Radiographic and histologic evaluation of the effect of endotoxin on the periapical tissues of the cat

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The periapical tissue reaction to three concentrations of *Escherichia coli* endotoxin solutions, to three detoxified *E coli* endotoxin solutions, and to a sterile saline control solution was examined in ten adult cats. The maxillary and mandibular canines were isolated with a rubber dam, and the pulps were extirpated. The solutions were deposited in the root canals of each cat, and the access cavities were sealed. The periapical tissues were examined histologically and radiographically at 2, 4, and 6 weeks. The radiographic and histologic results indirectly suggest that endotoxins have a part in initiating and perpetuating periapical inflammatory lesions in man.

The possibility that bacterial endotoxins are involved in the pathogenesis of periapical inflammatory lesions has received little attention in endodontic research. These lipopolysaccharide complexes are produced when the cell walls of gram-negative bacteria undergo lysis. They are structural components of the cell wall and are released when the cell integrity is interrupted.

Recent investigations of the root canal flora have discovered a significant number of gram-negative bacteria capable of releasing lipopolysaccharides (LPS) or endotoxins on cell lysis. The results obtained by Schein and Schilder[1] show a significant level of endotoxin in the root canal system. These investigators aspirated fluid from 40 teeth and analyzed it with the limulus lysate test. The teeth with necrotic pulps contained greater concentrations of endotoxin than those with vital pulps, and the symptomatic teeth contained more endotoxin than asymptomatic teeth. Furthermore, teeth with radiolucent periapical areas contained higher levels of endotoxin than teeth without such areas. Schein and Schilder concluded that bacterial endotoxins may be factors in producing pulpal and periapical disease.

On the other hand, Wesselink and others[2] tentatively concluded that the primary toxicity of endotoxin had no major part in the initiation or maintenance of chronic periapical inflammation. They investigated the role of endotoxin by using an experimental model simulating the root canal. Polyethylène tubes were filled with varying amounts of *E coli* endotoxin and implanted in the subcutaneous tissue of 27 rabbits.

Endotoxins have a wide variety of potent biologic properties, and man is more sensitive to their biologic effects than are other mammals.[3] Endotoxins act pharmacologically to increase capillary permeability and produce inflammation when injected intradermally. In addition, the endotoxins are strongly antigenic. The lipopolysaccharide portion is a potent antigen that can elicit antibody formation in submicrogram quantities.[4]

Several investigations have shown that the root canal system can act as a pathway for release of microbes and other potential antigens into the periapical tissues.[5-9] These studies indicate that when an antigen is placed in the root canal system it can egress into the periapical area and thus cause systemic sensitization and antibody formation. Because gram-negative bacteria have been isolated from the root canals of endodontically involved teeth, it is possible that their endotoxins affect the periapical tissues and have a role in the pathogenesis of inflammatory lesions of pulpal origin.

The purpose of this study was to investigate radiographically and histologically the effect of *E coli* endotoxin on periapical tissues of the cat.

MATERIALS AND METHODS

Animals

Other studies have indicated that the cat is a suitable experimental animal for investigating the immunopathogenesis of periapical lesions.[10,11] Some of the advantages of the cat model include availability, ease of handling and anesthetizing, and useful pulpal and periapical anatomy for endodontic experimentations. In this study, ten cats, each weighing 2.5 to 3.5 kg, were housed in individual cages and maintained on amounts of Purina Cat Chow and water.

Endotoxin

The bacterial endotoxin used in this study was extracted from *E coli* 0111:B4 and was supplied in a lyophilized form. To determine the possible mode of action of endotoxin on the periapical tissues, a portion of the *E coli* endotoxin was detoxified with 0.2 M NaOH at 37 C for 24 hours, dialyzed, and again lyophilized. Both of these endotoxin preparations were dissolved in sterile saline solutions to concentrations of 100 μg/ml. These solutions were diluted to make 10 μg/ml and 1 μg/ml concentrations of endotoxin and detoxified endotoxin. These dilutions were chosen to provide information regarding the effective range of endotoxin solution.

