Methods of experimental induction of periapical inflammation. Microbiological and radiographic evaluation

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Abstract

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Aim To evaluate the influence of coronal filling and apical perforation on the induction of periapical inflammation.

Methodology Fifty-eight root canals in the teeth of dogs were divided into four groups. Groups I and II: root canals were exposed for 180 days; groups III and IV: root canals were exposed for 7 days and then the access cavity filled for 53 days. The root apices of groups I and III were perforated after the coronal opening, whilst those of groups II and IV remained intact. Standard radiographs were taken before and after the experimental periods. Digital images of the radiographs were created and then analysed by three examiners. After induction of periapical inflammation, the root canal contents were collected using paper points. Microbiologic evaluation of the type of microorganism was carried out by culture in different growth media. The radiographic and microbiologic data were statistically analysed using ANOVA at a 5% significance level.

Results There were a greater total number of microorganisms in groups I and II ($P < 0.05$). The number of anaerobes was greater than the number of aerobes ($P < 0.05$). The size of the periapical radiolucencies were not significantly different between the experimental groups.

Conclusions The different methods analysed induced similar areas of periapical radiolucency in dogs with predominantly anaerobic bacteria. However, the time required for induction was less when the method with coronal filling was used.

Keywords: endodontics, microorganism, dental radiograph, periapical inflammation.

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Introduction

The presence of bacteria within necrotic pulp is essential for the establishment of periapical pathosis (Kakehashi *et al.* 1965, Siqueira 2002). Initially, when the pulp is necrotic, the bacteria are restricted to the root canal (Siqueira 2002). However, the dentinal tubules and canal ramifications (Hess & Zurcher 1994, Wada *et al.* 1998) are important areas of propagation, providing the opportunity for the establishment of a large quantity of bacterial morphotypes. In addition, the presence of necrotic pulp tissue and the loss of blood supply cause changes in the biochemical conditions of the pulp chamber, modifying the oxygen level and nutrient availability, making the environment suitable for microbiota changes. After this microbial shift, the

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predominance of obligate gram-negative anaerobic bacteria occurs (Fabricius *et al.* 1982, Tani-Ishi *et al.* 1994, Sundqvist *et al.* 1998).

The presence of gram-negative bacteria and consequently of endotoxins is fundamental for the establishment of periapical inflammation (Horiba *et al.* 1992, Silva *et al.* 2002, Tanomaru *et al.* 2003), which is only seen radiographically after the destruction of bone marrow and cortical bone (Schwartz & Foster 1971), reflecting a relatively long-term process.

Experimental induction of chronic periapical inflammation in the teeth of experimental animals is important so that the evaluation of new materials and techniques for clinical use can be carried out under conditions similar to those in humans (Watts & Patterson 1992). Periapical inflammation can be induced by different methods, for example, different time periods for contamination of root canals after oral exposure can be used as well as filling or not of the coronal opening during the process of periapical inflammation development. Several authors have used coronal filling after root canal contamination so that periapical inflammation is induced in a shorter period of time (Leonardo *et al.* 1994, Shabanhang *et al.* 1999, Tanomaru-Filho *et al.* 2002). Other researchers maintain the exposure of the root canal to the oral environment for longer periods of time (Jansson *et al.* 1993, Holland *et al.* 2003). Another variable that can be introduced in methods of periapical inflammation induction is related to the perforation of the foramen present in the root apex of dog teeth. Perforation of the apical foramen can be accomplished shortly after exposure of the root canal or the radicular apical delta can remain intact.

Because methodology is of fundamental importance for the interpretation of research results, the evaluation of periapical inflammation induced in the teeth of dogs using different methods is important in order to define the microbial flora of the root canal and evaluate the induced periapical inflammation in accordance with the technique used.

This study evaluated periapical inflammation and its correlation with root canal microbiota using several methods of inducing periapical inflammation.

Material and methods

The second, third and fourth mandibular premolars and second and third maxillary premolars of three dogs, totalling 58 root canals, were selected for treatment. The experimental protocols were in accordance to the Institutional Committee of Animal Experimentation.

The animals were anaesthetised intravenously with 3% sodium thiopental (Thionembutal, Abbot Laboratories, Rio de Janeiro, RJ, Brazil). Standard periapical radiographs were taken with the technique of Cordeiro *et al.* (1995).

After occlusal access cavities were prepared, the coronal pulp was removed and the root canals were explored with a size 20 K file (Dentsply Maillefer, Ballaigues, Switzerland). The pulp was then removed with a size 20 Hedstroem file.

