Photodynamic Therapy: A Shining Light in Periodontics

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Photodynamic therapy (PDT) uses a photoactive dye (photosensitizer [PS]) that activates by exposure to light of a specific wavelength in the presence of oxygen. The energy transfer from the activated PS to available oxygen leads to the formation of reactive oxygen species, such as singlet oxygen and free radicals. These chemical species are extremely reactive and can damage proteins, lipids, nucleic acids, and other components of the bacterial cell wall. Bacterial biofilms are widely implicated in their role in the causation of gingivitis and periodontitis. Prophylactic and therapeutic regimens for dental plaque related diseases include the usage of various chemotherapeutic agents. Since it is difficult to maintain therapeutic concentrations of these agents in the oral cavity and they run the risk of being rendered ineffective by bacterial resistance mechanisms, the need for an alternative antimicrobial approach in the treatment and prevention of dental plaque related diseases was felt. Many studies have reported the killing of bacteria via lethal photosensitization including both Gram-positive and Gram-negative bacteria. Photosensitization leads to bacterial elimination, with minimal chances of microbial resistance and with no adverse effects on host tissues and resident microflora. In dentistry, PDT has found use in the treatment of oral cancers, bacterial and fungal infections, and also in the detection of malignancies. PDT is free from genotoxic and mutagenic effects; another important factor for long-term safety. The ease of accessibility of the oral cavity to illumination makes it a suitable target for PDT.

Keywords: Laser, Periodontitis, Photodynamic therapy, Photo sensitizer

INTRODUCTION

Latest technological advances have led to an evolution of newer approaches for the treatment of periodontal diseases. The objective of periodontal treatment is to restore biological compatibility of periodontally diseased root surfaces. The need to find further optimal treatment protocols for periodontal disease has been a long-term goal for periodontal researchers and clinicians. When mechanical scaling and root planning fail to achieve complete removal of bacterial deposits, systemic, and local antibiotics are occasionally administered, and their frequent use has a potential risk of producing antibiotic-resistant microorganisms. A unique non-invasive photochemical approach for infection control, namely photodynamic therapy (PDT), has found increased usage in the treatment of oral diseases.

In the early 1900’s exposure of acridine dye to visible light was shown to be lethal to protozoa (Paramecium caudatum).¹

In 1904 it was termed a photodynamic reaction due to the phenomenon’s ability to excite oxygen molecules, like singlet oxygen.² Singlet oxygen (1O₂) is not a free radical but rather a highly reactive oxygen species (ROS) that can be involved in generation of radicals.³ More recently photodynamic therapies have been developed for the treatment of various malignancies. These include bladder, brain, breast, skin, gynecological, colorectal, thoracic, oral, and head and neck cancers.⁴ The therapeutic use of photodynamics continues to expand for the management of various non-oncological diseases like macular degeneration, prevention of arterial restenosis, treatment of autoimmune disorders, and epidermal/dermal pathologies.⁵ Although originally employed in the treatment of cancer during the last decade an increasing number of studies on PDT application have been published in periodontics. PDT is an effective and innovative microbicidal method, which involves the combination of a non-toxic dye (photosensitizer [PS]) and a visible light source. It shows a great microbicidal effect in addition to better access to sites that are inaccessible to conventional therapy.

HISTORICAL BACKGROUND

The concept of cell death being induced by the interaction of light and chemicals has been recognized for over 100 years and...
was first reported in 1900 by Oscar Raab, a medical student of Professor Herman von Tappeiner in Munich. Oscar Raab observed that acridine orange was lethal to paramecia in the presence of light. In 1904, it was termed a photodynamic reaction due to the phenomenon’s ability to excite oxygen molecules, like singlet oxygen. Singlet oxygen \((1O_2)\) is not a free radical but rather a highly ROS that can be involved in the generation of radicals. Von Tappeiner in collaboration with a dermatologist named Jesionek used this discovery to treat skin cancers with a combination of eosin and white light and noted a beneficial effect with this therapy. He also went on to demonstrate the requirement of oxygen in photosensitization reactions and in 1907 introduced the term “photodynamic action” to describe the phenomenon.

