INDIAN JOURNAL OF DENTAL ADVANCEMENTS

Journal homepage: www. nacd. in

REVIEW

Quick Response Code

doi: 10.5866/3.4.732

Photodynamic Theraphy in Controlling Bio-film - A Novel Therapeutic Approach

Gopalakrishnan S[1], Umasudhakar[2]

Department of .....
Thai Moogambigai Dental College,
Muggapair west Garden, Chennai

Senior lecturer[1]
Professor[2]

Article Info
Received: July 12, 2011
Review Completed: August, 15, 2011
Accepted: September, 16, 2011
Available Online: January, 2012

ABSTRACT:

Photodynamic therapy (PDT), also known as photoradiation therapy, phototherapy, or photochemo-therapy, involves the use of a photoactive dye (photosensitizer) that is activated by exposure to light of a specific wavelength in the presence of oxygen. The transfer of energy from the activated photosensitizer to available oxygen results in the formation of toxic oxygen species, such as singlet oxygen and free radicals. These very reactive chemical species can damage proteins, lipids, nucleic acids, and other cellular components. Applications of PDT in dentistry are growing rapidly: the treatment of oral cancer, bacterial and fungal infection therapies, and the photodynamic diagnosis (PDD) of the malignant transformation of oral lesions. Photodynamic antimicrobial chemotherapy (PACT) has been efficacious in the treatment of bacterial, fungal, parasitic, and viral infections. The absence of genotoxic and mutagenic effects of PDT is an important factor for long-term safety during treatment. PDT also represents a novel therapeutic approach in the management of oral biofilms. Disruption of plaque structure has important consequences for homeostasis within the biofilm. Studies are now leading toward selective photosensitizers, since killing the entire flora leaves patients open to opportunistic infections. Dentists deal with oral infections on a regular basis. The oral cavity is especially suitable for PACT, because it is relatively accessible to illumination.

Key words: ***Photosensitizers, photodynamic Antimicrobial Chemotherapy therapy (PACT), Photodynamic Therapy (PDT). Nanoparticles, Biofilm.***

INTRODUCTION

Photodynamic therapy (PDT) is a medical treatment that utilizes light to activate photosensitizing agent (photosensitizer) in the presence of oxygen. The exposure of the photosensitizer to light results in the formation of oxygen species, such as singlet oxygen and free radicals, causing localized photo damage and cell death. Clinically, this reaction is cytotoxic and vasculotoxic. Depending on the type of agent, photo sensitizers may be injected intravenously, ingested orally, or applied topically. The relative simplicity of the mechanism of activation of photosensitizers has stimulated considerable interest in PDT. The antimicrobial activity of photosensitizers is mediated by singlet oxygen, which, because of its high chemical reactivity, has a direct effect on extracellular molecules. Thus, the polysaccharides present in EMP of a bacterial biofilm are also susceptible to photodamage. Such dual activity, not exhibited by antibiotics, represents a significant advantage of PACT. Breaking down biofilms may inhibit plasmid exchange involved in the transfer of antibiotic resistance, and disrupt colonization.

Mechanism of photodynamic therapy action

After absorption of a photon of light, a molecule of the photosensitizer in its ground singlet state is excited to the singlet state (S) and receives the energy of the photon. The life time of the S* state is in the nano second stage, which is too short to allow significant interactions with the surrounding molecules. The S* state molecule may decay back to the ground state by

Email for correspondence:
gopalakrishnan_perio@ymail.com

emitting a photon as light energy (fluorescence) or by internal conversion with energy lost as heat. Alternatively, the molecule may convert into an excited triplet state (T) molecule via intersystem crossing that involves a change in the spin of an electron . The life time of the T state is in the microsecond to the millisecond range. Molecules in the T state can emit light (phosphorescence) by returning to the state or can react further by one or both of two pathways, both of which require oxygen. The type I reaction involves electron transfer reactions from the photosensitizer triplet state with the participation of a substrate to produce radical ions can react with oxygen to produce cytotoxic species, such as superoxide, hydroxyl and lipid derived radicals. The Type II reaction involves energy transfer from the photosensitizer triplet state to ground state molecular oxygen (triplet) to produce excited state singlet oxygen, which can oxidize many biological molecules, such as proteins, nucleic acids and lipips, and lead to cytotoxity.