Procedure

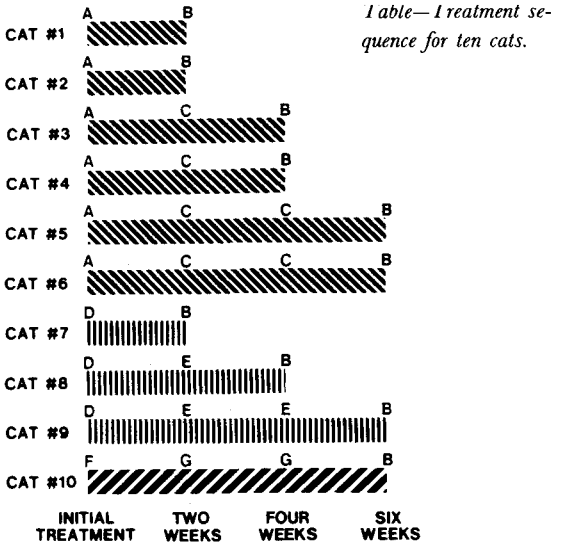
Each cat was anesthetized with an intramuscular injection of ketamine hydrochloride (40 mg/kg), combined with xylazine (2 mg/kg). Preoperative radiographs of the maxillary and mandibular canines were taken. Using an aseptic technique and rubber dam, the incisal portions of these teeth were removed, exposing the pulps. With successively larger Hedstroem files, the access openings were enlarged to the extent that medium-sized broaches could be easily inserted into the root canals. The pulps were extirpated with the broaches, and the canals were irrigated with sterile saline solution and dried with sterile paper points. Sterile, disposable 1-ml syringes with 1.25-inch, 23-gauge needles were used to deposit 0.1 ml of endotoxin or saline solution in excess into the root canals so that the four canines of six cats were injected with either 100 μg/ml, 10 μg/ml, or 1 μg/ml of endotoxin, or saline solution. The openings were sealed with IRM (L. D. Caulk Co.).

To investigate the possible mechanisms involved in pathogenesis of periapical reactions, three cats were treated in the previously described manner, but with the detoxified endotoxin solutions (100 μg/ml, 10 μg/ml, and 1 μg/ml), and saline solution was used as a control. As an additional control, in one cat all four canines were injected with saline solution initially, and again at two and four weeks. The Table describes the treatment sequence for all ten cats.

At 2, 4, and 6 weeks, cats were killed with an overdose of sodium barbiturate. In the six cats injected with endotoxin and saline solutions, 5 ml of blood was collected from the jugular vein. The double immunodiffusion (Ouchterlony) method was used to determine the presence of antibodies to endotoxin in the blood of treated cats. The test was performed by first pouring molten agar into petri dishes and allowing it to harden. Then a central hole and six circumferential holes were punched out of the agar and removed by suction with a pipette attached to a vacuum line. The central well was filled with endotoxin solution (100 μg/ml), and each of the peripheral wells was filled with serum from one of the cats. The plates were stored in a refrigerator at 10 C and observed daily for lines of precipitate between antigen and antiserum wells. No double immunodiffusion tests were performed on cats that were treated with detoxified endotoxin or on the cat whose canines were injected with sterile saline solution.

Block sections containing the maxillary or mandibular canines and the surrounding tissues were removed from all the cats and radiographed. The specimens were placed in 10% buffered formalin, decalcified in 20% formic acid, dehydrated, and embedded in paraffin. Sections at 6 μ were prepared and stained with hematoxylin and eosin for histopathologic examination. The sections were examined by two veterinary pathologists, who were not aware of the nature of the project, for the type of cellular inflammatory infiltrate, the degree of inflammation, and the presence of osteoclastic and osteoblastic activities.

The radiograph of each specimen removed from each of the ten cats was placed in a projector-viewer, and a clear plastic sheet was placed over the viewer screen. On this clear sheet, the outline of the radiographic lesion was traced independently by three observers on different days. A clear plastic grid containing 3.5-mm squares was then placed over the drawing, and the grid intersections inside the representations of the lesions were counted. These data were averaged to produce figures that allowed ranking of the radiolucent areas according to size for each



Table—Treatment sequence for ten cats.

cat. Rank 1 indicated the smallest radiolucent area, with the ranking increasing up to 4 for the largest. When there was a difference of less than two intersections between two specimens, the rank was averaged.

