

Radiographic Evaluation of the Effect of Endotoxin (LPS) Plus Calcium Hydroxide on Apical and Periapical Tissues of Dogs

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The aim of this study was the radiographic evaluation of the apical and periapical region of dog teeth submitted to intracanal bacterial endotoxin (lipopolysaccharide, LPS), associated or not with calcium hydroxide. After removal of the pulp, 60 premolars were divided into four groups and were filled with bacterial endotoxin (group 1), bacterial endotoxin plus calcium hydroxide (group 2), saline solution (group 3), or periapical lesions were induced with no treatment (group 4), for a period of 30 days. Similar periapical lesions were observed in groups 1 and 4. The lamina dura was intact in groups 2 and 3. Bacterial endotoxin (LPS) caused radiographically visible periapical lesions, but when associated with calcium hydroxide, this endotoxin was detoxified.

A fundamental role in the cause and maintenance of periapical lesions has been attributed to the bacterial endotoxin, lipopolysaccharide (LPS) (1–3). When released during bacterial multiplication or death, this endotoxin, composed of lipopolysaccharides, causes a series of important biological effects, which lead to an inflammatory reaction (4) and periapical bone resorption (2).

During the past 20 yr, a high prevalence of Gram-negative anaerobic microorganisms (5, 6) and the chemical structure of bacterial endotoxin commonly found in root canals with radiographically visible periapical lesions has been reported (7). The types of microorganisms and the level of intracanal endotoxin have also been correlated with specific clinical findings (1, 8). However, little research has evaluated the effect on periapical tissues of the inoculation of endotoxin into root canals (9–12).

The medical and dental literature have emphasized research attempting to obtain a medication that could inactivate bacterial endotoxin: examples are caustic soda (9), formocresol (13), 1.2% chlorhexidine (14), and sodium hypochlorite (15). Use of most of these products is limited due to their lack of efficiency or high toxicity, which causes undesirable effects when in contact with tissue.

During in vitro research, Safavi and Nichols (16, 17), Barthel et al. (3), and Olsen et al. (18) modified the concept of intracanal dressings, showing that calcium hydroxide hydrolyzes lipid A, which is the toxic part of endotoxin. Recently, we reported the histopathological finding of inactivation of the toxic effects of bacterial endotoxin by calcium hydroxide, in vivo (19). However, there are no in vivo radiographic reports of this inactivation. Thus, the purpose of this study was to evaluate radiographically the effect of endotoxin and endotoxin plus calcium hydroxide on the apical and periapical region in dogs.

MATERIAL AND METHODS

The methods used for this radiographic study were similar to those used for the histopathologic evaluation of the effect of endotoxin plus calcium hydroxide (19). In a laminar air flow, 100 mg of *Escherichia coli* endotoxin (lipopolysaccharide B, *E. coli* 055:B5-Lipid A, 9.2%; Difco, Bacto, Detroit, MI) was suspended in 10 ml of phosphate buffered saline. Half of the 10-mg/ml suspension was kept in sterile carpules and the other half was mixed with 2.75 g of calcium hydroxide p.a. (550 mg/ml, Merck, Whitehouse Station, NJ) and also kept in sterile carpules.

The second, third, and fourth mandibular premolars and the second and third maxillary premolars of three dogs (age: 12–18 months; weight: 8–15 kg) were selected for treatment (total: 60 root canals). Twenty roots were used for each of the two experimental groups (groups 1 and 2) and 10 for each of the control groups (groups 3 and 4).

The animals were anesthetized intravenously with sodium thiopental (30 mg/kg body weight, Thionembutal, Abbott Laboratories, São Paulo, SP, Brazil), and standardized radiographs were taken by using a Heliodont RX machine (Siemens, Erlanger, Germany) with 60 kVp, 10 mA, and 0.4 s exposure. Ultraspeed periapical film (Eastman Kodak, Corp., Rochester, NY) was used, and the radiographs were processed by the time/temperature method.

