Revista Odonto Ciência
Journal of Dental Science

ORIGINAL ARTICLE

Rev Odonto Cienc 2013; 28(3)
http://revistaseletronicas.pucrs.br/ojs/index.php/fo

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

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Abstract

Objective: 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.

Methods: 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 21 days after endodontic treatment, and the lesion area was determined in mm^2. Unpaired Student *t* test was used to verify the differences between groups.

Results: 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. These data were confirmed by histological evaluation of maxillas, indicating the applicability of CBTC in the rat model of apical periodontitis.

Conclusion: The rat model and CBCT was found to be a useful tool to study *in vivo* the effects of intracanal medications and their influence in periapical lesions healing.

Keywords: Apical periodontitis; intracanal medication; cone beam; rats

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

Resumo

Objetivo: Descrever uma metodologia para avaliar a eficácia das medicações intracanal em ratos, utilizando a Tomografia Cone Beam (CBCT) para acompanhar as lesões periapicais.

Métodos: 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.

Resultados: A área da lesão foi 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.

Conclusão: O modelo em ratos 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

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Received: September 23, 2012
Accepted: November 6, 2012

Conflict of Interests: The authors state that there are no financial and personal conflicts of interest that could have inappropriately influenced their work.

ISSN: 1980-6523

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.

Methods

Animals

Six male Wistar rats (220-250 g) 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 (100 mg/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. Figure 1 provides a representative image of periapical lesion, in a non-treated tooth.

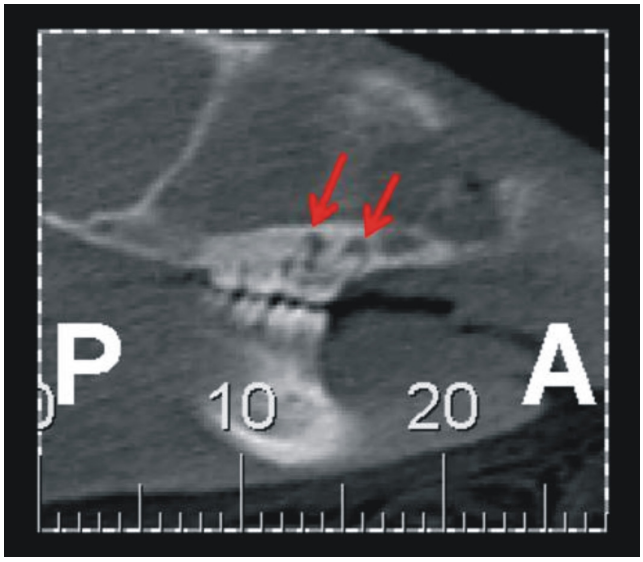

Fig. 1. 1.CBCT showing apical periodontitis in rat superior untreated molar (arrow).

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 × 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 analysis 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 mm2 in the calcium hydroxide-treated teeth, and from 7.9 to 12.1 mm2, in the non-treated control side, according to assessment by CBCT. 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 (Fig. 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 (Fig. 4).

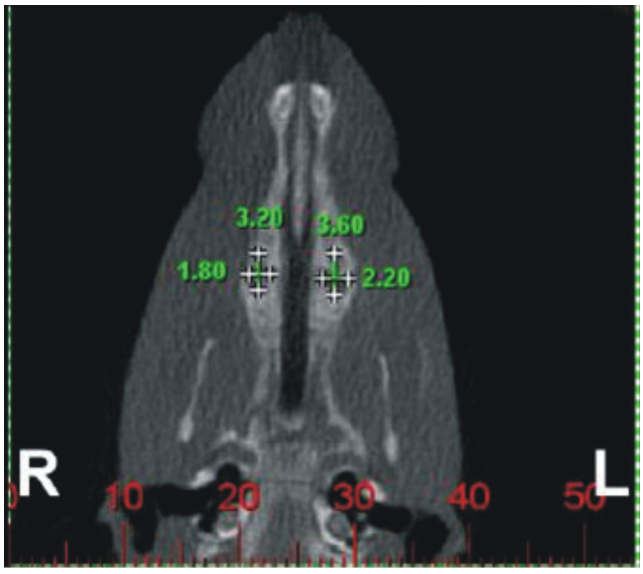

Fig. 2. Representative CBCT showing the apical lesion area in the control (left side) and the treated group (right side).

