Antimicrobial comparison on effectiveness of endodontic therapy and endodontic therapy combined to photo-disinfection on patients with periapical lesion. A 6-month follows up.

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ABSTRACT

This study compares the antimicrobial effect of photodynamic therapy (PDT) combined to endodontic treatment with conventional endodontic treatment alone in patients with necrotic pulp and has a 6-month radiographic follow up comparing the healing of periapical lesions. Fifteen patients with periapical lesion and requiring root canal treatment were selected. Microbiological samples were taken after accessing the root canal, conventional manual endodontic therapy (group 1 n=5) and after accessing the canal, endodontic therapy and PDT (group 2 n=10). All the root canals were filled with a calcium hydroxide paste for 1 week. Radiographs were taken after obturation and following 6 months. Endodontic therapy alone presented an 87% reduction in microorganisms while the combination with PDT had a 95% reduction. Radiographic follow up showed 32% higher reduction in the lesion area in PDT group. Results suggest that the use of PDT added to conventional endodontic treatment leads to a further major reduction of microbial load. PDT is an efficient alternative to chemical antimicrobial agents. It is a non-cumulative local treatment, which may be an appropriate approach for the treatment of infections in the oral cavity.

Keywords: Photodinamic therapy, endodontic treatment, polyethylenimine (PEI) and chlorin(e6), antimicrobial therapy, low intensity laser therapy, diode laser.

1- INTRODUCTION

Elimination of the pathogenic micro-flora from the root canal system during endodontic therapy is one of the main goals of endodontic treatment. Microbial infection plays an important role in the development of necrosis in the dental pulp and the formation of periapical lesions ([1]). It is well established that the eradication of bacteria from root canals is difficult, and current endodontic techniques are unable to consistently disinfect the canal systems ([2]). Accepted treatment procedures to eliminate the infection include root canal debridement and mechanical shaping or smoothing ([3]), irrigation with a disinfectant agent such as sodium hypochlorite or hydrogen peroxide, the application of an inter-appointment dressing containing an antimicrobial agent and finally sealing of the root canal ([4]). In case of infection, the use of antibiotics and antiseptics is an alternative approach, but the long-term use of chemical antimicrobial agents, however, can be rendered ineffective by resistance developing in the target organisms ([5,6,7]).

Studies have shown that in cases when a negative microbiological culture has been obtained from the root canal at the time of obturation, there is a 94% success rate. On the other hand, when obturation is performed and the cultures are positive, the success rate is reduced to 68% confirming previous studies showing, in case of periapical lesion, that failure of healing is more likely when the canal is obtured in the presence of persistent infection ([8,9]).

Novel approaches to disinfecting root canals have been proposed recently, that include the use of high-power lasers ([10]) as well as photodynamic therapy (PDT) ([11,12]). High power lasers function by dose-dependent heat generation but, in addition to killing bacteria, they have the potential to cause collateral damage such as char dentine, ankylose roots, melt cementum, cause root resorption and periradicular necrosis if are used with erroneous parameters ([13]).

PDT is a new antimicrobial strategy that involves the combination of a non-toxic photosensitizer (PS) and a harmless visible light source ([14]). The excited photosensitizer reacts with molecular oxygen to produce highly reactive oxygen species, which induce injury and death of microorganisms ([15,16]). It has been established that PS, which possess a pronounced cationic charge can rapidly bind and penetrate bacterial cells and therefore these compounds demonstrate a high degree of selectivity for killing microorganisms compared to host mammalian cells ([17,18]). PDT has been studied as a promising approach to eradicate oral pathogenic bacteria ([19,20]) that cause such diseases as periodontitis ([21]), peri-implantitis ([22]) and caries ([23]). We recently reported on the use of PDT using a polyethyleneimine chlorin(e6) conjugate and fiber-optic delivered red light to combat endodontic infection caused by bioluminescent bacteria in an ex vivo model using extracted human teeth ([24]). When PDT followed conventional endodontic therapy there was significantly more killing and less bacterial regrowth than was seen following endodontic therapy alone. The aim of the present study therefore was to test this combination of conventional endodontic therapy followed by antimicrobial PDT in a clinical trial in patients requiring endodontic treatment.

2 - MATERIALS AND METHODS

2.1 - Endodontic PDT

Fifteen patients were selected at random and the same practitioner carried out this study in a private dental office in São Paulo. Brazil. The patients were in good health and between the ages of 26 and 49. All the teeth presented with symptoms of periapical periodontitis and apical bone lesion in consequence of necrotic pulp, all requiring root canal treatment on teeth with closed apices. The protocol was reviewed and approved by the Institutional Review Board of the São Paulo University and all trial procedures were conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from each subject.

Fifteen root canals from anterior teeth were treated. The root canals were divided at random in two groups. The first group (N=5) received the endodôntico treatment and the second group (n=10) received the endodôntico treatment associated with PDT.