After isolation with a rubber dam and disinfection of the operative field with 0.3% iodine/70% alcohol, access was made. The working length was determined to 2-mm short of the radiographic apex by using #30 K-files. The root pulp was removed and the root canal was irrigated with saline solution (Labormédica Industria

Farmacêutica Ltda., São José dos Campos, SP, Brazil) with a minimum volume of 3.6 ml at each instrument change. The apical foramen was enlarged by sequential use of #15 to #30 K-files (Maillefer, Ballaigues, Switzerland) to the radiographic apex (always with saline irrigation). Instrumentation was then performed to the working length up to a #50 K-file. A #30 K-file was used at the total length of the root to ensure that no dentin chips or other residue remained in the apical foramen. After irrigation, the root canals were dried by aspiration and sterile paper points, filled with 14.3% buffered EDTA (pH 7.4; Odahcan-Herpo Produtos Dentários Ltda., Rio de Janeiro, RJ, Brazil) for 3 min, and then irrigated with saline and dried.

Because all variables should be tested in the same animal and in the different quadrants, each hemiarch was submitted, in an alternate manner, to the experimental protocols. Group 1: 20 root canals were filled with 0.1 ml of the endotoxin by using a threaded syringe (S.S. White Artigos Dentários Ltda., Rio de Janeiro, RJ, Brazil) with a 27-gauge needle (Terumo, Tokyo, Japan). Group 2: 20 root canals were filled with 0.1 ml of the endotoxin and calcium hydroxide suspension by using a threaded syringe with a Calasept Kit needle (Scania Dental AB, Knivsta, Sweden). Group 3: 10 root canals were filled with saline by using a carpule syringe with a 27-gauge needle. After these procedures, the pulp chambers of groups 1, 2, and 3 were sealed with a sterile cotton pellet, and the teeth were sealed with zinc oxide-eugenol cement (IRM, Dentsply, Rio de Janeiro, RJ, Brazil) for a period of 30 days. Group 4: 10 root canals were exposed to the oral environment for 5 days to allow microbial contamination, after which, under general anesthesia, the pulp chamber was cleared of all debris and sealed with a cotton pledge and zinc oxide-eugenol cement to induce a periapical reaction (20).

Thirty days after the surgical procedures, the teeth were radiographed as described previously.

Radiographic Evaluation

The two sets of radiographs were evaluated, by three trained examiners, by analyzing the integrity of the lamina dura, the presence or absence of root resorption, and areas of periapical bone resorption.

Statistical Analysis

The results were analyzed statistically by the Mann-Whitney nonparametric test by using the software GMC7.7 (<http://www.forp.usp.br/restauradora/gmc/html>).

RESULTS

The 20 roots of group 1 (LPS) had radiographically visible periapical lesions with a loss of lamina dura integrity and extensive circumscribed areas of periapical bone resorption (Fig. 1).

The lamina dura was intact and there were no areas of periapical bone resorption in 17 roots of group 2 (LPS plus calcium hydroxide) (Fig. 2). Only slight thickening of the periodontal ligament occurred in the remaining three roots at the apical level.

There was slight thickening of the periodontal ligament at the apical level in only 1 of 10 roots of group 3 (saline). The other nine roots were normal (Fig. 3).

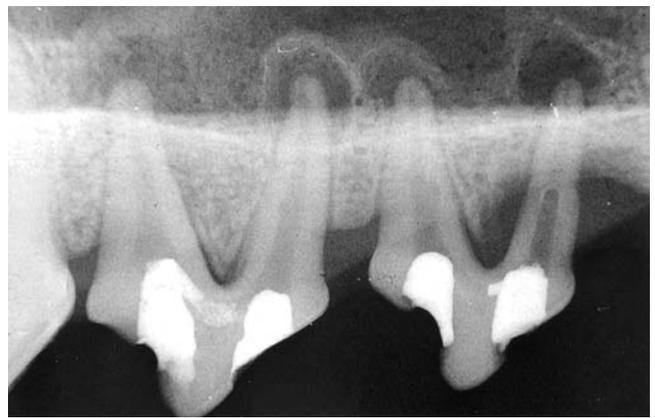


FIG 1. Radiograph, 30 days after filling root canals with LPS (group 1), showing extensive circumscribed radiolucent areas.