Advantages of PDT over the conventional treatments of cancer, such as surgery, radiotherapy, and chemotherapy, are summarized as

Potential Advantages of Photodynamic Therapy over Conventional Anti-cancer Therapies

- Is non-invasive and convenient for the patient
- Can be performed in outpatient or day-case (inpatient) settings
- Can be targeted accurately and selectively in early or localized diseases
- Although it cannot cure advanced disseminated disease, because illumination of the whole body is not possible, it can improve quality of life and lengthen survival
- Repeated doses can be given without the need for total-dose limitations
- Has moderate side-effects
- Can have excellent cosmetic results, and the healing process results in little or no scarring
- Can offer organ-sparing treatment worldwide, with very little investment in infrastructure

Light sources

PDT requires a source of light that activates the photosensitizer by exposure to low-power visible light at a specific wavelength. Human tissue transmits red light efficiently, and the longer activation wavelength of the photosensitizer results in deeper light penetration. Consequently, most photosensitizers are activated by red light between 630 and 700 nm, corresponding to a light penetration depth from 0.5 cm (at 630 nm) to 1.5 cm (at 700 nm)[1,2] This limits the depth of necrosis and/or apoptosis and defines the therapeutic effect. As a result, larger solid tumors cannot be uniformly illuminated, because of the limited depth of light penetration. The total light dose, the dose rates, and the depth of destruction vary with each tissue treated and with each photosensitizer .[3,4,5]

PHOTOSENSITZERS

Thousands of natural and synthetic photoactive compound have photosensitizing potential. They include degradation products of chlorophyll, polyacetylenes, thiophenes, quinones(cercosporin), anthraquinones (fagopyrin, hypericin), and 9-methoxypsoralen.[6] An ideal photosensitizer should be non-toxic, and should display local toxicity only after activation by illumination. The majority of the sensitizers used clinically belong to dyes, the porphyrinchlorin platform, and furocoumarins. The requirements of an optimal photosensitizer include photo-physical, chemical, and biological characteristics: (i) highly selective tumor accumulation; (ii) low toxicity and fast elimination from the skin and epithelium; (iii) absorption peaks in the low-loss transmission window of biological tissues; (iv) optimum ratio of the fluorescence quantum yield to the interconversion quantum yield (The first parameter determines the photosensitizer diagnostic capabilities, and plays a key role in monitoring the photosensitizer accumulation in tissues and its elimination from them; the second parameter determines the photosensitizer ability to generate singlet oxygen.); (v) high quantum yield of singlet oxygen production *in vivo*; (vi) cost effectiveness and commercial availability; (vii) high solubility in water, injection solutions, and blood substitutes; and (viii) storage and application light stability.

Photofrin® (dihematoporphyrin ether), available for 30 years in its commercial form, and hematoporphyrin derivatives (HPDs) are referred to as first-generation sensitizers. Photofrin® is the most extensively studied and clinically used photosensitizer. Second-generation photosensitizers include 5-aminolevulinic acid (ALA), benzoporphyrin derivative (BPD), lutetium texaphyrin, temoporfin (mTHPC), tinethyletiopurpurin(SnET2), and talaporfin sodium (LS11). Foscan® (mTHPC), the most potent second-

generation photosensitizer, has been reported to be 100 times more active than Photofrin® in animal studies. These photosensitizers have a greater capability to generate singlet oxygen; however, they can cause significant pain during therapy, and, because of their high activity, even dim light (60 Watt bulb) can lead to severe skin photosensitivity.The third agent, ALA, is an intrinsic photosensitizer that is converted *in situ* to a photosensitizer, protoporphyrin IX. Topical ALA and its esters have been used to treat pre-cancer conditions, and basal and squamous cell carcinoma of the skin[7] Third-generation photosensitizers include currently available drugs that are modified by targeting with monoclonal antibodies or with nonantibody- based protein carriers and protein/receptor systems, and conjugation with a radioactive tag .[8]

