Photodynamic Therapy Associated with Conventional Endodontic Treatment in Patients with Antibiotic-resistant Microflora: A Preliminary Report

Aguinaldo S. Garcez, PhD, Silvia C. Nuñez, PhD,† Michael R. Hamblim, PhD,‡§‖ Hideo Suzuki,* and Martha S. Ribeiro, PhD¶*

Abstract

Introduction: This study reports the antimicrobial effect of photodynamic therapy (PDT) combined with endodontic treatment in patients with necrotic pulp infected with microflora resistant to a previous antibiotic therapy. **Methods:** Thirty anterior teeth from 21 patients with periapical lesions that had been treated with conventional endodontic treatment and antibiotic therapy were selected. Microbiological samples were taken (1) after accessing the root canal, (2) after endodontic therapy, and (3) after PDT. **Results:** All the patients had at least 1 microorganism resistant to antibiotics. PDT used polyethylenimine chlorin(e6) as a photosensitizer and a diode laser as a light source (P = 40 mW, t = 4minutes, E = 9.6 J). Endodontic therapy alone produced a significant reduction in numbers of microbial species but only 3 teeth were free of bacteria, whereas the combination of endodontic therapy with PDT eliminated all drug-resistant species and all teeth were bacteria-free. **Conclusions:** The use of PDT added to conventional endodontic treatment leads to a further major reduction of microbial load. PDT is an efficient treatment to kill multi-drug resistant microorganisms. *(J Endod 2010;36:1463–1466)*

Key Words

Antibiotic resistant bacteria, endodontic re-treatment, laser, photodynamic therapy

From the *Centro de Pesquisa e Pós-Graduação São Leopoldo Mandic, Campinas, SP, Brazil; †CETAO, São Paulo, SP, Brazil; ‡Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts; §Department of Dermatology, Harvard Medical School, Boston, Massachusetts; ‖Harvard MIT Division of Health Science and Technology, Cambridge, Massachusetts; and ¶Center of Lasers and Applications, IPEN-CNEN/SP, São Paulo, SP, Brazil.

Address requests for reprints to Dr Aguinaldo Silva Garcez, Sao Leopoldo Mandic University, Campinas, SP, Brazil. E-mail address: garcez.segundo@terra.com.br.

0099-2399/$0 - see front matter

doi:10.1016/j.joen.2010.06.001

In the case of endodontic treatment failure, retreatment, surgical treatment, or extraction usually is carried out with the use of antibiotics and antiseptics as adjunctive therapies, but the long-term use of these agents can be rendered ineffective by resistance developing in the target organism (1). Currently, there is an emergence of bacteria with multiple resistances, and there is a need for alternative antimicrobial approaches (2–6).

The combination of conventional endodontic therapy and photodynamic therapy (PDT) has been shown as an effective approach in reducing bacterial load in *in vitro* and *in vivo* models (7–11).

This study investigated the combination of PDT with endodontic treatment in patients with necrotic pulp harboring microflora resistant to a previous antibiotic therapy.

Materials and Methods

Thirty teeth from 21 patients with periapical lesions who had been previously treated with endodontic treatment associated with antibiotic were selected. The patients were in good health and between the ages of 17 and 52 years. All the teeth presented sights and symptoms of periapical periodontitis and apical bone lesion detected by radiography, and some patients had pain by vertical percussion and/or local edema, all requiring root canal retreatment on teeth with closed apices. The same practitioner carried out this study in a private dental office in São Paulo, Brazil. The protocol was approved by the Institutional Review Board of the São Paulo University, and all procedures were conducted according to the principles of the Declaration of Helsinki.

Endodontic Treatment

Thirty root canals from anterior teeth were re-treated and received endodontic treatment followed by PDT. Microbiological samples were taken after accessing the root canal, after endodontic therapy, and after PDT. The first microbiological sample confirmed that all the patients had at least 1 microorganism resistant to antibiotic medication.

A periapical radiograph was taken for each case to determine the presence of apical lesion, the canal morphology, and its length.

The access to the pulp chamber was gained after installation of a rubber dam, and then the surrounding area received prophylactic asepsis and was irrigated with 5 mL of chlorhexidine solution at 2% to ensure that the crown of the tooth had minimal microbial load (8).