FIG 2. Radiograph, 30 days after filling root canals with LPS plus calcium hydroxide (group 2), showing normal apical and periapical tissues.



FIG 3. Radiograph, 30 days after filling root canals with saline (group 3), showing normal apical and periapical tissues.

There were radiographically visible periapical lesions in all 10 roots of group 4 (experimental lesion) with a loss of integrity of the lamina dura and extensive diffuse areas of bone resorption (Fig. 4).

There was no root resorption in any of the groups.

The loss of integrity of the lamina dura and the presence of periapical bone resorption were statistically similar ($p < 0.05$) in groups 1 and 4 and in groups 2 and 3 (group 1 = group 4 \neq group 2 = group 3).

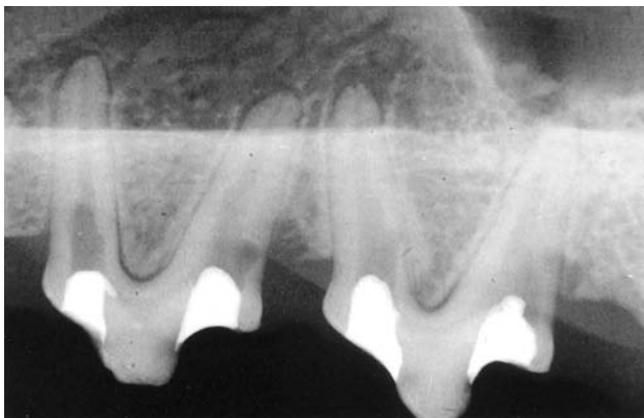


FIG 4. Radiograph, 30 days after the experimental induction of periapical lesions (group 4), showing diffuse radiolucent areas.

DISCUSSION

Despite the fact that the role of bacteria in the etiology of periapical lesions has already been proven, little research has been performed to evaluate the isolated effect of LPS on apical and periapical tissues (9–12). The results of this study, using dog teeth, are in agreement with those of Dwyer and Torabinejad (9), using cat teeth, Dahlén et al. (10), using monkey teeth, and Pitts et al. (11) and Mattison et al. (12), who also used dog teeth. We also found that after 30 days there were radiographically visible periapical lesions in 20 roots with loss of integrity of the lamina dura and extensive circumscribed areas of bone resorption (group 1), similar to the findings of Mattison et al. (12), and diffuse areas of resorption in group 4. The fact that after 30 days the lesions of group 4 were not circumscribed may be because the lesions were caused by bacteria, their products and subproducts, such as hyaluronidase, collagenase, and indole, which act in the dissociation of fibers and the collagen matrix leading to a diffuse lesion. In group 1, LPS adhered irreversibly to mineralized tissues (10), causing more localized extensive periapical bone resorption.

Even after their death, Gram-negative bacteria release endotoxin. Thus, from a clinical viewpoint, the use of medication that leads only to the death of bacteria for the treatment of teeth with pulp necrosis and chronic periapical lesion is not sufficient, but medication must also inactivate bacterial endotoxin. In 1993, Safavi and Nichols (16) reported that calcium hydroxide hydrolyzed lipid A in vitro, and in 1994, they concluded that after lipid A hydrolysis, this highly toxic agent releases free-hydroxy fatty acids that are nontoxic (17).

Furthering this line of research, Barthel et al. (3) and Olsen et al. (18) evaluated, in vitro, the capacity of neutralization of endotoxin by calcium hydroxide and reported that LPS was inhibited by calcium hydroxide. Using dog teeth in vivo, Silva et al. (19) reported histopathologically that calcium hydroxide inactivates the toxic effects of bacterial endotoxin.

In this study, 30 days after filling the root canals of dog teeth with a high concentration of LPS plus calcium hydroxide (group 2), the lamina dura was intact and there was no bone resorption. In most cases, there was no thickening of the ligament. These results were statistically similar to the teeth filled with saline (group 3). Comparison of these results with the current literature is not possible because there are no other in vivo studies. The results of

group 3, in which saline was used, were similar to those reported by others (9–12) with an intact lamina dura and absence of a periapical lesion.

