorhexidine gluconate represent the most used intracanal medications (2, 5, 10, 12, 24, 27-29). Nevertheless, calcium hydroxide is insufficient in the elimination of both facultative anaerobes and yeasts (2, 11), whilst chlorhexidine gluconate does not have the ability to dissolve tissues, and gels containing chlorhexidine cannot be easily removed from the canal spaces (2). In this context, innovative research in this area is highly necessary, and the use of antioxidant substances appears to be a reasonable alternative. For instance, it was previously demonstrated that antioxidant agents, such as propolis (13), green tea extract and its related compounds (23) or

tempol (30) displayed a significant reduction of periapical lesions, when administered systemically to rats.

In this study, we tested, for the first time, the effects of the antioxidant compound resveratrol as intracanal medication *in vivo*, and we obtained rather satisfactory results. A previous publication indicated that resveratrol was able to inhibit the adherence of the anaerobe bacteria *P. gingivalis in vitro* (19). Additionally, resveratrol was able to reduce the production of cytokines or VEGF expression induced by periodontal pathogens *in vitro* (21-22). Furthermore, several studies conducted in other research areas demonstrated that resveratrol exerts multiple health-promoting effects such as anti-inflammatory, anti-tumoral, anti-aging and anti-atherogenic effects (15-18), sustaining our results.

Herein, we tested the hypothesis that anti-inflammatory effects of resveratrol might help in the repair of periapical lesions. In fact, resveratrol was able to significantly reduce the extension of periapical lesions, when vehicled in either propyleneglycol or chlorhexidine, whereas it failed to promote periapical lesion healing when mixed with calcium hydroxide, or the anti-oxidant substances rice oil or vitamin E. As expected, in our study, calcium hydroxide displayed satisfactory results when tested alone. However, its association with resveratrol did not induce further significant diminishment of periapical lesions. This can be attributed to several reasons, but it can be explained by possible chemical interactions between the two substances.

The association of resveratrol with rice oil and vitamin E also failed to effectively reduce the length of periapical lesions. Vitamin E is a lipophilic antioxidant agent that can prevent the oxidative damage, and showed good results related to anti-inflammatory effects (31-36)**.** Thus, we expected an increased effectiveness of resveratrol when associated to vitamin E, since both have antioxidant and anti-inflammatory effects. On the basis of our results, there was no significant effect for that association. Additional studies using the

proposed combination of antioxidant substances are still needed. The rice bran oil contains high levels of antioxidant components, which could be useful for topical formulations(37), being recommended for treatment of hyperlipoproteinemia in humans (38). It is also used by cosmetic industry in sunscreen formulations, in topical aging-prevention products, and for treating skin diseases (37). This substance also attenuates hyperglycemia in patients with diabetes mellitus (38). Thus, it was supposed that rice oil could be an effective vehicle for resveratrol in our experimental paradigm. The Group 6, which received resveratrol plus rice oil as intracanal dressing, had a mean lesion area of 7.62 mm^2, whereas the control value was 8.17 mm^2. This difference was no statistically significant, but we might infer that prolonged times of treatment with this association would result in a better outcome. In addition, rice oil could be useful as vehicle for calcium hydroxide, which has been demonstrated to provide better results when mixed to oily vehicles (7, 11).

Concerning the use of resveratrol as intracanal medication, we might propose that this antioxidant compound might well be used as an alternative to the classical available intracanal medications, such as calcium hydroxide and chlorhexidine. As an advantage, resveratrol has been approved by the regulatory agencies for cosmetic and nutritional applications, presenting low adverse effects even when used systemically. It is tempting to suggest that resveratrol and other antioxidant agents could be added to Endodontics therapeutic arsenal.

References

1. Liu D, Lu F, Qin G, Fernandes SM, Li J, Davis AE, 3rd. C1 inhibitor-mediated protection from sepsis. J Immunol 2007;179(6):3966-3972.

2. Evans MD, Baumgartner JC, Khemaleelakul SU, Xia T. Efficacy of calcium hydroxide: chlorhexidine paste as an intracanal medication in bovine dentin. J Endod 2003;29(5):338-339.

3. LeCorn DW, Vertucci FJ, Rojas MF, Progulske-Fox A, Belanger M. In vitro activity of amoxicillin, clindamycin, doxycycline, metronidazole, and moxifloxacin against oral Actinomyces. J Endod 2007;33(5):557-560.

4. Siqueira JF, Jr., Rocas IN, Riche FN, Provenzano JC. Clinical outcome of the endodontic treatment of teeth with apical periodontitis using an antimicrobial protocol. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106(5):757-762.

5. Kontakiotis EG, Tsatsoulis IN, Papanakou SI, Tzanetakis GN. Effect of 2% chlorhexidine gel mixed with calcium hydroxide as an intracanal medication on sealing ability of permanent root canal filling: a 6-month follow-up. J Endod 2008;34(7):866-870.

6. Vianna ME, Horz HP, Conrads G, Zaia AA, Souza-Filho FJ, Gomes BP. Effect of root canal procedures on endotoxins and endodontic pathogens. Oral Microbiol Immunol 2007;22(6):411-418.

7. Kim SK, Kim YO. Influence of calcium hydroxide intracanal medication on apical seal. Int Endod J 2002;35(7):623-628.

8. Wang CS, Arnold RR, Trope M, Teixeira FB. Clinical efficiency of 2% chlorhexidine gel in reducing intracanal bacteria. J Endod 2007;33(11):1283-1289.

9. El Karim I, Kennedy J, Hussey D. The antimicrobial effects of root canal irrigation and medication. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103(4):560-569.

10. Gomes BP, Montagner F, Berber VB, Zaia AA, Ferraz CC, de Almeida JF, et al. Antimicrobial action of intracanal medicaments on the external root surface. J Dent 2009;37(1):76-81.

11. Sathorn C, Parashos P, Messer H. Antibacterial efficacy of calcium hydroxide intracanal dressing: a systematic review and meta-analysis. Int Endod J 2007;40(1):2-10.

12. Gomes BP, Vianna ME, Sena NT, Zaia AA, Ferraz CC, de Souza Filho FJ. In vitro evaluation of the antimicrobial activity of calcium hydroxide combined with chlorhexidine gel used as intracanal medicament. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102(4):544-550.

13. Ferreira FB, Torres SA, Rosa OP, Ferreira CM, Garcia RB, Marcucci MC, et al. Antimicrobial effect of propolis and other substances against selected endodontic pathogens. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104(5):709-716.

14. Ferguson JW, Hatton JF, Gillespie MJ. Effectiveness of intracanal irrigants and medications against the yeast Candida albicans. J Endod 2002;28(2):68-71.

15. Pirola L, Frojdo S. Resveratrol: one molecule, many targets. IUBMB Life 2008;60(5):323-332.

16. Sebai H, Ben-Attia M, Sani M, Aouani E, Ghanem-Boughanmi N. Protective effect of resveratrol in endotoxemia-induced acute phase response in rats. Arch Toxicol 2009;83(4):335-340.

17. El-Mowafy AM, Alkhalaf M. Resveratrol activates adenylyl-cyclase in human breast cancer cells: a novel, estrogen receptor-independent cytostatic mechanism. Carcinogenesis 2003;24(5):869-873.

18. Liang YC, Tsai SH, Chen L, Lin-Shiau SY, Lin JK. Resveratrol-induced G2 arrest through the inhibition of CDK7 and p34CDC2 kinases in colon carcinoma HT29 cells. Biochem Pharmacol 2003;65(7):1053-1060.

19. Park HJ, Jeong SK, Kim SR, Bae SK, Kim WS, Jin SD, et al. Resveratrol inhibits Porphyromonas gingivalis lipopolysaccharide-induced endothelial adhesion molecule expression by suppressing NF-kappaB activation. Arch Pharm Res 2009;32(4):583-591.

20. Bazzo KO, Souto AA, Lopes TG, Zanin RF, Gomez MV, Souza AH, et al. Evidence for the Analgesic Activity of Resveratrol in Acute Models of Nociception in Mice. J Nat Prod 2012.

21. Nunez MJ, Novio S, Balboa J, Seoane J, Suarez JA, Freire-Garabal M. Effects of resveratrol on expression of vascular endothelial growth factor in human gingival fibroblasts stimulated by periodontal pathogens. Acta Odontol Scand 2010;68(4):239-247.

22. Rizzo A, Bevilacqua N, Guida L, Annunziata M, Romano Carratelli C, Paolillo R. Effect of resveratrol and modulation of cytokine production on human periodontal ligament cells. Cytokine 2012;60(1):197-204.

23. Lee YL, Hong CY, Kok SH, Hou KL, Lin YT, Chen MH, et al. An extract of green tea, epigallocatechin-3-gallate, reduces periapical lesions by inhibiting cysteine-rich 61 expression in osteoblasts. J Endod 2009;35(2):206-211.

24. Metzger Z, Klein H, Klein A, Tagger M. Periapical lesion development in rats inhibited by dexamethasone. J Endod 2002;28(9):643-645.

25. Wagner C, Barth VC, Jr., de Oliveira SD, Campos MM. Effectiveness of the proton pump inhibitor omeprazole associated with calcium hydroxide as intracanal medication: an in vivo study. J Endod 2011;37(9):1253-1257.

