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Review Article

Photodynamic therapy in combating the causative microorganisms from endodontic infections

Bruna Paloma de Oliveira[1], Carlos Menezes Aguiar[1], Andréa Cruz Câmara[1]

Correspondence: Dr. Bruna Paloma de Oliveira,
Email: bruna_paloma@msn.com

[1]Department of Prosthodontics and Oral and Maxillofacial Surgery, Dental School, Federal University of Pernambuco, Brazil

ABSTRACT

Photodynamic therapy (PDT) is presented as a promising antimicrobial therapy that can eliminate microorganisms present in endodontic infections. This treatment is based on the use of a nontoxic photosensitizing agent followed by irradiation of a resonant light source being capable of generating highly reactive species that are harmful to microorganisms. The purpose of this paper is to review the dental literature about the main factors that encompass the use of PDT combined with endodontic treatment for decontamination of the root canal system. A literature search was performed using the following index databases: PubMed, ISI Web of Knowledge and MedLine, between 2000 and 2014, looking for studies regarding antimicrobial action of PDT and its application to endodontic therapy. It was observed that despite numerous promising results, it is still necessary to establish different parameters so that PDT can be used with maximum effectiveness in eliminating microorganisms that cause endodontic infections.

Key words: Endodontics, photodynamic therapy, root canal

INTRODUCTION

The success of endodontic treatment is based on the effective decontamination of the root canal system, whereas microbial agents are essential for the development and maintenance of pathological processes that damage the pulp and periapical region.[1]

Despite technological and scientific advances in endodontics, there are many cases that result in failure due to microbial factors. The persistence of endodontic infections depends on the ability of microorganisms to adapt to environmental changes. Many different mechanisms are used by bacteria, such as: Biofilm formation, physiological modifications, exchange of genetic material, and creation of cell subpopulations.[2]

One challenge that has motivated many researchers in recent years is to develop new technologies to eliminate these persistent microorganisms. Among the new technologies, photodynamic therapy (PDT), also known as photoactivated disinfection or photochemotherapy, has demonstrated to be a great ally to conventional endodontic treatment in eliminating microorganisms that remain viable in root canal system.[3,4] This treatment is based on the use of a nontoxic dye sensitive to light, followed by irradiation with a visible light source with a suitable wavelength in the presence of oxygen.[5]

Given the above, the purpose of this paper is to review the dental literature that describes the main factors involving antimicrobial effects of PDT combined with conventional endodontic treatment in the total disinfection of the root canal system.

The databases PubMed, ISI Web of Knowledge and MedLine were used with specific indexing of the following terms: PDT, photoactivated disinfection, photochemotherapy, endodontics and root canal, and a subsequent search with specific limits and criteria were performed. Relevant reports published between 2000 and 2014 were retrieved, followed by

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interpretation. The quality of each publication was assessed as high, moderate or low. The initial search process yielded 83 publications. All abstracts were read, and reference lists of relevant publications were searched. Seventy articles were read and interpreted in full. In total, 56 articles were included and evaluated in the review.

HISTORY

The first reports that the association between dye and light could generate antimicrobial effect date back over 100 years. In 1900 Oscar Raab and Hermann von Tappeiner realized that red acridine could absorb ambient light and cause toxic effect on cultures of protozoa.[6]

Years later, von Tappeiner and dermatologist Jesionek realized that it was possible to treat skin cancer lesions using a combination of topical eosin and white light. In 1904, von Tappeiner and Jodlbauer demonstrated that the presence of oxygen was required for the photosensitizing reaction to occur.[6]

Subsequently, research involving PDT studies concentrated on the area of combating cancer. However, in the decades of 80 and 90, due to the rapid increase in the appearance of antibiotic-resistant bacteria, a number of researchers[7-9] regained interest in the first study of Raab and von Tappeiner, studying elimination of microorganisms by PDT.

Currently, it is known that various microorganisms can be eliminated by activating a nontoxic photosensitizing using a resonant light source.

Principles of activation

The mechanism of action of PDT occurs when dye, acting as a photosensitizing agent, absorbs photons from the light source, and their electrons enter an excited state, also known as triplet state. In the presence of a substrate, such as oxygen, the photosensitizer, when return to its basic state, transfers the energy to substrate, forming free radicals of high cytotoxicity, such as superoxides and singlet oxygen.[10]

These highly reactive species can cause serious damage to microorganisms through irreversible oxidation of cellular components, causing damage to the cell membrane, to mitochondria, to nucleus, and to other microbial cell components.[11]

