Journal of Lasers in Medical Sciences

J Lasers Med Sci 2018 Summer;9(3):154-160

Review Article

http://journals.sbmu.ac.ir/jlms

doi 10.15171/jlms.2018.29

Antimicrobial Photodynamic Therapy: An Effective Alternative Approach to Control Bacterial Infections

Hassan Mahmoudi[1], Abbas Bahador[2], Maryam Pourhajibagher[3], Mohammad Yousef Alikhani[1*]

[1]Microbiology Department, Hamadan University of Medical Sciences, Hamadan, Iran
[2]Microbiology Department, Tehran University of Medical Sciences, Tehran, Iran
[3]Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

***Correspondence to**
Mohammad Yousef Alikhani, Department of Microbiology, Hamadan University of Medical Sciences, Hamadan, Iran.
Tel: +98-918-9539458;
Email: Alikhani43@yahoo.com

Published online 28 July 2018

Abstract

Introduction: The purpose of this review was to evaluate the available literature for in vitro and in vivo effectiveness of antimicrobial Photodynamic therapy (aPDT) in the field of bacteriology.

Methods: A review of the relevant articles carried out in PubMed and Scopus to determine the efficiency of aPDT used in the reduction of microbial infection. Thirty-one relevant documents retrieved from PubMed, Scopus by inserting "antimicrobial photodynamic therapy" and "bacterial infection" and "photodynamic therapy" keywords.

Results: According to different results, aPDT can be used as an adjuvant for the treatment of infectious diseases. The use of photosensitizer methylene blue, toluidine blue O (TBO), indocyanine green with light diode laser centered at (630±10 nm) and (650±10 nm) wavelengths have been shown to have significant results for the treatment of infectious diseases and bactericidal properties

Conclusion: These findings suggest that, aPDT can be an efficient method in the treatment of localized and superficial infections.

Keywords: Antimicrobial photodynamic therapy; Photosensitizer; Skin infections.

Introduction

Thebroad antibiotic resistance of hospital pathogens, inducing thigh index of human morbidity andmortality, as well as hospital costs.[1] The developed antibiotic resistant strains of bacteria to antibiotic therapy imply the demand for alternative treatments for infectious disease. One strategy that may lead to improved antimicrobial treatment is the application of antimicrobial photodynamic therapy (aPDT).[2] aPDT involves the use of a chemical photosensitizer or a nontoxic photoactivatable dye, visible light, and reactive oxygen. The therapy is based on the energy (absorbed as light via the intracellular photosensitizer) transferred to the oxygen molecules producing extremely reactive mediation, such as singlet oxygen and superoxide, that are noxious to the cells.[3,4]

History of Photodynamic Therapy

The origin of the light therapy found as an alternative treatment in medical profession and surgery from ancient to contemporary time. Phototherapy trace its root back to ancient Greece, Egypt and India, however not applied for centuries. Ultimately, it has been unearthed via the westerly society at the outset of the 20th century by a Danish physicist, Niels Finsen. He succeeded to prove the usage of photodynamic therapy by applying heat and light filtered through a carbon lamp for the treatment of cutaneous tubercles known as lupus vulgaris.[5] The idea of necrobiosis caused by action and reaction between light and chemicals were the earliest described by Raab in Munich. He found that chemical changes in the presence of a pigment called acridine light, inducing the death of a paramecium.[6] The next work that was done in the laboratory by Von Tappeiner, coined the term "Photodynamic action" was revealed that oxygen is essential for this operation. The next few years Thomas Dougherty et al at the Roswell Park Cancer Institute in clinical examined PDT. Photodynamic therapy was confirmed by the Food and Drug Administration in 1999 to treat pre-cancerous skin lesions in the head and face.[7]

The Mechanism of Action of Photodynamic Therapy

PDT comprise the application of visible light, combined with a photosensitizer (PS) and with the oxygen (Figure 1).[8] PDT is based on the interplay of visible light and a photosensitizer agent which under photo-activation generate short lived cytotoxic species in site. After stimulation, the photosensitizer is converted from singlet to triplet state by an intersystem crossing process which, in turn, reacts with surrounding molecules to produce radical species and hydrogen peroxide, or transfers its

Please cite this article as follows: Mahmoudi H, Bahador A, Pourhajibagher M, Alikhani MY. Antimicrobial photodynamic therapy: an effective alternative approach to control bacterial infections. *J Lasers Med Sci.* 2018;9(3):154-160. doi:10.15171/jlms.2018.29.

energy to molecular oxygen to manufacture singlet oxygen. Oxygen species that are capable of eliminating target cells by oxidative stress to cell membranes and other cellular parts.[9]

Photosensitizers

An optimally photosensitizers ought to have favorable structural features including physical, chemical, and biological. Many optical photosensitizers for photodynamic therapy have been tested in vitro as well as in vivo conditions. Optimal photosensitizers for aPDT of porphyrins derivatives, chlorine and phthalocyanine are porphycenes and phenothiazines' colors such as toluidine blue O (TBO), methylene blue (MB) and Azure is applied. Optimal photosensitizers' cationic tetrapyrrole contain quaternary groups based on foundations such as porphyrin and phthalocyanine, and spherical C60 molecules are produced. The mechanism of all optimal photosensitizers operation or polymer photosensitizers conjugate to light absorption or self-promoted uptake pathway, respectively.

