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Endodontic Disinfection Using Photodynamic Therapy with Indocyanine Green dye (ICG)

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Abstract

Aim: To analyze the antimicrobial effect of photodynamic therapy (PDT) in association with endodontic treatment.

Methods: Thirty anterior teeth from patients were selected. Microbiological samples were taken 1) after access canal preparation, 2) endodontic therapy, and 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 Indocyanine green dye as a photo-sensitizer and a diode laser as a light source. The samples collected after endodontic therapy showed decreased bacterial load, whereas the combination of endodontic therapy with PDT showed remarkable decrease in the bacterial load.

Conclusion: The use of Indocyanine green 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.

Key words: Photodynamic therapy, Indocyanine green dye, Diode laser, Endodontic disinfection.

Introduction

Microorganisms play a crucial role in pulp death and periapical infections.[1]Successful endodontic therapy, which mainly depends on the elimination of microorganisms from the root canal system, is accomplished by means of biomechanical instrumentation of the root canal. Studies have shown, however, that complete removal of microorganisms from the root canal system is virtually impossible.[2,3]Although the bulk of the infecting microorganisms are removed during endodontic instrumentation, residual bacteria are readily detectable in approximately one-half of teeth at the time of placement of a filling material, despite extensive irrigation

with sodium hypochlorite (NaOCl).[4]

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. This effect was studied extensively using lasers such as CO2,[5,6] Nd:YAG,[7-10]excimer,[11,12] diode,[13] and Er:YAG.[14,15]

Photodynamic therapy is the application of a nontoxic compound, termed a photosensitizer (PS) or lightactivated 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.[16] 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 ($^1O_2$) (type II reaction). PDT has a number of advantages over conventional antibiotics. Firstly, 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.[17] Secondly, 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 [18,19] that cause diseases such as periodontitis,[20] peri-implantitis,[21] and caries.[22] Use of PDT using a polyethyleneimine (PEI) chlorin (e6 [ce6]) conjugate and fiberoptic delivered red light to combat endodontic infection caused by bioluminescent bacteria in an ex vivo model using extracted human teeth.[23]

The photosensitizer used was Indocyanine green dye (ICG). It was procured and serially diluted to 5mg/ml(ICI industries,Madurai,India). ICG is a near infrared (NIR)-absorbing water-soluble tricarbocyaninedye, which has been approved by the United States Food and Drug Administration (US FDA) for medical diagnostic studies. ICG has a very low toxicity and a high absorption at wavelengths around 600-800 nm.[24]

Recently, PDT with ICG has been used to treat tumors.[25] However it hasnot been used for endodontic disinfection.

Materials &Methods

An invivo study was undertaken in the Department of Conservative Dentistry and Endodontics to evaluate the efficacy of endodontic disinfection using photodynamic therapy with indocyanine green dye (ICG)

Preparation of Samples

Thirty teeth from patients with periapical lesions were selected. All the teeth presented signs and symptoms of periapical abscess, and some patients had pain on vertical percussion and/or local edema, all requiring root canal treatment and teeth were with closed apices.

Endodontic Treatment

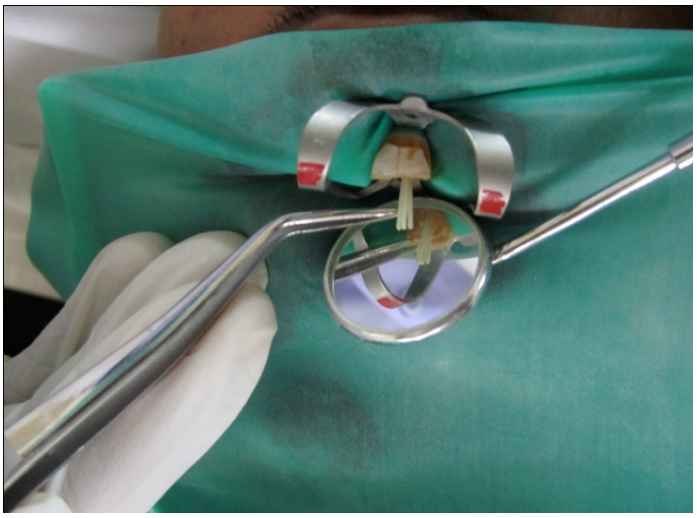
Thirty anterior teeth were treated with conventional endodontic treatment followed by PDT. Microbiological samples were taken after access canal preparation, after endodontic therapy, and after PDT. A periapical radiograph was taken for each case to determine the presence of apical lesion, the canal morphology, and its length.