The Friedman and Wilcoxon tests were used to analyze the ordinal data obtained through the ranking procedure to determine statistical differences between treatments.

RESULTS

Radiographic Findings

Relatively large apical radiolucent areas were noticed in the teeth injected with endotoxin when examined after two weeks, and these changes continued to be present in the specimens examined after four and six weeks. The teeth in which saline solution was injected either had no apical radiolucent areas or very small radiolucent areas compared with the other teeth in the same cat (Fig 1). In three of the cats in which 100 μg/ml endotoxin solution had been deposited, the periapical tissues showed the largest radiolucent areas. No radiographic changes were discernible in the periapical tissues of the cat in which all four teeth were injected with saline solution. Because this cat served as an additional control to see the possible effects of pulp extirpation and solution injections, it was not included in the statistical results. In the three cats in which detoxified endotoxin was injected, there was no difference between the saline solution control teeth and the experimental teeth. These cats were also excluded from statistical results.

Statistical Results

The Friedman test was performed on the ranked data to determine differences among the reactions to treatments. This test indicated that the treatments are not all equal in their effects.

The Wilcoxon test was used to analyze differences between the types of treatments according to differences in lesion size. These differences were determined for each animal, and the differences were ranked. In this test, saline solution was compared with each of the three endotoxin solutions, and the endotoxin solutions were compared with each other. The results of the Wilcoxon test indicate that there are significant differences ($P = .01$) between the effects of the three endotoxin solutions and that of the saline solution. However, there are no significant differences in effects when the different endotoxin treatments are compared with each other.

Histologic Observations

To determine the histological

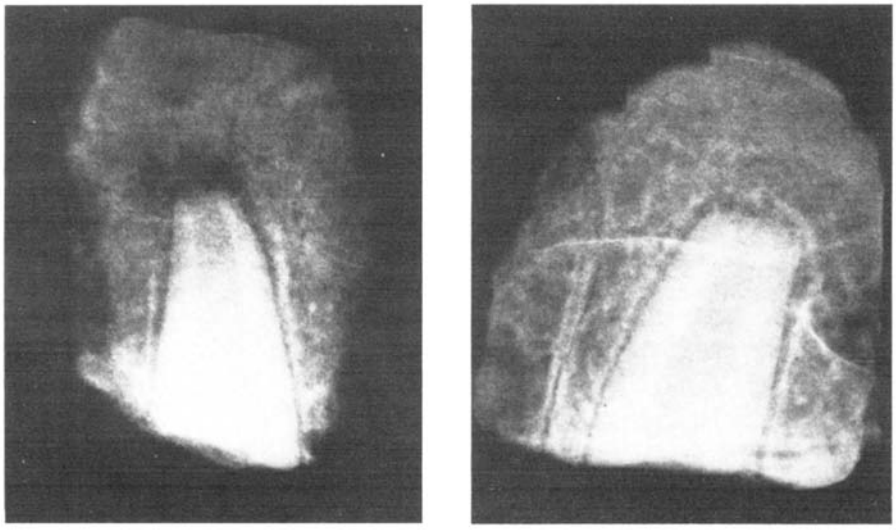


Fig 1—Left, radiograph of block section of maxillary canine removed after three intracanal injections of 100 μg/ml of endotoxin. Bone resorption is present at periapical region. Right, radiographic block section of contralateral canine removed after three intracanal injections of sterile saline solution. The lamina dura and periodontal membrane space appear intact around tooth apex.