There are two mechanisms that explain how photosensitizer in the triplet state can react with biomolecules. The type I reaction involves the transfer of electrons from excited photosensitizer molecules of the substrate, leading to production of free radicals that react rapidly with oxygen, resulting in the production of superoxide, hydroxyl radicals and hydrogen peroxide. In the type II reaction, the excited photosensitizer transfers energy to oxygen, leading to the production of electronically animated molecules known as singlet oxygen.[12]

In PDT is difficult to distinguish between the two types of reaction mechanisms. The reaction of type II; however, is accepted as the main means of microbial cells destruction.[11]

Advantages

Due to its selective antimicrobial action, PDT appears as a promising therapy to eradicate pathogenic bacteria, since, in low concentrations, it kills microorganisms without causing injury to human normal cells.[13] Several studies have shown that the dose required to cause bacterial death is lower the dose needed to cause damage to keratinocytes and fibroblasts.[14-17]

Xu *et al*.[18] suggested that PDT can be used as an adjunct to endodontic disinfection without damaging the cells of periapical region in their evaluation of the *in vitro* effects of methylene blue at 50 μg/mL and irradiation with diode light amplification by stimulated emission of radiation (LASER) at 665 nm for 5 min on human gingival fibroblasts and osteoblasts.

One of the advantages of photosensitization compared to traditional antimicrobials is that as the interaction of highly reactive oxygen with organic molecules is not specific, any macromolecule within microbial cell may become a potential target, thus hindering the development of mechanisms of microbial resistance. Furthermore, the procedure can be repeated several times, as it there are no reports of cumulative effects.[19,20]

Photodynamic therapy has other attributes that make it an excellent tool in intracanal bacterial reduction, such as: It is safe for human tissues, it has the ability to eradicate pathogens in biofilms, it is easy to apply, painless, and cheap when compared to high-intensity LASER.[21]

Bonsor *et al*.[22] evaluated the antimicrobial efficacy of PDT as an adjunct to disinfect root canals of patients with symptoms of irreversible pulpitis or periradicular periodontitis. The authors concluded that the use of toluidine blue O dye at 12.7 mg/L irradiated for

120 s using a diode LASER at 100 mW connected to a delivery fiber was effective to remove completely the remaining microorganisms of the chemomechanical preparation.

Garcez *et al*.[23] evaluated the antimicrobial action of PDT combined with endodontic retreatment and intracanal medication in anterior teeth with periapical lesions in patients who had undergone antibiotic therapy. The total elimination of intracanal microbial load was observed when PDT was used with a conjugate between polyethylenimine and chlorin (e6), and diode LASER irradiation at a wavelength of 660 nm and 40 mW of power associated with a fiber optics.

Ok *et al*.[24] evaluated the *in vitro* effect of PDT on the bond strength of AH Plus sealer to dentin in root canals obturated with lateral condensation technique. The authors concluded that the final disinfection using PDT (0.01% toluidine blue O and light-emitting diode (LED) irradiation with 625-635 nm wavelength) did not affect negatively the bond strength of the cement to root dentin.

Characteristics of photosensitizers

In order for PDT be successful, it is essential that the selected photosensitizer possesses particular characteristics such as it must be biologically stable, minimally toxic to healthy tissues, photochemically efficient, and resonant with the wavelength emitted by the light source.[6]

Various types of photosensitizers may be associated to the LASER. According to Wainwright,[25] the applicability of each dye is conditioned to its characteristics, such as maximum absorption of wavelength and intensity of light absorption.

The photosensitizing agents used in PDT belong to different groups of compounds and most of them are activated by light between 630 nm and 700 nm. In the literature, photosensitizers derived from group of phenothiazines, such as toluidine blue and methylene blue dyes have been the most used in research involving antimicrobial action in root canals.[26-28]

According to Fimple *et al*.[29] increasing the concentration of methylene blue and the light energy fluence (J/cm²), causes an increase in the antibacterial capacity of PDT.

Ng *et al*.[30] used freshly extracted teeth with pulp necrosis to compare the intracanal microbial reduction obtained by conventional chemomechanical debridement using sodium hypochlorite 6% only or added to PDT. Methylene blue was used at a concentration of 50 μg/mL and irradiation with diode LASER with a power of 100 mW/cm² and wavelength of 665 nm connected to an optical fiber. The results indicated that chemomechanical debridement followed by PDT was able to eliminate microorganisms totally in 86.5% of the canals, compared to 49% when PDT was not used.

Vaziri *et al*.[31] verified that the combination of sodium hypochlorite at 2.5% and PDT using toluidine blue at a concentration of 15 μg/mL and diode LASER with 200 mW/cm² of power and a wavelength of 625 nm was able to eliminate totally *Enterococcus faecalis* in single-rooted canals of freshly extracted teeth.