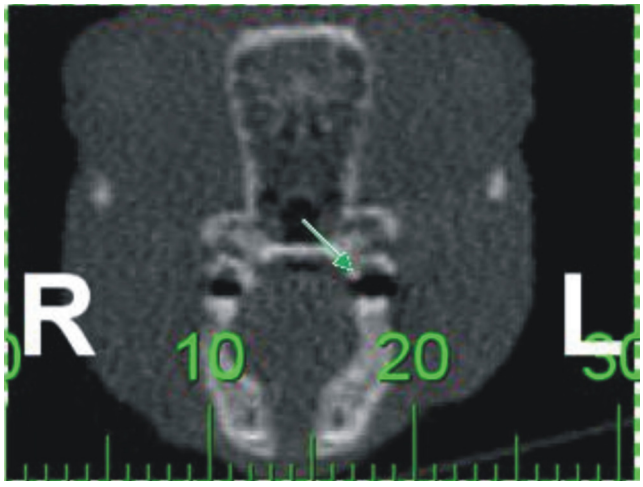

Fig. 3. Representative CBCT showing bursting of cortical in the control (left side, as indicated by an arrow), but not in the treated group (right group).

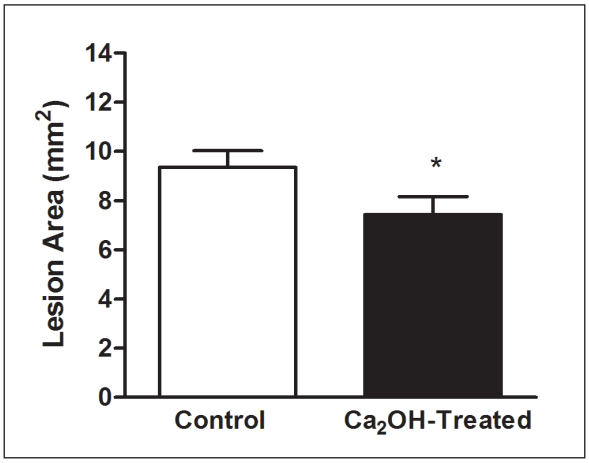

Fig. 4. Lesion areas (in mm^2) as determined by CBCT, in control (left upper molars) and calcium hydroxide-treated (right upper molars) groups. Each column represents the mean of 6 samples and the vertical lines show the SEM. *$P<0.05$ denotes the significance levels in comparison to control values.

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 (Fig. 5).

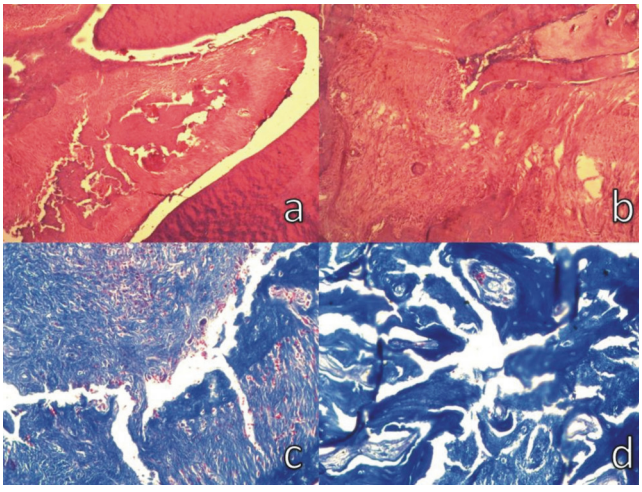

Fig. 5. Optical microscopy showing fewer and well-organized inflammatory infiltrate in the treated group (a), when compared to the control teeth (b). Teeth that received intracanal medication displayed more collagen fibers, offering support and organized connection between periapical tissues and endodontium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

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 [13,20]. 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 one 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.

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