26. Magnus, Larissa; Dutra, Vinícius; Bernardi, Lisiane; Campos, Maria Martha. Use of cone bean tomography to evaluate intracanal medications in a rat model of apical periodontitis Revista Odontociência 2012;Accept for publiccation in November, 2012.

27. Gomes BP, Souza SF, Ferraz CC, Teixeira FB, Zaia AA, Valdrighi L, et al. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against Enterococcus faecalis in bovine root dentine in vitro. Int Endod J 2003;36(4):267-275.

28. Manzur A, Gonzalez AM, Pozos A, Silva-Herzog D, Friedman S. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: a randomized clinical trial. J Endod 2007;33(2):114-118.

29. Paquette L, Legner M, Fillery ED, Friedman S. Antibacterial efficacy of chlorhexidine gluconate intracanal medication in vivo. J Endod 2007;33(7):788-795.

30. Brilhante Wolle CF, de Aguiar Zollmann L, Etges A, Vitalis GS, Leite CE, Campos MM. Effects of the antioxidant agent tempol on periapical lesions in rats with doxorubicin-induced cardiomyopathy. J Endod 2012;38(2):191-195.

31. Jiang XC, Tall AR, Qin S, Lin M, Schneider M, Lalanne F, et al. Phospholipid transfer protein deficiency protects circulating lipoproteins from oxidation due to the enhanced accumulation of vitamin E. J Biol Chem 2002;277(35):31850-31856.

32. Brenes A, Viveros A, Goni I, Centeno C, Sayago-Ayerdy SG, Arija I, et al. Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. Poult Sci 2008;87(2):307-316.

33. Naito Y, Yoshikawa T, Matsuyama K, Yagi N, Kasai K, Sugimoto N, et al. Effect of vitamin E in gastric mucosal injury induced by ischaemia-reperfusion in nitric oxide-depleted rats. Aliment Pharmacol Ther 1999;13(4):553-559.

34. Rizzo MR, Abbatecola AM, Barbieri M, Vietri MT, Cioffi M, Grella R, et al. Evidence for anti-inflammatory effects of combined administration of vitamin E and C in older persons with impaired fasting glucose: impact on insulin action. J Am Coll Nutr 2008;27(4):505-511.

35. Schneider M, Verges B, Klein A, Miller ER, Deckert V, Desrumaux C, et al. Alterations in plasma vitamin E distribution in type 2 diabetic patients with elevated plasma phospholipid transfer protein activity. Diabetes 2004;53(10):2633-2639.

36. Yoshida N, Yoshikawa T, Manabe H, Terasawa Y, Kondo M, Noguchi N, et al. Vitamin E protects against polymorphonuclear leukocyte-dependent adhesion to endothelial cells. J Leukoc Biol 1999;65(6):757-763.

37. Bernardi DS, Pereira TA, Maciel NR, Bortoloto J, Viera GS, Oliveira GC, et al. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. J Nanobiotechnology 2011;9:44.

38. Chou TW, Ma CY, Cheng HH, Chen YY, Lai MH. A rice bran oil diet improves lipid abnormalities and suppress hyperinsulinemic responses in rats with streptozotocin/nicotinamide-induced type 2 diabetes. J Clin Biochem Nutr 2009;45(1):29-36.

FIGURES

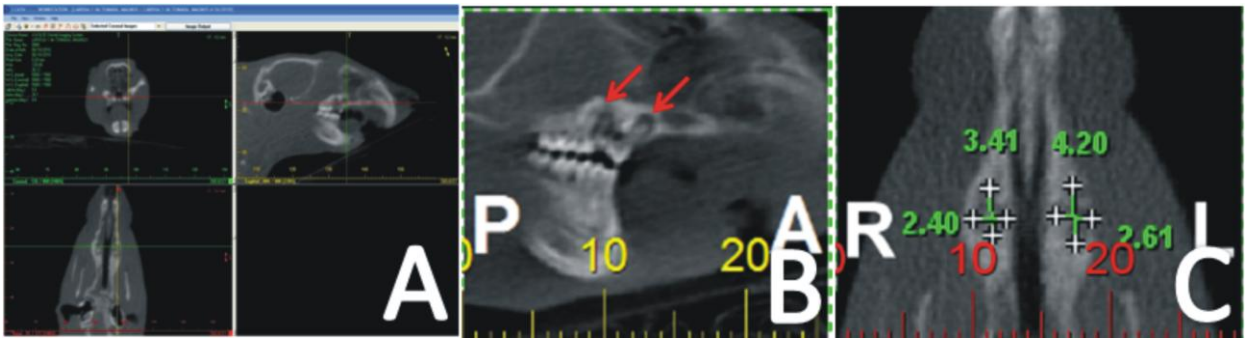

Figure 1. Representative image of cone beam tomography. Panel A shows an overall image as acquired using the program i-Cat vision. Panel B shows a lateral view of apical lesions (arrows). Panel C demonstrates the method used for calculating the periapical lesion areas of resveratrol-treated tooth (right side) and the respective control tooth (left side).

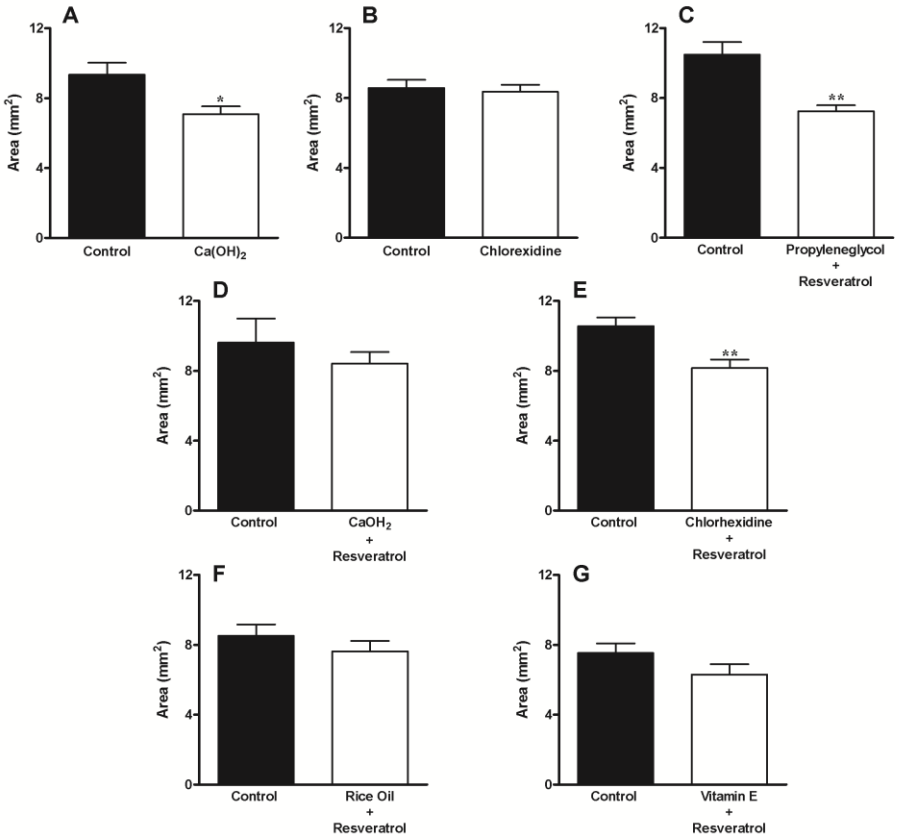

Figure 2. Comparison of the effects of calcium hydroxide paste (Panel A), 2% chlorhexidine gluconate (Panel B), resveratrol vehicled in propyleneglycol (Panel C), resveratrol vehicled in calcium hydroxide paste (Panel D), resveratrol vehicled in 2% chlorhexidine gluconate (Panel E), resveratrol vehicled in rice oil (Panel F), or resveratrol vehicled in vitamin E (Panel F), on the size of periapical lesions in rats: tomography analysis. The columns represent the mean of 6 experiments and the vertical lines indicate the standard error mean. *P<0.05; **P<0.001.