Effects of Photodynamic Antimicrobial Chemotherapy (PACT) on Oral Biofilms

The oral cavity is colonized by complex, relatively specific, and highly interrelated micro-organisms, including aerobic and anaerobic Gram-positive and Gram-negative bacteria, fungi, mycoplasma, protozoa, and viruses. Dental plaque can be defined as the diverse community of micro-organisms found on the tooth surface as a biofilm, embedded in an extracellular matrix of polymers (EMP) of host and microbial origin. In the biofilm, bacteria exhibit increased resistance to antibiotics, environmental stresses, and host immune defense mechanisms. Two of the most common bacterial diseases that afflict humans are dental caries and periodontal diseases. Both result originally from a build-up of plaque biofilms on the teeth and soft tissues of the mouth. Mechanical removal of plaque, good oral hygiene, and antimicrobial agents are the most common treatments for periodontitis. Nevertheless, the limited access of topical agents to the plaque and the development of antibiotic-resistance create the necessity for alternative strategies to control plaque and to treat gingivitis and periodontal diseases. The antimicrobial activity of photosensitizers has a direct effect on extracellular molecules. With all three photosensitizers, PACT was increasingly more effective as the biofilm age increased, suggesting that "young" biofilms are less susceptible than "older" biofilms [9] In contrast, [10] using TBO as a photosensitizer, reported that younger biofilms of *S. mutans* are more sensitive to PACT. The reasons for this difference are unclear, but may be due to the properties of the photosensitizers used or the differences in extracellular matrix composition. Thus, the polysaccharides present in extracellular matrix of polymers (EMP) of a bacterial biofilm are also susceptible to photodamage. Breaking down biofilms may inhibit plasmid exchange involved in the transfer of antibiotic resistance, and disrupt colonization. In addition to treatment for periodontitis, the use of PACT for peri-implantitis and endodontic treatment has also come into focus.S. Peri-implantitis is a multifactorial process involving bacterial contamination of the implant surface and the formation of biofilms. Bacterial plaque on implants leads to inflammatory changes in the adjacent soft tissues. An application of TBO (Toluidine blue O) on implant surfaces in 15 patients with peri-implantitis, followed by illumination with a diode soft laser (690 nm), significantly reduced the numbers of *A.actinomycetemcomitans*, *P. gingivalis*, and *P. Intermedia*.[11]

Photodynamic therapy and the use of nanoparticles to control oral biofilms

Photodynamic therapy (PDT) is very well-suited for the control of bacteria in oral plaque biofilms where there is relatively easy access for the application of the photosensitizing agent and light sources to areas requiring treatment.[12] The killing of micro-organisms with light depends upon cytotoxic singlet oxygen and free-radical generation, which are formed by the excitation of a photactivatable agent or sensitizer. The result of excitation is that the sensitizer moves from an electronic ground state to a triplet state that then interacts with microbial components to generate cytotoxic species .[13] One of the advantages of light-activated killing is that resistance to the action of singlet oxygen is unlikely to become widespread, in comparison with that experienced with more traditional chemical antimicrobial agents. A sensitizer ideally should absorb light at red to near-infrared wavelengths, because these wavelengths are able to penetrate more deeply. The most commonly tested sensitizers on bacteria have been tricyclic dyes (for example, methylene blue, erythrosine), tetrapyrroles (for example, porphyrins), and furocoumarins (for example, psoralen). The use of nanoparticles with in this area is now under investigation. For example, a complex of biodegradable and biocompatible poly(lactic-co-glycolic acid) (PLGA) and colloidal gold nanoparticles, loaded with methylene blue and exposed to red light at 665 nm, has been tested against planktonic *E. Faecalis* and in experimentally infected root canals.[14] Some studies suggest that sulfur-containing proteins/key

enzymes in the membrane or inside the cells and phosphorus-containing elements, such as DNA, are likely to be the preferential binding sites for silver nanoparticles. The contribution of silver ion release to overall antimicrobial activity remains unclear. It is suggested that a bacterial cell in contact with silver nanoparticles will take up silver ions, which possibly in turn will inhibit respiratory enzymes, and so help facilitate reactive oxygen species/free radical generation and subsequent damage to the cell membrane (Kim *et al*., 2007).[15]

Most work on light-activated killing has been performed with suspensions of planktonic bacteria, with relatively few studies observing biofilm grown micro-organisms. *In vitro* biofilm- grown *Streptococcus mutans* cells demonstrated a 3-log reduction when treated with erythrosine and white light (500- 650 nm).[16] while an approach with antibody and erythrosine-labeled nanoparticles has shown the potential for targeting specific bacterial species in oral plaque biofilms. These *in vitro* studies, with constant-depth film fermenters with gold nanoparticles conjugated to erythrosine and antibody to either *Streptococcus mutans* or *Lactobacillus casei*, have shown specific killing of target organisms in mixed-biofilm cultures. An understanding of the interface between biological systems and nanomaterials should enable design features to be used to control the exposure, bioavailability, and biocatalytic activities. Several possible approaches are starting to be identified (Nel *et al*., 2009)[17], including changing ability to aggregate, application of surface coatings, and altering charge density and oxidative state. However, this may well compromise the intended selective toxicity of antimicrobial nanoparticles. The full impact of potential mammalian toxicity issues on the use of nanotechnology in the control of oral biofilms remains to be determined. Considerations in relation to the therapeutic use of light activated killing of biofilms on host surfaces include: (1) direct toxicity of the sensitizer, (2) indirect toxicity of the sensitizer in terms of 'bystander' damage to adjacent host cells, (3) penetration into the biofilm, (4) light exposure time required to kill bacteria within *in vivo* biofilms, and (5) widespread relatively non-specific bacterial killing.[18] The photosensitizer erythrosine has an advantage over some other dyes because it is currently used in dentistry to visualize dental plaque *in vivo,* and so its lack of toxicity in the host is well established.