Komine and Tsujimoto[32] evaluated the relation between the amount of singlet oxygen generated by different concentrations of activated methylene blue and the bactericidal effect of PDT in suspensions of *E. faecalis*. They concluded that methylene blue at a concentration of 0.01%, when activated by diode LASER with a wavelength of 660 nm and 200 mW of power, was able to generate the greatest amount of singlet oxygen and consequently result in a large reduction in the number of colony-forming units of the micro-organism.

The use of encapsulated nanoparticles with photoactive drugs has also been tested to improve the ability of antimicrobial PDT. Pagonis *et al*.[33] verified that irradiation of poly (lactic-*co*-glycolic acid) nanopaticles loaded with methylene blue (50 μg/mL) showed elimination of approximately $1\log_{10}$ colony-forming units of *E. faecalis* in experimentally infected root canals.

Dentin staining caused by photosensitizing agents has been indicated as one of the main inconveniences of the use of PDT in root canals.[21,34] The effectiveness of some chemical compounds have been evaluated in an attempt to overcome this disadvantage. Carvalho *et al*.[34] concluded that the use of 2.5% of sodium hypochlorite, associated or not to endo-PTC cream, was effective in preventing tooth stains caused by the use of methylene blue during PDT.

Irradiation parameters

In PDT, light must be of a specific wavelength to ensure maximum effectiveness of the treatment. Thus, the LASER system used should be chosen according to the selected photosensitizer.[6]

Low-power LASERS, such as Helium-Neon (He-Ne) and diodes are the most used sources of radiation in PDT for microbial reduction of various cultures of bacteria and fungi in the oral cavity.[35-37]

Helium-Neon LASERS show positive results in the microbial reduction of various cultures of microorganisms using toluidine blue and methylene blue dyes. Currently, diode LASERS have been the most used because are more compact and easier to handle, less costly, more versatile and well absorbed by biological tissues.[20] This latter is an advantage, because in PDT the effects obtained are not due to increase in temperature,[38] but by photochemical reactions between photosensitizer, light source, and substrate.

Pinheiro *et al*.[39] evaluated the antimicrobial action of PDT in deciduous teeth with pulp necrosis after chemomechanical instrumentation of root canals. PDT was performed using toluidine blue in a concentration of 0.005% mg/L and diode LASER irradiation at 100 mW of power and a wavelength of 660 nm. The results demonstrated that chemo-mechanical instrumentation has led to a reduction of 82.59% of viable cells, and after PDT, the significant microbial reduction observed was 98.37%.

Recently, sources of nonlaser light, such as LEDs, have been successfully applied as alternative energy sources in PDT because of their low cost, flexibility, and light weight.[11,40,41]

Rios *et al*.[42] observed that the association between sodium hypochlorite at 6% and PDT (using toluidine blue O and LED lamp at 628 nm) resulted in low survival rate of *E. faecalis* (0.1%) in root canals of extracted teeth. When sodium hypochlorite or PDT was employed separately, microbial survival rate was observed to be 0.66% and 2.9%, respectively.

Light amplification by stimulated emission of radiation light used in PDT can be directed through an optical fiber. When employed in the elimination of microorganisms from root canal, this accessory can enhance the effectiveness of therapy. This is due to the capacity of optical fiber to distribute light evenly 360° around the root canal system with minimal losses, and compatible with the dimensions of the root canal.[29,43] With the aid of the fiber, the effect of LASER can be extended to areas of difficult access, and can easily reach the apical third, even in curvatures of molars, as well as to external biofilm of the root apex.[20] Is important that during use of optical fiber, helical movements be performed from apical third toward cervical, allowing the irradiation beam to reach the full extent of the canal during activation of the photosensitizer dye.[44,45]

After comparing the antimicrobial effects of PDT against *E. faecalis* in root canals, Garcez *et al*.,[46] suggested that the use of an optical fiber/diffusor, when used for endodontic treatment, had better results than when LASER light was used directed to access of the pulp cavity.

Another important factor for the success of PDT is the elapsed time between application of the photosensitizer and its activation by light.[6,21] At the moment of activation, photosensitizer must be next to its target so that the formation of toxic species occurs at the desired local. In antimicrobial PDT applications, it is important that at the moment of activation by the light source, the dye be attached to microorganism or has overtaken the barrier of its cell membrane. It is imperative that in this period the photosensitizer does not undergo degradation before it can be activated by the light source.

Queiroga *et al*.[47] evaluated *in vitro* the efficacy of PDT to eliminate suspensions of *Candida* species using methylene blue (300 μg/mL) and irradiation with diode LASER (660 nm, 40 mW) employing three energy dosages (60 J/cm^2, 120 J/cm^2, 180 J/cm^2). The evaluated dosages resulted in significant inactivation of *Candida* spp., and the dose of 180 J/cm^2 was the most effective, reducing about 78% of the number of colony-forming units.