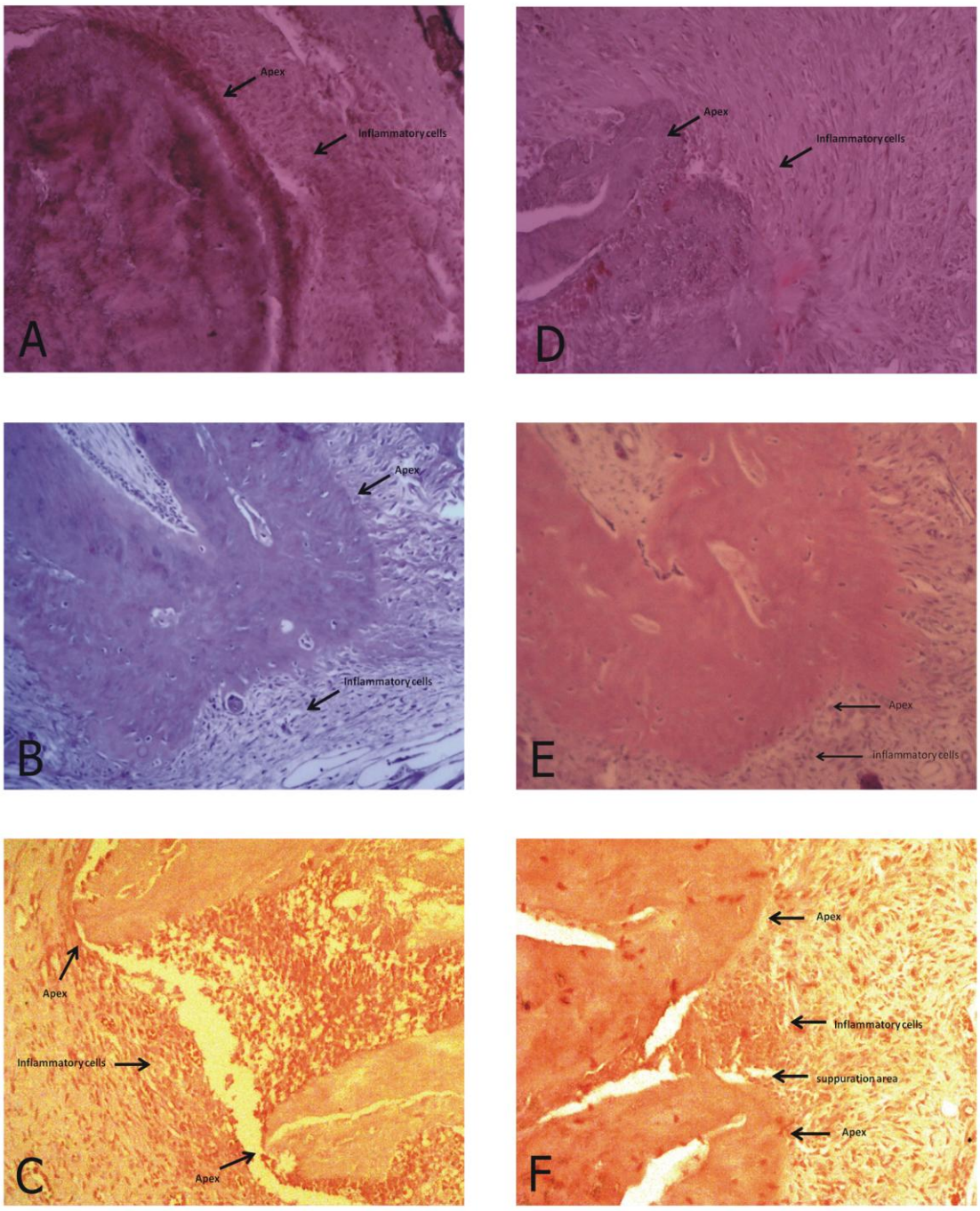

Figure 3. Microscopy slides stained with H&E (100x). Reduced inflammatory cell infiltration in the experimental groups: calcium hydroxide paste (Panel A), resveratrol vehicled in propyleneglycol (Panel B), resveratrol vehicled in 2% chlorhexidine gluconate (Panel C). Marked periapical bone resorption associated with chronic inflammation and suppuration in the corresponding control groups (Panels D, E and F). The arrows indicate the evaluated parameters and the anatomical areas.

Capítulo 3

Evaluation of amoxicillin as a component of intracanal medication in a rat model of apical periodontitis

Submetido ao Periódico Journal of Endodontics

Evaluation of amoxicillin as a component of intracanal medications in a rat model of apical periodontitis

Larissa Magnus[1], Luciana de Aguiar Zollmann[2,5], Adriana Etges[3], Vinicius Dutra[4], Maria Martha Campos[1,,5]

[1]Postgraduate Program of Dental College, Pontifical Catholic University of Rio Grande do Sul, PUCRS, Porto Alegre, RS, Brazil; [2]Institute of Toxicology and Pharmacology, PUCRS;; [3]Faculty of Dentistry, UFPEL; [4]Radiology Service, Mãe de Deus Hospital; [5]Institute of Toxicology and Pharmacology, PUCRS.

Corresponding Author: Maria Martha Campos, School of Dentistry, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, Partenon, 90619-900, Porto Alegre, Brazil. Phone number: 55 51 3320 3562; Fax number: 55 51 3320 3626; e-mail: camposmmartha@yahoo.com; maria.campos@pucrs.br

Acknowledgments

L.A.Z. held undergraduate grants from the Institutional BPA Program (PUCRS). L.M. is a PhD student in Dentistry supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). We would like to thank Ms. Silvana Pereira de Souza, Ms. Raquel Oliveira and Mr. Juliano Soares for their excellent technical assistance. This study was financially supported by CNPq, PUCRS, and the FINEP research grant "Implantação, Modernização e Qualificação de Estrutura de Pesquisa da PUCRS" (PUCRSINFRA) # 01.11.0014-00. The authors deny any conflicts of interest related to this study.

ABSTRACT

Objectives*:** This study evaluated the effects of the β-lactamic antibiotic amoxicillin, as intracanal medication, in periapical lesions in rats. ***Methods: Periapical lesions were induced in the first upper molars of 18 male Wistar rats. After 21 days, the endodontic instrumentation was performed. Intracanal medication was applied into the right molars, whereas the left molar received no medication, and it was used as the negative control group. The following experimental groups were evaluated: (1) amoxicillin vehicled in propyleneglycol; (2) amoxicillin plus chlorhexidine gluconate; (3) amoxicillin combined to calcium hydroxide paste. The extent of periapical lesions was determined quantitatively by cone-bean tomography, and qualitatively by H&E and Mallory histological analysis, after an additional period of 21 days. ***Results:*** Amoxicillin vehicled in propyleneglycol produced an inhibition of 47 ± 5% of periapical lesion areas, and a similar degree of reduction was observed in the group that received amoxicillin plus chlorhexidine gluconate (46 ± 5%). Notably, the combination of amoxicillin and calcium hydroxide paste resulted in a marked inhibition of periapical lesions (65 ± 10 %). Qualitative H&E histological analysis showed that inflammatory infiltrate ranged from mild to moderate in the test groups, and the control samples presented intense inflammatory infiltrate, with visible resorption areas. Regarding the Mallory staining, it was possible to observe a massive presence of fibroblasts, surrounded by intact and well-bonded collagen fibers. Otherwise, the control groups showed inflammatory pouch, with intense inflammatory infiltrate and areas of abscess. ***Conclusions:*** The local use of amoxicillin proved to be effective in rats, especially when combined to calcium hydroxide paste. This might well represent an attractive alternative in clinics.

Keywords: antibiotics, amoxicillin, calcium hydroxide paste, 2% chlorhexidine gluconate, periapical lesions, rats.

INTRODUCTION

Endodontic treatment, through biomechanical cleaning and shaping, complemented by intracanal medication represents a quite appropriate option to achieve healing, in the cases of periapical lesions (1-7). The endodontic therapy is based on clinical and radiographic findings, and it is supported by the use of intracanal medications and irrigating solutions, in order to complement the disinfection of the root canal system (1, 5, 8). However, despite the clear advantages, the intracanal dressings currently available in the clinics have some limitations (5-6, 9-11).

Among the existing medications, calcium hydroxide and chlorhexidine gluconate stand out presently as the most used agents in the clinical set (12-22). However, both have limitations and further studies in the area of intracanal medications are important for the evolution of endodontics. It is well known that calcium hydroxide displays limitations in the elimination of facultative anaerobes and yeasts (1, 4). In addition, chlorhexidine does not have the ability to dissolve tissues, and removing chlorhexidine-containing gels from the canal spaces can be a difficult process (4, 6).

Given the complexity of the root canal system, the great difficulty of endodontic treatment is the complete removal of microorganisms from dentinal tubules, lateral and accessory canals, and from the side and apical delta (10, 18, 22-23). The available intracanal medications do not reach all these regions, and systemic antibiotics are often used as adjuvant alternatives in endodontics therapy (10, 24-26). However, the concentration of the antibiotics reaching the canal system after systemic administration is minimal and unlikely able to inhibit the bacterial growth (14, 24-26). For this reason, intracanal medications with antibiotics in their composition could be effective in the treatment of apical lesions. Therefore, the purpose of this study was to evaluate amoxicillin as intracanal medication in periapical lesions in rats,

when used alone or vehicled in combination with the classical intracanal dressings, calcium hydroxide and chlorhexidine gluconate.

MATERIAL AND METHODS

Animals

Eighteen male Wistar rats (220-250g) were used in this study. The animals were maintained in controlled temperature (22 ± 2 ºC) and humidity (60 - 70 %), under a 12 h light-dark cycle. Food and water were available *ad libitum*. All animal procedures were performed according to the "Principles of Laboratory Animal Care" from NIH publication No. 85-23 and Ethical Guidelines for Investigation of Experimental Pain in Conscious Animals. The Institutional Animal Ethics Committee approved all the experimental protocols. The number of animals was the minimum necessary to demonstrate the consistent effects of the drug treatments. The sample size calculation was based on previous studies (27-29) considering a statistic power of 95% and a level of significance of 5%, what indicated a number of six animals per experimental group.

