ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 02 | February - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

THE DYANAMICS OF LIGHT- THE PHOTODYANAMIC THERAPY. ARE WE FORGETTING IT!

Dentistry

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ABSTRACT

Plaque biofilms is the primary etiology for periodontitis. Mechanical debridement procedures to treat periodontitis still remain the gold standard but adjunctive antimicrobial therapy is also required in certain cases. However the use of antibiotics has their own disadvantages of creating resistant bacterial forms as well as systemic side effects. Photodynamic therapy (PDT) is a novel non-invasive therapeutic approach which overcomes these problems. Photodynamic therapy (PDT) or photochemotherapy has increased pathogen specificity as well as site specificity and hence no systemic effects. PDT involves the use of a photosensitiser which on exposure to light of specific wavelength, gets activated, to form toxic oxygen species which further cause a localised photodamage and cell death. As PDT is with no localised as well as systemic adverse effects, its application in periodontal disease treatment continues to evolve into a mature clinical treatment modality.

KEYWORDS

Inflammation; Photodynamic therapy; Photosensitizer; Periodontitis

INTRODUCTION

Biofilms are adherent to a solid surface in which bacteria grow and multiply forming the microcolonies. They are embedded in an extracellular polymeric matrix, which includes water and nutrient channels.(1) The most complex of all the biofilms in nature are the ones present on the tooth surfaces and epithelial cells lining the periodontal pocket/gingival sulcus (subgingival dental plaques). Enzymes, endotoxins and cytotoxic factors released from the bacteria present in the biofilms lead to tissue destruction and initiate chronic inflammation. Currently the mechanical scaling and root planing procedure to remove the periodontal pathogens and their endotoxins is the gold standard for treatment. Moreover, some periodontopathogens reside in soft tissues thus escaping their removal. Thus the adjuvant use of antibiotics is also known to be effective.(2, 3) However wide use of antimicrobial agents has led to the development of resistance mechanisms in periodontal pathogens.(4-6) Also in biofilms, up to 1500-fold higher drug levels may be necessary to have an efficacy comparable to conditions in culture broth.(7) So the inability to disrupt the bacteria in the biofilms and maintenance therapeutic concentrations of antibiotics in the oral cavity are some drawbacks for their use.(8)

Decontamination of periodontal pockets with the use of dental lasers is also being performed. Owing to high bactericidal properties of lasers it results in effective killing of oral pathogenic bacteria associated with peri–implantitis and periodontitis. However the use of lasers present two major drawbacks, firstly it can lead to irreversible thermal damage to the surrounding periodontal tissues and secondly it can lead to excessive ablation/thermal coagulation, carbonization/necrosis of root, gingival connective tissues, bone and pulp tissues, depending on the type of laser employed.(9)

The use of low level laser light to treat periodontal disease in a non invasive manner has been employed - the photodynamic therapy (PDT). PDT is pathogen and site specific, which makes it better than high energy laser therapy. In Photodynamic therapy reactive oxygen species lead to the eradication of the target cells. The dyes which are sensitive to light of suitable wavelength, bring about photolethal sensitisation, which is damage of the target cells by making them sensitive to the light of specific wavelength.(10)

The Photodyanamic Action

Since long time light alone or in combination with different suitable chemicals is used to get the therapeutic benefits eg. the use of ultraviolet radiations for treatment of skin tuberculosis or rickets.(11) After it was known that some dyes have fluorescence properties after absorbing light of suitable wavelength, it became clear that dye excitation by light exerts destructive action in the target biological systems. The photodynamic action is described as a mechanism in which light after being absorbed by the dyes, sensitizes target microorganisms and cells for visible light inducing cell damage. Application of light to the dye results in the production of free oxygen radicals which are cytotoxic and destroy cellular constituents.(12)