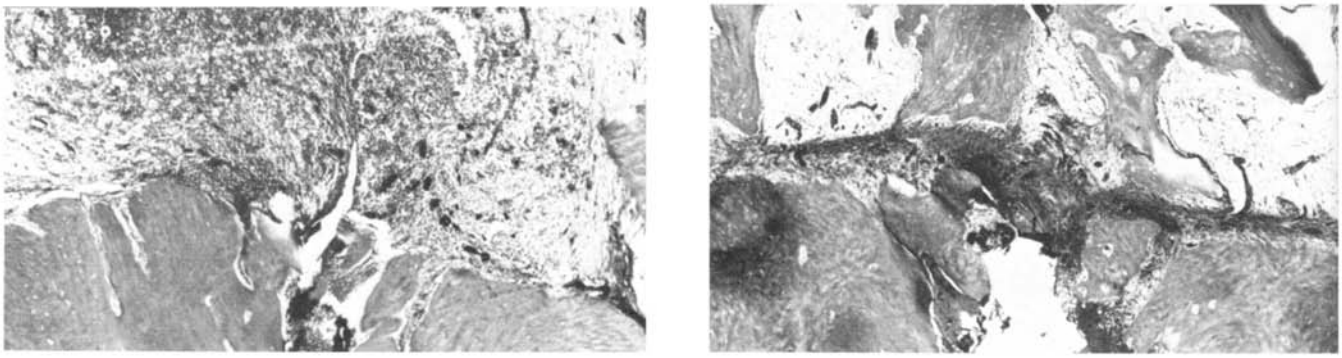


Fig 2—Left, photomicrograph of block section of maxillary canine 42 days after intracanal injection of 100 μg/ml of endotoxin solution. Dense inflammatory infiltrate is present around the tooth apex (H&E, orig mag ×50). Right, photomicrograph of block section of maxillary canine 42 days after intracanal injection of sterile saline solution. Insignificant infiltration of periapical tissues with inflammatory cells (H&E, orig mag ×50).

characteristics of the periapical tissues, the sections taken at 2, 4, and 6 weeks were examined microscopically. A relatively consistent histologic pattern developed in the periapical tissues in the teeth injected with the endotoxin solutions when compared with the teeth injected with saline solution. The inflammation was generally more intense (more inflammatory cells per unit area) in the endotoxin-injected teeth than in those treated with saline solution or detoxified endotoxin (Fig 2).

The cellular inflammatory infiltrate consisted primarily of polymorphonuclear (PMN) leukocytes, although macrophages, plasma cells, and lymphocytes were also present (Fig 3). Multinucleated cells having the morphologic appearance of osteoclasts were frequently seen at the periphery of the surrounding bone, and areas of bone resorption were associated with these cells (Fig 4).

Antigen-Antibody Reactions

The double immunodiffusion technique demonstrated no antibody formation in the serum collected from six cats at 2, 4, or 6 weeks. No banding or precipitin line was present in any of the samples.

DISCUSSION

Endotoxin derived from *E coli* was selected for use in this investigation even though the coliform bacilli are not principal constituents of the oral flora in man. Mergenhagen[12] states that, in toxicity tests, the lipopolysaccharide endotoxins of oral bacteria closely resemble endotoxins derived from *E coli*.

When the ranking procedure was carried out and the calculations for the Friedman test completed, it was determined that the effects of the three different experimental solutions and sterile saline solution were not equal. When the Wilcoxon test was used, there was a significant difference between the effects of the endotoxin solutions and the sterile saline solution. However, the Wilcoxon test showed that there was no significant statistical difference between the effects of the endotoxin solutions when compared with each other. This may have occurred because the endotoxin solutions were too toxic at the concentrations used in this experiment.

Histologically, the teeth injected with saline solution had milder periapical inflammatory reactions compared with those injected with endotoxin. All the lesions had an acute inflammatory infiltrate and did not resemble chronic lesions. Repeated injections of solutions may have caused this reaction.

There are three basic mechanisms that could give endotoxin a role in producing periapical lesions. One would involve the activation of the complement system through the formation of antigen-antibody complexes as activating agents. However, because there was no evidence of antibody formation during the six-week study period, it appears that this mechanism was not a factor in the production of periapical inflammatory lesions in this experiment.