Induction of periapical lesions

Periapical lesions were induced in right and left first upper molars, as described previously (27-28). Briefly, the animals were anesthetized by a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg), given by i.p. route. Pulp exposure was performed at the distal fossae of the right and the left maxillary first molars, by using a 1011 diamond round bur. The exposed pulps were left open to the oral environment for twenty one days, to allow the lesion formation

Endodontic treatment

After 21 days of pulp exposure, the animals were anesthetized as described before, and the endodontic treatment was performed in the right and left maxillary first molars, according to the method described before (30). The exploration of the root canals was done with an 8-type K file. The working length was established at 3 mm, according to the anatomy of the rat upper first molars. The technique consisted of manual instrumentation from the 8-type to the 20-type K file (all 21-mm long). The change of each instrument was performed under 0.2 ml sodium hypochlorite irrigation. At the end of instrumentation, the smear layer was removed by the application of 0.2 ml of EDTA, followed by irrigation with 0.2 ml of sodium hypochlorite. Intracanal medication was applied into the right molars, whereas the left molar received no medication, and it was used as the negative control group. The medications were:

Group 1: amoxicillin + propyleneglycol;

Group 2: amoxicillin + chlorhexidine gluconate 2%;

Group 3: amoxicillin + calcium hydroxide paste .

Teeth were restored with glass ionomer (Vidrion R, SS White Duflex, RJ, Brazil). The rats were maintained for an additional period of 21 days, in order to determine the effectiveness of the intracanal dressings.

Determination of periapical lesion areas

The areas of periapical lesions were measured by using cone beam tomography as described before (31). For these procedures, the animals were re-anesthetized and positioned in a modified cage equipped with an adaptor for the head, to permit the standardization of image takings. A 3D i-CAT tomography apparatus (Imaging Sciences, Hatfield, PA, USA) was used, and the images (0.2 mm voxel size) were analyzed by using the equipment's

software. A radiologist who was blinded to the groups performed the measurements. The area (length x height) of periapical lesions was provided in mm^2.

Histological analysis

Histological analysis was performed to complement the results of cone beam tomography, in order to confirm the intracanal medication effects. After tomography procedures, euthanasia was performed by deep inhalation of isoflurane. Immediately after, the maxillas were removed and placed in recipients containing 10% formaldehyde in 0.1 M phosphate buffer, for subsequent decalcification and histological processing. The histological slides were stained with Hematoxylin & Eosin (H&E) to verify the inflammatory infiltrate. Mallory staining was performed to verify the presence of fibroblasts and collagen fibers. Two slides of each tooth were analyzed by a pathologist blind to the experimental groups. Histological data is presented in a qualitative manner.

Statistical analysis

The results from tomography are presented as the mean ± standard error mean of 6 animals (left and right superior molars). Histological results were described in a qualitative manner. The statistical comparison of the data was performed by using Student's *t* test. *P*-values less than 0.05 ($P < 0.05$) were considered significant.

RESULTS

Data depicted in Figure 1A demonstrates that amoxicillin vehicled in propyleneglycol was able to significantly reduce the area of periapical lesions, according to cone-bean tomography evaluation, with an inhibition rate of 47 ± 5 %. A similar effect was observed when amoxicillin was associated to chlorhexidine gluconate (46 ± 5%) (Figure 1B). Of note,

in our experimental paradigm, chlorhexidine gluconate did not elicit a significant reduction of periapical lesions extent, when used alone (results not shown). However, when amoxicillin was tested in combination with calcium hydroxide paste, it was possible to observe a great reduction of periapical lesion area, which corresponded to 65 ± 10 % of inhibition (Figure 1C).

The qualitative histological analysis confirmed and extended tomography data, showing that amoxicillin, when used locally, alone or in combination with other medications, produced a general reduction of inflammation intensity in periapical areas. Data on H&E staining revealed that inflammatory infiltrate ranged from mild to moderate in all the test groups (Figure 2A to 2C), whereas in the control groups, the inflammatory infiltrate was intense, with visible areas of resorption (Figure 2D). For the Mallory staining, it was possible to observe a massive number of fibroblasts, surrounded by well-bonded and intact collagen fibers in the treated groups (Figure 2A-2C). Otherwise, the control groups showed inflammatory pouch, with intense inflammatory infiltrate and areas of abscess (Figure 3D).

DISCUSSION

Systemic antibiotic therapy is not usually recommended to treat root canal failures or periradicular lesions (32). When systemic antibiotic is used as adjuvant in endodontics therapy, the concentration of drug reaching the canal system after systemic administration is minimal, and unlikely to inhibit bacterial growth (14, 24-26). Therefore, this paper was designed to investigate the potential of the broad-spectrum β-lactamic antibiotic amoxicillin as intracanal medication in periapical lesions in rats, when used alone or vehicled in combination with the classical intracanal dressings, calcium hydroxide paste and chlorhexidine gluconate.

In all the treated groups, there was a large reduction of periapical lesions, what was most evident in the group where amoxicillin was mixed to calcium hydroxide. Amoxicillin is an antibiotic with extended spectrum of action, and this might explain the great difference between controls and treated animals, as evidenced in the tomography analysis. The extent of inhibition obtained with amoxicillin alone or even in combination with chlorhexidine gluconate was quite similar. Of note, we observed only a marginal effect for chlorhexidine gluconate when used alone in the same experimental model (data not shown). Therefore, it is possible to suggest that amoxicillin is the main responsible for the observed inhibitory effect in the group that received this antibiotic combined to chlorhexidine gluconate.

Regarding the combination of amoxicillin and calcium hydroxide, there was a marked reduction in the extent of periapical lesions, as revealed by cone bean tomography. It is tempting to suggest that the use of amoxicillin plus calcium hydroxide could increase the success rate of endodontic treatment. Noteworthy, both substances display a satisfactory safety profile, and the reduction of calcium hydroxide concentrations by associating amoxicillin might well minimize any potential periapical tissue damage caused by its high alkalinity. Furthermore, considering that a major limitation of calcium hydroxide paste is the

lack of effectiveness against *E. faecalis* (1, 4, 12, 14, 18), we might infer that this restriction could be overtaken by the association with amoxicillin. In fact, a recent *in vitro* study showed the ability of amoxicillin plus clavulanate to reduce *E. faecalis* biofilm, whilst calcium hydroxide was found marginally effective (17). Furthermore, clinical isolates of *E. faecalis* were demonstrated to be sensitive to β-lactamic antibiotics, supporting that notion (33). However, additional microbiological investigations need to be addressed to confirm this hypothesis. Nevertheless, several reasons can be pointed out to justify the use of this combination in the clinical set.

Qualitative histological analysis was performed to complement data on cone beam tomography. The H&E staining showed a visible reduction of inflammatory infiltrate in all the treated groups, in comparison to the control samples. Furthermore, the Mallory staining revealed that amoxicillin, even when used alone or in combination with other intracanal medications, promoted the healing of periapical lesions, as observed by an increased number of fibroblasts and by the presence of well-organized collagen fibers. When analyzed in concert, tomography and histological findings clearly demonstrate that intracanal dressings containing amoxicillin represent effective alternatives able to reduce both the extent and the cellularity of periapical lesions in rats. Interestingly, amoxicillin and calcium hydroxide have different mechanisms of action, what might additionally support the rational use of this combination in Endodontics. In the literature, there are some previous reports employing triple antibiotic paste (metronidazole, ciprofloxacin, and minocycline) as intracanal dressing (14). The present study is allied to the strategy of using antibiotics as local medications, and proposes a novel therapeutic approach to the currently available endodontics arsenal.

REFERENCES

1. Basrani B, Tjaderhane L, Santos JM, Pascon E, Grad H, Lawrence HP, et al. Efficacy of chlorhexidine- and calcium hydroxide-containing medicaments against Enterococcus faecalis in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96(5):618-624.

2. Carvalho FB, Goncalves M, Tanomaru-Filho M. Evaluation of chronic periapical lesions by digital subtraction radiography by using Adobe Photoshop CS: a technical report. J Endod 2007;33(4):493-497.

3. Estrela C, Decurcio DdA, Alencar AHGd, Sydney GB, Silva JA. Efficacy of calcium hydroxide dressing in endodontic infection treatment: a systematic review. Revista Odonto Ciência 2008;23(1):82-86.

4. Evans MD, Baumgartner JC, Khemaleelakul SU, Xia T. Efficacy of calcium hydroxide: chlorhexidine paste as an intracanal medication in bovine dentin. J Endod 2003;29(5):338-339.

5. Ferguson JW, Hatton JF, Gillespie MJ. Effectiveness of intracanal irrigants and medications against the yeast Candida albicans. J Endod 2002;28(2):68-71.

6. Gomes BP, Souza SF, Ferraz CC, Teixeira FB, Zaia AA, Valdrighi L, et al. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against Enterococcus faecalis in bovine root dentine in vitro. Int Endod J 2003;36(4):267-275.

7. Kontakiotis EG, Tsatsoulis IN, Papanakou SI, Tzanetakis GN. Effect of 2% chlorhexidine gel mixed with calcium hydroxide as an intracanal medication on sealing ability of permanent root canal filling: a 6-month follow-up. J Endod 2008;34(7):866-870.

8. El Karim I, Kennedy J, Hussey D. The antimicrobial effects of root canal irrigation and medication. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103(4):560-569.