In 1960, researchers at the Mayo Clinic observed that injection of hematoporphyrin led to preferential fluorescence of neoplastic lesions. Following this discovery, new photosensitizers were developed which were designed to target cancerous tissue. Among these, a porphyrin derivative called “hematoporphyrin derivative” (HPD) was found to have superior localizing and photosensitizing properties compared to the parent hematoporphyrin or other porphyrins. Systematic studies in tumor-bearing animals started only in the mid-1970’s following a landmark paper in 1972 in the Lancet by Diamond et al., where the authors studied the effect of light activation of hematoporphyrin in an experimental rat glioma. Almost a century after Raab’s original observations, great strides have been made in realizing the clinical potential of PDT. In 1993, Photofrin\(^6\) (porfimer sodium), a refined and purified form of HPD, was approved for PDT of recurrent superficial papillary bladder cancer by the Canadian Health Agency. This was the first official approval of PDT in the world and a milestone in PDT history. The next major breakthrough was achieved when Photofrin was accepted by the US Food and Drug Administration (FDA) in 1998 for treatment of esophageal and lung cancer. In 2003, Photofrin received approval from FDA for the ablation of high-grade dysplasia in Barrett’s esophagus, a condition that can lead to esophageal adenocarcinoma. This approval is of significant importance as it marks the first instance of PDT being considered as the primary treatment option for an early-stage neoplastic condition.

In 2001, meso tetra hydroxyphenyl chlorin received approval in the European Union for the treatment of advanced head and neck cancer.

**ANTIMICROBIAL PDT (APDT)**

Among all the alternative treatments to antimicrobial agents, APDT seems to be a promising one. Two main advantages have to be highlighted about PDT. On one hand, it has been demonstrated that drug-resistant microorganisms are as susceptible to APDT as their native counterparts, or even more susceptible. On the other hand, it has not been possible to artificially induce resistance to APDT yet, presumably because of the short-lived species related to the photodynamic effect and the non-specific nature of the photo-oxidative damage that leads to cell death.

**MOLECULAR BASIS OF PDT**

PDT involves the administration of a photoactive dye that is able to produce ROS upon irradiation with light. Thus, when the dye absorbs a photon, an electron is promoted from its ground state to an electronically excited state that returns the energy through three main pathways:

a) Non-radiative processes: The excited state species release the excess of energy as heat by three different processes:
   - Vibrational relaxation (VR): the excited molecule decreases its vibrational energy within a single electronic state.
   - Internal conversion: Transition between two electronic states with the same spin multiplicity, generally followed by VR.
   - Intersystem crossing: Transition between two electronic states with different spin multiplicity, generally followed by VR.

b) Radiative processes: The excited state species return the excess of energy as electromagnetic radiation. Divided into two kinds of processes:
   - Fluorescence: spontaneous emission of radiation upon transition between two electronic states with the same spin multiplicity.
   - Phosphorescence: spontaneous emission of radiation upon transition between two electronic states with different spin multiplicity.

c) Other deactivation processes: The excited state molecules can undergo photochemical or photophysical reactions or photosensitization.

Photosensitization is the process by which a photochemical or photophysical alteration occurs in one molecular entity \((a)\) as a result of initial absorption of radiation by another entity called PS. It can schematically be represented as follows:

\[
\text{PS} + h\nu \rightarrow \text{PS}^* 
\]

**Photochemical:** \(\text{PS}^* + A \rightarrow \text{PS} + \text{A} \)

**Photophysical:** \(\text{PS}^* + A \rightarrow \text{PS} + \text{A}^* \)

When molecular oxygen is involved in photosensitization, such process is termed “photodynamic action” and two different mechanisms are possible:

Type I mechanism: The PS in its singlet or triplet excited state reacts with a substrate \(\text{via} \ (a) \) electron transfer or \(b) \) hydrogen abstraction to yield free radicals, which will...
readily react with oxygen to form peroxides radicals, and in turn starting a radical chain reaction.

\[ \text{PS} + \text{hv} \rightarrow \text{PS}^* \]

(a) \( \text{PS}^* + \text{R} \rightarrow \text{PS}^* + \text{R} \) 
(b) \( \text{PS}^* + \text{RH} \rightarrow \text{PSH} + \text{R} \)
\[ \text{PS} + 3\text{O}_2 \rightarrow \text{PS} + \text{O}_2^- + \text{R} \rightarrow \text{ROO} \]
\[ \text{R} + \text{O}_2^- \rightarrow \text{ROO} + \text{RH} \rightarrow \text{ROOH} + \text{R} \]
\[ \text{PS} = \text{Photo Sensitizer, R = Substrate, E = Electron, } ^* \text{ = Excited State, hv = Light} \]

Type II mechanism: In this process, the sensitizer in its excited state (commonly in its triplet state) transfers its energy to ground-state molecular oxygen, giving rise to the PS in its ground state and singlet oxygen (\( \text{O}_2^* \)), a very ROS towards electron rich substrates such as alkenes, aromatic rings, phenols, amines, and thioethers.\(^{17} \)

\[ \text{PS} + \text{hv} \rightarrow \text{PS}^* \]
\[ \text{PS}^* + 3\text{O}_2 \rightarrow \text{PS} + \text{O}_2 \]

In general, in biological media, the photodynamic effect occurs simultaneously by either the two mechanisms. The relative importance of one mechanism over the other depends, among other factors, on the substrate and oxygen concentrations and on the distance between the PS and the substrate. However, both mechanisms can produce the photooxidation of relevant biomolecules, such as amino acids, nucleic bases, and lipids, which leads to damage on proteins, DNA and membranes, i.e. leading to cell death.