Current concepts in PDT

Biophysical means such as ultrasonic irradiation[19] and electric fields[20] known as the 'bioacoustic' effect and the 'bioelectric' effect respectively, have been employed to enhance the efficacy of various agents in killing biofilm microorganisms. Photomechanical waves are unipolar compression waves generated by lasers[21] and are one of the latest technology platform for drug delivery. Antibodies conjugated with photosensitizers have been used to target staphylococcus aureus [22] and selective killing of P.gingivalis was achieved using a murine monoclonal antibody against P.gingivalis lipopolysaccharide conjugated with toluidine blue O. The therapeutic potential of these approaches for targeting is based on their ability to demonstrate minimal damage to host cells.

Current Limitations in PDT

Even the best currently available systemic photosensitizers accumulate to a certain degree in other organs, particularly in the skin, causing prolonged photosensitivity after exposure to light. Thus, an ideal photosensitizer should be administered easily and safely, targeted appropriately, illuminated and activated at clinically useful wavelengths, pain-free, and obtained easily. For use in periodontitis, the dye needs to be applied subgingivally prior to fiber-optic laser light activation. However, in disease the periodontal site has a marked flow of gingival crevicular fluid into the pocket, and most photosensitizers lose some activity in the presence of extraneous protein. Also, some have virtually no effect in the presence of saliva and other body fluids. This is because the agents complex with proteins and host cells in the gingival crevicular fluid, and effectively compete for binding to bacteria. The use of nanoparticles as applied to PDT may help to overcome some of the issues associated with serum constituents. Although PDT was originally considered as a local treatment, limited to sites where the light activates a photosensitizer, it is now realized that PDT can initiate regional and systemic immune responses.[23] Despite all these limitations, the existing photosensitizers and light sources have achieved significant clinical success, allowing PDT to expand. The future of PDT will depend on the interactions between clinical applications and technological innovations. Allison *et al*. have described PDT as the therapy that "is truly the marriage of a drug and a light", and, as a result, only interdisciplinary research approaches can overcome all the difficulties and challenges of PDT.

CONCLUSION

A successful PDT should overcome the limitations of currently used light sources. In the past, many of the applications of PDT used a laser source for illumination of the target area, particularly for internal use. Recently, non-laser light sources, such as light-emitting diodes (LEDs), are making an impact on PDT. The further development of therapy- and cost-effective light sources for PDT would be of benefit for both research and clinical applications.