The root canals were divided into four groups on the basis of the time of placement of the coronal filling and apical foramen perforation. In groups I and II, root canals were exposed to the oral cavity for 180 days. A total of 120 days after the procedures in groups I and II, the root canals of the other quadrants of the same dog were exposed to the oral cavity for 7 days, after which the coronal opening was restored for 53 days. These canals were included in groups III and IV.

In groups I and III, following pulpectomy, the foramen was perforated using sequential sizes 20, 25 and 30 K-files to create a standardized apical opening. All experimental procedures were tested in the same animal, and were performed in alternate quadrants. Sixty days after the coronal opening for groups III and IV and 180 days for groups I and II, standard periapical radiographs were taken to detect the development of periapical pathosis (apical radiolucency) and samples of material from the root canals were collected for microbiological evaluation. The operative procedures were undertaken under rubber dam isolation. The teeth and rubber dam were disinfected with 2% chlorhexidine solution. After removing the coronal filling (groups III and IV), or the pulp chamber residue (groups I and II), the microbiological samples were obtained from the root canals, using three sterile absorbent paper points that were transferred to test tubes containing 1.5 mL of reduced transport fluid (RTF).

Microbiological laboratory procedures

The contaminated paper points were placed in test tubes with RTF and sent for microbiological processing. At the microbiology laboratory, four to six glass beads and a sterile metal wing were added to the test tubes containing the samples. The tubes were agitated for 2 min in a Mixtron mixer (Toptronix, São Paulo, Brazil) at maximum speed. Subsequently, serial dilutions up to 10^{-5} were made in Sorensen phosphate buffer (PBS)

under laminar airflow. A volume of 0.05 mL of the pure samples and of each dilution was seeded, with a sterile calibrated pipette, onto plates containing blood agar (As), Mitis Salivarius agar (Ms; Difco, Detroit, MI, USA) and blood agar supplemented with 5.0 μg mL^{-1} haemin and 1.0 μg mL^{-1} menadione (Ask; Sigma Chemical Co., St Louis, MO, USA).

Ask plates were incubated anaerobically using the GasPak system for 7–10 days, Ms and SB_{20} plates microaerobically by the candle jar system for 2–3 days, and the As plates aerobically for 24–48 h, at 37 °C. After incubation, colonies were counted with a stereomicroscope (Nikon, Yokohama, Japan) under reflected light and the CFU mL^{-1} were counted.

Radiographic procedures

Periapical radiographs were obtained with a Heliodent dental X-ray machine (Siemens, Malvern, PA, USA) using a positioner for dogs recommended by Cordeiro *et al.* (1995). Radiographic processing was performed by the temperature–time method (Leonardo *et al.* 1994). The digital images were obtained with a Scanner Sprint Scan 35 (Polaroid, Waltham, MA, USA) and were saved as tiff extension, 30 × 40 mm in 300 dpi special resolution. The radiographic images were then imported to VixWin 2000 Versão 1.2 software (Gendex Dental Systems, Lake Zurich, IL, USA) and analysed individually by three examiners, two endodontists and one radiologist, using a mouse to outline the periapical images on the computer monitor. The examiners were blinded as to the experimental groups. The programme determined the area that corresponded to the radiographic image of the periapical radiolucency.

To analyse the results of the effect of periapical inflammation induction with and without root apex perforation, multiple comparisons were performed with the ANOVA test at a 5% significance level.

Results

Microbiological evaluation

The values of the colony forming units (CFU) of microorganisms were transformed into logarithms

