0099-2399/92/1804-0152/$03.00/0
JOURNAL OF ENDODONTICS

Printed in U.S.A.
VOL. 18, NO. 4, APRIL 1992

A Bacteriological and Histological Evaluation of 58 Periapical Lesions

Blake E. Wayman, DDS, MS, Steven M. Murata, DDS, MS, Roy J. Almeida, MS, DPH, and Craig B. Fowler, DDS

Periapical tissue from 58 cases requiring periapical surgery was examined histologically and cultured for the presence of microbes. Twenty-nine had a possible oral cavity communication and 29 did not. Approximately one-half of each biopsy was submitted for culture while the other portion was examined histologically. Cultures were positive for the presence of bacteria in 51 of 58 cases while bacteria were seen histologically in only 8 of 58 cases. A total of 50 different species of bacteria were isolated from the 58 cultures of periapical tissue. Of 133 isolates, 87 were strict anaerobes, 37 were facultative anaerobes, and 9 were aerobes. Bacteroides species were found in 17 cultures, always with additional bacteria. Seventeen of 58 biopsies contained foreign particulate matter thought to be root canal sealer. Bacteria were found in periapical granulomas, radicular cysts, and a periapical abscess. According to our data, bacteria, foreign material, missed canals, vertical root fractures, and periodontal disease may all contribute to the chronic, nonhealing periradicular lesion.

Through the years there has existed a difference of opinion regarding the presence of microorganisms in periapical tissue. Stewart (1), in 1947, and later Hedman (2), in 1951, supported the concept that bacteria were present in periapical tissue. In Hedman's study (2), which was accomplished prior to present techniques for culturing anaerobic bacteria, bacteria were reported in 68% of 82 periapical lesions. In a study similar to Hedman's (2), Shindell (3) found that only 5% of 62 periapical lesions contained bacteria. However, Winkler et al. (4), using a modified Gram stain for periapical tissue, were able to demonstrate the presence of bacteria in 87% or 13 of 15 cases examined under a light microscope.

Sabiston et al. (5) and Brook et al. (6) isolated numerous facultative and anaerobic microorganisms from periapical abscesses. With advances in microbiological techniques, anaerobic microorganisms can now be cultured. Earlier investigators could culture only aerobic microorganisms which did not thrive on tissues with reduced oxygen such as periapical tissue. Langeland et al. (7) could not consistently demonstrate the presence of microorganisms in periapical granulomas. In their 1977 study, 35 specimens were examined histologically for the presence of bacterial colonies. Five of the 35 cases were observed to contain bacteria, but only one contained bacteria in the "disintegrating part of the root canal and periapical tissue."

Quoting Kronfeld (8), Grossman stated that "a tooth with a granuloma may have an infected root canal, but sterile periapical tissue. In gram-stained sections through infected pulpless teeth in situ, bacteria were always found in abundance within the root canal, but granulation tissue and cysts attached to the apices of these teeth were often free from microorganisms," and that "a granuloma is not an area in which bacteria live, but in which they are destroyed."

Recently, Tronstad et al. (9) investigated the presence of periapical microbial flora of eight cases which had not healed with nonsurgical endodontic treatment. In all eight cases bacterial growth was evident on culture. Three samples from each case were cultured and Tronstad et al. (9) state that "our study clearly showed that anaerobic bacteria are able to survive and maintain an infectious disease process in periapical tissue." Five of the eight cases contained mixed flora but were dominated by anaerobes while two cases were found to contain anaerobes exclusively. Haapasalo et al. (10) treated a case in which nonsurgical endodontics, calcium hydroxide, systemic erythromycin, and, finally, a regimen of systemic metronidazole failed to resolve the draining fistula associated with a maxillary lateral incisor. Following periapical surgery, the lesion resolved. A mixed infection of anaerobic and facultative anaerobic microorganisms was cultured from the root canal and the periapical lesion.

Iwu et al. (11) studied the bacterial content of 16 periapical granulomas obtained under as aseptic a technique as possible. These specimens were homogenized and cultured. Fourteen of the 16 cultures (88%) yielded positive growth. There were 47 isolates of which 26 (55%) were facultative anaerobes and 21 (45%) were strict anaerobes. Using an indirect immunofluoresence technique, Barnett et al. (12) also demonstrated the presence of *Bacteroides intermedius* in the tissue of a periapical granuloma.

With current techniques to culture both facultative anaerobes and obligate anaerobes, it appears that some periapical granulomas, cysts, and abscesses do indeed contain microorganisms. The periradicular tissues from 58 cases which had not resolved following conventional endodontic treatment were examined in order to identify the bacteria, to compare the histological and microbiological findings, and to correlate the periapical microbiology with clinical signs and symptoms in these cases.