Another mechanism for the production of periapical pathology would be the activation of complement by endotoxin. Morrison and Kline[13] have demonstrated the activation of the classical and alternate pathways of complement by bacterial LPS. They say that the lipid A region of the LPS is responsible for properdin (alternate) pathway activation. When the complement com-

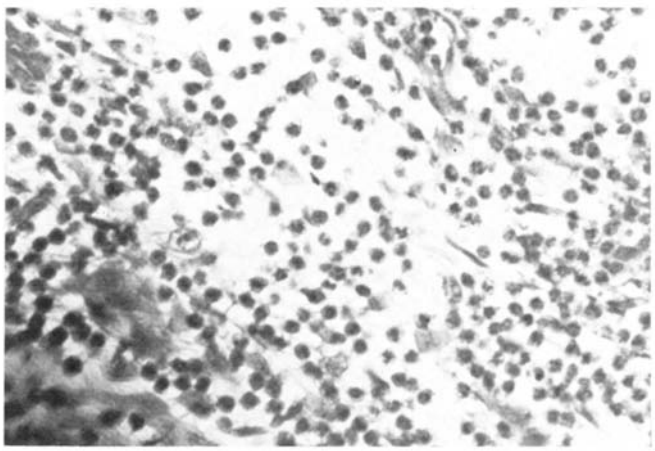


Fig 3—Higher magnification of inflammatory infiltrate at periapical region in Figure 2, left, shows presence of PMN leukocytes, macrophages, lymphocytes, and plasma cells (H&E, orig mag ×400).

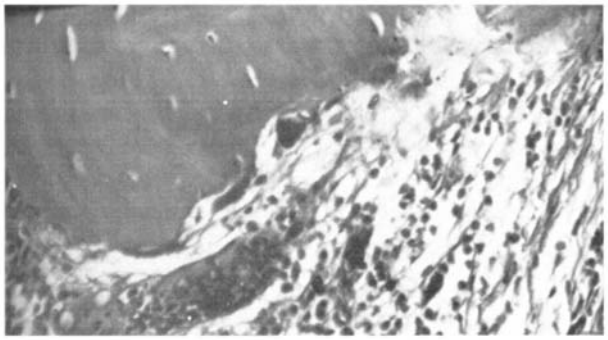


Fig 4—Peripheral edge of periapical lesion with cells having morphologic appearance of osteoclasts (H&E, orig mag ×100).

ponents are activated and cleaved by endotoxin, biologically active peptides are released that mediate a number of inflammatory reactions, including vascular permeability, chemotactic attraction of PMN leukocytes and macrophages, degranulation of mast cells, and cell lysis.[14] Complement activation by endotoxin releases biologic fragments which may lead to inflammatory changes and also possibly to bone resorption in the periapical region.

By yet another mechanism, the biologic properties of endotoxin make it a potent inflammatory agent, and when introduced locally into a number of species, including man, endotoxin induces a dramatic, acute inflammatory response. Mergenhagen[15] states that intradermal injection of small amounts of endotoxin leads to the rapid accumulation of PMN leukocytes. Besides being an inflammatory agent, endotoxin has biologic properties that produce bone resorption. Hausman and others[16] demonstrated that the LPS of bacterial endotoxin stimulated osteoclastic activity in tissue cultures of fetal rat bones. Hausman and others[17] also demonstrated that as little as 1 μg/ml of endotoxin from an oral *Bacteroides* caused significant bone resorption of fetal rat bone in tissue culture. They reported that endotoxin caused a proliferation of osteoclasts and the removal of matrix. Rizzo and Mergenhagen[18] inoculated the palatal gingiva of rabbits with endotoxin from an oral *veillonella.* A single injection of 50 μg of this endotoxin produced an acute inflammatory lesion accompanied by bone resorption.

In this study, because the detoxified endotoxin solution did not produce a significant amount of periapical change compared with saline solution, apparently it is through its inflammatory and toxic properties that endotoxin participates in production of periapical inflammatory lesions.

SUMMARY

The cat was used as a model in this study to determine the effects of endotoxin on the periapical tissues. Radiographic and histologic observations were made to determine the effects of three concentrations of nondetoxified and detoxified endotoxins and a saline control solution. The results indicate that nondetoxified bacterial endotoxins placed in the root canal system of the cat caused greater pathologic periapical changes than did detoxified endotoxins or saline solution. The results support the conclusion that bacterial endotoxin may have a role in the production of periapical inflammatory lesions.

This paper won first place in the Graduate Research Seminar, AAE Convention, Los Angeles, April, 1980.

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