Bacteremia after Endodontic Procedures in Patients with Heart Disease: Culture and Molecular Analyses

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Abstract

Introduction: Infective endocarditis (IE) is still associated with high mortality, and antibiotic prophylaxis strategies are under intense debate. We evaluated the incidence of bacteremia after root canal preparation in teeth with necrotic pulps and apical periodontitis. **Methods:** Blood samples were taken before and 5 and 30 minutes after endodontic treatment in teeth with apical periodontitis from individuals at high (n = 21) or no risk (n = 11) for IE. The former received prophylactic antibiotic therapy. Bacteriologic samples were taken from root canals before chemomechanical preparation to confirm pulp infection. Samples were subjected to aerobic and anaerobic culture and quantitative real-time polymerase chain reaction (qPCR), the latter to determine the total bacterial and streptococcal levels. **Results:** Culture revealed no bacteremia in all individuals. Analysis by qPCR showed that bacterial DNA occurred in all root canal samples. qPCR showed a similar incidence of bacteremia between patients who received or did not receive prophylactic antibiotic therapy (P > .05). In blood samples taken 5 minutes after endodontic procedures, bacteria were detected in 2 of 11 (18%) individuals not taking antibiotics and in 4 of 21 (19%) patients under prophylaxis. After 30 minutes, the incidence of bacteremia decreased to 2 of 21 (10%) in patients taking antibiotics and was undetectable in patients at no risk of IE. The incidence of bacteremia by streptococci was identical as that for total bacteria. **Conclusions:** No detectable bacteremia was evident by culture after treatment of infected root canals. Molecular analysis revealed bacterial DNA and streptococci in blood from some patients without a significant difference between individuals receiving or not receiving antibiotic prophylaxis. *(J Endod 2016;42:1181–1185)*

Key Words

Bacteremia, endodontic treatment, infective endocarditis, prophylactic antibiotic therapy

Significance

Bacteremia incidence after endodontic procedures in patients with heart disease is low, irrespective of antibiotic prophylaxis. Endodontic therapy should be the treatment of choice for infected teeth, and the need for antibiotic prophylaxis before endodontic intervention should be re-evaluated.

Infective endocarditis (IE) is a disease characterized by microbial colonization of the endothelial surface of the heart, prosthesis, or implantable cardiac devices. Its characteristic lesion (vegetation) consists of an amorphous mass of platelets and fibrin, with colonies of microorganisms and inflammatory cells (1). Despite advances in diagnosis and treatment, IE is still responsible for noticeably high rates of morbidity and mortality (2), and predisposed patients should be evaluated for antibiotic prophylaxis whenever they undergo medical and dental procedures that may cause bacteremia (3, 4).

There are not many studies evaluating the incidence of bacteremia after endodontic treatment procedures. Baumgartner et al (5, 6) reported that nonsurgical root canal treatment resulted in a lower incidence of bacteremia (3%, as a result of overinstrumentation) than surgical flap reflection (83%), periradicular surgery (33%), and tooth extraction (100%). The incidence of bacteremia has been shown to be greater when endodontic instruments are used beyond the apical foramen (7, 8). Debelian et al (7) observed the occurrence of bacteremia in 54% of the cases after instrumentation 1 mm beyond the apical foramen when compared with 31% when instrumentation was 1 mm short of the apical foramen. A similar incidence of bacteremia after instrumentation short of the apex was shown by Savarrio et al (9). By causing bacteremia, endodontic treatment might increase the risk for occurrence of IE in patients with predisposing valve conditions (10).

Although most previous studies used culture for bacterial detection and identification (6–8), the low sensitivity and inability of this method to detect the presence of difficult-to-grow or as-yet-uncultivated bacteria may lead to underestimation of the real incidence of bacteremia. Molecular methods have been shown to be more accurate and reveal a more diverse bacterial population during bacteremia than culture (11).

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Furthermore, previous studies evaluated the incidence of bacteremia, but the bacterial load, another important factor that may determine the outcome of bacteremia, has not been assessed.

The study of bacteremia after dental procedures is crucial to the development of IE prophylaxis guidelines, but few studies investigated the occurrence of bacteremia after endodontic treatment procedures. In addition, no previous study has investigated the magnitude of bacteremia after endodontic intervention, which is information necessary to infer the possible systemic repercussion of bacteremia. The aim of the present study was to evaluate the incidence of bacteremia after endodontic procedures in patients receiving or not receiving a prophylactic antibiotic therapy regimen by means of culture and real-time quantitative polymerase chain reaction (qPCR). The levels of total bacteria and *Streptococcus* species in the blood and the root canal were also quantified by qPCR.

Materials and Methods

Patients

This study was approved by the Institutional Ethics Committee (#0298/11.11.2010) and followed the standards and guidelines established by the Brazilian National Commission for Research Ethics. All patients included in the study signed informed consent forms after the risks and benefits of participation were described to the subjects.

