Photodynamic Therapy in Periodontics: A Review

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Received 20 December 2015; Accepted 28 December 2015; Available online 31 December 2015

Abstract
The use of photodynamic therapy has been encouraged as one of the substitutes to antimicrobial agents for suppressing subgingival species and for treatment of periodontitis. Bacteria loaded with dense biofilms such as those encountered in dental plaque or peri implant surfaces are becoming resistant due to the injudicious use of antibiotics, recently. The present review elucidates the evolution and use of photodynamic therapy with the emphasis on application of photosensitizing dyes and their excitation by visible lights that enables effective killing of periodontopathogens.

Keywords: Microbial resistance; Photodynamic therapy; Photosensitizers; Antibiotics

Introduction
The presence of bacterial species, particularly the periodontopathogenic species on the tooth or root surface is major cause of gingivitis or periodontitis (Kolenbrander 2000; Page et al. 1997; Chen 2001). It is well accepted that mechanical removal of dental biofilms is basis of any adjunctive periodontal therapy as microbial biofilms in the oral cavity involved in etiology of various oral conditions including caries, periodontal and endodontic disease, oral malodour, denture stomatitis, candidiasis and dental implant failures (Singh et al. 2014; Cortelli et al. 2014). In general, it is recognised that the growth of bacteria in biofilms imparts a substantial decrease in susceptibility to antimicrobial agents compared with culture growth in suspension (Soukos and Goodson 2011; Silva et al. 2012; Bjarnsholt et al. 2013; Singh et al. 2014). Therefore, dental plaque, a naturally occurring biofilm (Marsh 2005), display increased resistance to antimicrobial agents (Anderson and Toole 2008; Fux et al. 2005). Currently, various treatment modalities with least possible side effects have been increasingly searched. Local and systemic administration of antibiotics may lead to resistance, gastrointestinal and other disorders besides patient compliance which is often an added problem (Pallasch 2003; Quirynen et al. 2003; Rodrigues et al. 2004).

Seeking the alternative to antibiotic treatment, periodontal researchers have found that PDT is advantageous for suppressing anaerobic bacteria that lead to Periodontal Diseases (Pfitzner et al. 2002). Michael Prethman, the president of AAP 2003 said- “antibiotics may be used as an adjunctive therapy for periodontal diseases”. Recent number of reports about bacterial strains becoming resistant to frequent doses of antibiotics, leads to development of alternative antimicrobial concept. PDT could be an alternative to conventional periodontal therapeutic measures (Malik et al. 2010). The present literature aims to discuss the PDT from a periodontal perspective.

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History

The use of photodynamic therapy for inactivating microorganisms was first demonstrated more than 100 years ago, in 1900 when Oscar Rabb (Rabb, 1900) reported the lethal effects of acridine hydrochloride and visible light on Paramecia caudatum. PDT was introduced in medical therapy in 1904, as the light induced inactivation of cells, microorganisms or molecules (Von Tappeiner and Jodlbauer, 1904). In 1913, Friedrich Meyer Betz, the German physician performed the pioneering study which was at first called Photo-radiation Therapy with porphyrins. He tested the effect of hematoporphyrins on his own skin (Moan and Peng, 2003). It was John Toth who acknowledged the photodynamic chemical effect of therapy with early clinical argon due lasers and wrote the first white paper renaming the therapy as photodynamic therapy. Thomas Dougherty formed international photodynamic association in 1986. PDT was first approved by drug and food administration in 1999 to treat precancerous skin lesions on face and scalp (Babilas et al., 2005). The photodynamic therapy in curing human infections is based on the conception that an agent which absorbs light known as a photosensitizer, is preferentially taken up by bacteria and subsequently photon of light; a molecule of the photosensitizer is activated by light of the appropriate wavelength in the presence of oxygen to generate oxygen and free radicals that are cytotoxic to microorganisms (De Melo et al., 2013). Because of the primitive molecular nature of singlet oxygen, it is unlikely that microorganisms would develop resistance to the cytotoxic action (Soukos and Goodson, 2011). Photodynamic therapy has emerged as an alternative to antimicrobial regimes and mechanical means in eliminating dental plaque of species owing to the groundbreaking work of Professor Michael Wilson and colleagues (Wilson, 1993) at the Eastman Dental Institute, University College London, UK.

Principles behind the photodynamic therapy

PDT is based on the principle that a photoactivable substance (photosensitiser) binds to the target cell can be activated by light of suitable wavelength. During this process, free radicals are formed (among them singlet oxygen) which then produce an effect that is toxic to the cells. For bactericidal effect on the cells, the respective photosensitiser needs to have selectivity for prokaryotic cells. Some authors have reported the possibility of lethal photosensitisation of bacteria in vivo and in vitro (Martinetto et al., 1986; Wilson, 1993; De Simone, 1999; Bertoloni et al., 1992).