Evolution And Medical Application

PDT has been used in various medical applications for over a decade especially for treatment of some form of cancers, macular degeneration, and various dermatological applications.(13) Raab studied this reaction showing the killing of protozoa in the presence of acridine dye. The dye got activated when irradiated with light in the visible range of the spectrum.(14)The term photodynamic was coined by Jodlbaner and Von Tappeneir in 1904. They described oxygen dependant chemical reactions induced by photosensitisation, which could inactivate the bacteria.(15) Then technique was used successfully for treatment of different cancers and pre cancerous lesions. Further progress made in this field is connected with the work by Straub, Hausmann , Meyer-Betz.(16-18) Blum and later Spikes confined the definition of the photodynamic action to sensitized photoreactions in which molecular oxygen participates, i.e., dye-sensitized photo-oxidation. For the treatment of various types of cancers, it has been found that photosensitizers have a selective affinity for tumor or vascular tissue, after excitation by light they produce cytotoxic effects, which may lead to cell death or tissue destruction by necrosis or apoptosis.(19-21)

Mechanism Of Action

3 non – toxic ingredients are required for the photodyanamic action to take place (1) Visible harmless light (2) photosensitiser (3) oxygen.(10,22)

Photosensitiser

The dye used in photodyanamic therapy is referred to as photosensitizer because after absorbing the light, it sensitizes the microorganisms or target cells to visible light inducing damage. The light of suitable wavelength when applied to the dye, results in the production of free oxygen radicals. There are various chemicals that have been used as photosensitizing agents in PDT such as Phenothiazine dyes, Phthalocyanine dyes, Chlorines, Porphyrines etc.(23) Both gram positive and gram negative bacteria are responsible for periodontal disease and Methylene blue and Toluidine blue O dyes are very effective against both of them. They both can bind to the outer membrane of gram-negative bacteria and penetrate bacterial cells by having a cationic charge on photoactivation. Thus they have a selective killing mechanism for periodontopathic bacterial cells as compared to other cells.(24-27) Therefore, toluidine blue O and methylene blue have been the photosensitizers of choice in the treatment of periodontitis and peri-implantitis. Elimination of A. actinomyce-temcomitans, P. gingivalis and Fusobacterium nucleatum has been

demonstrated to be more effectively achieved whilst using toluidine blue O than methylene blue.(28) Tetracyclines, the commonly used antibiotics, also act as effective photosensitizers and can produce singlet oxygen.(29) They absorb light in the near UV, copper complexes of tetracyclines at ca. 400 nm.(30)

Based On Chemical Structure The Photosensitizers Can Be Classified As:

- Tricyclic dyes (methylene blue, toludine blue O, and acridine orange)
- Phthalocyanines (aluminum disulfonatedphthalocyanine and cationic Zn(II) phthalocyanine)
- Chlorines: Chlorine e6, stannous (IV) chlorine e6, chlorine e6-2.5 N-methyl-d-glucamine (BLC1010), polylysine and polyethyleneimine conjugates of chlorine e6
- Porphyrines: HematoporphyrinHCl, photofrin, and 5-aminolevulinic acid (5-ALA), benzoporphyrin derivative (BPD)
- Xanthenes: Erythrocine
- Monoterpene: Azulene. (10,21,31)

Photofrin and hematophyrin derivatives are referred to as first generation sensitizers. Second generation photosensitizers include 5–aminolevulinic acid (ALA), benzoporphyrin derivative, texaphyrin, and temoporfin (mTHPC). Third generation include Biologic conjugates (e.g. Antibody conjugate, liposome conjugate).(32)

Light Sources

A light source is required to activate the photosensitisers. Initially the laser systems used as light source were complex and expensive. To overcome this, diode laser systems were developed that are easy to handle, portable and cost effective. More recently visible light sources began to be employed. When irradiated with light of specific wavelength the photosensitiser undergoes transition to an excited singlet state from a low – energy ground state. After this either the photosensitizer may come back to its ground state or may go to a higher energy level: the triplet state. In this triplet state two different pathways can be followed to react with the target cells.(10,21)