In this process, at first the cationic molecules, Mg^{+2} and Ca^{+2} are displaced in outer membrane, and then followed by the light photosensitizer absorption via permeability of outer membrane, and finally of the cell membrane is destructed Table 1.[10-12]

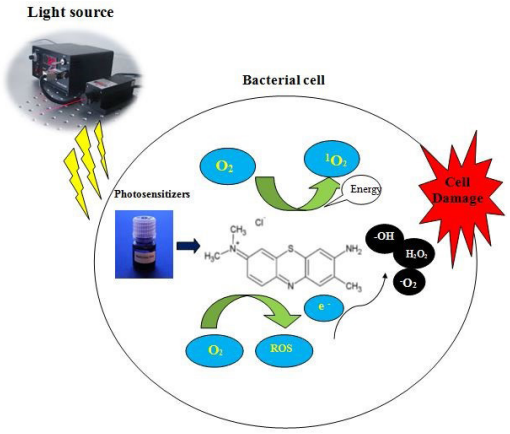
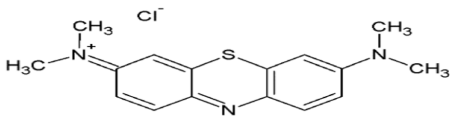
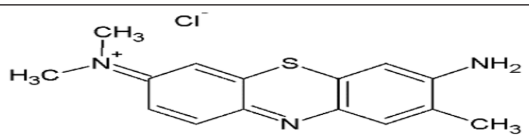
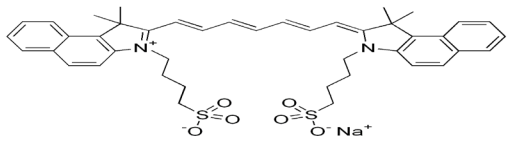
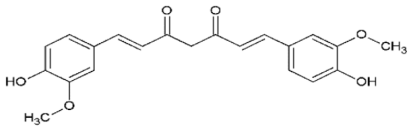
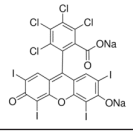
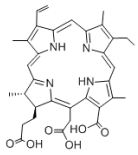


Figure 1. The Mchanism of Antibacterial Photodynamic Therapy. Photosensitizers can be preferentially uptaken by bacteria, accumulating inside the bacteria and in the cytoplasm membranes, or in the proximity. The photosensitizer in its ground singlet state is exposed to light of a appropriate wavelength and attract a photon. Then, the photosensitizer transferral energy from light to molecular oxygen to produce singlet oxygen and free radicals that are cytotoxic to cells.

Light Sources Used in Photodynamic Therapy

Photodynamic therapy requires a light source for triggering the photosensitizer exposures with a low power visible light at a particular wavelength. More the optical

Table 1. Photosensitizers Used in Antimicrobial Photodynamic Therapy

Photosensitizer	Chemical Structure	Referance
Methylene blue		(4, 13)
Toluidine blue		(4, 13)
Indocyanine Green		(14)
Curcumin		(15)
Rose bengal		(4, 13)
Chlorin(e6)		(16)

photosensitizers are actuated by red light in 630 nm and 700 nm wavelengths. Corresponding to a light influence depth of 0.5 cm to 1.5.[17,18]

Methods

A review of pertinent literatures was carried out in PubMed and Scopus to determine the efficiency of aPDT applied in the reduction of microbial infection. Sixty relevant documents were retrieved from PubMed, Scopus by inserting "Antimicrobial photodynamic therapy" and "Bacterial infection" and "photodynamic therapy" keywords.