Once the canal was accessed, a Hedström file #15 (Maillefer Instruments SA, Ballaigues, Switzerland) was inserted inside the canal to remove the gutta-percha and root canal sealer obturation; then the root canal was irrigated with 1 mL of sterile saline solution. The canal was dried with 3 sterile paper points (Dentsply Latin America, Petropolis, Brazil) and left inside the root canal for 1 minute each. All 3 paper points were combined for microbiological analysis. This procedure was the first microbiological sampling representing the initial contamination. The paper points were deposited in a fresh sterile bottle with sterile nutrient broth.

The canals were prepared with manual instrumentation by K files (Maillefer Instruments SA) by using a standard crown-down technique working to 1 mm short of the working length (file #45 was the average apical preparation diameter). Ten milliliters of sodium hypochlorite at 2.5% and hydrogen peroxide at 3% was alternated between each instrumentation by using an endodontic needle (27-gauge). At the end of the procedure the root canals were irrigated with 5 mL of 17% ethylenediaminetetraacetic acid followed by irrigation with 5 mL of phosphate-buffered saline (PBS) solution to remove the smear layer (12).

The canal was irrigated with 5 mL of sterile saline solution to remove the antimicrobial agent and dried with another 3 paper points by using the same methodology cited above (second microbiological sample).

Photosensitizer

The photosensitizer used was a conjugate between polyethylenimine (PEI) and chlorin(e6), which has been previously described in detail (13). The photosensitizer was used in a PBS solution at 60 μmol/L.

Light Source

The illumination was performed with a disposable 200-μm diameter fiber-coupled diode laser (MMOptics, Sao Paulo, Brazil). The laser delivered 660 nm light at a total power of 40 mW out of the fiber. The fiber was placed in the apical portion of the root canal at a point where resistance to the fiber was just felt (usually 1 mm from the apex), and spiral movements, from apical to cervical, were manually performed to ensure even diffusion of the light inside the canal lumen (14, 15).

After the endodontic procedure, the canal was irrigated with 0.5 mL of the photosensitizer and left inside the root canal for 2 minutes as an incubation time. The root canal was then irradiated for 240 seconds (total energy, 9.6 J), and the fiber was changed between each patient. The root canal was again irrigated with 10 mL of sterile saline solution to remove the photosensitizer and dried as before (third microbiological sample).

A calcium hydroxide paste (Ultradent Products, South Jordan, UT) was placed into the canals; cotton was placed in the pulp chamber, and the teeth were dressed with temporary restorative material (IRM; Dentsply Latin America).

One week later, a second session of each therapy was performed without microbiological sampling. Thereafter, root canal was sealed by using conventional techniques with Sealer 26 (Dentsply Petropolis), and the tooth was restored with a composite resin Z250 (3M, Sumaré, Brazil). This 1-week interappointment dressing approach was used by Garcez et al (8). Briefly, the pH in the environment is increased; consequently, the live-time of reactive oxygen species increases, and the photodynamic effect is improved at the second session.

Microbiological Analyses

The samples were sent in a sterile bottle with fresh sterile nutrient broth (Viability Medium Göteborg Agar III) to a private microbiological facility for identification and to antibiogram analyses. The bacterial species were identified on the basis of Gram stain, aerotolerance, colony morphology, esculin hydrolysis, nitrate reduction, indole production, (alpha)-glycosidase and N-benzoyl-DL-arginine-2-naphthylamide (BANA) hydrolysis, oxidase and catalase activities. The antibiogram tested 17 different antibiotics by using the Kirby-Bauer method (16).

Results

The first samples showed that all teeth harbored at least 1 resistant microorganism, indicating unsuccessful previous treatment and/or antibiotic therapy. The number of multi-drug resistant bacterial species did vary widely between individual teeth, with a mean value of 2.16 species per root canal sample (range, 4–1). This was probably due to differences in the geometry of the root canal systems and initial contamination. The mean values of the number of species for each step are given in Fig. 1.