This radiographic evaluation of the effect of LPS combined with calcium hydroxide shows that calcium hydroxide detoxifies the bacterial endotoxin LPS in vivo and thus should be the medication of choice for intracanal dressings in teeth with pulp necrosis and a periapical lesion.

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References

- Schein B, Schilder H. Endotoxin content in endodontically involved teeth. *J Endodon* 1975;1:19–21.
- Yamasaki M, Nakane A, Kumazawa M, Hashioka K, Horiba N, Nakamura H. Endotoxin and Gram-negative bacteria in the rat periapical lesions. *J Endodon* 1992;18:501–4.
- Barthel CR, Levin LG, Reisner HM, Trope M. TNF-alpha in monocytes after exposure to calcium hydroxide treated *Escherichia coli* LPS. *Int Endod J* 1997;30:155–9.
- Rietschel ET, Brade H. Bacterial endotoxins. *Sci Am* 1992;267:26–33.
- Sundqvist G. Ecology of the root canal flora. *J Endodon* 1992;18:427–30.
- Assed S, Ito IY, Leonardo MR, Silva LAB, Lopatin D. Anaerobic microorganisms in root canals of human teeth with chronic apical periodontitis detected by immunofluorescence. *Endod Dent Traumatol* 1996;12:66–9.
- Kumada H, Haishima Y, Umemoto T, Tanamoto KI. Structural study on the free lipid A isolated from lipopolysaccharide of *Porphyromonas gingivalis*. *J Bacteriol* 1995;177:2098–106.
- Horiba N, Maekawa Y, Abe Y, Ito M, Matsumoto T, Nakamura H. Correlations between endotoxin and clinical symptoms or radiolucent areas in infected root canals. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1991;71:492–5.
- Dwyer TG, Torabinejad M. Radiographic and histologic evaluation of the effect of endotoxin on the periapical tissues of the cat. *J Endodon* 1981;7:31–5.
- Dahlén G, Magnusson BC, Moller A. Histological and histochemical study of the influence of lipopolysaccharide extracted from *Fusobacterium nucleatum* on the periapical tissues in the monkey *Macaca fascicularis*. *Arch Oral Biol* 1981;26:591–8.
- Pitts DL, Williams BL, Morton TH Jr. Investigation of role of endotoxin in periapical inflammation. *J Endodon* 1982;8:10–8.
- Mattison GD, Haddix JE, Kehoe JC, Progulske-Fox A. The effect of *Eikenella corrodens* endotoxin on periapical bone. *J Endodon* 1987;13:559–65.
- Sant'anna AT, Ramalho LTO, Spolidório DMP. Effect of the formocresol on bacterial LPS in mouse's subcutaneous tissue [Abstract]. *J Dent Res* 2000;79:1084.
- Aibel K, Stevens R. Effect of chlorhexidine on IL-6 induction by LPS [Abstract]. *J Endodon* 1999;25:282.
- Buttler TK, Crawford JJ. The detoxifying effect of varying concentrations of sodium hypochlorite on endotoxins. *J Endodon* 1982;8:59–66.
- Safavi KE, Nichols FC. Effect of calcium hydroxide on bacterial lipopolysaccharide. *J Endodon* 1993;19:76–8.
- Safavi KE, Nichols FC. Alteration of biological properties of bacterial lipopolysaccharide by calcium hydroxide treatment. *J Endodon* 1994;20:127–9.
- Olsen MH, Difiore PM, Dixit SN, Veis A. The effects of calcium hydroxide inhibition on LPS induced release of IL-1 β from human monocytes in whole blood [Abstract]. *J Endodon* 1999;25:289.
- Silva LAB, Nelson-Filho P, Leonardo MR, Rossi MA, Pansani CA. Effect of calcium hydroxide on bacterial endotoxin in vivo. *J Endodon* (in press).
- Leonardo MR, Silva LAB, Leonardo RT, Utrilla LS, Assed S. Histological evaluation of therapy using a calcium hydroxide dressing for teeth with incompletely formed apices and periapical lesions. *J Endodon* 1993;19:348–52.