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

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

Once the canal was accessed, a K file #10 (Maillefer Instruments SA, Ballaigues, Switzerland) was inserted inside the canal, approximately until the apical portion of the canal. The file was moved backwards and forwards to remove the necrotic tissue, then the root canal was irrigated with 1 mL of sterile saline solution and the canal was dried with 3 sterile paper points (Dentsply Latin America, Petropolis, Brazil), left inside the root canal for 1 minute each one. All the 3 paper points were combined for CFU determination. This procedure consisted in the first microbiological

sample representing the initial contamination of the root canal. 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) using a standard Crown-down technique working to 1 mm short of the working length (file #40 was the average apical preparation diameter, enough to allow the optical fiber to achieve the working length). Ten-mL of sodium hypochlorite at 2.5% and hydrogen peroxide at 3% was alternated between each instrumentation using an endodontic needle (27 G). At the end of the procedure the root canals were irrigated with 5 mL of a 17% EDTA followed by irrigation with 5 mL of PBS solution to remove the smear layer ([25]).

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

In group 2, after the endodôntico procedure, the solution of the photosensitizer was injected (the root canal was irrigated with 0.5 mL) with an endodontic needle and left inside the root canal for 2 minutes as a pre-irradiation time. After this time, the root canal was irradiated with the diode laser coupled with the optical fiber for 240 s (total energy 9.6 J) and the fiber was changed between each patience. The root canal was again irrigated with 10 mL of sterile saline solution to remove the photosensitizer and dried with another 3 paper points (third microbiological sample).

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

At a subsequent visit one week later, a second session of each therapy was performed. Then each root canal was sealed using conventional techniques with Sealer 26 (Dentisply Petropolis, Brazil) and the tooth restored with photopolymerized resin Z250 (3M Sumaré, Brazil).

A periapical radiograph was taken for each case after the treatment and after 6 month to evaluate the decrease of the periapical lesion area. To measure the area of the lesion was used the software Image J (National Institute of Health, USA).

2.2 - Microbiological analyses

The method of culture was selected to assess the microbial load of common aerobes, facultative anaerobes and microaerobes such as *Enterococcus sp*, *Candida sp, Lactobacillus sp* and *Porphyromonas sp* found in infected root canals. However no attempt was made to identify the specific microbial flora during the process (2).

Once arrived at the microbiological facility, the paper points were removed from the reduced transport broth media (VGMA III), placed inside a 1.5-mL microcentrifuge with BHI broth and vortexed for 30 s. One-hundred-µL 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 ([26]). The plates were placed inside a microaerophilic chamber with 5% oxygen, 15% carbon dioxide and 80% nitrogen and incubated for 72 h at 37ºC ([27]). At each stage of the treatment (initial, after endodontic treatment and after PDT) the CFUs were measured. Survival fractions were determined from the CFUs in the initial inoculums.

2.3 - Photosensitizer

The Photossensitizer used was a conjugate between polyethylenimine (PEI) and chlorin(e6) and the synthesis and characterization has been previously described in detail ([24, 28]). It was used in a PBS solution at 60 µM.

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 initially placed in the apical portion of the root canal at a point where resistance to the fiber was just felt and spiral movements, from apical to cervical, were manually performed to ensure even diffusion of the light inside the canal lumen ([29, 30]). These movements were repeated approximately ten times per minute.

2.4 - Statistical Analysis

Values are given as means and error bars are standard deviations. Statistical comparisons between means were performed with 2-tailed unpaired t-test assuming unequal variation of standard deviations using Microsoft Excel.

3 - RESULTS

The radiographic examination confirmed the diagnosis of necrotic pulp and periapical lesions for all the patients selected and the analysis of the first microbiological sample corroborated the presence of infection in all teeth.

Initial infectious burden did vary widely between individual teeth. This variation was probably due to differences in the internal anatomy and geometry of the individual root canal systems. However, the initial contamination for both groups is not significantly different (P=0.0005).

Figure 1 shows the mean values of infectious burden over the two groups for each stage of the study and Figure 2 the percentage of microbial reduction. After the initial endodontic therapy the mean infectious burden was reduced to 87% for group 1 and 88% for group 2. After the subsequent PDT the mean infectious burden was reduced to 95% and this was significantly greater than that achieved by endodontic therapy alone (p = 0.0005).

