International Journal of Innovative Research and Review ISSN: 2347 – 4424 (Online)
An Online International Journal Available at http://www.cibtech.org/jirr.htm
2014 Vol. 2 (4) October-December, pp.9-17/Reddy et al.
Review Article

PHOTODYNAMIC THERAPY: A NOVEL APPROACH FOR PERIODONTAL PATHOGENS

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ABSTRACT
Periodontal disease is a polymicrobial infectious disease, which is characterized by loss of periodontal tissue support and in extreme cases, which leads to the loss of alveolar bone with ultimate loss of the tooth. Mechanical therapy and the use of antibacterial disinfectants or various antibiotics have been considered as gold standard non surgical periodontal therapy. Incomplete removal of plaque and pathogenic bacteria from inaccessible areas, antibiotic resistance and side effects can be overcome by using a new antimicrobial concept called photodynamic therapy. Photodynamic therapy (PDT) is a novel approach to kill the periodontal microorganisms by using low power laser and photosensitizer. PDT could be a valuable adjunct to mechanical therapy as well as antibiotics in eradicating periopathogenic bacteria and helps in maintaining the healthy periodontium.

Keywords: *Antibiotic Resistance, Lasers, Mechanical Therapy, Photodynamic Therapy, Photosensitizer*

INTRODUCTION
Periodontal diseases are consider to be the most common oral diseases, which results from inflammation of supporting structure of the teeth in response to chronic infection caused by various periodontopathogenic bacteria. The gold standard treatments as non-surgical approach for periodontal diseases are based on its mechanical therapy by scaling and root planning to remove bacterial deposits, calculus, and necrotic cementum. Inaccessibility of deeper pockets by non surgical therapy for complete removal of plaque and pathogenic bacteria associated with periodontal disease become questionable. Although systemic and local antibiotics have been used as an adjuvant to conventional therapy because of its undesirable side effects and development of drug resistance, outcome of periodontal therapy become ineurtable. In order to overcome these limitations and to provide better results, newer mode of non-invasive and effective therapy has been put forward in 1960 by Lipson named photodynamic therapy (photo radiation therapy, photo chemotherapy).This approach emerged in recent years as a non-invasive therapeutic modality for various infection caused by bacteria, virus and fungi.

Historical Backround
Phototherapy has been used by humans for 3000 years and was recognized by the Egyptians, the Indians and the Chinese. Herodotes of Greece, called it ‘heliotherapy ’and suggested it for ‘restoration of health’ in the 2nd century BC. The effect of sunlight on rickets was known in the 18th century.
The therapeutic effect of sunlight on ‘scrofula’, rickets, rheumatism, scurvy, paralysis and muscle weakness was authenticated by Carvin in 1815. The importance of sun exposure for the prevention of rickets was recorded by a Polish physician, Sniadecki in 1822. Later, in 1903, Dane and Niels, was awarded the Nobel Prize for their work on the therapeutic use of light from the carbon arc in to treat lupus vulgaris (skin tuberculosis) and was recognized as the founder of modern phototherapy (Moan and Peng)
Professor Hermann, director of the Pharmacological Institute of the Ludwig-Maximilians University in Munich, one of the pioneers of photobiology, coined the term ‘photodynamic action’ (photodynamische wirkung) (Sruthima, 2012). Oscar Raab reported the idea of cell death induced by the interaction of light and chemicals.
Application PDT to treat tumors and other skin diseases, such as lupus of the skin and chondylomata of the female genitalia, were first done by the group of von Tappeiner in 1903-1905 and they discovered that

oxygen was essential for the photodynamic effect.[1] Scherer manufactured the first hematoporphyrin compound (in an impure form). He removed iron from dried blood by treating it with sulfuric acid in 1841. Thudichum in 1867, described the spectrum of this red substance, as well as its fluorescence, and Hoppe-Seyler named it as 'hematoporphyrin' in 1871.

Photobiological experiments with hematoporphyrin, demonstrating how it sensitized paramecia, erythrocytes, mice, guinea pigs and humans to light be done in large numbers in the period of 1908-1913. Hans Fischer, the Nobel Prize winner for his work on porphyrins, reported that uroporphyrin was equally phototoxic as hematoporphyrin. A pioneering study which was at first called photo radiation therapy' (PRT) was done by a German physician Friedrich Meyer-Betz with porphyrins in 1913. He tested the effects of hematoporphyrin-PRT on his own skin. Thomas Dougherty aided in expanding clinical trials and they formed the International Photodynamic Association, in 1986.