MATERIALS AND METHODS

Periradicular tissue from 58 consecutive surgical cases was submitted for histological diagnosis and microbiological culturing. The surgical procedures were performed by endodontic or general dentistry residents and the endodontic staff members. All teeth had previously been treated endodontically, yet the periradicular areas continued to show evidence of pathoses.

Data, such as symptoms, presence of fistulous tracts, periodontal pockets greater than 3 mm, and size of the lesion radiographically, were recorded.

Each patient was instructed to rinse twice daily with ½ oz of Peridex 0.12% Oral Rinse (Procter & Gamble, Cincinnati, OH) beginning 2 days before surgery. Following local anesthesia of the surgical site, the area was isolated with sterile sponges and then lavaged with Betadine (Goldline Laboratories, Ft. Lauderdale, FL). This was followed by a sterile saline rinse immediately before surgery. The flap was reflected and cultures and biopsies were obtained as quickly as possible. Approximately one-half of the biopsy was placed in a Vacutainer (Becton Dickson and Co., Rutherford, NJ), an anaerobic specimen carrier. The remaining portion was immediately placed in 10% buffered formalin and submitted to the Department of Oral Pathology for histological diagnosis.

Tissue sections for histological examination were obtained from paraffin-embedded tissue. The second specimen was submitted to the microbiology laboratory for culture to determine the presence or absence of anaerobic and aerobic microbes. All microbiology specimens were placed in a Vacutainer Anaerobic Specimen Collector as soon as they were collected. The specimens were delivered to the microbiology lab within 4 h of the time of collection. Once the specimen arrived at the Anaerobic Section, initial inoculation was performed in an anaerobic chamber (Anaerobe Systems, San Jose, CA) containing a gas mixture of 5% carbon dioxide, 10% hydrogen, and 85% nitrogen. The anaerobic media included the following agar plates (13): CDC blood agar, phenylethyl alcohol, blood agar, Bacteroides bile esculin agar, and Kanamycin-vancomycin laked blood agar. The aerobic media included the following agar plates (13): CDC blood agar, phenylethyl alcohol blood agar, chocolate agar, MacConkey agar, and trypticase soy agar with 5% sheep blood. A chopped meat broth (13) tube was also incubated as a backup culture. Both groups were incubated at 37°C in their respective atmospheric environments and read after 48 h. Colonies were then restreaked for isolation. Preliminary identification was derived from pure culture based on aerotolerance, Gram stain, and spot tests (catalase, indole, oxidase). Aerobic speciation was performed on the MicroScan Walkaway (Baxter Healthcare Corp., West Sacramento, CA) or the AutoMicrobic System (Vitek Systems Inc., Hazelwood, MO). Both of these instruments are automated bacterial identification systems. Anaerobic speciation was performed using the RapID ANA II system (Innovative Diagnostic Systems, Inc., Decatur, GA). This is a manual anaerobic bacterial identification system that utilizes conventional and chromogenic substrates.

RESULTS

There were 58 periapical lesions investigated in this study, submitted by seven practitioners. Of the 58 lesions, 29 had no detectable communication with the oral cavity. The remaining 29 communicated with the oral cavity through either a vertical root fracture or fistula, or had periodontal pockets of 4 mm or greater. Seven of the 58 lesions demonstrated "no growth" on culture. In one of these cases, the patient had received antibiotic therapy for 1 wk before the surgery.

Microbiological Findings

In the 29 lesions with no detectable communication with the oral cavity, 24 (83%) demonstrated growth of microorganisms on culture. Of these 24 positive cultures, 9 contained obligate anaerobes, 6 had only facultative anaerobic bacteria, 7 contained both anaerobic and facultative anaerobic bacteria, and 2 contained only aerobic bacteria. Five produced no growth. A single microorganism was grown in 13 cultures, the other 11 were polymicrobial with two to six microorganisms isolated. The average number of isolates per case was 1.72. The five most commonly isolated microbes, in descending order, were *Staphylococcus epidermidis, Fusobacterium nucleatum, Propionibacterium acnes, Peptostreptococcus micros*, and *Bacteroides gracilis* (Table 1).