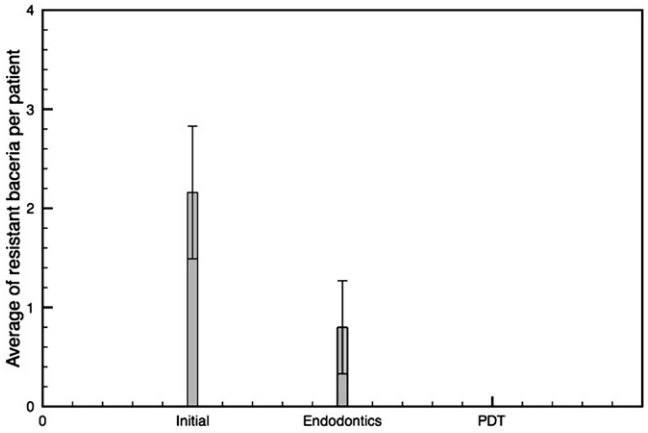

Figure 1. Means and standard deviations of multi-drug resistant bacteria inside root canal in each step of the treatment.

Among the initial samples, 33% were gram-negative, and 67% were gram-positive bacteria; moreover, 57% were facultative anaerobes, and 43% were obligate anaerobes.

After the endodontic therapy the infectious burden was reduced to 0.8 species per root canal (range, 2–0). After PDT, microorganism growth was not detected on any of the samples from any of the root canals. Ten of the root canals treated had 100% bacterial elimination after endodontic treatment, whereas all 30 teeth showed total absence of microorganisms after the combination.

The multi-drug resistant bacteria found in the initial samples were, in order of prevalence, *Enterococcus* sp, *Prevotella* sp, *Actinomyces* sp, *Peptostreptococcus* sp, *Streptococcus* sp, *Fusobacterium* sp, *Porphyromonas* sp, *Enterobacter* sp, and *Propionibacterium* sp. After the endodontic therapy, the species found were *Enterococcus* sp, *Actinomyces* sp, *Peptostreptococcus* sp, *Fusobacterium* sp, and *Porphyromonas* sp. All teeth were completely free of bacteria after the 2 combination therapies. Fig. 2 shows the number of bacterial species that grew in each sample from each stage of the therapy.

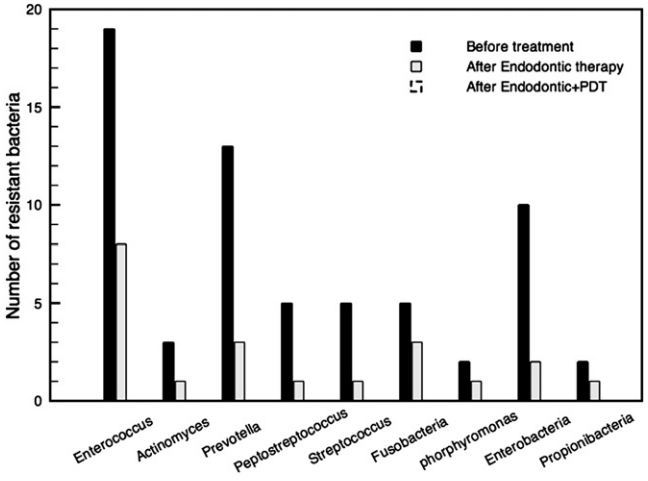

Figure 2. Bacteria species per patient in each step of the treatment.

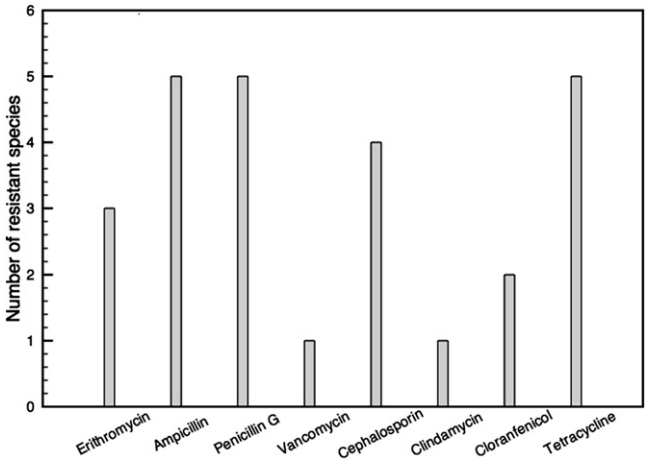

Figure 3. Number of species resistant to each type of antibiotic.

The antibiogram showed bacteria resistant to ampicillin, penicillin G, vancomycin cephalosporin, clindamycin, chloramphenicol, erythromycin, and tetracycline. Fig. 3 shows the number of species that were resistant to each antibiotic.