9. Jacinto RC, Gomes BP, Ferraz CC, Zaia AA, Filho FJ. Microbiological analysis of infected root canals from symptomatic and asymptomatic teeth with periapical periodontitis and the antimicrobial susceptibility of some isolated anaerobic bacteria. Oral Microbiol Immunol 2003;18(5):285-292.

10. LeCorn DW, Vertucci FJ, Rojas MF, Progulske-Fox A, Belanger M. In vitro activity of amoxicillin, clindamycin, doxycycline, metronidazole, and moxifloxacin against oral Actinomyces. J Endod 2007;33(5):557-560.

11. McBain AJ, Bartolo RG, Catrenich CE, Charbonneau D, Ledder RG, Gilbert P. Effects of a chlorhexidine gluconate-containing mouthwash on the vitality and antimicrobial susceptibility of in vitro oral bacterial ecosystems. Appl Environ Microbiol 2003;69(8):4770-4776.

12. Farhad AR, Barekatain B, Allameh M, Narimani T. Evaluation of the antibacterial effect of calcium hydroxide in combination with three different vehicles: An in vitro study. Dent Res J (Isfahan) 2012;9(2):167-172.

13. Ximenes M, Cardoso M. Assessment of diffusion of hydroxyl and calcium ions of root canal filling materials in primary teeth. Pediatr Dent 2012;34(2):122-126.

14. Taneja S, Kumari M. Use of triple antibiotic paste in the treatment of large periradicular lesions. J Investig Clin Dent 2012;3(1):72-76.

15. Guerreiro-Tanomaru JM, Chula DG, de Pontes Lima RK, Berbert FL, Tanomaru-Filho M. Release and diffusion of hydroxyl ion from calcium hydroxide-based medicaments. Dent Traumatol 2012;28(4):320-323.

16. Panzarini SR, Trevisan CL, Brandini DA, Poi WR, Sonoda CK, Luvizuto ER, et al. Intracanal dressing and root canal filling materials in tooth replantation: a literature review. Dent Traumatol 2012;28(1):42-48.

17. Saber Sel D, El-Hady SA. Development of an intracanal mature Enterococcus faecalis biofilm and its susceptibility to some antimicrobial intracanal medications; an in vitro study. Eur J Dent 2012;6(1):43-50.

18. Gondim JO, Avaca-Crusca JS, Valentini SR, Zanelli CF, Spolidorio DM, Giro EM. Effect of a calcium hydroxide/chlorhexidine paste as intracanal dressing in human primary teeth with necrotic pulp against Porphyromonas gingivalis and Enterococcus faecalis. Int J Paediatr Dent 2012;22(2):116-124.

19. Pereira MS, Faria G, Bezerra Da Silva LA, Tanomaru-Filho M, Kuga MC, Rossi MA. Response of mice connective tissue to intracanal dressings containing chlorhexidine. Microsc Res Tech 2012.

20. Valera MC, Maekawa LE, Chung A, de Oliveira LD, Carvalho CA, Koga-Ito CY, et al. Effectiveness of castor oil extract on Escherichia coli and its endotoxins in root canals. Gen Dent 2012;60(4):e204-209.

21. Prado M, Gusman H, Gomes BP, Simao RA. Effect of disinfectant solutions on gutta-percha and resilon cones. Microsc Res Tech 2012;75(6):791-795.

22. Vilanova WV, Carvalho-Junior JR, Alfredo E, Sousa-Neto MD, Silva-Sousa YT. Effect of intracanal irrigants on the bond strength of epoxy resin-based and methacrylate resin-based sealers to root canal walls. Int Endod J 2012;45(1):42-48.

23. Pannuti CM, Lotufo RF, Cai S, Saraiva Md Mda C, de Freitas NM, Falsi D. Effect of a 0.5% chlorhexidine gel on dental plaque superinfecting microorganisms in mentally handicapped patients. Pesqui Odontol Bras 2003;17(3):228-233.

24. Fouad AF, Barry J. The effect of antibiotics and endodontic antimicrobials on the polymerase chain reaction. J Endod 2005;31(7):510-513.

25. Henry M, Reader A, Beck M. Effect of penicillin on postoperative endodontic pain and swelling in symptomatic necrotic teeth. J Endod 2001;27(2):117-123.

26. Siqueira JF, Jr., Rocas IN, Riche FN, Provenzano JC. Clinical outcome of the endodontic treatment of teeth with apical periodontitis using an antimicrobial protocol. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106(5):757-762.

27. Lee YL, Hong CY, Kok SH, Hou KL, Lin YT, Chen MH, et al. An extract of green tea, epigallocatechin-3-gallate, reduces periapical lesions by inhibiting cysteine-rich 61 expression in osteoblasts. J Endod 2009;35(2):206-211.

28. Metzger Z, Klein H, Klein A, Tagger M. Periapical lesion development in rats inhibited by dexamethasone. J Endod 2002;28(9):643-645.

29. Sobrinho AP, Barros MH, Nicoli JR, Carvalho MA, Farias LM, Bambirra EA, et al. Experimental root canal infections in conventional and germ-free mice. J Endod 1998;24(6):405-408.

30. Wagner C, Barth VC, Jr., de Oliveira SD, Campos MM. Effectiveness of the proton pump inhibitor omeprazole associated with calcium hydroxide as intracanal medication: an in vivo study. J Endod 2011;37(9):1253-1257.

31. Magnus Larissa; Dutra, Vinicius; Bernardi, Lisiane; Campos, Maria Martha. Use of cone bean tomography to evaluate intracanal medications in a rat model of apical periodontitis Revista Odontociência 2012;Accept for publiccation in November, 2012.

32. Krithikadatta J, Indira R, Dorothykalyani AL. Disinfection of dentinal tubules with 2% chlorhexidine, 2% metronidazole, bioactive glass when compared with calcium hydroxide as intracanal medicaments. J Endod 2007;33(12):1473-1476.

33. Rams TE, Feik D, Mortensen JE, Degener JE, van Winkelhoff AJ. Antibiotic Susceptibility of Periodontal Enterococcus Faecalis. J Periodontol 2012.

FIGURES

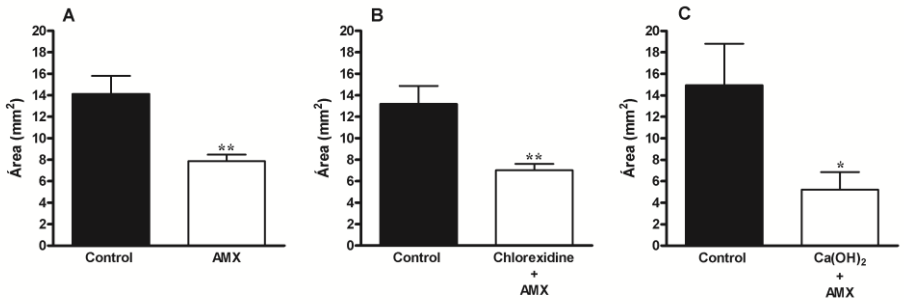

Figure 1. Tomography analysis showing the effects of amoxicillin vehicled in propyleneglycol (panel A), amoxicillin plus chlorhexidine gluconate (panel B) and amoxicillin plus calcium hydroxide paste (panel C). The columns represent the mean of 6 experiments and the vertical lines indicate the standard error mean. *P<0.05; **P<0.001.

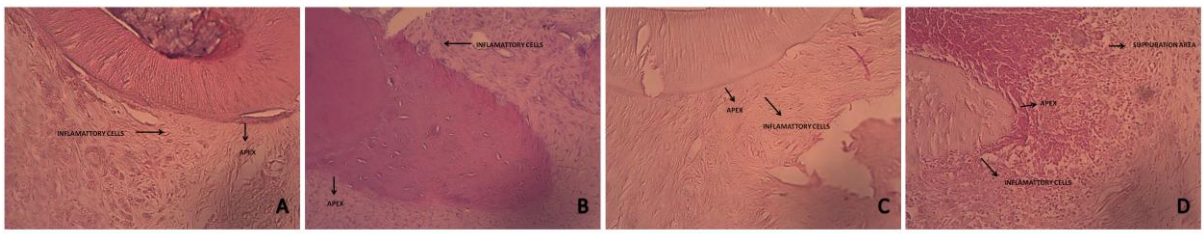

Figure 2. Histological images showing H&E staining (100x). Reduced inflammatory cell infiltration in the experimental groups: Amoxicillin + propileneglycol (Panel A); Amoxicillin with Calcium Hydroxide (Panel B); and Amoxicillin with chlorhexidine gluconate (Panel C). Marked periapical bone resorption associated with chronic inflammation and suppuration in the control groups (Panel D). The arrows indicate the evaluated parameters and the anatomical areas.

