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Photodynamic Therapy – Coupling Of Light And Dye – A Review

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ABSTRACT

The oral cavity is colonized by a large number and highly diversified communities of micro-organisms. Bacterial biofilm present on tooth or root surface is a major cause of gingivitis and periodontitis. Chemical antimicrobial agents are widely used in prophylactic and therapeutic regimens for dental plaque related diseases. As these agents are difficult to maintain at therapeutic concentrations in the oral cavity and can be rendered ineffective by resistance development in target organisms, there is a need for an alternative antimicrobial approach. A novel approach, photodynamic therapy (PDT), could be a solution to these problems. Lethal photosensitization of many bacteria, both Gram positive and Gram negative was found in many studies. The advantage of this new approach includes rapid bacterial elimination, minimal chance of resistance development and safety of adjacent host tissue and normal microflora.

KEY WORDS: Antimicrobial Therapy, Bacterial Resistance, Periodontal Disease, Photodynamic Therapy, Photosensitizers.

INTRODUCTION

Periodontitis is the inflammation of the supporting structures of the teeth caused by various pathogenic microorganisms. The main objective of periodontal therapy is the elimination of bacteria and bacterial niches by removing the supragingival and subgingival plaque. Plaque removal is done by means of mechanical debridement, which is a non-surgical therapy.

SRP may fail to completely eradicate subgingival pathogens in areas such as deep pockets, root concavities, and furcations where mechanical access is problematic. In these difficult-to-reach sites, bacterial persistence can lead to residual periodontal pocketing and persistent or rebound inflammation after mechanical debridement. Antimicrobial therapy combined with SRP may suppress these pathogens, but systemic antimicrobial agents have potential side effects such as gastrointestinal disturbance, allergic reactions, and drug resistance.

Photodynamic therapy (PDT) has emerged recently as a new non-invasive treatment modality. It is based on light-induced inactivation of bacteria and has two essential components: a photosensitizing agent (e.g., toluidine blue and methylene blue) and light energy. A photosensitizer that absorbs light binds to the outer membrane of target bacteria and induces the formation of reactive oxygen species (ROS), causing localized photo damage and cell death. [1]

In dentistry, PDT has been proposed as a novel disinfection method, which may be a potential treatment for several infectious diseases by eradicating microorganisms. This mode of usage is often mentioned as antimicrobial PDT (a-PDT). Due to the high antibacterial potential, PDT has been proposed in the treatment of chronic periodontitis, peri-implantitis and endodontic infections.

One advantage of PDT, when compared to antibiotics, is that bacteria do not develop resistance to ROS. Treatment of periodontitis is aimed at decreasing the bacterial load by removing plaque and calculus. Among non-surgical approaches used, scaling and root planing (SRP) leads to the greatest improvement in several clinical and biologic parameters of the disease and is considered the principal method for the management of periodontal disease. [2]

BACKGROUND OF LASER

Based on Albert Einstein's theory of spontaneous and stimulated emission of radiation, Maiman (1960) developed the first laser prototype.

The first application of a laser to dental tissue was reported by Goldman et al. (1964) and it was suggested by Myers and Myers (1985) that a Nd:YAG laser could be used for oral soft tissue surgery. Myers (1989) published the first article on the pulsed Nd:YAG laser in periodontal surgery. PDT uses laser as light source to activate the photosensitizer.

HISTORY OF PHOTODYNAMIC THERAPY

It was at the beginning of the 20th century that Oscar Raab, a medical student of Professor Herman von Tappeiner in Munich, first examined photosensitized reactions in a scientific way and introduced the subject to Western medicine. Von Tappeiner's initial interest was in identifying the process by which quinine was effective in malaria whereas other chemicals, in particular acridine, were more toxic against the protozoan in vitro but were not effective in vivo. [3]

Hence, von Tappeiner investigated the properties of acridine (a coal tar derivative), initially demonstrating its potency in vitro with serial dilutions. However, Oscar Raab, who was performing the experiment, came upon an apparent paradox at the lowest limit of concentration.