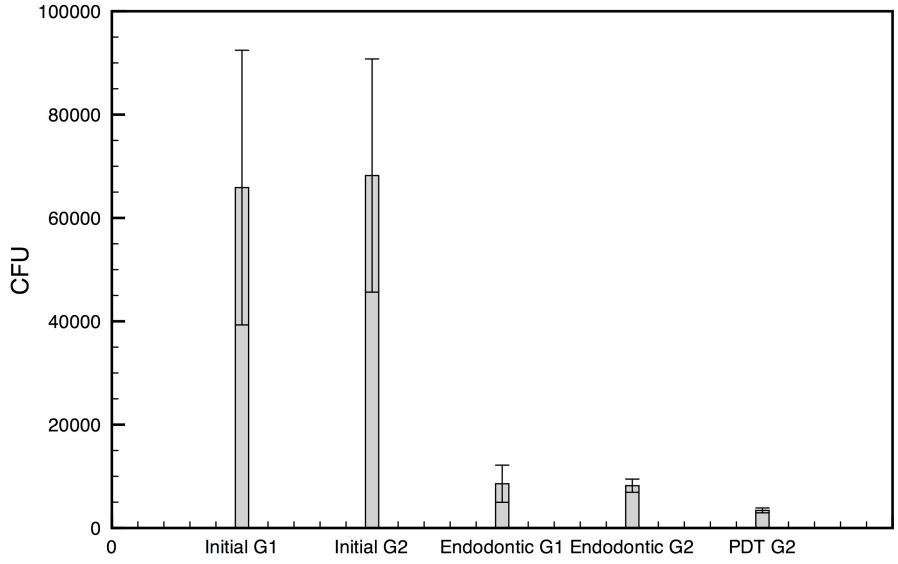

FIGURE 1: Total of CFU at each stage of the treatment for both groups.

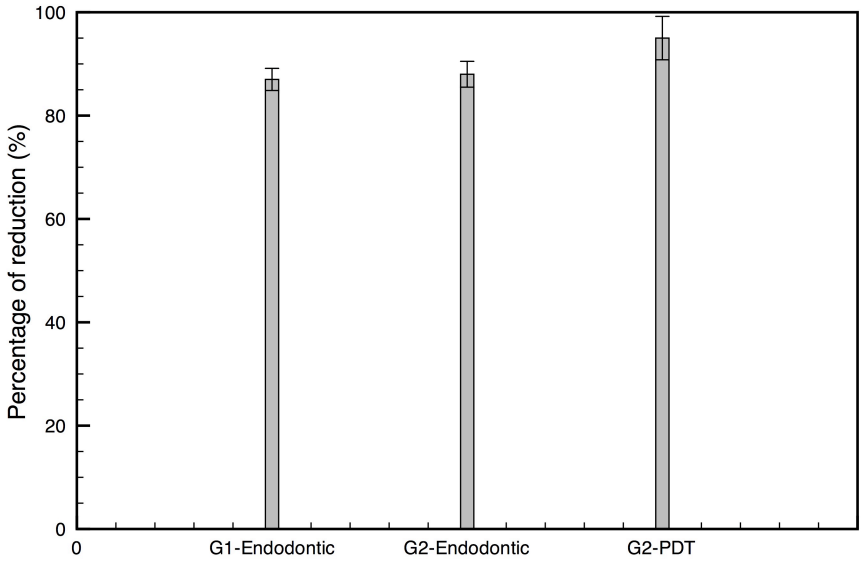

FIGURE 2 : Percentage of microbial reduction after eachstage of the treatment for both groups.

The 6-month radiographic follow up showed a decrease of the lesion area for all teeth in both groups. The decrease of the lesion area did vary widely between individual teeth, probably for the same reasons the contamination vary and also due the individual variation on healing for each patient.

As we can see at figure 3, group 1 showed approximately 46% of the reduction at the periapical lesion area while the reduction in group 2 was 68%. Figure 3 shows the area reduction after 6 month of radiographic follow up.

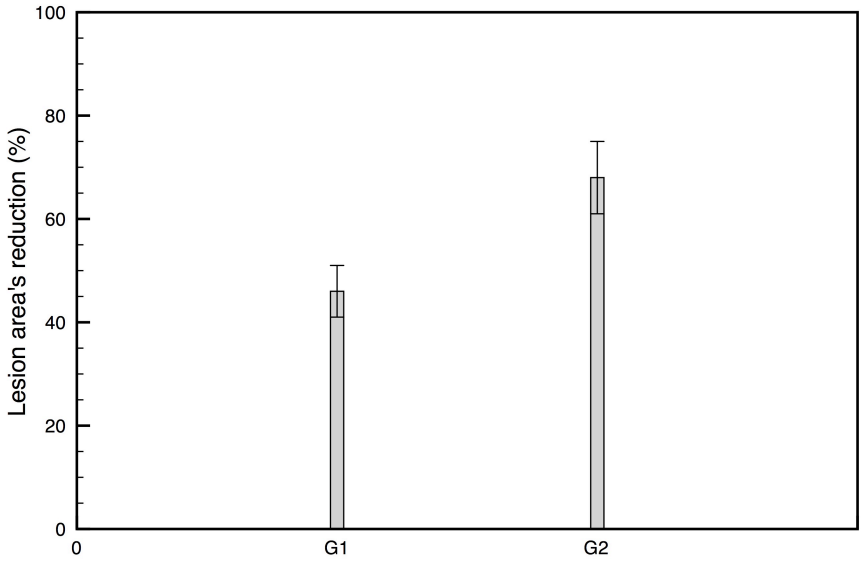

FIGURE 3: Reduction of the periapical lesion area in percentage for each group after 6 month of radiographic follow up.