In the remaining 29 lesions where there was a possible communication between the oral cavity and the periradicular lesion, there were 27 (93%) positive cultures containing one or more bacteria. Two had no growth. Eleven of 27 contained only anaerobic bacteria with 1 or more isolates, 5 had only facultative anaerobes, 10 grew both anaerobic and facultative anaerobic bacteria, and 1 contained aerobic microbes. Eight contained one microorganism and the remaining 19 were polymicrobial with two to eight isolates. The average number of isolates per case in this group was 2.86. Four of the five most commonly isolated bacteria were anaerobes (Table 1). Bacteroides spp. was cultured from 17 of the 58 cases, it was never a single isolate but always presented with one or more additional bacteria.

Histological Findings

Histologically, of the 58 biopsies submitted, 41 were diagnosed as periapical granulomas, 16 were periapical cysts, and 1 was a periapical abscess. Of the 58 cases which were treated surgically, the etiology of the nonhealing lesion was not determined at the time of surgery in 35 teeth. There were 23 cases in which treatment irregularities or the location of the lesions may have contributed to the nonresolution following conventional endodontic care: seven teeth had vertical root fractures, six teeth had missed canals, four teeth were perforated, two had the root apex and lesion perforating into the maxillary sinus, two contained failing silver points which were not retreatable, and two had severe periodontal disease.

In the 58 cases for which both the microbiological specimen and the biopsy were submitted, bacteria were detected microbiologically and histologically in only 8 cases or 14%, whereas 51 cases had positive cultures for 88%. There were seven cases (12%) in which no bacteria were detected by either method.

Particulate foreign material thought to be root canal sealer was detected in 17 of the 58 biopsies.

TABLE 1. Bacterial species recovered from periapical lesions with possible oral cavity communication (C) and with no obvious communication (N)

Species	Anaerobes		Facultative Anaerobes		Aerobes	
	C	N	C	N	C	N
Gram-positive cocci						
Streptococcus salivarius			1			
S. mitis			1			
S. mutans			1			
S. constellatus			1			
S. M G intermedius			1			
S. intermedius	3			3		
S. anginosus constellatus			1	1		
S. milleri			1			
S. sanguis II			2	1		
Group F β-streptococcus					1	
Staphylococcus hominis					1	
S. auricularis				1		
S. epidermidis			5	7		
S. aureus			1			
S. capitis			1	2		
S. warneri			1			
Peptostreptococcus micros	10	4				
P. anaerobius	1					
P. magnus	1					
Gemella morbillorum			1	1		
Gram-negative cocci						
Viellonella parvula	1	3				
Moraxella osloensis					1	
Gram-positive rods						
Eubacterium lentum	3					
Actinomyces odontolyticus	2	1				
A. meyeri		1				
A. israeli	2					
Propionibacterium acnes	4	4				
Corynebacterium species					4	1
C. pyogenes					1	
Lactobacillus acidophilus	1					
L. casei		1				
Bacillus pumilus				1		
B. species				1		
Bifidobacterium		1				
Gram-negative rods						
Fusobacterium nucleatum	8	5				
F. necrophorum	2					
F. varium	1					
F. species		1				
Bacteroides intermedius	6					
B. gracilis	4	3				
B. melaninogenicus	2	1				
B. buccae	4					
B. oris	1					
B. distasonis		1				
B. porphomonas gingivalis		1				
B. fragilis		1				
B. loescheii		1				
CDC Group M-S		1				
Pseudomonas aeruginosa	—	—	—	—	1	1
Total	56	30	18	18	9	2

The estimated area of the periradicular rarefactions seen on the periapical radiographs ranged from 4 to 100 mm^3 for all but one case in which the estimated area was 300 mm^3. This particular lesion contained three anaerobes, root canal sealer, and had no obvious communication with the oral cavity.

DISCUSSION

Many parameters were investigated in this study of 58 periapical lesions which were refractory to nonsurgical endodontic treatment. Care was taken to prevent contamination of the periapical specimens as described in "Materials and Methods."

Our results corroborate the results of Tronstad et al. (10), Iwu et al. (11), and Barnett et al. (12). In the periapical tissue of the 29 cases in which there was no obvious communication with the oral cavity, 24, or 83%, contained microorganisms. There was a total of 50 microbial isolates cultured from these periapical lesions, of which 30 were anaerobes, 18 were facultative anaerobes, and 2 were aerobes (Table 1). Of the 29 cases with a possible communication with the oral cavity, 27, or 93% contained microorganisms. There was a total of 83 microbial isolates. Fifty-six were anaerobes, 18 were facultative anaerobes, and 9 were aerobes (Table 1). The possibility of contamination is a plausible explanation for the presence of aerobes. However, the fact that there were 7 samples of the 58 in which no growth occurred would seem to verify that the technique used was reliable and avoided contamination. The presence of bacteria in these periapical lesions would provide an explanation for nonresolution of some nonsurgical treatment cases. There was neither a preponderance of cocci nor rods with both being present. Neither were there many more Gram-positive microbes than Gram negative ones.