" Von Tappeiner took over Raab's research and, with a dermatologist named Jesionek, published clinical data using eosin as a photosensitizer in the treatment of skin cancer, lupus of the skin and chondylomata of female genitalia." In 1904 von Tappeiner and Jodlbauer reported that the presence of oxygen was a requirement for photosensitization. [4]

In 1907 these experiments were collated into a book in which von Tappeiner coined the term 'photodynamic therapy' to describe the phenomenon of oxygen-dependent photosensitization. The term 'photodynamic therapy' was used to distinguish these reactions from the phenomenon of photosensitization of photographic plates, which was popular at the time and which was the basis for modem photography. Von Tappeiner is probably the most important early pioneer of photodynamic therapy, clearly predicting the photochemotherapeutic application of photosensitizers as early as 1900.

PRINCIPLES OF PHOTODYNAMIC THERAPY

PDT is based on the principle that a photoactivable substance (the photosensitizer) binds to the target cell and can be activated by light of a suitable wavelength. During this process, free radicals are formed (among them singlet oxygen), which then produce an effect that is toxic to the cell.

By irradiation with light in the visible range of the spectrum, the dye (photosensitizer) is excited to its triplet state, the energy of which is transferred to molecular oxygen. The product formed is the highly reactive singlet oxygen capable of reacting with biological systems and destroying them. Only the first excited state with the energy of 94 kJ/mol (22 kcal/mol) above the ground state is important and the second excited state does not react. [5]

MECHANISM OF ACTION

The three components of PDT are oxygen, photosensitizer, and light. [6]

When a photosensitizer is administered to the patient and irradiated with a suitable wavelength, it goes to an excited state from its ground state. This excited state can then decay back to its ground state or form the higher energy triplet state. The triplet state photosensitizer can react with biomolecules in two different pathways - type I and II (figure 2)

Main mechanism involves the use of a selective photosensitizer which is activated photosensitizer reaches the target tissues to bind to the bacterial cell wall and in some instances, penetrates into the cytoplasm.

Type I: It involves electron/hydrogen transfer directly from the photosensitizer, producing ions, or electron/hydrogen removal from a substrate molecule to form free radicals. These radicals react rapidly with oxygen, resulting in the production of highly reactive oxygen species (superoxide, hydroxyl radicals, and hydrogen peroxide).

Type II: In type II reaction, the triplet state photosensitizer reacts with oxygen to produce an electronically excited and highly reactive state of oxygen, known as singlet oxygen ($^1O_2$) which can interact with a large number of biological substrates inducing oxidative damage on the cell membrane and cell wall. Microorganisms that are killed by singlet oxygen include viruses,

bacteria, and fungi. Singlet oxygen has a short lifetime in biological systems and a very short radius of action (0.02 mm). Hence, the reaction takes place within a limited space, leading to a localized response; thus making it ideal for application to localized sites without affecting distant cells or organs. Thus, the type II reaction is accepted as the major pathway in microbial cell damage.

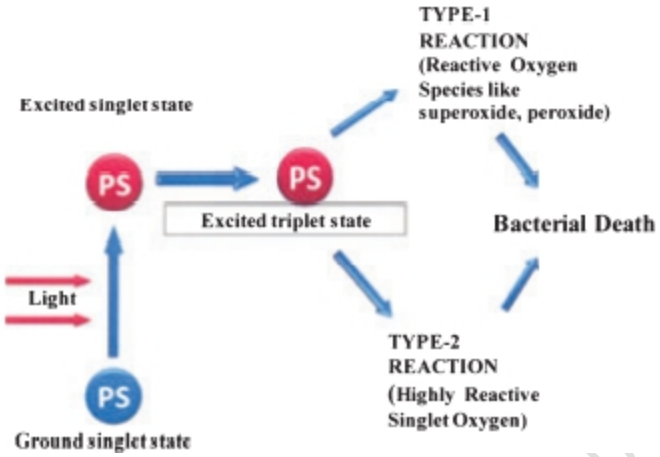

Figure 2: mechanism of PDT

PHOTOSENSITIZERS

An optimal photosensitizer must possess photo-physical, chemical, and biological characteristics.[7] Most of the sensitizers used for medical purposes belong to the following basic structure:

• **Tricyclic dyes with different meso-atoms**. E.g.: Acridine orange, proflavine, riboflavin, methylene blue, fluorescein, and erythrosine.

• **Tetrapyrroles.** E.g.: Porphyrins and derivatives, chlorophyll, phylloerythrin, and phthalocyanines

• **Furocoumarins**. E.g.: Psoralen and its methoxy derivatives, xanthotoxin, and bergaptene.

VARIOUS APPLICATIONS OF PHOTOACTIVATED DISINFECTION [8]

– PDT can be used in Non-surgical treatment of aggressive periodontitis

- Treating periodontal pockets.