The first pathway involves hydrogen atom abstraction or electron transfer reactions. It occurs between the excited state of the photosensitiser and an organic substrate molecule of the cells, which produces free radicals and radical ions. There is reaction between these highly reactive free radical species and endogenous molecular oxygen and the result of this interaction is the production of highly reactive oxygen species such as superoxides, hydroxyl radicals and hydrogen peroxide. These highly reactive oxygen species are harmful to the cell membrane integrity. The second pathway involves the production of singlet oxygen. It is a highly reactive state of oxygen which results due to the reaction of the triplet state photosensitiser with oxygen. This singlet oxygen has high chemical reactivity and thus can react with large number of biological substrates thus inducing oxidative damage and ultimately lethal effects upon the bacterial cell by damaging the cell membrane and cell wall.(21) Both the radius of action and the lifetime in biological systems for singlet oxygen is very short ie. 0.02 μm and less than 0.04 μs respectively.(10,15) These two properties make it ideal for application to local sites without affecting distant molecules, cells and organs. The bactericidal effect of singlet oxygen can be attributed either due to DNA damage or due to damage caused to cytoplasmic membrane of bacteria by cytotoxic oxygen species such as inactivation of membrane transport systems, and enzymes, alteration of cytoplasmic membrane proteins, disturbance of cell wall synthesis and loss of potassium ions from bacterial cells. (33)

Photodyanamic Therapy In Dentistry

Application of photodynamic therapy has led to significant advances in dentistry because the delivery of light is more accessible and topical application of the photosensitizer is more feasible in the oral cavity. The application of photodynamic therapy includes the treatment of different types of oral solid tumors, treatment of superficial precancerous oral lesions, such as oral leukoplakia, oral erythroleukoplakia and oral verrucous hyperplasia have been widely performed.(34-36) In addition to this, photodynamic therapy has been effectively applied in the treatment of lichen planus.(37,38) Some other potential applications for PDT are caries, viral diseases such as herpes, stomatitis , fungal infections by Candida species.(13, 39,40) Photodyanamic therapy is also used in the sterilization of endodontic root canals in patients who are being treated for necrotic pulp and periapical lesions.PDT can be combined with the usual mechanical debridement and chemical antimicrobials such as hypochlorite and hydrogen peroxide. Garcez et al. used this approach and found decrease in bacterial load during endodontic treatment.(41) Pinheiro et al. also found similar results using TBO + urea peroxide preparation and red light in addition to mechanical instrumentation to sterilize root canals with necrotic pulps.(42)

Application In Periodontitis

The two most common diseases of the oral cavity are the dental caries and periodontal diseases. Periodontitis can be effectively treated by changing the local environment of the oral cavity and thus suppressing the growth of periodontal pathogens. In the oral biofilms bacterial polysaccharides are present which are highly sensitive to singlet oxygen and thus the biofilms and the pathogenic bacteria are susceptible to photodamage. Damaging or breaking the biofilms inhibits plasmid exchange involved in transfer of antibiotic resistance and disrupts colonization.(43) Even in antibiotic resistant bacteria, PDT is very effective. Bacteria produce antioxidant enzymes which may protect them against some oxygen radicals, but not against singlet oxygen.(44)

There is reduced oxygen consumption and venous stagnation in the tissues during inflammation in periodontal tissues. This change in pH due to decrease in oxygen level may enhance the growth of anaerobic species. There is reduction in the venous congestion and improved tissue blood flow in the microcirculatory system in the gingival tissues due to the application of PDT. This also leads to increase oxygenation of gingival tissues which thus normalizing oxygen metabolism in periodontal tissues and improves the local environment.(45) The bacterial virulence factors play a major role in periodontal disease pathogenesis.