Differences of susceptibility among micro-organisms

The microbial reduction by photodynamic effect faces different challenges when used against Gram-positive bacteria, Gram-negative bacteria and fungi. In general, the literature shows that Gram-positive bacteria are more susceptible to PDT action compared to Gram-negative. This is due to differences in the physiology of these microorganisms, because Gram-positive bacteria have a relatively porous outer membrane, formed by a thick layer of peptidoglycan and lipoteichoic acid. This characteristic allows a greater diffusion of the photosensitizer into the bacterium, so that various types of dyes and lower doses of irradiation can remove it.

On the other hand, the outer membrane of Gram-negative bacteria is thinner and more complex, formed by a heterogeneous composition that includes proteins with a porin function, lipopolysaccharides

and lipoproteins, which act as an effective barrier limiting the penetration of some substances.[10,13]

The photosensitization of bacteria is related to the photosensitizer charge. Because it has characteristics such as a positive charge, low molecular weight and hydrophilicity, methylene blue is capable of interacting with anionic lipopolysaccharide macromolecules and penetrate the outer membrane of Gram-negative bacteria.[48]

As regards the fungi, these have a cell wall constituted by a thick layer of beta glucan and chitin, which promotes an intermediate permeability barrier between Gram-positive and Gram-negative bacteria.[49]

Soukos *et al*.[50] investigated the effects of PDT in root canals of extracted teeth experimentally infected with endodontic pathogens. Methylene blue was used at a concentration of 25 μg/mL and exposure to diode LASER with a wavelength of 665 nm and energy fluence of 30 J/cm^2 coupled to an optical fiber. Following this protocol, all bacterial species were completely eliminated, except for *E. faecalis* (53% of death). However, by increasing the energy fluence to 222 J/cm^2, they eliminated 97% of *E. faecalis* is also possible to observe differences in susceptibility of PDT when microorganisms are organized in the form of biofilm and when they are arranged as isolated cells. The challenge is greater when microorganisms are organized in biofilms, because they are then protected within a matrix, showing, thus, less susceptibility to antimicrobial therapy.[11,51]

Bergmans *et al*.[52] tested the bactericidal effect of PDT on strains of *Streptococcus anginosus*, *E. faecalis* and mixed cultures containing *E. faecalis* and *Fusobacterium nucleatum* inoculated in root canals of extracted teeth. The authors verified that when microorganisms were organized in individual cells or monolayers, PDT easily eliminated them. Whereas when microorganisms were arranged in biofilm, the bacterial eradication was substantially reduced in the deeper layers.

Upadya and Kishen[53] compared the efficacy of PDT in planktonic suspensions and mono-species biofilms containing *Pseudomonas aeruginosa* and *E. faecalis*. The authors concluded that modifications in the formulation of the photosensitizer increased the antibacterial efficacy of PDT in biofilms.

The reduced susceptibility of biofilms to PDT is attributed to the low penetration of the photosensitizer.[20] Accordingly, various methods have been studied aiming to increase the potential penetration of the photosensitizer in biofilms.

According to George and Kishen[54] the inclusion of an oxidant and an oxygen carrier in the formulation of methylene blue enables an increase in the potential of photo-oxidation and generation of singlet oxygen of PDT, facilitating the disruption of the biofilm matrix of *E. faecalis* in root canals *in vitro*.

Kishen *et al*.[55] concluded that the use of a specific microbial efflux pump inhibitor added to methylene blue was able to increase the efficacy of PDT in eliminating biofilms formed by *E. faecalis* in polystyrene plates.

Stojicic *et al*.[56] compared the *in vitro* efficacy of conventional PDT (methylene blue at 15 μmol/L and irradiation with diode LASER with 40 mW and 660 nm) and modified PDT (methylene blue at 100 μmol/L, hydrogen peroxide at 0.5%, ethylenediaminetetraacetic acid at 0.05%, chlorhexidine at 0.05% and LASER irradiation) in the elimination of *E. faecalis* and mixed bacterial plaque in suspensions and biofilms. The authors concluded that modified PDT was able to remove up to twenty times more bacterial biofilms than conventional PDT.

Thus, research protocols on LASER light intensity, photosensitizers concentrations and activation methods are still being developed, showing different results and susceptibilities of microorganisms to treatment.

Concluding remarks

It could be concluded that PDT is presented as an important auxiliary tool to antimicrobial substances commonly used in endodontic treatment. However, this therapy presents different challenges regarding its susceptibility to different microorganisms, according to their physiology. Thus, for PDT to be employed with maximum effectiveness is important that further studies be performed in order to determine appropriate parameters for energy dosage used, photosensitizer concentration, time of preirradiation, and exposure.

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