Endotoxin content in endodontically involved teeth

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Fluid was aspirated from the root canals of 40 endodontically involved teeth. This fluid was assayed for endotoxin with the limulus lysate test. Pulpless teeth contained greater concentrations of endotoxin than those with vital pulps. Symptomatic teeth also contained more endotoxin than asymptomatic teeth.

Gram-negative bacteria are commonly found in the oral cavity. The cell walls of these gram-negative microorganisms are composed of or contain endotoxin, a lipopolysaccharide complex. When these bacteria die, their cell walls are lysed and endotoxin is released.

Endotoxins are potent biological agents that are capable of initiating various biological responses. These include pyrogenicity, local and generalized Schwartzman reactions, primary inflammatory reactions on skin, vascular and hemodynamic effects, abortion, immunotolerance, and others.[1,2] A relationship between oral endotoxin levels and the severity of clinical gingival inflammation has been suggested.[3,4] Mergenhagen[5] postulated that the destructive action of endotoxin is mediated by activation of the complement system. Subsequently, there is a cleavage of C5, which is a substance that enhances vascular permeability, causes vasodilation, and chemotactically attracts polymorphonuclear leukocytes and macrophages. Endotoxins also have been found to stimulate bone resorption in tissue cultures[6,7] and to possess the ability to attract osteoclasts to bone.[8]

There are various methods for the detection and quantification of endotoxins.[9,10] Recently, a reliable biological method that uses the lysed amebocytes of the horseshoe crab *(Limulus polyphemus)* has been described.[11-14] The most sensitive and accurate biologic determination of endotoxin is the limulus lysate technique.[15,16]

Since some of the symptoms and reactions produced by endotoxins resemble certain symptoms and reactions associated with endodontically involved teeth, it is postulated that endotoxins may play a role in pulpal and periapical disease. No association of endotoxin with endodontic pathosis has been reported. The current study was undertaken to detect and to quantitate the levels of endotoxin in the fluid aspirated from the root canals of teeth that were opened for endodontic treatment.

METHODS AND MATERIALS

Forty patients for this study were chosen at random from the patient population at Boston University School of Graduate Dentistry. They were assigned to four clinical groups and two radiographic groups. The clinical groupings were 1, teeth with vital pulps, symptomless; 2, teeth with vital pulps showing symptoms; 3, pulpless teeth, symptomless; and 4, pulpless teeth showing symptoms. The radiographic groupings were A, teeth with no radiolucent areas that showed on a periapical radiograph, and B, teeth with radiolucent areas that showed on a periapical radiograph.

Collection of samples

All instruments, materials, glassware, and diluents were pyrogenfree. The tooth to be investigated was isolated with a rubber dam and the field was disinfected with Mercresin tincture. All carious dentin was removed with burs and excavators. Access to the pulp chamber was attained with a new, sterile no. 6 round bur. A sterile pyrogenfree syringe was used to deposit 0.1 ml of pyrogenfree saline solution in the pulp chamber. Five apical filing strokes were made with a no. 1 Kerr file. With the use of a new pyrogenfree syringe with negative pressure, 0.1 ml of fluid was aspirated from the root canal. Each collected sample was deposited in a test tube containing 1 ml of pyrogenfree saline solution.

Processing of samples

The samples were boiled at 100 C for 30 seconds to denature the protein content, especially of the globulins. This was necessary since globulins might interfere with the limulus lysate test. Boiling did not affect the endotoxin activity of the sample since the endotoxin molecule is heat stable.

The sample was sonicated for ten seconds at the lowest power of a W85 Sonicator.*

Limulus lysate test

Aliquots of each of the processed samples were subjected to the limulus lysate test for endotoxin as described by Reinhold and Fine.[14] For this test, 0.1 ml of limulus lysate was reacted with 0.1 ml of the collected sample, and with serial twofold dilutions of the same sample. Before this reaction, the activity (end point) of the lysate was determined by using a known stock solution of endotoxin. The end point of the lysate used in this study was 0.001 μg of endotoxin; that is, the lysate could detect as little as 0.001 μg of endotoxin. No lysate was added to the last dilution in the event that further dilutions would become necessary.