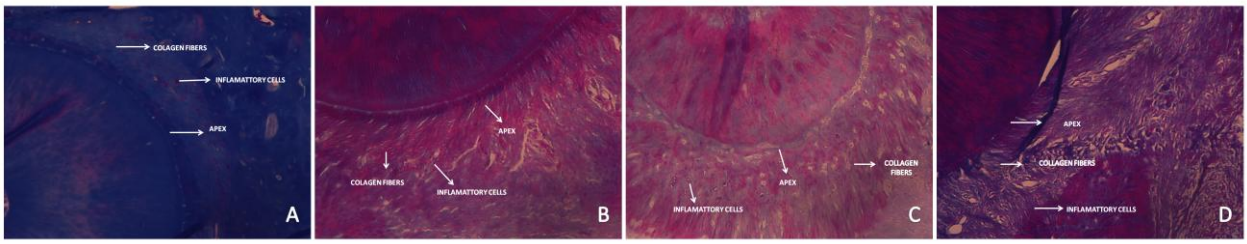

Figure 3. Histological images from Mallory-stained slides showing a large of number of fibroblasts, with intact and well-bonded collagen fibers in the test groups: Amoxicillin + propileneglycol (Panel A); Amoxicillin with Calcium Hydroxide (Panel B); and Amoxicillin with chlorhexidine gluconate (Panel C). The control groups showed inflammatory pouch, with intense inflammatory infiltrate and areas of abscess (Panel D). The arrows indicate the evaluated parameters and the anatomical areas.

Capítulo Final
Discussão Geral e Considerações Finais

9. DISCUSSÃO GERAL

Nos últimos anos, a Endodontia evoluiu muito, possibilitando o acesso a materiais e técnicas avançadas, as quais facilitaram os procedimentos e trouxeram maior conforto e segurança para os tratamentos. Salienta-se, porém, que um profissional de saúde, especialista numa área tão delicada quanto Endodontia, deve sempre alicerçar sua conduta clínica em bases biológicas, fundamentando seus tratamentos em preceitos básicos de histologia, farmacologia e, patologia, entre outros. O docente de Endodontia, por sua vez, deve ter o cuidado de apresentar para os seus alunos as razões pelas quais os princípios biológicos devem estar aliados à tecnologia na busca do sucesso terapêutico, para que a Endodontia moderna não deixe de ser, antes de tudo, uma disciplina baseada em evidências.

Ressalta-se que o uso de uma medicação intracanal faz-se importante, principalmente, como suporte para os procedimentos realizados durante o preparo químico mecânico, aumentando as chances de reparo(1, 67, 69, 83-84). Na prática Endodôntica, o hidróxido de cálcio e a clorexidina são referências por suas propriedades, sendo as medicações de demora mais utilizadas na atualidade (1, 10, 13, 80). Sabe-se, porém, que estas apresentam limitações, o que tem estimulado pesquisas sobre novas medicações, como o presente trabalho. As substâncias naturais vêm sendo estudadas há algum tempo na área da Endodontia, mostrando resultados interessantes(4, 53). O resveratrol é amplamente utilizado na medicina oriental e mostrou-se efetivo em modelos de câncer e inflamação(45-46, 48), o que motivou sua aplicação como medicação intracanal. Os resultados com resveratrol veiculado em propilenoglicol foram bastante animadores, uma vez que a clorexidina isolada, no tempo testado, não foi diferente do controle. Num futuro próximo, pode-se vir a testar esta formulação de outras maneiras, até que ensaios clínicos cegos randomizados possam ser

executados e, quem sabe, permitam que o resveratrol seja, de fato, considerado um curativo de demora em Endodontia.

Também foi avaliado o efeito da amoxicilina, um agente antimicrobiano que já possui seu uso sistêmico consagrado em Odontologia, devido a seu amplo espectro(20, 33, 40). Foi avaliado se ação local desta promoveria o reparo por atingir os microrganismos de maneira mais direta e, em maiores concentrações. Novamente, os resultados foram muito promissores, especialmente quando da associação com hidróxido de cálcio. A histologia corroborou os achados tomográficos. Tanto a coloração por H&E, quanto por Mallory, mostraram resultados também animadores, confirmando a ideia de que a aplicação local de amoxicilina pode ser uma estratégia interessante na clínica.

O presente trabalho trouxe resultados que podem ser relevantes para a Endodontia como especialidade. Sabe-se, entretanto que muitos estudos precisam ainda ser realizados, pois só a partir de dados de ensaios clínicos randomizados cegos e controlados qualquer uma das medicações poderá de fato ser considerada curativo de demora. Salienta-se por fim que as bases biológicas aqui apresentadas representam um passo importante para que medicações intracanal sejam desenvolvidas e melhoradas.

10. CONSIDERAÇÕES FINAIS

- ✓ A tomografia computadorizada cone beam mostrou-se efetiva para medir lesões periapicais em ratos, mostrando ser uma metodologia interessante em trabalhos que têm por objetivo medir o reparo das lesões periapicais, após o uso de medicação intracanal.

- ✓ O Resveratrol pode ser uma alternativa interessante como medicação intracanal, podendo, futuramente, vir a integrar o arsenal de medicações utilizadas em Endodontia.

- ✓ A amoxicilina apresentou ação local marcante no reparo da região periapical. Tendo em vista que a mesma mostrou-se eficaz, tanto quando isolada, como em conjunto com hidróxido de cálcio e clorexidina, sugere-se que o uso local deste antibiótico em Endodontia pode ser interessante para potencializar o reparo da região periapical.

REFERÊNCIAS

1. Basrani B, Tjaderhane L, Santos JM, Pascon E, Grad H, Lawrence HP, et al. Efficacy of chlorhexidine- and calcium hydroxide-containing medicaments against Enterococcus faecalis in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003 Nov;96(5):618-24.

2. Estrela C, Decurcio DdA, Alencar AHGd, Sydney GB, Silva JA. Efficacy of calcium hydroxide dressing in endodontic infection treatment: a systematic review. Revista Odonto Ciência. 2008;23(1):82-6.

3. Evans MD, Baumgartner JC, Khemaleelakul SU, Xia T. Efficacy of calcium hydroxide: chlorhexidine paste as an intracanal medication in bovine dentin. J Endod. 2003 May;29(5):338-9.

4. Ferreira FB, Torres SA, Rosa OP, Ferreira CM, Garcia RB, Marcucci MC, et al. Antimicrobial effect of propolis and other substances against selected endodontic pathogens. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007 Nov;104(5):709-16.

5. Gomes BP, Montagner F, Berber VB, Zaia AA, Ferraz CC, de Almeida JF, et al. Antimicrobial action of intracanal medicaments on the external root surface. J Dent. 2009 Jan;37(1):76-81.

6. Gomes BP, Souza SF, Ferraz CC, Teixeira FB, Zaia AA, Valdrighi L, et al. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against Enterococcus faecalis in bovine root dentine in vitro. Int Endod J. 2003 Apr;36(4):267-75.

7. Kontakiotis EG, Tsatsoulis IN, Papanakou SI, Tzanetakis GN. Effect of 2% chlorhexidine gel mixed with calcium hydroxide as an intracanal medication on sealing ability of permanent root canal filling: a 6-month follow-up. J Endod. 2008 Jul;34(7):866-70.

8. Vianna ME, Horz HP, Conrads G, Zaia AA, Souza-Filho FJ, Gomes BP. Effect of root canal procedures on endotoxins and endodontic pathogens. Oral Microbiol Immunol. 2007 Dec;22(6):411-8.

9. Siqueira JF, Jr., Rocas IN, Riche FN, Provenzano JC. Clinical outcome of the endodontic treatment of teeth with apical periodontitis using an antimicrobial protocol. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008 Nov;106(5):757-62.

10. Paquette L, Legner M, Fillery ED, Friedman S. Antibacterial efficacy of chlorhexidine gluconate intracanal medication in vivo. J Endod. 2007 Jul;33(7):788-95.

11. Wang CS, Arnold RR, Trope M, Teixeira FB. Clinical efficiency of 2% chlorhexidine gel in reducing intracanal bacteria. J Endod. 2007 Nov;33(11):1283-9.

12. El Karim I, Kennedy J, Hussey D. The antimicrobial effects of root canal irrigation and medication. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007 Apr;103(4):560-9.

13. Sathorn C, Parashos P, Messer H. Antibacterial efficacy of calcium hydroxide intracanal dressing: a systematic review and meta-analysis. Int Endod J. 2007 Jan;40(1):2-10.

14. Siqueira JF, Jr., Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J. 1999 Sep;32(5):361-9.

15. Ercan E, Dalli M, Dulgergil CT. In vitro assessment of the effectiveness of chlorhexidine gel and calcium hydroxide paste with chlorhexidine against Enterococcus faecalis and Candida albicans. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006 Aug;102(2):e27-31.

16. Gomes BP, Vianna ME, Sena NT, Zaia AA, Ferraz CC, de Souza Filho FJ. In vitro evaluation of the antimicrobial activity of calcium hydroxide combined with chlorhexidine gel used as intracanal medicament. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006 Oct;102(4):544-50.

17. Lambrianidis T, Kosti E, Boutsioukis C, Mazinis M. Removal efficacy of various calcium hydroxide/chlorhexidine medicaments from the root canal. Int Endod J. 2006 Jan;39(1):55-61.

18. Leonardo MR. Endodontia: Tratamento de Canais Radiculares Princípios Técnicos e Biológicos. São Paulo, Brasil: Editora Artes Médicas LTDA; 2008.