**Possible Applications of APDT**

There are many different possible applications of APDT against a wide range of pathogenic microorganisms, some of them in clinical trials. Herein, a brief summary with examples of possible applications for different microbial cells is presented:

- APDT: Dental infections are the largest growth area of clinical antibacterial PDT. Indeed, three different companies in North America, Austria, and UK use APDT with phenothiazinium dyes and red light to treat periodontitis, endodontics, and caries.
- Antifungal PDT: Teichert et al. demonstrated in a mouse model that methylene blue mediated PDT can efficiently inactivate *Candida albicans* upon irradiation with red light in order to treat mucocutaneous oropharyngeal candidiasis.\(^{18} \)
- Also, APDT was used clinically by Calzavara-Pinton *et al.* to treat interdigital mycosis of the feet by *Candida* or *Tricophyton* species by means of an aminolevulinic acid preparation and red light.\(^{19} \)
- Antiprotozoal PDT: The most important application in this field is the use of APDT to treat leishmaniasis. PDT was shown to be more effective than topical paromomycin and methylbenzethonium chloride in the therapy of cutaneous leishmaniasis.\(^{20-22} \)
- Antiviral PDT: Although, the virus are not microorganisms because they are not considered living cells, it has been demonstrated the efficiency of APDT against them.\(^{23} \)
- Diseases such as herpesvirus infections and papillomatosis are susceptible to be treated with APDT. However, the most general application in this field is blood sterilization. Mohr *et al.* reported the inactivation of hepatitis B and C viruses, human immunodeficiency virus, parvovirus B19 and west nile virus with phenothiazinium dyes in blood products or plasma.\(^{24,25} \)

**PDT IN PERIODONTAL DISEASE**

Periodontal diseases can involve severe inflammation of the periodontium and chronic infections caused by a mixture of Gram-positive and Gram-negative bacteria growing as a biofilm (Jori *et al.* 2006). Biofilms that colonize tooth surfaces and epithelial cells lining the periodontal pocket (subgingival) are among the most complex biofilms that exist in nature (Soukos *et al.* 1998, Merchant *et al.* 1996). The presence of primarily Gram-negative obligate anaerobes, capnophiles and spirochetes at threshold levels within the subgingival biofilms, along with a wide range of host-compatible species for prolonged periods of time (Soukos *et al.* 1998) are strongly associated with tissue degradation (Wilson 2003, Soukos *et al.* 1998, AAP 2005, Socransky and Haffajee 1994, Jori *et al.* 2006). These subgingival biofilms exist in complexes (a matrix of polymeric material) that provide protection against antimicrobial agents and host defense mechanisms (Jori *et al.* 2006, Soukos* et al.* 1998, Millson* et al.* 1996).

Several studies have demonstrated that Gram-positive bacteria are susceptible to APT (Meisel and Kocher 2005, Soukos *et al.* 1998, 2005, Hamblin *et al.* 2002). On the other hand, Gram-negative bacteria have been resistant to APT action but have shown enhanced susceptibility to APT following the application of a PS with a cationic charge (Jori *et al.* 2006, Meisel and Kocher 2005, Soukos *et al.* 1998, 2005, Hamblin *et al.* 2002) like toluidine blue O and methylene blue (Soukos *et al.* 2005).

Methylene blue has been studied for decades (Meisel and Kocher 2005) and was proven to penetrate deeper in the plaque biofilm (Wood *et al.* 1999) and increase the killing rate (Meisel and Kocher 2005). Although most studies demonstrated a log reduction in bacteria, a confocal laser scanning micrograph of a biofilm after exposure to APT revealed that in some of the biofilm stacks, lethal photosensitization occurred predominantly in the outer layers of the stack leaving some of the innermost bacteria
alive (O’Neil et al. 2002) which may allow for bacterial recolonization. In vitro studies have established that several associated periodontopathogens in the subgingival biofilms like Porphyromonas gingivalis, Fusobacterium nucleatum, Staphylococcus spp. are efficiently eradicated by photodynamic treatment, both in aqueous suspension and in biofilm (Jori et al. 2006, Wilson 2003). Moreover, in vivo animal studies showed that phenothiaziniums like toluidine blue-PDT can selectively kill P. gingivalis in the oral cavity and significantly decrease the level of alveolar bone loss in rats and dogs affected by periodontitis (Milanezi de Almeida et al. 2007, R. de Oliveira et al. 2007, Komerik et al. 2003, Hayek et al. 2005).