This study included patients who presented with heart disease with either a high or insignificant risk for IE. Thirty-two samples were collected from 27 patients (16 men and 11 women) with a mean age of 52 years (ranging from 30–75 years). The patients were distributed as follows: 18 patients with valvular heart disease and high risk for IE requiring prophylactic antibiotic therapy and 9 patients with coronary artery disease, considered as having no significant risk for IE and not requiring prophylactic antibiotics. All patients were referred for endodontic treatment of teeth with necrotic pulps, which was confirmed by pulp sensibility tests and radiographic evidence of apical periodontitis. All teeth were asymptomatic. Multirooted teeth, teeth with vital pulps, teeth with no apical periodontitis lesion, teeth previously subjected to endodontic treatment, and patients with periodontal pockets deeper than 4 mm were excluded from the study. Three patients contributed 2 teeth each, and 1 patient contributed 3 teeth. The time elapsed between each treatment in the same patient was at least longer than 5 months. In short, 32 teeth with necrotic pulps and apical periodontitis were included in the study, 21 from patients at risk for IE and 11 from patients considered to be at no risk for IE.

Prophylactic Antibiotic Therapy

Prophylactic antibiotic therapy consisted of 2 g amoxicillin 1 hour before the endodontic intervention as recommended by the Brazilian Cardiology Society guidelines for patients at risk for IE (12). These guidelines extend the indication of prophylactic antibiotics to patients presenting with rheumatic heart valve disease, mitral valve prolapse with mitral regurgitation, and bicuspid or degenerative aortic valve disease (12). In Brazil, rheumatic valve disease is among the most prevalent cardiac risk factors for IE (12, 13). Patients considered as having no significant risk for IE did not receive prophylactic antibiotics.

Endodontic Procedures and Sample Collection

Before endodontic treatment, the oral cavity was rinsed with a solution of 0.12% chlorhexidine digluconate for 1 minute. Each tooth was isolated with a rubber dam, and the operative field was cleaned with 3% hydrogen peroxide and disinfected with 2.5% sodium hypochlorite (NaOCl). The access cavity was prepared with sterile burs, and the tooth, clamp, and rubber dam were once again disinfected with 2.5% NaOCl. Negative control samples were taken from the disinfected tooth crown using sterile paper points and evaluated by qPCR using universal 16S ribosomal RNA gene-based primers (see later). For inclusion in the study, these cases had to show negative results for bacteria. Next, samples were taken from the root canal using sequentially 3 sterile paper points placed up to 1 mm short of the apex as determined by radiographs. Each paper point remained in the canal for 1 minute and was then transferred to cryotubes containing Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.6) and immediately stored at −20°C until qPCR analysis was performed.

Cleaning and shaping of the root canal were performed with the ProTaper Universal System (Dentsply Maillefer, Ballaigues, Switzerland) up to the F3 instrument under 5.25% NaOCl irrigation. The working length was established 1 mm short of the radiographic apex. In sequence, an intracanal dressing with calcium hydroxide was placed, and the tooth was temporized. The root canal was obturated at a subsequent appointment.

Before blood sample collection, the skin on the site of the median cubital vein was disinfected with 2% chlorhexidine gluconate, and 20 mL blood was collected immediately before and 5 and 30 minutes after the endodontic intervention. The samples obtained were immediately processed for aerobic (9 mL) and anaerobic culture (9 mL), and 2 mL blood was stored at −80°C for further molecular microbiology analysis.

Blood Culture Analysis

Peripheral blood samples were cultured using the BacT/ALERT 3D system (BioMérieux Inc, Durham, NC). Blood was collected in bottles containing aerobic and anaerobic media and was incubated for 5 and 15 days, respectively. Bottles were monitored, and if bacterial growth was evident, they were subcultured onto 5% sheep blood agar plates supplemented with 5 mg/L hemin (Sigma-Aldrich, St Louis, MO) and 1 mg/mL menadione (Sigma-Aldrich). Plates were incubated in aerobic, capnophilic (5% CO_2) and anaerobic (85% N_2, 5% H_2, and 5% CO_2) atmospheres for 48 hours at 37°C. For anaerobiosis, samples were handled and incubated inside an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI).

qPCR Analysis

Real-time qPCR using primers to the 16S ribosomal RNA gene was used to quantify the total bacterial load and the prevalence and levels of *Streptococcus* species in the root canal and blood samples. Analysis was performed in a total volume of 20 μL containing 2 μL DNA extracted from each clinical sample, primers (0.5 μmol/L each), and Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) in an ABI 7500 thermocycler (Applied Biosystems). The primers were as described previously (14, 15), and their sequences and annealing temperatures are listed in Table 1. Samples were dispensed into 96-well plates (MicroAmp Optical, Applied Biosystems), sealed, and centrifuged. qPCR was run according to the following settings: 95°C/10 minutes followed by 40 amplification cycles at 95°C/1 minute, annealing temperature (Table 1)/1 minute, and 72°C/1 minute. After each cycle, polymerase chain reaction (PCR) products were monitored for the increase in fluorescence of SYBR Green. All measurements were performed in triplicate for both samples and controls. For the negative control, ultrapure water was used replacing the clinical sample. To determine the specificity of the amplified products, a melting curve was obtained from 60°C to 95°C, with continuous fluorescence measurements for each 1% increase in temperature. Data acquisition and analyses were performed using the ABI 7500 software, version 2.0.6 (Applied Biosystems).

Bacterial counts were determined for each sample based on standard curves. The standard curve for both universal and *Streptococcus*