Correlations between endotoxin and clinical symptoms or radiolucent areas in infected root canals

Naoki Horiba, DDS, Yoshinori Maekawa, DDS, Yoshie Abe, DDS, Masato Ito, DDS, Toru Matsumoto, DDS, PhD, and Hiroshi Nakamura, DDS, PhD, Nagoya, Japan

THE DEPARTMENT OF ENDODONTICS, SCHOOL OF DENTISTRY, AICHI-GAKUIN UNIVERSITY

Samples were collected from the root canals of 30 teeth of patients with apical periodontitis and assayed for endotoxin content. The detection rates of endotoxin and endotoxin content were higher in symptomatic teeth, teeth with radiolucent areas, and teeth with exudation than in those without them.
(ORAL SURG ORAL MED ORAL PATHOL 1991;71:492-5)

The cause of apical periodontitis may be bacterial, chemical, mechanical, or of another nature; however, it appears that bacteria are the most important factor. In view of this fact, there are many reports with respect to the isolation and identification of bacteria found in the root canal.[1-4] However, we often do not know how the bacteriologic status compares with the clinical appearance. Recently it has become possible to obtain higher rates of detection of strict anaerobic bacteria from infected root canals because of improvements in techniques for culturing anaerobic bacteria. As a result, gram-negative bacteria predominantly were identified and isolated from specimens taken from infected root canals.

Gram-negative bacteria contain endotoxin, a lipopolysaccharide complex that has many biologic activities including fever induction, adjuvant activity, Shwartzman reaction, cytotoxicity, and the like.[5-9] Therefore it is very important to evaluate the endotoxin content of infected root canals.

Morishima et al.[10] and Schein and Schider[11] have suggested that there is a correlation between endotoxin content and symptomatic teeth or radiolucent areas. However, they have not reported a correlation between endotoxin content and degree of exudation observed frequently in infected root canals.

7/15/16545

The purpose of the present study was to evaluate the correlation between endotoxin content and clinical symptoms such as spontaneous pain, percussion pain, exudations, or radiolucent areas.

MATERIAL AND METHODS

Experimental teeth

The material consisted of 30 teeth of 27 patients in whom root canal treatment had been performed by members of the endodontics staff at the School of Dentistry, Aichi-Gakuin University. None of the patients reported in this study received antibiotic therapy during the course of root canal treatment or 6 months before treatment.

Sampling of the substance in the root canals

After intraoral examination the outline form of the endodontic preparation in the teeth was made with a high-speed bur (No. 1557, Maillefer Co., Zurich, Switzerland). The teeth, all of which had a single canal, were isolated with a rubber dam and sterilized with tincture of iodine and 70% ethyl alcohol. Subsequently, access was gained to the pulp chamber and instrumentation with a No. 20 or No. 25 hand file (Sybron/Kerr Co., Romulus, Mich.) was carried out. A fresh, sterile absorbent point (No. 20, GC Co., Tokyo, Japan) saturated with physiologic salt solution was inserted into the apical portion of the root canal. The absorbent point was allowed to remain in the canal for 1 minute to absorb periapical exudate and mi-

Table I. Correlation between symptoms and endotoxin content

	Symptomatic	*Asymptomatic*
No. of cases	16	14
No. of teeth with endotoxin	16 (100%)†	4 (28.6%)
Endotoxin content (μg/ml)	8.7 ± 4.7*	0.7 ± 1.6

Symptomatic, Teeth with spontaneous and/or percussion pain; *asymptomatic*, teeth without either spontaneous or percussion pain.
*$p < 0.005$.
†$p < 0.001$ as compared with asymptomatic teeth (Fisher's exact probability test and Student's *t* test).

Table II. Correlation between radiolucent areas and endotoxin content

	Radiolucent areas	
	Present	*Absent*
No. of cases	22	8
No. of teeth with endotoxin	19 (86.4%)*	1 (12.5%)
Endotoxin content (μg/ml)	5.9 ± 5.2†	0.8 ± 2.1

*$p < 0.01$.
†$p < 0.005$ as compared with teeth without radiolucent areas (Fisher's exact probability test and Student's *t* test).

Table III. Correlation between exudation in root canal and endotoxin content

	Exudation	
	Present	*Absent*
No. of cases	20	10
No. of teeth with endotoxin	19 (95.0%)†	1 (10.0%)
Endotoxin content (μg/ml)	6.8 ± 5.0*	0.1 ± 0.2

*$p < 0.005$.
†$p < 0.001$ as compared with teeth without exudation (Fisher's exact probability test and Student's *t* test).