REFERENCES

1. Salva KA (2002). Photodynamic therapy: unapproved uses, dosages, or indications. *Clin Dermatol* **20**:571-581.
2. Kübler AC (2005). Photodynamic therapy. *Med Laser Appl* **20**:37-45.
3. Grant WE, Speight PM, Hopper C, Bown SG (1997). Photodynamic therapy: an effective, but non-selective treatment for superficial cancers of the oral cavity. *Int J Cancer* **71**:937-942.
4. Biel MA (2002). Photodynamic therapy in head and neck cancer. *Curr Oncol Rep* **4**:87-96.
5. Allison RR, Cuenca RE, Downie GH, Camnitz P, Brodish B, Sibata CH (2005). Clinical photodynamic therapy of head and neck cancers–a review of applications and outcomes. *Photodiagn Photodyn Ther* **2**:205-222.
6. Ebermann R, Alth G, Kreitner M, Kubin A (1996). Natural products derived from plants as potential drugs for the photodynamic destruction of tumor cells. *J Photochem Photobiol B* **36**:95-97.
7. Peng Q, Warloe T, Berg K, Moan J, Kongshaug M, Giercksky KE, *et al*. (1997). 5-aminolevulinic acid-based photodynamic therapy. Clinical research and future challenges. *Cancer* **79**:2282-2308.
8. Vrouenraets MB, Visser GW, Snow GB, van Dongen GA (2003). Basic principles, applications in oncology and improved selectivity of photodynamic therapy. *Anticancer Res* **23**:505-522.
9. Wood S, Metcalf D, Devine D, Robinson C (2006). Erythrosine is a potential photosensitizer for the photodynamic therapy of oral plaque biofilms. *J Antimicrob Chemother* **57**:680-684.
10. Zanin IC, Gonçalves RB, Junior AB, Hope CK, Pratten J (2005). Susceptibility of *Streptococcus mutans* biofilms to photodynamic therapy: an in vitro study. *J Antimicrob Chemother* **56**:324-330.
11. Dortbudak O, Haas R, Bernhart T, Mailath-Pokorny G (2001). Lethal photosensitization for decontamination of implant surfaces in the treatment of peri-implantitis. *Clin Oral Implants Res* **12**:104-108.
12. MacRobert AJ, Bown SG, Phillips D (1989). What are the ideal photoproperties for a sensitizer? *Ciba Found Symp* **146**:4-12.
13. Pagonis TC, Chen J, Fontana CR, Devalapally H, Ruggiero K, Song X, *et al*. (2010). Nanoparticle-based endodontic antimicrobial photodynamic therapy. *J Endod* **36**:322-328.
14. Sondi I, Salopek-Sondi B (2004). Silver nanoparticles as an antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *J Colloid Interface Sci* **275**:177-182.
15. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, *et al*. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine* **3**:95-101.
16. Allison RR, Bagnato VS, Cuenca R, Downie GH, Sibata CH (2006). The future of photodynamic therapy in oncology. *Future Oncol* **2**:53-71.
17. Nel AE, Madler L, Velegol D, Xia T, Hoek EM, Somasundaran P, *et al*. (2009). Understanding biophysicochemical interactions at the nano-bio interface. *Nat Mater* **8**:543-557.
18. Allaker RP, Douglas CW (2009). Novel anti-microbial therapies for dental plaque-related diseases. *Int J Antimicrob Agents* **33**:8-13.
19. Qian Z, Sagers RD, Pitt WG. T he effect of ultrasonic frequency upon enhanced killing of P.aeruginosa biofilms Ann Biomed Eng 1997:**25**: 69-76.
20. Costerton JW, Ellis B, Lam k, Johnson F,Khoury AE, Mechanism of electrical enhancement of efficacy of antibiotics in killing biofilm bacteria. Antimicrob Agents Chemother 1994: **38**: 2803-2809.
21. Doukas AG, Flotte TJ.Physical characteristics and biological effects of laser -induced stress waves. Ultrasound Med Biol 1996:**22**: 151-164.
22. Embleton MI, Nair SP,Cookson BD,Wilson M.Antibody - directed photodynamic therapy of methicillin resistant Staphylococcus aureus.Microb Drug Resist 2004:**10**:92-97.
23. Dougherty TJ, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbelik M, *et al*. (1998). Photodynamic therapy. *J Natl Cancer Inst* **90**:889-905.

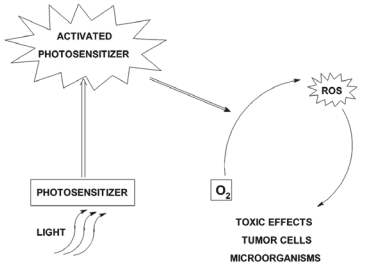

Figure 1. Schematic representation of photodynamic reaction and photodynamic therapy (Konopka and Goslinski J Dent Res 2007)

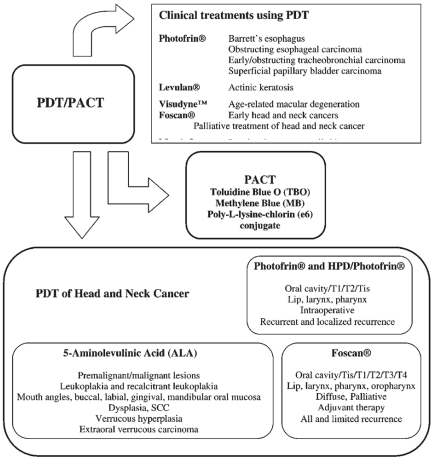

Figure 2. Photosensitizers used in the different clinical applications of photodynamic therapy (PDT) and photodynamic antimicrobial chemotherapy (PACT).(Konopka and Goslinski J Dent Res 2007)