The access to the pulp chamber was gained after installation of a rubber dam, andthen 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.[26]

After access canal preparation, a K file #15 (Dentsply Maillefer, Switzerland) was placed into the canal and the canal patency was checked. Then the canal was irrigated with 1ml of normal saline. 3 sterile paper points were placed in the canal and left inside for 1 minute each (Fig 1). The paper points were then deposited in a fresh sterile test tube with nutrient broth.

Fig 1 – Sample collection

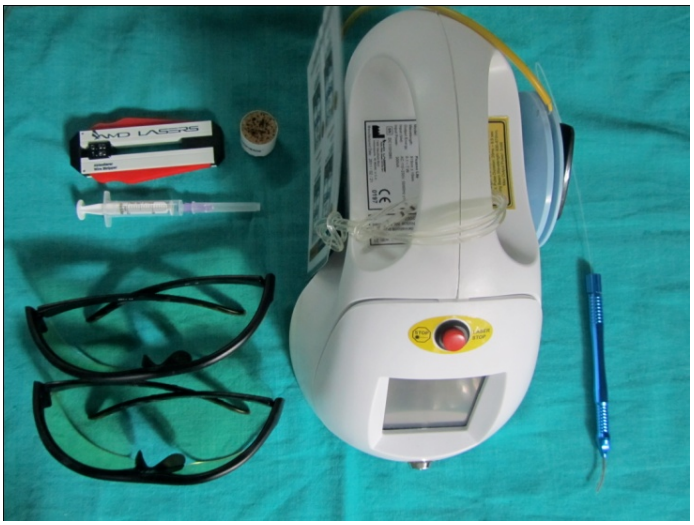


The canals were prepared with manual instrumentation by K files (Maillefer Instruments SA) by using a standard crown-down technique (file #45 was the average apical preparation diameter). 5 ml of sodium hypochlorite at 5.23% was used to irrigate between each instrumentation by using an endodontic needle (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.[27] The canal was dried with another 3 paper points by using the same methodology cited above (second microbiological sample).

Photodynamic Procedure

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 (Fig 2). 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.[28,29] After the endodontic procedure, the canal was filled with 0.5 ml of the Indocyanine green dye (photosensitizer). 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 was placed into the canals; cotton was placed in the pulp chamber, and the teeth were restored with temporary restorative material.

Fig 2 - Laser and Dye used in the study



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). This 1-week inter appointment dressing approach was used by Garcez et al.[26] 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. Moreover Srikanth K et al [30] in their study showed that

laseractivated ICG dye may enhance the potential benefits of SRP and can be used as an adjunct to nonsurgical periodontal therapy

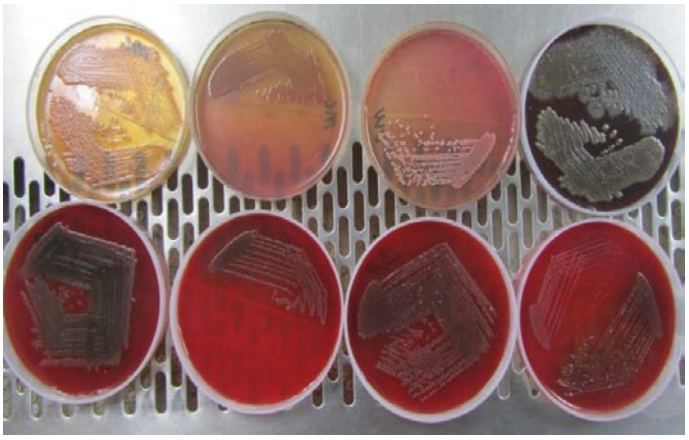
Microbiological Analyses

The method of culture was selected to assess the microbial load of common aerobes, facultative anaerobes, and microaerophilic such as Enterococcus sp, Candida sp, Lactobacillus sp, and Porphyromonasspfound in infected root canals. However no attempt was made to identify the specific microbial flora during the process.[31]

Once they arrived at the microbiological facility, the paper points were removed from the transport medium, placed inside a 1.5 mlmicrocentrifuge with brain-heart infusion (BHI) broth, and positioned in a vortex for 30 seconds (Fig 3). 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.[32] The plates were placed inside a microaerophilic chamber with 5% oxygen, 15% carbon dioxide, and 80% nitrogen and incubated for 72 hours at 37°C.[33] At each stage of the treatment (initial, after endodontic treatment, and after PDT), the CFUs were counted. Survival fractions were calculated from each tooth taking into account its initial bacterial load (Fig 4).