Table IV. Correlation between pyogenic exudation and endotoxin content

	Pyogenic exudation	
	Present	*Absent*
No. of cases	7	13
No. of teeth with endotoxin	7 (100%)	12 (92.3%)
Endotoxin content (μg/ml)	9.4 ± 4.9	5.5 ± 4.8

croorganisms. Exudate containing pus was termed pyogenic, and that containing translucent solution was called serous exudation. Radiolucency was evaluated from roentgenograms of the teeth.

Detection of endotoxin

The point bearing the collected substance was put into 2.0 ml of sterilized physiologic salt solution (endotoxin free). The samples were mixed for 30 seconds with a vortex mixer (Scientific Industries, Bohemia, N.Y.). The vortexed suspension was heated at 100° C for 30 minutes and centrifuged at 2000 rpm for 10 minutes (Sakuma R 90-23, Sakuma Co., Tokyo, Japan). Serial twofold dilutions of this supernatant were made with sterile distilled water. A 0.1 ml volume of each dilution of the suspension and 0.1 ml of distilled water were added to a Pregel ampoule (Teikokuzoki Co., Tokyo, Japan). After incubation for 4 hours at 37° C and for 5 minutes at room temperature, endotoxin was detected by gel formation in the ampoule. *Escherichia coli* 0111:B4 lipopolysaccharide (LPS) (Sigma Co., St. Louis, Mo.) at 10^{-3} μg/ml was used as a positive control and distilled water and physiologic salt solution as negative controls. The glassware used for the detection of endotoxin was sterilized in a dry air box at 220° C for 2 hours.

Statistical analysis

The correlation between clinical symptoms or radiolucent areas and the detection rates of endotoxin was evaluated statistically with Fisher's exact probability test. The correlation between those and endotoxin content was evaluated by means of Student's *t* test.

RESULTS

Correlation between symptomatic teeth and endotoxin content

Endotoxin was detected in all 16 of the teeth with symptoms and in 4 (28.6%) of 14 teeth without symptoms, and this difference in percentage was highly significant ($p < 0.001$) (Table I). Endotoxin content was higher in symptomatic teeth (average value 8.0 μg/ml) than in asymptomatic teeth (0.7 μg/ml) ($p < 0.005$).

Correlation between radiolucent area and endotoxin content

Endotoxin was detected in 19 (86.4%) of 22 teeth having a radiolucent area (Table II). In terms of endotoxin content, there was a significant difference between teeth with a radiolucent area (5.9 μg/ml) and those without it (0.8 μg/ml) ($p < 0.005$). The detection rate of endotoxin was higher in teeth with a radiolucent area than in those without it ($p < 0.01$).

Correlation between exudation and endotoxin content

Endotoxin was detected in 19 (95.0%) of 20 teeth with exudation (both pyogenic and serous exudations) (Table III). The detection rates of endotoxin and the average endotoxin content were higher in teeth with exudation than in those without it ($p < 0.001$ and $p < 0.005$, respectively). No significant difference in endotoxin content was found when the data derived from seven teeth with pyogenic exudates were compared with those of 13 teeth having serous exudates (Table IV).

DISCUSSION

Our results showed that teeth with clinical symptoms contain high levels of endotoxin. A few investigators have demonstrated a correlation between clinical symptoms and endotoxin content.[2, 10, 11] Morishima et al.[10] and Schein and Schider[11] reported the endotoxin level detected in symptomatic teeth to be 0.098 to 20.978 μg/ml and 0.256 to 2.048 μg/ml, respectively. In our study the average levels of endotoxin detected in samples from symptomatic and asymptomatic teeth were 8.0 μg/ml and 0.7 μg/ml, respectively. These facts indicate that the endotoxin plays an important role in the appearance of symptoms.

With respect to the presence of radiolucent areas, Morishima et al.[10] and Schein and Schider[11] reported that the endotoxin content of teeth with radiolucent areas was five times as great as that from teeth without such areas. In our study, the average concentration of endotoxin was 5.9 μg/ml in samples from teeth with a radiolucent area and 0.8 μg/ml in those without it. Thus our results are similar to theirs. Schonfeld and coworkers[12] reported that endotoxin was detected in 75% of human apical granulomas and in 20% of both human apical scars and cysts. Hausman et al.[13] reported the effect of lipopolysaccharides on bone resorption in tissue culture. In addition, Norton and associates[14] showed that in vitro bone growth was inhibited in the presence of endotoxin. These facts suggest that endotoxin may be one of the factors involved in the resorption of alveolar bone.[15-18]

We could find no report in the literature of association between the presence of exudations and endotoxin content. In our study endotoxin was detected in 19 (95.0%) of 20 teeth with exudation. There was a significant difference in endotoxin level between teeth with exudation and those without it. Although the average endotoxin content detected in samples from teeth with pyogenic exudation was 1.7 times greater than that from those with serous exudation, the difference was not statistically significant. However, our results indicate that the endotoxin may be associated with the production of exudate.