By irradiation with light in visible spectrum, the dye (photosensitizer) is excited to its triplet state, the energy of which is transferred to molecular oxygen. The product formed is highly reactive singlet oxygen capable of reacting with biological systems and destroying them, while only the first excited state with energy of 94 kJ/mol (22 kcal/mol) above the ground state is important, the second excited state does not react. The bactericidal effect of photo-dynamic therapy can be explained by two potential, but different, mechanisms- one is DNA damage and the other is the damage caused to the cytoplasmic membrane of the bacteria by cytotoxicity (Bertoloni et al., 1992).

Species generated by antimicrobial photodynamic therapy lead to events such as inactivation of membrane transport system, inhibition of plasma membrane enzyme activities, lipid peroxidation and others (Kennedy, 1990; Mohr et al., 1993). The mechanism of action can be briefly described as follows- after irradiation of light of specific wavelength (lasers), the
photosensitizer at ground state are activated to highly energized triplet state. The extended life of triplet state enables the interaction of excited photosensitiser that leads to generation of cytotoxic species produced during PDT. Two different pathways are followed by the triplet state photosensitizer involving Type I and Type II reactions—

**Type I reactions**

Type I reaction involves the hydrogen atom abstraction or electron transfer reaction between the excited state of photosensitizer and an organic substrate molecule of the cells which produces free radical and radical ions. These free radical species are generally highly reactive and interact with endogenous molecular oxygen to produce highly reactive oxygen species such as superoxide, hydroxyl radicals and hydrogen peroxide that harm cell membrane integrity causing irreparable biological damage (Rajesh et al. 2011; Khurana et al. 2014).

**Type II reaction**

In type II reaction, the triplet state photosensitizer reacts with oxygen to produce an electronically excited singlet oxygen which can interact with a large number of biological substrate as a result of its high chemical reactivity inducing oxidative damage and damaging the cell membrane and cell wall (Foote et al. 1991; Sharman et al. 1999). Microorganisms that are killed by singlet oxygen includes viruses, bacteria, protozoa and fungi. Sites of initial cell damage from PDT are closely related to the localization of photosensitizer. Thus, the reaction takes place in limited space, leading to a localized response and making it ideal for application at localized sites without affecting distant molecules, cells or organs (Moan et al. 1991; Peng et al. 1996).

A schematic diagram of mechanisms involved during Type I and Type II reactions in photodynamic therapy is presented in Fig 1.

**Photosensitizers**

More than 400 compounds are known with photosensitizing properties including dyes, drugs, cosmetics, chemicals and many natural substances (Santamaria et al. 1972). Most of the sensitizers used for medical purposes belong to the following basic structures:

1. Tricyclic dyes with different meso atoms, acridine orange, proflavine, riboflavin, methylene blue, fluorescein, eosin, erythrosine, rose Bengal.
2. Tetrapyrroles, porphyrins and derivatives, chlorophyll, phylloerythrin, phthalocynins.
3. Furocoumarins, psoralen and its methoxy derivatives- xanthotoxin, bergaptene.

Based on the advantages and characteristics of anti-microbial photodynamic therapy, it has been anticipated that periodontal and peri-implant diseases are potential targets of this novel antimicrobial photo-chemotherapy. Antimicrobial photodynamic therapy is expected to resolve the difficulties and problems of conventional antimicrobial therapy and can work as an adjunctive to conventional mechanical treatments.

The photosensitizer is located directly in the periodontal and peri-implant pocket and the liquid agent easily contact the whole root or implant surface before activation by the laser light through placement of the optical fiber directly in the pocket. Due to the
Fig 1. The schematic representation of Type I and Type II reactions in photodynamic therapy (Adapted from Khurana et al. 2014).

After exposure to light, the activated photosensitizer during excited triplet state, tracks either of the two pathways. Type I pathway includes electron-transfer reactions from the photosensitizer triplet state by the participation of a substrate to produce radical ions which reacts with oxygen to produce cytotoxic species. Type II pathway includes energy transfer from the photosensitizer triplet state to the ground state molecular oxygen (triplet) to produce excited state singlet oxygen, which oxidizes biological molecules.

hv = photon energy; PS = photosensitizer.