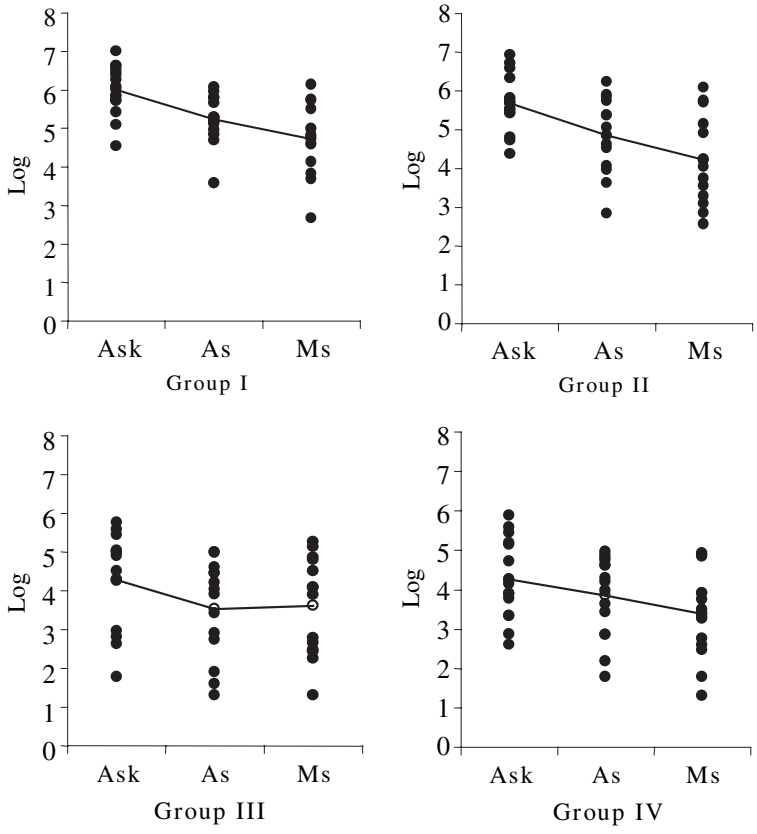

Figure 1 Graphic representation of the logarithms of microbial counts (CFU) of groups I, II, III and IV. Anaerobes: Ask (blood agar supplemented with 5.0 μg mL^{-1} haemin and 1.0 μg mL^{-1} menadione); aerobes: As (blood agar); microanaerobes: Ms (Mitis Salivarius agar)]. The horizontal lines join the means.

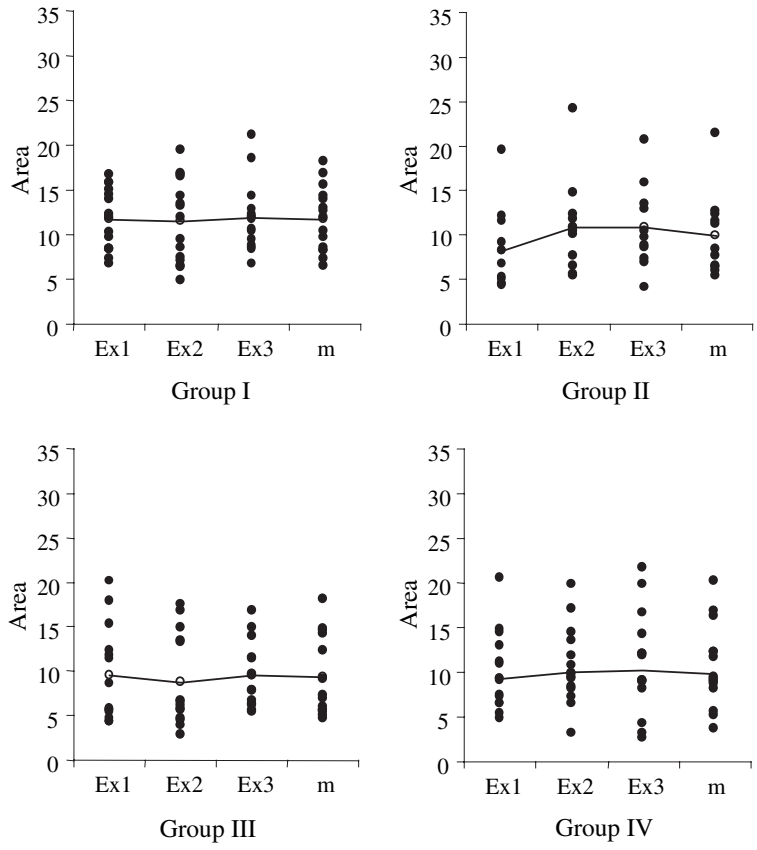

Figure 2 Graphic representation of the areas of the lesions (mm^2) of groups I, II, III and IV by the three examiners (Ex1, Ex2 and Ex3) and the mean of the evaluations (m).

(Fig. 1). The number of anaerobic microorganisms was greater than the number of aerobic microorganisms in all groups ($P < 0.05$). Statistical analysis with ANOVA showed that the variable 'foramen perforation' did not influence the type of microbiota isolated from the root canals. The method used to induce periapical inflammation without coronal filling (groups I and II) had a greater number of microorganisms (Fig. 1 – aerobes and anaerobes) in the root canals ($P < 0.05$).

