

Comparative Evaluation of Endodontic Disinfection Using PDT and MTAD - An *In Vivo* Study

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Abstract

Introduction: Antimicrobial effect of photodynamic therapy (PDT) and MTAD in association with endodontic therapy was shown in this study.

Methods: Thirty anterior teeth from patients were selected. Microbiological samples were taken Group 1 after endodontic therapy and irrigation with 5.23% NaOCl (Control group), Group 2 endodontic therapy and irrigation with MTAD, and Group 3 after PDT. After the three samples were collected, the root canal was filled with Calcium hydroxide, and after 1 week, a second session of the therapies was performed.

Results: PDT used Methylene blue (MB) dye as a photo-sensitizer and a diode laser as a light source. The samples collected after endodontic therapy in combination with MTAD showed decreased bacterial load, whereas the combination of endodontic therapy with PDT showed remarkable decrease in the bacterial load.

Conclusion: The use of Methylene blue dye as a photo-sensitizer in PDT along with endodontic treatment leads to an enhanced decrease of bacterial load and may be an appropriate approach for the treatment of root canal.

Keywords: Photodynamic Therapy; MTAD; Diode Laser; Methylene Blue Photo-Sensitizer

Introduction

Successful endodontic therapy depends on the elimination of microorganisms from the root canal system, by means of biomechanical instrumentation of the root canal. However complete removal of microorganisms from the root canal system is virtually impossible [1]. The bulk of the infecting microorganisms are removed during endodontic instrumentation, bacterial residue is readily detectable at the time of placement of a filling material, despite extensive irrigation with sodium hypochlorite (NaOCl) [2].

In various laser systems used in dentistry, the emitted energy can be delivered into the root canal system by a thin optical fiber (Nd:YAG, erbium, chromium: yttrium scandium-gallium-garnet [Er,Cr:YSGG], argon and diode) or by a hollow tube (CO₂ and Er:YAG). Thus, the potential bactericidal effect of laser irradiation can be used effectively for additional cleansing of the root canal system following biomechanical instrumentation [3].

In Photodynamic therapy (PDT), the application of a nontoxic compound, termed a photosensitizer (PS) or light activated antimicrobial agent (LAAA), which can be activated by light of an appropriate wavelength to produce reactive oxygen species (ROS) (i.e. singlet oxygen and free radicals) which can then exert a microbicidal effect [4]. Light of the appropriate wavelength excites the PS molecule into a triplet state which reacts with either a substrate to produce radical ions which in turn react with oxygen to produce cytotoxic species such as superoxide and hydroxyl radicals (type I reaction), or reacts directly with molecular oxygen to produce singlet oxygen (type II reaction). Advantages of PDT over conventional antibiotics are, as the mechanism of killing is non-specific, with reactive oxygen species causing damage to many bacterial components, resistance is unlikely to develop from repeated use and also both the PS and the light are applied locally to the target tissue; therefore, reducing the risk of adverse systemic effects. PDT has been studied as a promising approach to eradicate oral pathogenic bacteria that cause diseases such as periodontitis, peri-implantitis, and caries [5].

The introduction of MTAD, an aqueous solution of 3% doxycycline, 4.25% citric acid, and 0.5% polysorbate 80 (polyethylene sorbitol ester) detergent, represents a clinical effective endodontic irrigation technique. When MTAD is used as directed, it is proven to effectively remove the smear layer with less erosion to the dental structure than EDTA [6].

Materials and Methodology

Thirty teeth from patients with periapical lesions were selected. All the teeth presented signs and symptoms of periapical abscess, and some patients had pain by vertical percussion and/or local edema, all requiring root canal treatment on teeth with closed apices. Thirty anterior teeth were treated with conventional endodontic treatment followed by PDT. After access canal preparation, after endodontic therapy and after PDT, microbiological samples were taken. A periapical radiograph was taken for each case to determine the presence of apical lesion, the canal morphology, and its length.

After installation of a rubber dam, access to the pulp chamber was gained and was irrigated with 5 mL of chlorhexidine solution at 2% to ensure that the crown of the tooth had minimal microbial load. Access cavity preparation was done, a K file #15 (Maillefer Instruments SA) was placed into the canal and the canal patency was checked. Then the canal was irrigated with 1 ml of normal saline. The canals were prepared with manual instrumentation by K files (Maillefer Instruments SA) by using a standard crown-down technique (file #40 was the average apical preparation diameter). In group 1 (Control group), 5 ml of sodium hypochlorite at 5.23% was used to irrigate 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 normal saline solution to remove the smear layer. Three sterile paper points (Dentsply Latin America) were placed in the canal and left inside for 1 minute each. The paper points were then deposited in a fresh sterile test tube with nutrient broth. (First microbiological sample). In group 2, canal preparation was done in the same way as above and 5 ml of MTAD irrigating solution (Tetracycline isomer, 4.25% citric acid, and detergent Tween 80) was used for root canal disinfection. At the end of the procedure the root canals were irrigated with 5 mL of normal saline solution to remove the smear layer. Three sterile paper points (Dentsply Latin America) were placed in the canal and left inside for 1 minute each. The paper points were then deposited in a fresh sterile test tube with nutrient broth. (Second microbiological sample). The illumination was performed with a disposable 200-mm diameter fiber-coupled diode laser. 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. After the endodontic procedure, the canal was filled with 0.5 mL of the Methylene blue

(photosensitizer). The root canal was then irradiated for 240 seconds (total energy, 9.6J) 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 was placed into the canals; cotton was placed in the pulp chamber, and the teeth were restored with temporary restorative material.