- Plaque-infected cervical regions of teeth and implants.

- Disinfecting oral tissues prior to and during surgery.

- Treating oral candidiasis in immunocompromised patients.

- Guided bone regeneration (as an adjunct in minimizing any bacterial contamination) success enhanced following Photodynamic antimicrobial chemotherapy (PACT).

- Photodynamic therapy in implantology: Laser PDT can be used in implantology to promote osseointegration and to prevent peri- implantitis.

STUDIES ON USE OF PHOTODYNAMIC THERAPY FOR PERIODONTAL THERAPY [13]

Antimicrobial PDT can be considered as an adjunctive to conventional mechanical therapy. The liquid photosensitizer 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. As a result of the technical simplicity and the effective bacterial killing, the application of PDT in the treatment of periodontal diseases has been studied extensively.

A randomized controlled clinical study compared the effects of PDT alone without sub gingival SRP to sub gingival SRP in subject with aggressive periodontitis. At three months following the therapy, both treatment yielded comparable outcomes in terms of reduction of bleeding on probing and probing depth (PD), gains in clinical attachment level (CAL), thus suggesting a potential clinical benefits of PDT.

Christodoulides et al. evaluated the clinical and microbiologic effects of the adjunctive use of PDT to non - surgical periodontal treatment. Twenty four subjects with chronic periodontitis

were randomly treated with scaling and root planing followed by a single episode of PDT. The additional application of a single episode of PDT to scaling and root planing failed to result an additional improvement in terms of pocket depth reduction and clinical attachment level gain, but it resulted in a significant reduction in bleeding scores compared to scaling and root planing alone.

Bhatia et al. demonstrated that the optimal concentration of toluidine blue O to kill P. gingivalis was 12.5 µg/ml with helium-neon laser irradiations. This was caused by the disruption of outer membrane proteins of these bacteria. **Chan and Lai** showed that the presence of methylene blue at the wavelength of 632.8 nm (helium-neon laser) and 665 and 830 nm (diode laser) has a high bactericidal effect on periodontal pathogens.

In an in vitro study, **Hass et al**. examined the efficacy of PDT in killing bacteria associated with peri - implantitis which adhered to titanium plates with different surface characteristics. Scanning electron microscopic analysis showed that antimicrobial photodynamic therapy led to bacterial cell destruction without damaging the titanium surface. Similar antimicrobial results were obtained by **Shibli et al.** who reported that PDT could reduce the bacterial count of P. intermedia, P. nigrescens, Fusobacterium spp. in ligature induced peri - implantitis of dogs.

On interpreting the data from the various controlled clinical studies, it becomes obvious that in patients with chronic periodontitis, aggressive periodontitis and peri implantitis, the adjunctive use of PDT to scaling and root planing may result in greater clinical attachment level gains, reduction in bleeding on probing and probing pocket depths. PDT has advantage such as reducing the treatment time, no need for anesthesia, destruction of bacteria, inactivation of endotoxins, and unlikely development of resistance by the target bacteria and no damage to the adjacent host tissues.

ADVANTAGES

Benefits to be derived from the adjunctive use of PDT in providing treatment of conditions of a bacterial origin may be summarized as follows:

- Straightforward clinical technique. [9]

-Development of resistance to the PDT is less as singlet oxygen and other free reactive oxygen

species interact with several cell structures and different metabolic pathway.

- Non-surgical protocol required for the application of the photosensitizer. - Use as an adjunct to restorative/endodontic/surgery site pathogen reduction, PACT can disrupt plaque biofilm thus making it an adjunctive for use with ultrasonics and surfactant cleansers.

- Facilitates access into deep/limited access sites.

-Reduced need for surgery/direct flap approach. Patient comfort enhanced.

- Photodynamic therapy in implantology: Laser PDT can be used in implantology to promote osseointegration and to prevent peri- implantitis.

- There is no need to prescribe antibiotics, therefore the possibility of side effects is avoided.

- There is no need to anaesthetize the area and destruction of bacteria is achieved in a very short period (<60 seconds)

DRAWBACKS

The risk and side effects of antimicrobial PDT are basically classified into two categories.