19. Ferguson JW, Hatton JF, Gillespie MJ. Effectiveness of intracanal irrigants and medications against the yeast Candida albicans. J Endod. 2002 Feb;28(2):68-71.

20. LeCorn DW, Vertucci FJ, Rojas MF, Progulske-Fox A, Belanger M. In vitro activity of amoxicillin, clindamycin, doxycycline, metronidazole, and moxifloxacin against oral Actinomyces. J Endod. 2007 May;33(5):557-60.

21. Kakehashi S, Stanley HR, Fitzgerald RJ. The Effects of Surgical Exposures of Dental Pulps in Germ-Free and Conventional Laboratory Rats. Oral Surg Oral Med Oral Pathol. 1965 Sep;20:340-9.

22. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. Int Endod J. 2009 Apr;42(4):288-302.

23. Moller AJ, Fabricius L, Dahlen G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res. 1981 Dec;89(6):475-84.

24. Sundqvist G. Ecology of the root canal flora. J Endod. 1992 Sep;18(9):427-30.

25. Kim SK, Kim YO. Influence of calcium hydroxide intracanal medication on apical seal. Int Endod J. 2002 Jul;35(7):623-8.

26. Costa JR, Jr., Abizaid A, Feres F, Costa R, Seixas AC, Maia F, et al. EXCELLA First-in-Man (FIM) study: safety and efficacy of novolimus-eluting stent in de novo coronary lesions. EuroIntervention. 2008 May;4(1):53-8.

27. Filho FJdS. Antimicrobial effect and pH of Chlorhexidine Gel and Calcium Hydroxide alone and associated with other materials. Brazilian Dental Journal. 2008;19(1):28-33.

28. Krithikadatta J, Indira R, Dorothykalyani AL. Disinfection of dentinal tubules with 2% chlorhexidine, 2% metronidazole, bioactive glass when compared with calcium hydroxide as intracanal medicaments. J Endod. 2007 Dec;33(12):1473-6.

29. Fachin EVF, Nunes LSdS, Mendes AF. Intracanal Medication indicated for pulpar necrosis with apical osteites. *Revista Odonto Ciência* 2006;21(54):351-7.

30. Gomes BP, Sato E, Ferraz CC, Teixeira FB, Zaia AA, Souza-Filho FJ. Evaluation of time required for recontamination of coronally sealed canals medicated with calcium hydroxide and chlorhexidine. Int Endod J. 2003 Sep;36(9):604-9.

31. Molander A, Dahlen G. Evaluation of the antibacterial potential of tetracycline or erythromycin mixed with calcium hydroxide as intracanal dressing against Enterococcus faecalis in vivo. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003 Dec;96(6):744-50.

32. Neelakantan P, Sanjeev K, Subbarao CV. Duration-dependent susceptibility of endodontic pathogens to calcium hydroxide and chlorhexidene gel used as intracanal medicament: an in vitro evaluation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007 Oct;104(4):e138-41.

33. Henry M, Reader A, Beck M. Effect of penicillin on postoperative endodontic pain and swelling in symptomatic necrotic teeth. J Endod. 2001 Feb;27(2):117-23.

34. Lin S, Levin L, Peled M, Weiss EI, Fuss Z. Reduction of viable bacteria in dentinal tubules treated with clindamycin or tetracycline. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003 Dec;96(6):751-6.

35. Jacinto RC, Montagner F, Signoretti FG, Almeida GC, Gomes BP. Frequency, microbial interactions, and antimicrobial susceptibility of Fusobacterium nucleatum and Fusobacterium necrophorum isolated from primary endodontic infections. J Endod. 2008 Dec;34(12):1451-6.

36. Pinheiro ET, Gomes BP, Drucker DB, Zaia AA, Ferraz CC, Souza-Filho FJ. Antimicrobial susceptibility of Enterococcus faecalis isolated from canals of root filled teeth with periapical lesions. Int Endod J. 2004 Nov;37(11):756-63.

37. Khemaleelakul S, Baumgartner JC, Pruksakorn S. Identification of bacteria in acute endodontic infections and their antimicrobial susceptibility. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002 Dec;94(6):746-55.

38. Pinheiro ET, Gomes BP, Ferraz CC, Teixeira FB, Zaia AA, Souza Filho FJ. Evaluation of root canal microorganisms isolated from teeth with endodontic failure and their antimicrobial susceptibility. Oral Microbiol Immunol. 2003 Apr;18(2):100-3.

39. Jacinto RC, Gomes BP, Ferraz CC, Zaia AA, Filho FJ. Microbiological analysis of infected root canals from symptomatic and asymptomatic teeth with periapical periodontitis

and the antimicrobial susceptibility of some isolated anaerobic bacteria. Oral Microbiol Immunol. 2003 Oct;18(5):285-92.

40. Fouad AF, Barry J. The effect of antibiotics and endodontic antimicrobials on the polymerase chain reaction. J Endod. 2005 Jul;31(7):510-3.

41. Pirola L, Frojdo S. Resveratrol: one molecule, many targets. IUBMB Life. 2008 May;60(5):323-32.

42. Sebai H, Ben-Attia M, Sani M, Aouani E, Ghanem-Boughanmi N. Protective effect of resveratrol in endotoxemia-induced acute phase response in rats. Arch Toxicol. 2009 Apr;83(4):335-40.

43. Larrosa M, Yanez-Gascon MJ, Selma MV, Gonzalez-Sarrias A, Toti S, Ceron JJ, et al. Effect of a low dose of dietary resveratrol on colon microbiota, inflammation and tissue damage in a DSS-induced colitis rat model. J Agric Food Chem. 2009 Mar 25;57(6):2211-20.

44. Park HJ, Jeong SK, Kim SR, Bae SK, Kim WS, Jin SD, et al. Resveratrol inhibits Porphyromonas gingivalis lipopolysaccharide-induced endothelial adhesion molecule expression by suppressing NF-kappaB activation. Arch Pharm Res. 2009 Apr;32(4):583-91.

45. Cucciolla V, Borriello A, Oliva A, Galletti P, Zappia V, Della Ragione F. Resveratrol: from basic science to the clinic. Cell Cycle. 2007 Oct 15;6(20):2495-510.

46. David JM. Resveratrol açôes e beneficios a saúde humana. Revista da rede de ensino FTC. 2007;Maio (10):1-11.

47. Liu D, Lu F, Qin G, Fernandes SM, Li J, Davis AE, 3rd. C1 inhibitor-mediated protection from sepsis. J Immunol. 2007 Sep 15;179(6):3966-72.

48. El-Mowafy AM, Alkhalaf M. Resveratrol activates adenylyl-cyclase in human breast cancer cells: a novel, estrogen receptor-independent cytostatic mechanism. Carcinogenesis. 2003 May;24(5):869-73.

49. Liang YC, Tsai SH, Chen L, Lin-Shiau SY, Lin JK. Resveratrol-induced G2 arrest through the inhibition of CDK7 and p34CDC2 kinases in colon carcinoma HT29 cells. Biochem Pharmacol. 2003 Apr 1;65(7):1053-60.

50. Bazzo KO, Souto AA, Lopes TG, Zanin RF, Gomez MV, Souza AH, et al. Evidence for the Analgesic Activity of Resveratrol in Acute Models of Nociception in Mice. J Nat Prod. 2012 Dec 28.

51. Nunez MJ, Novio S, Balboa J, Seoane J, Suarez JA, Freire-Garabal M. Effects of resveratrol on expression of vascular endothelial growth factor in human gingival fibroblasts stimulated by periodontal pathogens. Acta Odontol Scand. 2010 Jul;68(4):239-47.

52. Rizzo A, Bevilacqua N, Guida L, Annunziata M, Romano Carratelli C, Paolillo R. Effect of resveratrol and modulation of cytokine production on human periodontal ligament cells. Cytokine. 2012 Oct;60(1):197-204.

53. Lee YL, Hong CY, Kok SH, Hou KL, Lin YT, Chen MH, et al. An extract of green tea, epigallocatechin-3-gallate, reduces periapical lesions by inhibiting cysteine-rich 61 expression in osteoblasts. J Endod. 2009 Feb;35(2):206-11.

54. Metzger Z, Klein H, Klein A, Tagger M. Periapical lesion development in rats inhibited by dexamethasone. J Endod. 2002 Sep;28(9):643-5.

55. Wagner C, Barth VC, Jr., de Oliveira SD, Campos MM. Effectiveness of the proton pump inhibitor omeprazole associated with calcium hydroxide as intracanal medication: an in vivo study. J Endod. 2011 Sep;37(9):1253-7.

56. Magnus Larissa; Dutra, Vinicius; Bernardi, Lisiane; Campos, Maria Martha. Use of cone bean tomography to evaluate intracanal medications in a rat model of apical periodontitis Revista Odontociência. 2012;Accept for publiccation in November, 2012.

57. Manzur A, Gonzalez AM, Pozos A, Silva-Herzog D, Friedman S. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: a randomized clinical trial. J Endod. 2007 Feb;33(2):114-8.

58. Brilhante Wolle CF, de Aguiar Zollmann L, Etges A, Vitalis GS, Leite CE, Campos MM. Effects of the antioxidant agent tempol on periapical lesions in rats with doxorubicin-induced cardiomyopathy. J Endod. 2012 Feb;38(2):191-5.