The Food and Drug Administration approved PDT in 1999 to treat pre-cancerous skin lesions of the face or scalp. PDT has been used to a great extent in treating cancers and certain other diseases (Rajvir, 2010). Goldman *et al.,* in 1964 reported the first application of a laser to dental tissue. Myers and Myers (1985) proposed that a Nd: YAG laser may be used for oral soft tissue surgery (Rajesh, 2011).

Components of Photodynamic Therapy

A source of light which activates the photosensitizer by exposure to low-power visible light at a specific wavelength is essential for PDT. Human tissue transmits red light efficiently, and the activation wavelength of the photosensitizer for a longer duration results in deeper light penetration.

Light Sources

Accordingly, red light between 630 and 700 nm activates most photosensitizers corresponding to a light penetration depth from 0.5 cm (at 630 nm) to 1.5 cm (at ~ 700 nm). This restricts the depth of necrosis and/or apoptosis and the therapeutic effect is defined. So larger solid tumors cannot be uniformly illuminated, as the depth of penetration is limited. The total light dose, the dose rates, and the depth of destruction vary depending upon the tissue treated and the photosensitizer used. A variety of light sources, such as argon-pumped dye lasers, potassium titanyl phosphate (KTP) or neodymium: yttrium aluminum garnet (Nd/YAG)-pumped dye lasers, and gold vapor- or copper vapor-pumped dye lasers were used for the activation of photosensitizer in the earlier days. But they were complex and highly priced. Currently diode laser systems that are easy to handle, portable, and economic are used predominantly. For treatment of larger areas, tungsten filament, quartz halogen, xenon arc, metal halide, and phosphor-coated sodium lamps, which are non-coherent light sources are used. In recent times non-laser light sources, such as light-emitting diodes (LED), have also been used in PDT. They are much economical and are small, lightweight, and highly flexible (Konopka 2007; Raghavendra, 2009).

Three light systems are available for the therapy:

1. Diode laser systems: They are handled with much ease, portable, and economical.
2. Non-coherent light sources: it is preferred to treat large areas and include tungsten filament, quartz halogen, xenon arc, metal halide, and phosphor-coated sodium lamps.
3. Non-laser light sources include light-emitting diodes (LEDs). They are cost effective, light weight, and highly flexible (Shivakumar, 2012; Dortbudaka, 2001).

Photosensitizers

Thousands of natural and synthetic photoactive compounds possess photosensitizing potential. Degradation products of chlorophyll, polyacetylenes, thiophenes, quinines (cercosporin), anthraquinones (fagopyrin, hypericin), and 9- methoxypsoralen are some of them. An ideal photosensitizer should be non-toxic, and should display local toxicity only after activation by illumination. The greater part of the sensitizers used clinically belong to dyes, the porphyrinchlorin platform, and furocoumarins.

An ideal photosensitizer includes photo-physical, chemical, and biological characteristics:

(i) Highly selective tumor accumulation;
(ii) Less toxic and quickly eliminated from the skin and epithelium;
(iii) Absorption is more in the low-loss transmission window of biological tissues;

(iv) Should possess photosensitizer diagnostic capabilities, which plays a key role in monitoring the photosensitizer accumulation in tissues and its elimination from them;
(v) Should have photosensitizer ability to generate singlet oxygen.
(vi) High quantum yield of singlet oxygen production in vivo;
(vii) Economical and easily available.
(viii) Highly soluble in water, injection solutions, and blood substitutes; and
(ix) Storage and application light stability (Konopka 2007).

Classification of Photosensitizers

Photosensitizers are classified as
I. *First-Generation Sensitizers*:
1. Hematoporphyrin derivatives (HPDs)
Eg: Photofrin (dihematoporphyrin ether)
II. *Second-Generation Photosensitizers*:
1. 5-aminolevulinic acid (ALA),
2. Benzoporphyrin derivative (BPD),
3. Lutetium texaphyrin,
4. Temoporfin (mthpc), (foscan)
5. Tinethyletiopurpurin (snet2), and
6. Talaporfin sodium (LS11).
III. *Third-Generation Photosensitizers*:
1. Biologic conjugates.
Eg: Antibody conjugate and liposome conjugate
(Aisling, 2009; Alexandra, 2013; Aleksander, 2003; Alparslan, 2013; Charlej and Paszkoa, 2011; Ethan, 1998; Konopka 2007; Mathias, 2011; Rovaldi, 2000; Allison, 2004).