Our study indicates a positive correlation between endotoxin and clinical symptoms or radiolucent areas. Such correlations suggest that an increase in endotoxin level in infected root canals may be associated with an increased degree of periapical disease.

SUMMARY

Endotoxin content of samples obtained from the single root canals of 30 teeth displaying apical periodontitis was measured. A correlation between endotoxin content and clinical symptoms or radiolucent areas was sought.

The rates of detection of endotoxin and endotoxin content in symptomatic teeth exceeded those in asymptomatic teeth. These rates for teeth with radiolucent areas differed significantly, as was also noted for teeth with exudations. Endotoxin was present in all seven samples from teeth with pyogenic exudations and in 12 of 13 samples from teeth with serous exudations. The concentration of endotoxin was essentially the same for teeth with either type of exudate.

REFERENCES

1. Kants WE, Henry CA. Isolation and classification of anaerobic bacteria from intact pulp chambers of nonvital teeth in man. Arch Oral Biol 1974;9:91-6.
2. Dahlen G, Bergenholtz G. Endodontic activity in teeth with necrotic pulps. J Dent Res 1980;59:1033-40.
3. Toyoshima U, Fukushima H, Yamamoto K, et al. A bacteriological study of necrotic pulp with periapical radiolucent areas. Jpn J Oral Biol 1986;28:182-91. (In Japanese.)
4. Yoshida M, Fukushima H, Yamamoto K, Ogawa K, Toda T, Sagawa H. Correlation between clinical symptoms and microorganisms isolated from root canals of teeth with periapical pathosis. J Endod 1987;13:24-8.
5. Niwa M, Harada T. Molecular constitution and biological activities of endotoxin. In: Kato I, ed. Studies of the bacterial toxin—the recent steps. Tokyo: Kyoritsu Pub. Co.,1976:188-98.
6. Mergenhagen SE, Hampp EG, Scherp HW. Preparation and biological activity of endotoxins from oral bacteria. J Infect Dis 1961;108:304-10.
7. Sveen K. The capacity of lipopolysaccharides from *Bacteroides, Fusobacterium,* and *Veillonella* to produce skin inflammation in rabbits. J Periodont Res 1977;12:340-50.
8. Sveen K, Hofstad T, Milner KC. Lethality for mice and chick embryos, pyogenicity in rabbits and ability to gelate lysate from amoebocytes of *Limulus polyphemus* by lipopolysaccharides from *Bacteroides, Fusobacterium,* and *Veillonella.* Acta Pathol Microbiol Scand Sect B 1977;85:388-96.
9. Mashimo J, Yoshida M, Ikeuchi K, et al. Fatty acid composition and Shwartzman activity of lipopolysaccharides from oral bacteria. Microbiol Immunol 1985;29:395-403.
10. Morishima M, Tanaka T, Takei T. Detection of endotoxin and microorganisms from root canals. Aichi-Gakuin J Dent Sci 1980;18:88-93. (In Japanese.)
11. Schein B, Schider H. Endotoxin content in endodontically involved teeth. J Endod 1975;1:19-21.
12. Schonfeld SE, Greening AB, Glick DH, Frank AL, Simon JH,

Herles SM. Endodontic activity in periapical lesions. ORAL SURG ORAL MED ORAL PATHOL 1982;53:82-7.
13. Hausman E, Weinfeld N, Miller N. Effects of lipopolysaccharides on bone resorption in tissue culture. Calcif Tissue Res 1972;9:272-82.
14. Norton LA, Proffitee WR, Moore RR. In vitro bone growth inhibition in the presence of histamine and endotoxins. J Periodontol 1970;41:153-7.
15. Sveen K, Skaug N. Bone resorption stimulated by lipopolysaccharides from *Bacteroides, Fusobacterium,* and *Veillonella,* and by lipid A and the polysaccharide part of *Fusobacterium* lipopolysaccharide. Scand J Dent Res 1980;88:535-42.
16. Raisz LG, Nuki K, Alander CB, Craig RG. Interactions between bacterial endotoxin and other stimulators of bone resorption in organ culture. J Periodont Res 1981;16:1-7.
17. Johnson NW, Iino Y, Hopps RM. Bone resorption in periodontal diseases: role of bacterial factor. Int Endod J 1985; 18:152-7.
18. Mattison GD, Haddix JE, Kehoe JC, Progulske-Fox A. The effect of *Eikenella corrodens* endotoxin on periapical bone. J Endod 1987;13:559-65.

Reprint requests to:
Naoki Horiba, DDS
Department of Endodontics
School of Dentistry
Aichi-Gakuin University
2-11 Suemori-dori
Chikusa-ku
Nagoya 464, Japan