All test tubes were incubated in a water bath and then were read. The gel formation indicated a positive reaction. The endotoxin activity of the sample could then be determined from the last positive dilution.

Suitable positive and negative controls were used in all phases of the study. Random samples were rerun to ensure the reproducibility of the results.

RESULTS

The results for the clinical groups are shown in Table 1.

A statistical analysis was performed to see if there was a significant difference between the groups. After a logarithmic transformation of each measurement was made, the analysis of variance test was used. A significant difference was demonstrated ($P<0.001$) when teeth with vital pulps were compared to pulpless teeth. The comparison of teeth with symptoms also showed a significant difference ($P<0.001$).

The findings for the radiographic groups are shown in Table 2.

The Spearman rank correlation of these data indicated that the results were significant ($P<0.001$) when comparing teeth without radiolucent areas on a periapical radiograph to teeth showing radiolucent areas.

Table 1 • Limulus lysate test results for endotoxin in clinical groups.

Groups	No. of samples	Mean (μg Et/ml)	Range
1	10	0.007	0.000-0.032
2	10	0.075	0.001-0.256
3	10	0.192	0.064-0.512
4	10	1.070	0.256-2.048

Table 2 • Limulus lysate test results for endotoxin in radiographic groups.

Groups	No. of samples	Mean (μg Et/ml)	Range
A	23	0.057	0.000-0.256
B	17	0.717	0.032-2.048

DISCUSSION

The results showed that pulpless teeth contain high levels of endotoxin. This is not surprising since the endotoxin level probably mirrors the gram-negative flora of root canals with necrotic pulps. Most oral gram-negative bacteria are strict anaerobes. The necrotic tissue provides an excellent anaerobic environment. The more elaborate methods for isolation of anaerobes that have been applied to intestinal flora have not been used for root canal samples. However, studies are in progress at Boston University to determine the role of anaerobic bacteria in periapical disease.

The fact that there was a positive correlation of increased endotoxin level with clinical symptoms is probably a result of the inflammatory potential of endotoxin. The release of histamine, serotonin, and vasoactive polypeptidelike substances after injection of endotoxins has been reported.[1,2] These substances are believed to be pain producing.[17] Also, the endotoxic lipopolysaccharides may act in a manner that is similar to immune complexes in activating the complement system. This is central in the mediation of inflammatory reactions that are initiated by antibodies.[5]

During endodontic therapy, viable gram-negative microorganisms may be sealed in the root canals. If bacteria within the canals succumb to the intracanal medication, the endotoxin released from their cell walls may cause a periapical tissue response. If gram-negative bacteria are present at the periapex, and if the periapical inflammatory response as a result of instrumentation and medication is sufficient to cause lysis of these bacteria, the endotoxin released may also cause a periapical response. In both instances, an acute clinical inflammation may follow.

When the radiographic groups were compared, teeth with radiolucent areas contained a higher level of endotoxic lipopolysaccharides than teeth without these areas. Since endotoxins stimulate bone resorption in tissue cultures,[6,7] this current finding suggests that endotoxins stimulate bone resorption in vivo as well as in vitro.

Schilder[18] postulated that periodontal and periapical diseases are similar biologic processes. He suggested that the vector for the former is through the crevice of the diseased gingiva and the vector for the latter comes through the diseased root canal. The increase of endotoxin levels in

the gingival crevice exudate has been associated with an increased degree of periodontal inflammation.[3,4] On the basis of these previous periodontal studies and of the current study, Schilder's assumption may be substantiated.

Since endotoxins are capable of producing a great number of biologic reactions, the recovery of endotoxins from root canal fluid is a significant finding. The positive correlation of clinical and radiographic signs indicates the possible role of these toxic substances in pulpal and periapical diseases.

SUMMARY

It has been demonstrated that measurable quantities of endotoxin can be recovered from endodontically involved teeth. The endotoxin content of fluid aspirated from root canals of teeth that were opened for endodontic treatment was assayed and correlated with clinical symptoms and radiographic appearance. Tests of pulpless teeth, symptomatic teeth, and teeth with radiolucent areas on periapical radiographs gave the highest readings. Endotoxic lipopolysaccharides that are produced by oral gram-negative microorganisms may be factors in pulpal and periapical diseases.

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