Photosensitizers in Gram Negative Microorganisms

When PS are administrated along with outer membrane disrupting agents such as CaCl2, EDTA or polymixin B nonapeptide which are able to promote electrostatic repulsion with destabilization of the structure of the cell wall they bring deleterious effects against gram –ve bacteria. This allows considerable concentrations of the PS to penetrate the cytoplasmic membrane which can be photosensitized after light activation of the PS (Eliana, 2009; Drulis-Kawa, 2005; Fontana, 2009; Polansky, 2009).

Mechanism of Action

Photophysics

Light absorption and energy transfer are the two principle aspects of PDT. The ground state PS has two electrons with opposite spins (this is known as singlet state) in the low energy molecular orbital. Subsequent to the absorption of light (photons), one of these electrons is boosted into a high-energy orbital but on the other hand keeps its spin (first excited singlet state). This is a short-lived (nanoseconds) species and can lose its energy by emitting light (fluorescence) or by internal conversion into heat. Most PS are fluorescent and this has led to the development of sensitive assays to quantify the amount of PS in cells or tissues, and allows in vivo fluorescence imaging in living animals or patients to measure the pharmacokinetics and distribution of the PS. The excited singlet state PS may also undergo 'intersystem crossing' a process where the spin of the excited electron inverts to form the relatively long-lived (microseconds) excited triplet-state that has electron spins parallel. The loss of energy by emission of light (phosphorescence) is a ''spinforbidden'' process as the PS would move directly from a triplet to a singlet-state. This explains the long lifetime of thr PS triplestate (Castanoa, 2004).

Photochemistry

The PS excited triplet can go through two kinds of reactions. Initially, in a Type 1 reaction, it reacts directly with a substrate, such as the cell membrane or a molecule and forms a radical anion or radical cation, respectively by transferring a proton or electron. These radicals may produce reactive oxygen species by reacting with oxygen. In a Type 2 reaction, the triplet PS transfers its energy directly to molecular oxygen (itself a triplet in the ground state), and forms excited state singlet oxygen. Both Type 1

and Type 2 reactions can occur concurrently, and the ratio between these processes relies on the type of PS used, the substrate concentrations and oxygen (Figure 1).

Type 1 pathways involve initial production of superoxide anion frequently by electron transfer from the triplet PS to molecular oxygen (monovalent reduction). Superoxide by itself is not particularly reactive in biological systems and does not cause much oxidative damage. But it can react with itself to produce hydrogen peroxide and oxygen by a reaction known as ‘‘dismutation’’ and the enzyme superoxide dismutase (SOD) catalyzes the reaction. Hydrogen peroxide is important in biological systems as it passes readily through cell membranes and cannot be excluded from cells. Hydrogen peroxide is essential for the proper functioning of many enzymes, and hence is vital for health (like oxygen itself). Superoxide is also crucial in the production of the highly reactive hydroxyl radical (HO•).

In this process, superoxide in fact acts as a reducing agent, not as an oxidizing agent. This is because superoxide gives one electron to reduce the metal ions (such as ferric iron or Fe3+) that act as the catalyst to convert hydrogen peroxide (H_2O_2) into the hydroxyl radical (HO•). This reaction, known as Fenton reaction, was discovered over a hundred years ago. This is significant in biological systems as most cells have some amount of iron, copper, or other metals, which can catalyze this reaction. The reduced metal (ferrous iron or Fe2+) then catalyzes the breaking of the oxygen—oxygen bond of hydrogen peroxide to produce a hydroxyl radical (HO•) and a hydroxide ion (HO). Superoxide can react with the hydroxyl radical (HO•) to form singlet oxygen, or with nitric oxide (NO−) (also a radical) to produce peroxynitrite (OONO−), another highly reactive oxidizing molecule.

HO• travels easily through membranes and cannot be kept out of cells in the same way as H_2O_2. Hydroxyl radical damage is ‘‘diffusion rate-limited’’. This highly reactive radical can add to an organic (carboncontaining) substrate (represented by R below), for example, it can be a fatty acid which could form a hydroxylated adduct that is itself a radical. The hydroxyl radical can also oxidize the organic substrate by ‘‘stealing’’ or abstracting an electron from it. The resulting oxidized substrate is again itself a radical, and can react with other molecules in a chain reaction. For example, it could react with ground-state oxygen to produce a peroxyl radical (ROO•). The peroxyl radical again is highly reactive, and can react with another organic substrate in a chain reaction. Chain reaction of this type is common in the oxidative damage of fatty acids and other lipids, and demonstrates the damage caused by radicals such as the hydroxyl radical.