4 - DISCUSSION

The combination of conventional endodontic therapy followed by antimicrobial PDT was highly effective in reducing bacterial load in an ex vivo model of infected root canals in extracted teeth([24]). In that study we used bioluminescent bacteria and a conjugate between polyethyleneimine and chlorin(e6) (PEI-ce6) as the PS. PEI-ce6 has been designed to bind and penetrate both Gram-positive and Gram-negative bacterial cell walls, while not binding strongly to host mammalian cells. Experiments were carried out by illuminating inside the root canal for periods of 1, 2, 3 and 4 minutes and measuring the contamination using bioluminescent images after each minute of illumination (2.4 J/minute). That study shown that there was a fluence-dependent reduction in contamination until an energy of 9.6 J (240 s) was reached when further light delivery ceased to have a noticeable effect and this fluence was therefore chosen for the clinical PDT treatment. The positive results of the pre-clinical study encouraged us to test this novel combination therapy in a clinical trial.

Some studies ([2,5,8]) have shown that culturing of root canal microflora is complicated and demand microbiological facilities in close proximity to the dental office to ensure that microorganisms do not die in transit. However, it is the most effective short-term means of evaluating the disinfection of root canals *in vivo* ([2]). To avoid this problem canal samples were cultured within one hour after the sample had been taken. It was decided that a quantitative method to count the total of microorganisms assessed inside the root canal would be appropriate since the aim of this study was to verify the number of microorganisms present after endodontic treatment and subsequent antimicrobial PDT.

In conventional endodontic treatment of infected root canals, reducing the bacterial count is accomplished by a combination of mechanical instrumentation, various irrigation solutions, and antimicrobial medicaments or dressings placed into the canal ([27]). PDT is a treatment that can be delivered as an addition to conventional endodontic therapy and produces a remarkable additional reduction in bacterial burden.

Comparing our results with *in vitro* studies, Seal et al ([12]) and Lee et al ([11]) have reported results using PDT in root canal treatments; both the authors have used phenothiazinium-based PS and low intensity red lasers against Gram-positive bacteria, but did not use an optical fiber to access the root canal lumen. Seal et al ([11]) found that 3% sodium hypochlorite irrigation killed more *Streptococcus intermedius* in the endodontic biofilms than PDT with 100 µg/mL toluidine blue and 21 J of 632-nm laser light. Garcez *et al* ([30]) used *E. faecalis*, a more relevant endodontic pathogen, and an optical fiber to access the root canal and the same methodology for irradiation, achieved better results than the cited authors. These results undoubtedly indicate the use of an optical fiber to improve the irradiation in root canals. The fiber probably distributes homogeneously the light inside the root canal guaranteeing a better photoreaction; also the technique of irradiation using helicoidal movements contributes to the results.

Literature about antimicrobial PDT shows surprisingly few reports of its use to treat localized infections *in vivo*. The *in vivo* studies of Bonsor and coworkers ([2,31]) using tolonium chloride as the photosensitizer and a diode laser coupled with an optical fiber as a light source was successful in eliminating all the microorganisms found in the initial root canal infection. The use of a chelating agent after instrumentation, in our case EDTA instead of citric acid used by Bonsor, acts as a cleaner and disrupter of the biofilm expanding the access of the PS to the canal system.

Working *in vivo* is more complex since the variance of root canal anatomy is higher than a controlled *in vitro* experiment. However, the results *in vivo* for the combined treatments were even better compared to the *ex vivo* study with extracted teeth. It is possible that *in vivo* the surrounding tissue could promote light back scattering thus increasing the number of photons available to the photoreaction.

Six month after the treatments all the patients were asymptomatic and all the teeth had returned to then normal mastigatory function.

The decrease of the periapical lesion area indicated the healing of the surround bone consequence of the success of the treatment. Both groups presented decrease of the lesion area indicating that as is well know the endodôntico treatment is effective against this pathology, however, the decrease in group 2 was higher than group 1. This finding could be explained by the hypothesis that a higher microbial reduction increase de healing process and/or that inside the root canal where the photosensitizer was, the photodynamic action was happening, on the other hand out side the root canal where there wasn't any PS, the light was acting as a low level laser therapy, stimulating the healing process.

In conclusion 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 a second PDT is even more effective than the first. Antimicrobial PDT offers an efficient non-toxic means of destroying microorganisms remaining inside the root canal system after using conventional endodontic chemo-mechanical therapy.

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