Discussion

Previous studies from our group (7, 8) and from other groups (9, 17, 18) showed that a combination of conventional endodontic therapy followed by antimicrobial PDT was effective in reducing bacterial load in *ex vivo* root canals (for planktonic and biofilm endodontic microorganisms) and in patients. In both studies we used the same photosensitizer, a conjugate between PEI and chlorin(e6) (PEI-ce6) that has been designed to have a broad-spectrum antimicrobial effect under illumination (19).

This study shows for the first time, *in vivo*, the susceptibility of drug-resistant bacteria in root canal infections to PDT. The literature reports that endodontic therapy will have a 94% success rate when a negative microbiological culture is obtained from the root canal at the time of obturation. On the other hand, when obturation is performed and the cultures are positive, the success rate is reduced to 68%; in the case of a periapical lesion, the failure of healing is more likely when the canal is obturated in the presence of persistent infection (20, 21). Treatment procedures to eliminate the infection include root canal debridement and mechanical shaping or smoothing (22), irrigation with a disinfectant agent, the application of an interappointment dressing, and sealing of the root canal (23). In case of infection, the use of antibiotics and antiseptics is an alternative approach, but the long-term use of antimicrobial agents, however, can be rendered ineffective by resistance developing in the target organisms (24–26). Our results confirm that the long-term use of antibiotics can lead to development of resistance in microorganisms.

Endodontic treatment alone had an effect in reducing the number of multi-drug resistant species in root canals and produced a total bacterial elimination in 10 of 30 teeth, but the addition of PDT produced a reduction in bacterial burden leading to total elimination in all teeth.

Previous studies compared photodynamic antimicrobial therapy of multi-drug resistant bacteria with wild-type strains. Maisch et al (27) found identical killing of methicillin-resistant *Staphylococcus aureus* (MRSA) and native strain. Wainwright et al (28) showed that PDT killed MRSA somewhat less efficiently than the native strain; also Embleton et al (29) used a phage delivery system to carry out PDT with the photosensitizer Sn-ce6 and again found that MRSA was susceptible. Tang et al (30) showed that PDT with a polylysine-chlorin(e6) killed multi-drug resistant and native *Escherichia coli* strains equally and killed MRSA better than the sensitive strain.

Our results showed that the combination of endodontic therapy and PDT killed all 9 multi-drug resistant bacterial species found in root canal infections. Therefore, PDT not only kills multi-drug resistant bacteria *in vitro* but is also effective in eliminating species resistant to diverse antibiotics in patients.

The samples showed that the multi-drug resistant bacteria found consisted of facultative and obligate anaerobic species. However, it is well-known that aerobic microorganisms can deal better with reactive oxygen species, and the greater susceptibility of anaerobes to the reactive oxygen species produced during PDT could explain the 100% reduction of multi-drug resistant bacteria after the combination therapy.

Furthermore, the majority of the species found were gram-positive, and the literature has shown that PDT is more efficient in killing these microorganisms (6, 7, 13, 17). Nevertheless, the photosensitizer used in this study (PEI-ce6) has also a high efficacy in killing gram-negative species compared with alternative photosensitizers such as toluidine blue (31). In fact, despite several attempts to induce resistance, the use of PDT to kill bacteria has not resulted in the generation of any PDT resistance among treated species, suggesting that bacteria do not find it easy to develop defenses against the reactive oxygen species generated during PDT (32).

In addition, the literature has showed that it is safe to use PDT against microorganisms near normal cells, for example, cells from apical region. George and Kishen (33) showed that cytotoxicity was significantly less in PDT compared with conventional antimicrobial irrigation. In an *in vitro* experiment, *Enterococcus faecalis* were killed at a faster rate than normal fibroblasts. PDT produced 97.7% bacterial killing and only 30% fibroblast dysfunction. Also Xu et al (34) suggested that there is a safe therapeutic window whereby PDT can inactivate endodontic pathogens without affecting host cell viability.

Conclusions

Our results suggest that the use of PDT as an adjuvant to conventional endodontic treatment leads to a significant further reduction of bacterial load and is effective against multi-drug resistant bacteria. PDT offers an efficient means of destroying multi-drug resistant bacteria remaining inside the root canal system after using conventional endodontic chemomechanical therapy.

Acknowledgments

The authors deny any conflicts of interest.

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