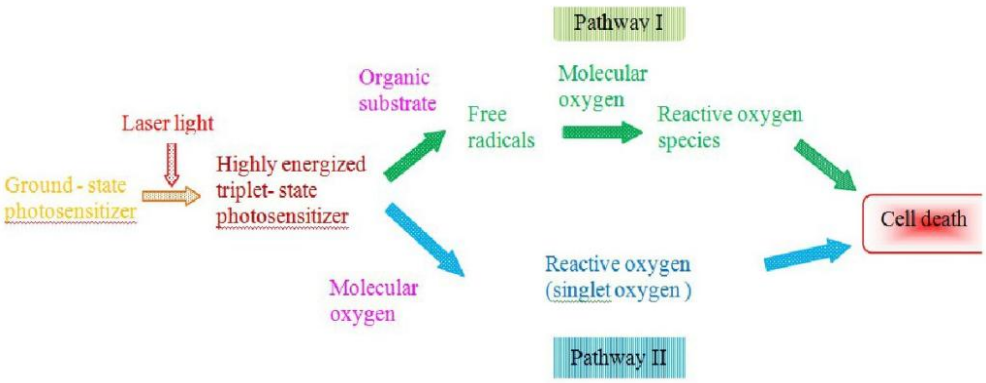

Figure: 1 mechanism of action

These ROS, along with singlet oxygen produced via Type 2 pathway, are oxidizing agents. These oxidizing agents can directly react with many biological molecules. Cysteine, methionine, tyrosine, histidine, and tryptophan which are Amino acid residues in proteins are important targets. These amino acids are the primary target of an oxidative attack on proteins due to their reactivity. The reaction mechanisms are complex and as a rule lead to a number of final products. Cysteine and methionine are oxidized mostly to sulfoxides, a thermally unstable endoperoxide is yielded by histidine, tryptophan reacts by a complicated mechanism to give N-formylkynurenine, tyrosine undergoes phenolic oxidative

coupling. Unsaturated lipids normally undergo ene-type reactions to give lipid hydroperoxides (LOOHs derived from phospholipids and cholesterol).

Oxidative damage to DNA can occur at both the nucleic bases (the individual molecules that make up the genetic code) and at the sugars that link the DNA strands by oxidation of the sugar linkages, or cross-linking of DNA to protein (a form of damage particularly difficult for the cell to repair). Even though all cells are capable of repairing oxidative damage to proteins and DNA to a certain extent, excess damage can cause mutations or cell death. Guanine, of the four bases in nucleic acids is mainly susceptible to oxidation by O2. The reaction mechanism has been studied at length in connection with oxidative cleavage of DNA. The initial step is a cycloaddition to the C-4 and C-8 carbons of the purine ring leading to an unstable endoperoxide. The following complicated sequence of reactions and the final products depend on whether the guanine moiety is bound in an oligonucleotide or a double stranded DNA.

Owing to the high reactivity and short half life of singlet oxygen and hydroxyl radicals, PDT directly affects the molecules and structures that are only proximal to the area of its production (areas of PS localization). The half-life of singlet oxygen in biological systems is < 40 ns, and, hence, the radius of the action of singlet oxygen is of the order of 20nm (Castanoa, 2004; Maria, 2002; Robertson, 2009)

Application of Photodynamic Therapy in Periodontics

PDT can be considered as an adjunctive to conventional mechanical therapy. The liquid photosensitizer when placed directly in the periodontal pocket can easily access the whole root surface before activation by the laser light through an optical fiber placed directly in the pocket.

Owing to the technical simplicity and the effective bacterial killing, the application of PDT in the treatment of periodontal diseases has been studied widely. PDT not only kills the bacteria, but might also bring about the detoxification of endotoxins such as lipopolysaccharide. These lipopolysaccharides treated by PDT do not stimulate the cells. Thus, PDT inactivates endotoxins by diminishing their biological activity (Komerik, 2000; Rafael, 2007; Ricardo, 2005; Qin, 2008; Jamil, 2003; juliano, 2008; Bernd, 2005; braun, 2008).

Table 1: Study Reports

Study	Material And Methods	Results	Conclusion
Rovaldi *et al.*, (2000)	Porphyrin derivatives (chlorin e6)	Chlorin e6 conjugated to pentalysine showed in vitro activity against all oral microorganisms tested, including P.gingivalis, A.A comitans, B. forsythus, C. rectus, E.corrodens, F. nucleatum subsp. polymorphum, A. viscosus, and the streptococci.	Chlorin e6–pentalysine conjugate to more effectively reduce the pathogenic bacteria in the periodontal pocket may be a significant tool for the treatment of periodontal disease.
Dortbudaka *et al.*, (2001)	Toluidine blue "'O", and Diode laser with a wavelength of 690 nm for one minute.	Combined treatment resulted in a significant bacterial reduction by up to 4 legs ($p < 0.001$), but complete elimination of all three microorganisms was achieved in none of the cases and this treatment seems to be more effective in reducing black pigmented microorganisms such as P. gingivalis and P.intermedia	Photodynamic treatment is a valuable additional method without any side effects in the treatment of patients with periodontal disease