Fig 3 - Samples Stored in Incubator

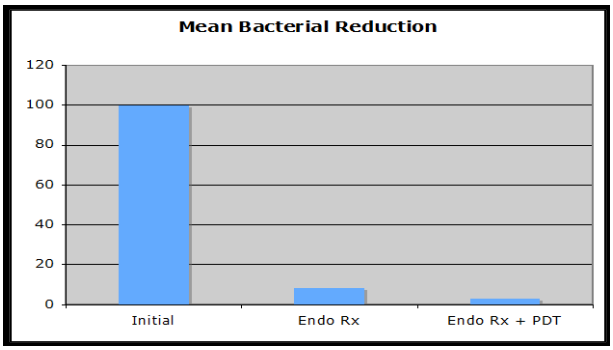
Fig 4 - Bacterial cultures



Statistical Analyses

Values are given as means, and error bars are standard deviations. Statistical comparisons between means were performed with a paired ttest using Microsoft Excel (Redmond, WA) (Fig 5).

Fig 5 - Mean Bacterial Reduction



Results

The initial infectious burden did vary widely between individual teeth with a mean value of 45,280 CFU. This variation was probably caused by differences in the internal anatomy and geometry of the individual root canal systems and the duration of the infections and the presence or absence of infiltration or caries on the teeth in the beginning of the treatment. After the initial endodontic therapy, the mean infectious burden was reduced to 3,715 CFU, a mean log reduction of or 91.8%. The mean infectious burden after subsequent PDT was 1,360, a further mean log reduction of 97% this was significantly

greater than that achieved by endodontic therapy alone (p_0.0005) (Table 1). 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- Colony forming units during initial treatment, after endodontic treatment and after endodontic treatment combined with photodynamic therapy.

Treatment	**CFU/mL**	**Percentage Mean reduction**
Initial	45,280 $^{+}_{-}$ 56,890	0%
Post Endodontic Rx	3,715 $^{+}_{-}$ 6,360	91.8%
Post Endodontic Rx + PDT	1360 $^{+}_{-}$ 2365	97%

Discussion

Indocyanine green is a water-soluble anionic photosensitizer which is widely used in medical diagnosis. A number of *in vitro* and *in vivo* studies of the potential use of ICG-mediated PDT have been carried out. *In vitro* researches reported an inhibitory effect of photoactivated ICG on pancreatic cancer cells,[34] colonic cancer cells,[35] human (SKMEL 188) and mouse (S91) melanoma cells.[36] These studies suggested that ICG was a promising photosensitizer for clinical PDT but that further *in vivo* investigations were needed. Several authors have investigated ICG cyto-toxicity and photo-toxicity using *in vitro*,[37-40]*in vivo*,[41,42] and *ex vivo* models.[43]

In humans, ICG-mediated PDT has been used in the treatment of acne vulgaris. A recent pilot study was carried out by Tuchin*et al*, (2003) on the effects of ICG photodynamic and photothermolysis treatment on acne vulgaris. As no adverse effects were reported, the investigators concluded that such high light intensities and ICG concentrations were safe for use in humans.[44]

Previous studies 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.[45,46] In both studies 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.[47] This study shows, in vivo, 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. 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.[48,49]

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 medication or dressings placed into the canal.[29] PDT is a treatment that can be delivered as an addition to conventional endodontictherapy and produces a remarkable additional reduction in bacterial burden. Treatment procedures to eliminate the infection include root canal debridement and mechanical shaping or smoothing,[50] irrigation with a disinfectant agent, the application of an interappointment dressing, and sealing of the root canal.[51] Previous studies compared photodynamic antimicrobial therapy of multi-drug resistant bacteria with wild-type strains. Maisch et al[52] found identical killing of methicillin-resistant Staphylococcus aureus (MRSA) and native strain.

Furthermore, the majority of the species found were grampositive, and the literature has shown that PDT is more efficient in killing these microorganisms.[23] Nevertheless, the photosensitizer used in this study (Indocyanine green dye) has also a high efficacy in killing gram-negative species.[26] 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. 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 Kishenet al [53]showed that cytotoxicity was significantly less in PDT compared with conventional antimicrobial irrigation.

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

Conclusion

In conclusion the results of the study have shown that photosensitization using ICG as a photosensitizer in combination with conventional endodontic treatment has been effective against destroying both Gram-positive and Gram-negative bacteria present inside the root canal. Antimicrobial PDT offers an efficient nontoxic means of destroying microorganisms remaining inside the root canal system after using conventional endodontic chemomechanical therapy.

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