After one week, a second session of each therapy was performed without microbiological sampling. Thereafter, root canal was sealed by using conventional techniques, and the tooth was restored with a composite resin (3M ESPE). 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 culture method was selected to assess the microbial load of common aerobes, facultative anaerobes, and microaerophilic species such as *Enterococcus*, *Candida*, *Porphyromonas* and *Lactobacillus*, found in infected root canals. Although, there was no attempt was made to identify the specific microbial flora during the process [7].

On arrival of the culture at the microbiological facility, the paper points were removed from the transport medium, placed inside a 1.5-mL microcentrifuge with brain-heart infusion (BHI) broth, and positioned in a vortex for 30 seconds. One hundred-microliter aliquots were added to wells of a 96-well plate for serial dilution and streaking on square BHI agar plates for CFU enumeration according to the method of Jett., *et al.* The culture plates were placed inside a microaerophilic chamber with 5% oxygen, 15% carbon dioxide, and 80% nitrogen and incubated for 72 hours at 37°C [8]. In all the three microbiological samples, the CFUs were counted. Statistical comparisons between means were performed with a paired *t* test using Microsoft Excel (Redmond, WA).

Results

After the initial endodontic therapy, the mean infectious burden was reduced to 4,650 CFU/ml, a mean log reduction of or 89.8%. The mean infectious burden after subsequent PDT was 1,280 CFU/ml and with MTAD was 2,780 CFU/ml, further mean log reduction with PDT of 97.5% this was significantly greater than that achieved by endodontic therapy alone (p_0.0005). None of the root canals treated had 100% microbial reduction after endodontic treatment, whereas six teeth showed total absence of microorganisms after the combination of endodontic treatment and PDT (Table 1).

Treatment	CFU/mL	Percentage Mean reduction
Post Endodontic Rx	4,650 ± 7,860	89.8%
Post Endodontic Rx+MTAD	2,780 ± 3,860	95.6%
Post Endodontic Rx + PDT	1,280 ± 2,110	97.5%

Table 1: Number of colony forming units/ml.

Discussion

Considering that endodontic infections are of mixed Gram-positive and Gram-negative bacteria, amphiphilic PS such as MB (both hydrophobic and hydrophilic), seem to be the most appropriate for PDT in endodontics. The choice of PSs used in dentistry is also dependent on the light source used. The basic requirement for PDT light sources is that they match the activation spectrum (electronic absorption spectrum) of the PS (usually the longest wavelength peak) and generate adequate light potency at this wavelength [9]. Currently, light

sources of a specific wavelength (between 630 and 800 nanometres) mostly applied in PDT are helium-neon lasers (633 nm), gallium-aluminium-arsenide diode lasers (630 - 690, 830 or 906 nm) and argon lasers (488 - 514 nm). The wavelengths of these sources range from visible light to the blue of argon lasers or from the red of helium-neon and gallium-aluminium-arsenide lasers to the infrared area of some diode lasers (Klotz., *et al.* 1999).

Studies done previously from other groups 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 [10]. This *in vivo* study shows the susceptibility of 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. But 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 occurs more likely when the canal is obturated in the presence of persistent infection [11].

Endodontic therapy of infected root canals in general reduces the bacterial count is accomplished by a combination of mechanical instrumentation, various irrigation solutions, and antimicrobial medication or dressings placed into the canal. If PDT is a treatment is done in addition to conventional endodontic therapy it can produce a greater reduction in bacterial burden. Previous studies compared photodynamic antimicrobial therapy of multi-drug resistant bacteria with wild-type strains. Maisch., *et al.* found identical killing of methicillin-resistant *Staphylococcus aureus* (MRSA) and native strain [12].

Furthermore, the majority of the species found were gram positive, and the literature has shown that PDT is more efficient in killing these microorganisms [13]. 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. Moreover, the literature has showed that it is safe to use PDT against microorganisms near cells from apical region and also cytotoxicity was significantly less in PDT compared with conventional antimicrobial irrigation.

The effectiveness of Biopure MTAD in this study might be attributed to its anti-collagenase activity, low pH and its ability to be released gradually over time. In addition, presence of a detergent (Tween 80) reduces its surface tension and thus improves its penetration over the deeper layers of dentin. The cleaning ability of tetracycline based MTAD can be attributed to its ability to chelate calcium. Tetracyclines are broad spectrum antimicrobials. They can bind directly to the demineralized dentinal surfaces and maintain antimicrobial activity by being subsequently released [13].

The effectiveness of PDT might be mostly related to the following reasons, PS capability of interacting with the bacterial membrane; PS ability of penetration and action inside the cell; and reactive singlet oxygen formation around the bacterial cell by illumination of the PS. The resistance of Gram-negative bacteria against efficient killing by antibacterial PDT is due to the different outer membrane structures of Gram-negative bacteria and to the hydrophobic and charge effects of the PSs. In fact, the photosensitivity of bacteria appears to be related to the charge of the sensitizer. The cationic MB photosensitizer is capable of inactivating both Gram-positive and Gram-negative bacteria [14].

In vivo studies might be more complex because the variance of root canal anatomy is higher than in a controlled *in vitro* experiment. However, the results of the *in vivo* study for the combined treatments were even better than those obtained in the *ex vivo* study with extracted teeth. However, the surrounding tissue could promote light backscattering, thus increasing the number of photons available to the photoreaction, could be the main possibility in the *in vivo* study.

Conclusion

In conclusion the results of the study have shown that photodynamic therapy using methylene blue as a photosensitizer in combination with conventional endodontic treatment has been most effective against destroying both Gram-positive and Gram-negative bacteria present inside the root canal. Group 3 (PDT) and Group 2 (MTAD) are almost equally effective in the apical third, without much significant difference. Photodynamic therapy offers an efficient nontoxic means of destroying microorganisms remaining inside the root canal system after using conventional endodontic treatment modality.

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