1. Relates to the effect of light energy.

2. Relates to the photosensitizer and the photo chemical reaction.

In photodynamic therapy, Laser power employed is very low. But during treatment procedures, irradiation of the patient's eyes must be avoided by wearing protective glasses. Most of the dyes adhere strongly to the soft tissue surface of the pocket, and retention of the dyes in the pocket, even for a short period of time, may affect periodontal tissue attachment during wound healing or may show photosensitivity towards the day light causing irritation in the area of application for the patients. [10]

POTENTIAL RISKS OF USING PHOTODYNAMIC THERAPY

• Excessive tissue destruction by direct ablation and thermal side effects.

• Destruction of the attachment apparatus at the bottom of pockets

• Excessive ablation of root surface and gingival tissue within periodontal pockets.

• Thermal injury to the root surface, gingival tissue, dentin, pulp and bone tissue.

PRECAUTIONS BEFORE AND DURING IRRADIATION

• Use glasses for eye protection (patient, operator and assistant)

• Prevent inadvertent radiation (action in noncontact mode)

• Protect the patient's eyes, throat, and oral tissues outside the target site.

• Use of wet gauze packs to avoid reflection from shiny metal surfaces.

• Ensure adequate high- speed evacuation to capture the laser plume.

NEW FRONTIERS IN ORAL ANTIMICROBIAL PHOTODYNAMIC THERAPY

Since complex oral biofilms have limited susceptibility to antimicrobial photodynamic therapy the development of novel delivery and targeting approaches is essential. [11]

Recent innovations in the field of antimicrobial photodynamic therapy have been discussed below.

1. **Phototherapy**

In the oral black –pigmented species, the application of photosensitizer may not be required because photosensitizer occurs naturally in this species. Studies have shown that visible light ranging from 380 to 520 nm was able to achieve a threefold reduction in the growth of P. gingivalis, P. intermedia, P. nigrescens and P. melangencia in dental plaque samples obtained from human subjects diagnosed with chronic periodontitis. Inactivation of black-pigmented bacteria by visible light has also been reported by other investigators.

2. **Antibody-targeted antibacterial approaches using photodynamic therapy**

Antibodies conjugated with photosensitizer have been used to target staphylococcus aureus. Selective killing of P. gingivalis was achieved in the presence of streptococcus

sanguinis or in human gingival fibroblasts using a murine monoclonal antibody against P. gingivalis lipopolysaccharide conjugated with toluidine blue O. Recently gold nanoparticles were used as photo-thermal sensitizer which were conjugated to antibodies. During irradiation the energy absorbed by these particles during irradiation was quickly transferred into heat and accompanied bubble-formation phenomena around the clustered nanoparticles, leading to irreparable bacterial damage.

3. Nanoparticle –based antimicrobial photodynamic therapy

To overcome the incomplete penetration of methylene blue in oral biofilms has led to the development of new delivery systems that significantly improve the pharmacological characteristics of methylene blue. Researchers recently proposed the encapsulation of methylene blue within poly D,L-lactide-co-glycolide [PLGA] nanoparticle [150-200 nm in diameter] that may offer a novel design of nano-platform for enhanced drug delivery and photo destruction of oral biofilm. When the nanoparticles were incubated with cells, they showed a time-depended release of the PS, which then regained its phototoxicity and resulted in a activatable photodynamic therapy-nano agent. Nanoparticles were not internalized by microorganisms, but they were mainly concentrated on to their cell walls. This may have rendered the cell wall permeability to methylene blue released by the nanoparticles. This intracellular localization and the local surroundings of methylene blue influence the phototoxicity.

CONCLUSION

The periodontal disease remains a challenging clinical condition. Periodontitis is the chronic inflammation of supporting structures of teeth and is the major reason for tooth loss. The objective of periodontal therapy is to reduce the bacterial burden. Non-surgical therapy is considered as the gold standard technique of periodontal therapy. Phototherapy using low level lasers is called photodynamic therapy. Photodynamic therapy targets the specific pathogen without damaging the host tissue. This is effective for killing drug resistant pathogens and in treating multidrug resistant infection. [12]

This new strategy of using PDT is less traumatic and quicker but it is still in the experimental stage of development and testing. Development of new photosensitizers, more

efficient light delivery systems and further clinical studies are required to reinforce the beneficial effects of the PDT. The clinical applications of antimicrobial PDT have been slow but steady. Though limited clinical trials have been conducted for different diseases using PDT, its intensive use in periodontitis has given hope that it can be likewise used to clinically treat a number of other infectious diseases.

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