59. Jiang XC, Tall AR, Qin S, Lin M, Schneider M, Lalanne F, et al. Phospholipid transfer protein deficiency protects circulating lipoproteins from oxidation due to the enhanced accumulation of vitamin E. J Biol Chem. 2002 Aug 30;277(35):31850-6.

60. Brenes A, Viveros A, Goni I, Centeno C, Sayago-Ayerdy SG, Arija I, et al. Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. Poult Sci. 2008 Feb;87(2):307-16.

61. Naito Y, Yoshikawa T, Matsuyama K, Yagi N, Kasai K, Sugimoto N, et al. Effect of vitamin E in gastric mucosal injury induced by ischaemia-reperfusion in nitric oxide-depleted rats. Aliment Pharmacol Ther. 1999 Apr;13(4):553-9.

62. Rizzo MR, Abbatecola AM, Barbieri M, Vietri MT, Cioffi M, Grella R, et al. Evidence for anti-inflammatory effects of combined administration of vitamin E and C in older persons with impaired fasting glucose: impact on insulin action. J Am Coll Nutr. 2008 Aug;27(4):505-11.

63. Schneider M, Verges B, Klein A, Miller ER, Deckert V, Desrumaux C, et al. Alterations in plasma vitamin E distribution in type 2 diabetic patients with elevated plasma phospholipid transfer protein activity. Diabetes. 2004 Oct;53(10):2633-9.

64. Yoshida N, Yoshikawa T, Manabe H, Terasawa Y, Kondo M, Noguchi N, et al. Vitamin E protects against polymorphonuclear leukocyte-dependent adhesion to endothelial cells. J Leukoc Biol. 1999 Jun;65(6):757-63.

65. Bernardi DS, Pereira TA, Maciel NR, Bortoloto J, Viera GS, Oliveira GC, et al. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. J Nanobiotechnology. 2011;9:44.

66. Chou TW, Ma CY, Cheng HH, Chen YY, Lai MH. A rice bran oil diet improves lipid abnormalities and suppress hyperinsulinemic responses in rats with streptozotocin/nicotinamide-induced type 2 diabetes. J Clin Biochem Nutr. 2009 Jul;45(1):29-36.

67. Carvalho FB, Goncalves M, Tanomaru-Filho M. Evaluation of chronic periapical lesions by digital subtraction radiography by using Adobe Photoshop CS: a technical report. J Endod. 2007 Apr;33(4):493-7.

68. McBain AJ, Bartolo RG, Catrenich CE, Charbonneau D, Ledder RG, Gilbert P. Effects of a chlorhexidine gluconate-containing mouthwash on the vitality and antimicrobial susceptibility of in vitro oral bacterial ecosystems. Appl Environ Microbiol. 2003 Aug;69(8):4770-6.

69. Farhad AR, Barekatain B, Allameh M, Narimani T. Evaluation of the antibacterial effect of calcium hydroxide in combination with three different vehicles: An in vitro study. Dent Res J (Isfahan). 2012 Mar;9(2):167-72.

70. Ximenes M, Cardoso M. Assessment of diffusion of hydroxyl and calcium ions of root canal filling materials in primary teeth. Pediatr Dent. 2012 Mar-Apr;34(2):122-6.

71. Taneja S, Kumari M. Use of triple antibiotic paste in the treatment of large periradicular lesions. J Investig Clin Dent. 2012 Feb;3(1):72-6.

72. Guerreiro-Tanomaru JM, Chula DG, de Pontes Lima RK, Berbert FL, Tanomaru-Filho M. Release and diffusion of hydroxyl ion from calcium hydroxide-based medicaments. Dent Traumatol. 2012 Aug;28(4):320-3.

73. Panzarini SR, Trevisan CL, Brandini DA, Poi WR, Sonoda CK, Luvizuto ER, et al. Intracanal dressing and root canal filling materials in tooth replantation: a literature review. Dent Traumatol. 2012 Feb;28(1):42-8.

74. Saber Sel D, El-Hady SA. Development of an intracanal mature Enterococcus faecalis biofilm and its susceptibility to some antimicrobial intracanal medications; an in vitro study. Eur J Dent. 2012 Jan;6(1):43-50.

75. Gondim JO, Avaca-Crusca JS, Valentini SR, Zanelli CF, Spolidorio DM, Giro EM. Effect of a calcium hydroxide/chlorhexidine paste as intracanal dressing in human primary teeth with necrotic pulp against Porphyromonas gingivalis and Enterococcus faecalis. Int J Paediatr Dent. 2012 Mar;22(2):116-24.

76. Pereira MS, Faria G, Bezerra Da Silva LA, Tanomaru-Filho M, Kuga MC, Rossi MA. Response of mice connective tissue to intracanal dressings containing chlorhexidine. Microsc Res Tech. 2012 Aug 6.

77. Valera MC, Maekawa LE, Chung A, de Oliveira LD, Carvalho CA, Koga-Ito CY, et al. Effectiveness of castor oil extract on Escherichia coli and its endotoxins in root canals. Gen Dent. 2012 Jul-Aug;60(4):e204-9.

78. Prado M, Gusman H, Gomes BP, Simao RA. Effect of disinfectant solutions on gutta-percha and resilon cones. Microsc Res Tech. 2012 Jun;75(6):791-5.

79. Vilanova WV, Carvalho-Junior JR, Alfredo E, Sousa-Neto MD, Silva-Sousa YT. Effect of intracanal irrigants on the bond strength of epoxy resin-based and methacrylate resin-based sealers to root canal walls. Int Endod J. 2012 Jan;45(1):42-8.

80. Pannuti CM, Lotufo RF, Cai S, Saraiva Md Mda C, de Freitas NM, Falsi D. Effect of a 0.5% chlorhexidine gel on dental plaque superinfecting microorganisms in mentally handicapped patients. Pesqui Odontol Bras. 2003 Jul-Sep;17(3):228-33.

81. Sobrinho AP, Barros MH, Nicoli JR, Carvalho MA, Farias LM, Bambirra EA, et al. Experimental root canal infections in conventional and germ-free mice. J Endod. 1998 Jun;24(6):405-8.

82. Rams TE, Feik D, Mortensen JE, Degener JE, van Winkelhoff AJ. Antibiotic Susceptibility of Periodontal Enterococcus Faecalis. J Periodontol. 2012 Oct 29.

83. Cruz RM, Barbosa SV. Histologic evaluation of periradicular tissues in dogs treated with calcium hydroxide in combination with HCT20 and camphorated P-chlorophenol. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005 Oct;100(4):507-11.

84. de Chevigny C, Dao TT, Basrani BR, Marquis V, Farzaneh M, Abitbol S, et al. Treatment outcome in endodontics: the Toronto study--phases 3 and 4: orthograde retreatment. J Endod. 2008 Feb;34(2):131-7.

Anexos

Anexo A – Carta aceite da Revista Odontociência – Artigo 1

Revista OdontoCiência (Journal of Dental Science) – Editorial decision

Dear Dr. Larissa Magnus Klassmann,

We are pleased to inform you that your manuscript entitled 'Use of cone bean tomography to evaluate intracanal medications in a rat model of apical periodontitis' was accepted for publication in the Revista Odonto Ciência (Journal of Dental Science).

After editing the page proofs will be sent to your e-mail address for final approval before printing. The online issue will allow the download of full-text articles in pdf file, and a complimentary printed journal will be mailed to you.

We thank you again for considering our journal to publish your work.

Sincerely,

Rosemary Shinkai, DDS, PhD
Editor-in-Chief
Revista Odonto Ciência (Journal of Dental Science)

http://revistaseletronicas.pucrs.br/ojs/index.php/fo/index

ISSN 0102-9460 (print)
ISSN 1980-6523 (online)

E-mail: odontociencia@pucrs.br
Pontifícia Universidade Católica do Rio Grande do Sul

Anexo B – Carta Submissão – Artigo 2

March 7th 2013

Dear Dr. Campos,

Your submission entitled "Efficacy of different formulations containing the antioxidant compound resveratrol as intracanal medication in a rat model of periapical lesions" has been received by the Journal of Endodontics.

You will be able to check on the progress of your paper by logging on to the Journal of Endodontics web site as an author.

The URL is http://ees.elsevier.com/joe/

Your username is: maria.campos

If you need to retrieve password details,
please go to: http://ees.elsevier.com/joe/automail_query.asp

Your manuscript will be given a reference number once an Editor has been assigned.

Thank you for submitting your work to the Journal of Endodontics.

Kind regards,

Journal of Endodontics

Anexo C – Carta Submissão – Artigo 3

March 7th 2013

Dear Dr. Campos,

Your submission entitled "Evaluation of amoxicillin as a component of intracanal medications in a rat model of apical periodontitis" has been received by the Journal of Endodontics.

You will be able to check on the progress of your paper by logging on to the Journal of Endodontics web site as an author.

The URL is http://ees.elsevier.com/joe/

Your username is: maria.campos

If you need to retrieve password details,
please go to: http://ees.elsevier.com/joe/automail_query.asp

Your manuscript will be given a reference number once an Editor has been assigned.

Thank you for submitting your work to the Journal of Endodontics.

Kind regards,

Journal of Endodontics