technical simplicity of the method and the high effectiveness of bacterial killing, the use of antimicrobial photodynamic therapy in the treatment of periodontal and peri-implant diseases has extensively studied. The bactericidal effect of antimicrobial photo-dynamic therapy on periodontal pathogens has been demonstrated in several basic studies. In the early 1990s, Dobson & Wilson showed that low-level helium–neon laser irradiation with toluidine blue O or methylene blue was effective for killing *P. gingivalis*, *F. nucleatum*, *A. actinomyctemcomitans* and *S. sanguinis*. In comparison with other photosensitizers, toluidine blue O and methylene photosensitizers, toluidine blue O and methylene blue prove to be more effective for killing periodontal pathogens in antimicrobial photodynamic therapy (Wilson et al.1993). These authors also revealed that the most effective bactericidal effect was achieved with the combination of toluidine blue O and a helium–neon laser in a supragingival biofilm model study (Wilson M Dobson et al.1995). Bhatti et al. 2002 demonstrated that the optimal concentration of toluidine blue O to kill *P. gingivalis* was 12.5 lg/ml with helium–neon laser irradiation. In addition, they revealed, by transmission electron microscopic examination, that the bactericidal effect of light-activated toluidine blue O against *P. gingivalis* was caused by disruption of the outer membrane proteins of the bacteria. Additionally, it has been indicated that in the presence of methylene blue, the wavelength of 632.8 nm (helium neon) laser and 665 and 830 nm (diode) have a high bactericidal effect on periodontal pathogens (Chan and Lai 2002).

Moreover, the bactericidal effect of antimicrobial photodynamic therapy was demonstrated not only on pure culture of bacteria but also on plaque biofilm. Sarkar and Wilson (1993) reported that helium neon irradiation combined with toluidine blue O killed the oral bacteria within samples of subgingival plaque obtained from patients with chronic periodontitis. Sukos et al. (2003) demonstrated the bactericidal effect of PDT with poly-t-lysine chlorine conjugate and a diode laser
against subgingival plaque biofilm that comprised both gram positive and gram negative bacteria. They demonstrated that the bacteria present in deep layers of biofilm were killed by extensive penetration of the photosensitizer into the biofilm following antimicrobial photodynamic therapy. In black pigmented bacteria, the endogenous porphyrins present on bacteria may also act as photosensitizer. It seems that antimicrobial photodynamic therapy not only kills the bacteria but may also lead to the detoxification of endotoxins because it has been demonstrated that LPS stimulate the production of pro-inflammatory cytokines by mono nuclear cells (Kennedy et al. 1990). Consequently, PDT may possibly inactivate endotoxins such as lipopolysaccharides by decreasing their biological activity.

Use of antimicrobial PDT in treatment of peri-implant disease

It has been proven by the treatment of peri-implantitis that complete eradication of causative bacteria is responsible for development of periodontal disease and disinfection or detoxification of peri-implant pockets are essential to achieve effective healing with regeneration of lost bone around the affected implants (Mombelli, 1992, 1987). In one of the examples of in vitro study carried out by Hass et al (1997), the efficacy of antimicrobial PDT was examined in killing bacteria associated with Peri-implantitis such as A. actinomycetemcomitans, P. gingivalis or Provetella intermedia which adhered to titanium plates with different surface characteristics. Plates were incubated with those bacteria and subjected to four different treatments - (i) PDT (toluidine blue O + diode laser) (ii) no treatment (iii) laser light alone (iv) toluidine blue O alone. None of the plates subjected to PDT showed bacterial growth of any of the micro-organisms. While in the other treatment groups, all the three species of bacteria were detected after treatment. The scanning electron microscopic analysis revealed that antimicrobial PDT led to bacterial cell destruction without damage to the titanium surface.

Adverse effects

PDT has a potential of phototoxic or photoallergic unwanted side effects (Kubler et al. 2002). There can be impairment of benign oral flora which may lead to the overgrowth of single resistant species (Roberts et al. 2002). In order to avoid phototoxic reactions, it is more important to stain selectively the target leaving out the gingival mucosa or tongue. Burning sensation stinging or prickling is common complained experienced during PDT (Lui et al. 1993; Kalker et al 2002). It usually occurs in early part of light exposure. A clinically obvious scar is rarely observed. The histological evidence of scarring is evident (Fink et al 1998). Hyperpigmentation or hypopigmentation can occasionally be seen in treated areas and resolves within six months.

Conclusion

The antimicrobial photodynamic therapy is an interesting therapeutic approach in the direction of the treatment of periodontitis and peri-implantitis. Various studies suggests the effective and efficient bactericidal effect of antimicrobial PDT. However, further in vivo and clinical studies are necessary to determine the optimal conditions of this novel therapy. Low toxicity and rapidity of effects are the good qualities of PDT. Antimicrobial PDT may hold a promise as a substitute for currently available chemotherapy in the treatment of periodontal and peri-implant diseases.
Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgements

The authors would like to thank the Professors of the People’s Dental College, Tribhuvan University for providing necessary assistance and guidance.

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