You chan *et al.*, (2003)	Methylene blue (MB) and He-Ne laser (632.8 nm) with a 30 mW power output, a 100 mW diode laser at 665 nm, or a 100 mW diode laser at 830 nm,	The best PDT response rate was achieved with a 60 s (energy density 21.2 J/ cm2) exposure to the 665 nm wavelength diode laser in the presence photosensitiser. In this condition, approximately 95% of A.a.comitans and F. nucleatum, and 99–100% of the black-pigmented bacteria (P. gingivalis and P. intermedia) and S. sanguis were eliminated.	Using a diode laser of proper power and wavelength to deliver 60 s of irradiation could be a useful adjunct with mechanical debridement in the prevention of the re-colonisation of subgingival lesions by pathogenic microorganisms.
Anne *et al.*, (2004)	Chlorin e6, BLC 1010, and BLC 1014	The anaerobic bacteria Porphyromonas gingivalis, Fusobacterium nucleatum, and Capnocytophaga gingivalis can be photoinactivated completely by illumination with an intensity of 5.3 J/cm2 in the presence of 10 μM chlorin e6 and 10 μM BLC 1010	Photodynamic therapy with chlorin e6 and BLC 1010 is advantageous for suppressing periodontopathogenic bacteria.
Tatiana *et al.*, (2005)	Rose bengal (RB), toluidine blue O (TBO), and a poly-L-lysine chlorin(e6)	pL-ce6 was overall the most powerful photosensitizer, was equally effective with and without washing, and showed a strong dependence on cell concentration and TBO was less effective in all cases after washing, and the dependence on cell concentration was less pronounced and RB was ineffective after washing (except for S. aureus) but still showed a dependence on cell concentration and the overall order of susceptibility was S. aureus > E. coli > C. albicans, but C. albicans cells were 10 to 50 times bigger than the bacteria	The number and mass of the cells compete both for available dye binding and for extracellularly generated reactive oxygen species.
Nicos *et al.*, (2008)	Scaling and root planing followed by a single episode of PDT (test) or scaling and root planning alone (control)	At 3 and 6 months after treatment, there were no statistically significant differences between the groups with regard to CAL, PD, FMPS, or microbiologic changes	Significantly higher reduction in bleeding scores compared to scaling and root planing alone

Rafael *et al.*, (2009)	Photodynamic therapy or scaling and root planning,in a split mouth design on 0,1,7,30,and 90 days	PDT or SRP led to statistically significant reductions in TNF - α level 30 days following treatment	SRP and PDT had similar effects on crevicular TNF-α and RANKL levels in patients with aggressive periodontitis
Parand sorkhdini *et al.*, (2013)	Hydrosoluble chlorine– mediated aPDT	After both 3 weeks and 3 months, all treatment groups showed significant improvement in all clinical and immunologic parameters (P <0.001) and no significant differences were found between the four groups with regard to the measured parameters (P >0.05)	The adjunctive use of hydrosoluble chlorine–mediated apdt with the current setting has no additional effect on the clinical parameters or proinflammatory cytokine levels in ligature-induced periodontitis
Alparslan *et al.*, (2013)	Potassium–titanyl–phosphate (KTP) laser and PDT	all treatments yielded significant improvements in terms of BOP and PD decrease and CAL gain compared to baseline values (P <0.05) and Group C showed a greater reduction in PD compared to the other groups (P <0.05)	Patients with CP, clinical outcomes from conventional periodontal treatment of deeper pockets can be improved by using adjunctive KTP laser.

CONCLUSION

Though this new approach of using PDT is less traumatic and faster in the treatment of periodontal diseases it is still in the progressive stage of development and testing. In vitro studies on Photodynamic therapy have shown greater (>95%) reduction in micro-organisms. Clinical trial outcomes are also encouraging. In addition to reducing clinical parameters in periimplantitis cases, evidences show that PDT will also inactivate virulence factors of periodontal pathogens, enhancing post-treatment outcomes. PDT offers numerous advantages, particularly in avoiding emergence of antibiotic resistance species, requiring less technical skills and reducing operating time in comparison to manual scaling and root planing. To establish most favorable treatment parameters for PDT, development of new photosensitizers, more competent light delivery systems and further clinical studies are essential. This innovative approach of using PDT could be useful as an adjunct or conventional therapy during the maintenance period.

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