Radiographic evaluation

Statistical analysis with ANOVA of two factors was used for the comparison of the means of the areas of the periapical lesions obtained by the methods, with or without apical perforation, using 5% as the level of significance. There were no statistically significant differences in the area of the periapical radiolucency in the groups ($P > 0.05$) (Fig. 2). Figures 3 and 4 illustrate typical examples of the radiographs from groups with coronal filling after 60 days (Fig. 3) or without coronal filling (Fig. 4) after 180 days.

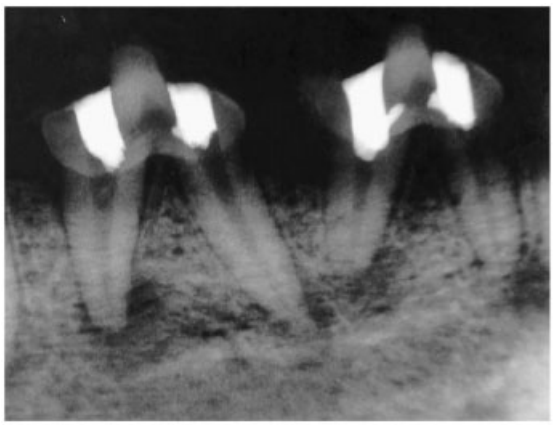

Figure 3 Typical radiographic findings after 60 days using the induction technique with coronal filling.

Discussion

In the study reported by Leonardo *et al.* (1994) for the induction of periapical inflammation in the teeth of dogs, the pulp cavity was exposed to the oral cavity for

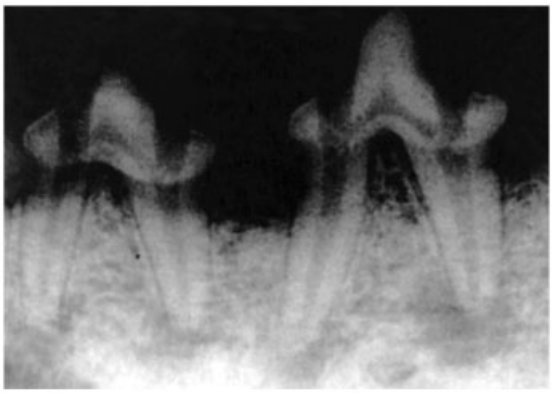

Figure 4 Typical radiographic findings after 180 days using the induction technique without coronal filling.

7 days in order to contaminate the root canals. After this period, coronal filling was performed and maintained until radiolucent periapical areas were observed, normally within 45–60 days.

This methodology has was also been used by others (Walton & Ardjamandi 1992, Shabanhang *et al.* 1999, Berbert *et al.* 2002, Tanomaru-Filho *et al.* 2002), who reported that after contamination of the root canal, the coronal restoration reduced the time necessary for the formation of radiolucencies. This was also observed in the present study.

Jansson *et al.* (1993), using two methods to induce periapical inflammation with and without coronal restoration, observed that when the pulp chambers were closed, formation of the radiolucencies was more rapid than when they were open, demonstrating that a closed environment favours the development of selective anaerobic gram-negative bacteria. In the four experimental groups in the present study, all teeth with apical radiolucencies had a large number of microorganisms in the root canals, of which the number of anaerobic microorganisms was greater than the number of aerobic microorganisms ($P < 0.05$). These findings are in agreement with other studies (Sundqvist 1976, Fabricius *et al.* 1982, Tani-Ishi *et al.* 1994, Siqueira & Roças 2003).

In the root canals that remained exposed to the oral cavity, the number of microorganisms isolated was higher than from the root canals that were closed after 7 days of contamination. This may be because of two important factors: higher nutrient availability and the niche produced by food residue in pulp chambers exposed for 180 days. In the root canals with the coronal filling, the food residues initially available were progressively consumed, thus controlling the intracanal microbiota.

The two methods of induction showed similar microbial species with anaerobic predominance in both methods; however, the time necessary to obtain this microbiota differed. Closed canals developed periapical lesions in a shorter period of time.

The radiographic evaluation of the size of the periapical radiolucencies did not show a statistical difference between the experimental groups. There was also no statistically significant difference in the size of the radiolucencies when the use of apical perforation was evaluated. This suggests that the apical anatomy of dog teeth, with various foramina forming the apical delta, does not prevent microorganisms or their by-products from reaching the periapical region to cause inflammation.

Conclusions

This study demonstrated that periapical inflammation (assessed by the development of radiolucencies) can be induced in the teeth of dogs in a predictable manner. Closing the coronal access cavity led to more rapid development of the radiolucencies and perforation of the apical foramen was not necessary.

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