Lipopolysaccharide possesses a wide spectrum of immunological and endotoxin activities such as activation of macrophages, production of interleukin-1, release of prostaglandin E_2 etc. Lipopolysaccharide is also stimulates bone resorption in inflammatory periodontal diseases. Endotoxins present on root surface inhibit periodontal fibre reattachment on cementum. The virulence factors secreted by micro-organisms are inactivated by the effect of PDT. The activity of lipopolysaccharide and IL-1 secretion from human peripheral mononuclear cells in periodontitis patients were significantly reduced following exposure to low energy He-Ne laser and TBO photosensitizer.(46) Also there was a substantial, lightdose dependent decrease in the proteolytic activity of P. Gingivalis.(47)

Periowave™ is a photodynamic disinfection system developed by Ondine Biopharma Corporation. It utilizes low intensity lasers and wavelength-specific, light-activated compounds to specifically target and destroy microbial pathogens.(48) The photosensitizer is topically applied in the gingival sulcus and the laser is used to activate it and complete the disinfection. Due to the recent rise in antibiotic resistance throughout the world, there has been renewed interest in alternative antimicrobial therapies. PDT or photodynamic antimicrobial chemotherapy (PACT) presents us with an option for these drug resistant pathogens. Drug resistant bacteria can be effectively eliminated by PDT.(49) For bacterial inactivation, the activity of PDT is found to be more effective for Gram-positive as compared to Gram negative bacteria. This difference arises due to structural differences in the cell wall structure of these two bacteria. A relatively porous layer of peptidoglycan and lipotechoic acid surrounds the cytoplasmic membrane of Gram positive bacteria which allows the photosensitizer to be absorbed easily.(50) Gram negative bacteria has a cell envelope which has an outer membrane and an inner cytoplasmic membrane that are separated by the peptidoglycan containing periplasm. This structure forms an effective permeability barrier and restricts binding and penetration of many photosensitizers.(51) Several approaches have been tried and applied to overcome this permeability barrier in Gram negative bacteria. A permeabilizing agent such as polymyxin nonapeptide or EDTA in combination with photosensitizer makes the treatment of photodyanamic therapy effective against Gram negative bacteria.(52,53) Photosensitizers can be made to produce broad spectrum effects if imparted with positive charges. In this state they can inactivate all classes of microorganisms: Gram-positive, Gram-negative bacteria, fungi, viruses, parasites and also the highly resistant life forms such as bacterial spores and the cystic stage of Acanthamoeba. (13,39,40,54,55)

PDT In Peri-Implantitis

With the increased placement of dental implants to replace the missing

teeth, the implants affected by peri-implantitis are also increased in numbers. The pathogens responsible for the development of peri-implantitis are similar to those of periodontitis.(56-58) Complete eradication of the causative bacteria and also the disinfection and detoxification of the diseased implant surface, as well as of the periimplant pockets, are essential to achieve effective healing with regeneration of the lost bone around the peri-implantitis cases. Recently, several studies have demonstrated bactericidal and detoxification effects of high-level lasers on contaminated dental implant surfaces. High-level lasers have been used successfully in the surgical management of peri-implantitis. However, in nonsurgical therapy, high-level lasers have shown limited clinical efficacy. (59-63) The application of high-level laser therapy have shown surface alterations (such as melting and carbonization) on the treated titanium surface.(64,65) Based on its successful application in the treatment of periodontitis, antimicrobial photodynamic therapy is used as an adjunctive treatment for bacterial elimination in the treatment of peri-implantitis. The effectiveness of antimicrobial photodynamic therapy in treating contaminated implant surfaces was examined by Do¨rtbudak et al. and he concluded decrease in levels of A. actinomycetemcomitans, P. gingivalis and P. Intermedia after application of toluidine blue O and laser light in comparison with baseline levels.(66)

CONCLUSION

In the field of periodontics and implant dentistry, antimicrobial photodynamic chemotherapy seems to be an attractive option. Use of antimicrobial photodynamic therapy can avoid the systemic administration of antibiotics in the treatment of localized infections and also has the advantage of low-cost of the treatment. PDT allows the use of a high concentration of the photosesitizer at the locus of infection and results in efficient bacterial elimination without inducing side effects on the host tissue. In periodontal therapy, in addition to conventional mechanical treatment to treat moderate to severe periodontal pockets adjunctive antimicrobial photodynamic therapy can be employed. Also PDT can be used as supportive therapy of the remaining pockets during the maintenance period. Application of antimicrobial photodynamic therapy can also be done to treat peri-implantitis as an adjunct following debridement in surgical therapy.

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