However, a number of investigations have demonstrated that bactericidal efficiency may be adversely affected by environmental factors such as the presence of saliva, serum, and pH (Komerik and Wilson 2002, Komerik et al. 2003, Bhatti et al. 1997). Serum-derived gingival crevicular fluid as well as blood may be confounders in treating periodontal pocket with APT. Matevski et al. 2003 identified the similarity between serum and blood kills was due to the presence of serum itself that provided protection to P. gingivalis from photo-activated TBO. The protection could be due to the presence of light scattering/absorbing proteins (Wilson and Pratten 1995) or perhaps the serum component bind to P. gingivalis which in turn protects it from the activated PS (Matevski et al. 2003). Upon evaluating clinical parameters Qin et al. reported a significant reduction in the total bacterial flora and, histologically, a large reduction in inflammatory cell infiltration after application of antimicrobial PDT (toluidine blue O + diode laser) in the treatment of experimentally induced periodontitis in rats. Sigusch et al. demonstrated that antimicrobial PDT (chlorin-e6 and BLC1010 + diode laser) was distinctly more advantageous than laser treatment alone or no treatment in reducing the periodontal signs of redness and bleeding on probing (BOP) in dogs.

Yilmaz et al. randomly assigned a total of ten patients to receive repeated application of scaling and root planing + PDT (methylene blue + 30 mW diode laser), scaling and root planing alone, PDT alone or supragingival oral hygiene instructions. After 32 days of healing, significant clinical, and microbiological improvements were only observed in the scaling and root planing + PDT and scaling and root planing alone groups. Andersen et al. observed that a combination of scaling and root planning + PDT resulted in significant improvements in the BOP, probing pocket depth and clinical attachment level (CAL) parameters over the use of scaling and root planing alone at all evaluation time points.

Clinical periodontal parameters such as plaque index, gingival index, BOP, probing depth, and CAL can be improved using adjunctive KTP laser in patients with CP, along with conventional periodontal treatment of deeper pockets (Dilsiz et al. 2013). The use of PDT with methylene blue dye 0.01% proved to be an effective adjuvant in periodontal therapy with manual debridement, accelerating the process of tissue regeneration, decontamination, reduction of pain, and significant improvement of parameters of periodontal health in patients with down syndrome (De Souza et al. 2011).

Also in HIV-associated periodontitis PDT used adjunctively to SRP promoted additional benefits in the treatment parameters (NoroFilho 2012). However, some clinical studies have failed to elicit any additional benefits of PDT in comparison to those obtained with full-mouth ultrasonic debridement used alone in patients with chronic periodontitis (Balata et al. 2013). In patients with CP, a single application of PDT (using a 638-nm laser and toluidine blue) did not provide any additional benefit to SRP in terms of clinical parameters or inflammatory markers 3 months following the intervention (Pourabbas et al. 2014).

When interpreting the available data, it should be kept in mind that the evidence from randomized controlled clinical studies, evaluating the potential clinical benefit of PDT in the treatment of periodontitis, is still limited. The main drawbacks may be related to the rather limited number of patients, the short-term duration of studies (i.e. 3 or 6 months), and the fact that the most effective protocol of antimicrobial PDT has not yet been established.

**CONCLUSION**

Antimicrobial PDT seems to be a unique and interesting therapeutic approach towards the treatment of periodontitis. The results of a number of in vitro studies clearly demonstrate the effective and efficient bactericidal effect of antimicrobial PDT. However, sufficient clinical and microbiological data that support the superior effects of the adjunctive use of PDT have not been demonstrated in vivo or clinically in either periodontal or peri-implant therapies. The discrepancy in the results obtained from previous clinical studies may be a result of the differences in treatment conditions and parameters. Therefore, further in vivo and clinical studies are necessary to determine the optimal conditions of this novel therapy. Furthermore, further randomized long-term clinical studies and meta-analyses are necessary to demonstrate the beneficial effects of antimicrobial photochemical therapy and their real advantages in comparison with conventional methods.
REFERENCES