Of the bacteria isolated from these lesions, most, if not all, have also been isolated from the root canal space. These microorganisms produce many products which are pathogenic. These microbial virulence factors destory many host resistance mechanisms, thus permitting the survival of these microbes in the periapical area (14).

Of interest is the frequency of the genus Bacteroides. Griffee et al. (15) state that *Bacteroides melaninogenicus* actually refers to a group of species which include *Bacteroides gingivalis* and *B. intermedius*. In addition, Sundqvist et al. (16) have listed *Bacteroides loescheii* as a species formerly classified *B. melaninogenicus*. In all instances, in our study in which a Bacteroides species was isolated, other microorganisms were also cultured. This supports their (16) thesis that other bacteria must be present in order for Bacteroides spp. to achieve their pathogenicity. Proteinases from these black-pigmented bacteria have also been shown to inactivate many factors in the body's defense system (14). In our results, we found Bacteroides in only 17 of these 58 lesions, which emphasizes that other bacteria may be as important as Bacteroides in refractory periapical lesions. Although the number of cases in our study was small, not all lesions with the Bacteroides spp. were symptomatic and a correlation between symptoms and the presence of this microbial species could not be made.

There was some concern that the facultative anaerobes such as *Staphylococcus epidermidis* were contaminants in the noncommunicating group. However, these microbes have been isolated from the root canal space (14). In their study, Tronstad et al. (9) also isolated many of these same microbes from nonhealing periapical lesions. Sabiston et al. (5) similarly isolated facultative anaerobes from pyogenic dental infections in which aspirates were taken and cultured.

The main difference between the 29 lesions communicating with the oral cavity and the 29 lesions with no obvious communication was the average number of microbes. The average number of microbial species isolated from the communicating group was 2.86 compared with 1.72 species in those cases with no communication. Possible means of communication with the periapex in these cases were periodontal pockets greater than 4 mm, a fistulous tract, or a vertical root fracture. Salivary contamination would account for the greater number of microorganisms in these cases as well as their persistence in these periradicular lesions. Of these 29 "communication cases," no microbes were cultured from two compared with five in those with "no communication."

No growth could be due to small numbers of bacteria present in these lesions, an inadequate specimen, death of the anaerobic microorganisms, systemic antibiotics, or type of culture medium. A small number of bacteria would also be difficult to detect histologically. Prior to the advent of current anaerobic culturing techniques, Langeland et al. (7) were able to demonstrate bacterial colonies in only 5 of 35 cases in which periapical tissue was examined under a light microscope. In our study, there were 58 cases in which specimens for culture and histological examination were submitted. Of these 58 biopsies examined under a light microscope and stained with hematoxylin and eosin, bacteria were seen in 8, or 14%, while bacteria were grown in 51 (88%). It is apparent that the best way to determine the presence of microorganisms in periapical tissues is through culturing of these lesions.

The presence of bacteria in 51 of 58 periapical lesions corresponds with the findings of Tronstad et al. (9) and Iwu et al. (11), both in the presence and types of microorganisms in these lesions. Our study also supports the earlier studies of Stewart (1) and Hedman (2), in which they reported finding bacteria in a significant percentage of periapical lesions.

The presence of particulate foreign material, believed to be root canal sealer, in 17 of the 58 biopsies is of interest. This sealer may act as a foreign body and contribute to the persistent nature of many of these lesions. These sealer remnants are known to be resorbed in some cases (17) and this occurs through the phagocytosis of these particles by macrophages which are derived from tissue histiocytes and blood-born monocytes (18).

This immune response to the sealer may contribute to the persistence of these lesions as well as the microbes which are there. Additionally, the cytotoxicity of freshly prepared root canal sealers has been demonstrated and may add to the problem of nonhealing (19).

Although the number of lesions in this study is small in comparison to the study by Bhaskar (20), granulomas were also the most common periradicular lesion identified. Bhaskar (20) reported that 48% of his 2308 cases were granulomas and 42% were cysts. Our results indicate that of the 58 biopsies submitted, 41 (71%) were granulomas and 16 (27%) were cysts.

In cases of nonhealing periradicular lesions, both aerobic and anaerobic bacteria and extruded sealer may contribute to the persistent pathosis. However, the results of this study demonstrate that the presence of bacteria and sealer in these lesions is not the only cause for failure to heal. Twenty-three of 58 cases had irregularities in treatment or location which may have contributed to the nonhealing after root canal treatment: seven had vertical root fractures, six contained missed canals, four had been perforated, two had lesions penetrating into the maxillary sinus, and two had severe periodontal disease. Seventeen of the lesions contained extruded sealer.

The authors wish to thank Mrs. Maria Garcia, anaerobic microbiologist, for her valuable technical assistance and Dr. William A. Walker III, Dr. William G. Schindler, Dr. Q. Michael Fuhs, Dr. Richard E. Rutledge, Dr. Daniel M. Kier, Dr. Garry Myers, and Dr. Richard Morgan for their clinical contributions.

The views expressed in this article are those of the authors and do not reflect the official policy of the Department of Defense or other departments of the U.S. government.

Dr. Wayman is chief, Endodontics Section, Department of General Dentistry, Wilford Hall USAF Medical Center, Lackland AFB, Texas. Dr. Murata is former chief, Endodontic Section, Department of General Dentistry, Wilford Hall USAF Medical Center. Dr. Almeida is chief, Microbiology Branch, Wilford Hall USAF Medical Center. Dr. Fowler is assistant chairman, Department of Oral Pathology, Wilford Hall USAF Medical Center. Address requests for reprints to COL Blake E. Wayman, Department of General Dentistry, Endodontics Section, Wilford Hall USAF Medical Center/SGDG, Lackland AFB, TX 78236-5300.

References

1. Stewart GG. A study of bacteria found in root canals of anterior teeth and the probable ingress. J Endod 1947;2:8.
2. Hedman WJ. An investigation into residual periapical infection after pulp canal therapy. Oral Surg 1951;4:1173–9.
3. Shindell E. A study of some periapical roentgenolucencies and their significance. Oral Surg 1961;14:1057–65.
4. Winkler TF III, Mitchell DF, Healey HJ. A bacterial study of human periapical pathosis employing a modified gram tissue stain. Oral Surg 1972;34:109–16.
5. Sabiston CB Jr, Grigsby BA, Segerstrom MT. Bacterial study of pyogenic infections of dental origin. Oral Surg 1976;41:430–5.
6. Brook I, Grimm S, Kielich RB. Bacteriology of acute periapical abscess in children. J Endodon 1981;7:378–80.
7. Langeland K, Block RM, Grossman LI. A histopathologic and histobacteriologic study of 35 periapical endodontic surgical specimens. J Endodon 1977;3:8–23.
8. Kronfeld R. Histopathology of the teeth and their surrounding structures. 2nd ed. Philadelphia: Lea & Febiger, 1939:209.
9. Tronstad L, Barnett F, Riso K, Slotz J. Extraradicular endodontic infections. Endod Dent Traumatol 1987;3:86–90.
10. Haapasalo M, Ranta K, Ranta H. Mixed anaerobic periapical infection with sinus tract. Endod Dent Traumatol 1987;3:83–5.
11. Iwu C, MacFarlane TW, Mackenzie D, Stenhouse D. The microbiology of periapical granulomas. Oral Surg 1990;69:502–5.
12. Barnett F, Stevens R., Tronstad L. Demonstration of *Bacteroides intermedius* in periapical tissue using indirect immunofluorescence microscopy. Endod Dent Traumatol 1990;6:153–6.
13. Phillips E, Nash P. Culture media. In: Lennette EH, Balows A, Hausler WJ, Shadomy HJ, eds. Manual of clinical microbiology. 4th ed. Washington, DC: American Society for Microbiology, 1985:1051–92.
14. Cohen S, Burns RC. Pathways of the pulp. 4th ed. St. Louis: CV Mosby, 1987:364–72.
15. Griffee M, Patterson S, Miller C, Kafraway A, deObarrio J. *Bacteroides melaninogenicus* and dental infections: some questions and some answers. Oral Surg 1982;54:486–9.
16. Sundqvist G, Johansson E, Sjogren U. Prevalence of black-pigmented *Bacteroides* species in root canal infections. J Endodon 1989;15:13–9.
17. Augsberger RA, Peters DD. Radiographic evaluation of extruded obturation materials. J Endodon 1990;16:492–7.
18. Cotran RS, Kumar V, Robbins SL. Robbins' pathologic basis of disease. 4th ed. Philadelphia: WB Saunders, 1989:61–3.
19. Pascon EA, Spangberg LS, Langeland K. Cytotoxicity of endodontic sealers. General Session IADR [Abstract 474]. J Dent Res 1987;66(special issue):200.
20. Bhaskar SN. Periapical lesions—types, incidence, and clinical features. Oral Surg 1966;21:657–71.