Results

Application of Photodynamic Therapy In Vitro

For this review, the summary of the selected studies, the authors' name, the general description of the studies, and the results are presented. The effect of photodynamic inactivation in 14 clinical strains *Staphylococcus aureus* resistant to methicillin (MSRA) and 26 clinical strains *Staphylococcus aureus* susceptible to methicillin (MSSA) were evaluated by Kashef et al. The results showed that the toluidine blue- and MB photodynamic inactivation on MRSA strains to reduce ranging from1-1.3 $\log_{10}$ – 0.7-1 $\log_{10}$ CFU/mL, respectively. In the case of MSSA strains the achieved results for toluidine blue- and MB photodynamic inactivation included viable count reduction ranging approximately from 1.1 to 1.3 $\log_{10}$ – 0.7- 0.9 $\log_{10}$ CFU/mL, respectively.[19] In another study, Maisch et al investigated the impact of photodynamic therapy with photosensitizers (XF73, XF70,CTP1) on strains of *S. aureus* resistant to methicillin. The findings showed that concentrations (0.005 µM) of photosensitizers, using light (13.7 J/cm^2) for 10 minutes to reduce a 3$\log_{10}$ (>99.9%) of bacteria.[20] Grinholc et al evaluated the bactericidal effects of the photodynamic inactivation with a porphoporphyrin photosensitizer at 624 nm wavelength with an energy density of (0.2 J/cm^2) on 40 clinical isolates of methicillin-resistant *S. aureus* and 40 clinical isolates of methicillin-sensitive *S. aureus* isolated patients admitted to the hospital in Gdansk. The results of the study indicated the reduction of 3$\log_{10}$ in the number of bacteria.[21] the effect of aPDT with Radachlorin photosensitizer on *S. aureus* was studied by Fekrazad et al. The findings showed that the number of viable *S. aureus* was decreased to 6.28 $\log_{10}$ in a study group that was used for photosensitizers with laser light.[22] Kashef et al studied the photodynamic inactivation influence on *Escherichia coli* (ATCC25922) and clinical resistant strains of *E. coli* using photosensitizers of MB and toluidine blue O (TBO). MB (50 µg/mL) with a laser light of red (163.8 J/cm^2) capable of reducing 53.1% and 37.6% in the number of viable *E. coli* (ATCC25922) and drug resistant *E. coli* (the initial number of bacteria was 10^4-10^5 Cfu/mL). Moreover, TBO (50 µg/mL) and a laser dose of 46.68 J/cm^2 killed 98.2% and 83.2% of *E. coli* (ATCC25922) and drug-resistant *E. coli*.[23] In another study, Ragas et al studied the photodynamic efficiency on the plankton condition in *A. baumannii*. The results showed that the decreasing in the number of *A. baumannii* in plankton condition was 2-3$\log_{10}$ reported after photodynamic inactivation with 2 photosensitizers of TBO and MB.[24] Kashef et al assessed the efficacy of photodynamic therapy on *A. baumannii*. The findings of the study showed the reducing in logarithmic growth of live cells after photodynamic inactivation with MB and TBO (TBO) for 5 strains of *Acinetobacter baumannii* was between (1.3$\log_{10}$), (3.5–2.4$\log_{10}$), (2.9-2.2$\log_{10}$) and (2.6$\log_{10}$). Furthermore, photodynamic inactivation reduced the minimum inhibitory concentrations of growth inhibitors into Azithromycin, Imipenem, Ciprofloxacin and Gentamicin antibiotics.[25] Donnelly et al performed a photodynamic test of 2 photosensitizers of TBO and meso-Tetra(N-methyl-4-pyridyl) porphine tetra tosylate (TMP) at a concentration of 5 mg/mL on 5 strains of *Pseudomonas aeruginosa* isolated from cystic fibrosis, and the laser light killed 99.99% of bacteria.[26] Garcia et al investigated the effect of photodynamic therapy using a Hypericin sensitizer against biofilms formed of methicillin-resistant and sensitive staphylococci. The results of this study showed planktonic forms destruction in 10 minutes exposed to laser light.[27] The study by Miyabe et al evaluated the impact of photodynamic therapy using photosensitizers MB (3mM) and gallium-aluminium lasers at a wavelength of 660 nm red light with an energy density and time of 35 mW, 10 J, and 285 seconds, on *S. aureus*. The findings showed the number of bacteria (4.89-6.83 $\log_{10}$ CFU/mL) decreased in relation to the initial concentration of bacteria.[28] Grinholc et al investigated the effect of photodynamic therapy using a protoporphyrin 25 µM photosensitizer with laser radiation at a wavelength of 624 nm on the methicillin-resistant (MRSA) and sensitive strains (MSSA) of *S. aureus*. The results of this study indicated that there was a reduction of 0-3 $\log_{10}$ and 0.2-3 $\log_{10}$ strains of MRSA and MSSA in the number of bacteria, respectively.[21] Sharma et al assessed the effectiveness of aPDT on *S. aureus* biofilm formation. In this study, a TBO photosensitizer was used at concentrations of 10-80 µm and light source diode laser with a 640 nm wavelength. The results showed that at 40 µM concentration, biofilm was destroyed and has the least cytotoxic effect in cells.[29] Li et al investigated the effect of photodynamic therapy with 5-aminolevulinic acid (ALA) photosensitizer and a concentration of 40 mM and an optical source laser with 635 nm wavelength for activating ALA at doses (0, 100, 200, 300 J/cm^2) used. The results of this study showed that ALA without exposure to light or red light does not affect bacterial biofilm. However, a significant number of cells in the biofilm was inactivated during radiation with different doses of red light in the presence of 5-aminolevulinic acid, and at the dosage of 300 J/cm^2, all bacteria (99.99%) were killed.[30]

Khalil et al reported the effect of laser light on MRSA strains using TBO photosensitizer at a concentration of 50 μg/mL and a HeNe laser light with a wavelength of 632.8 nm in 1, 5, 10 minutes. The findings of the study indicated that 100% of the bacteria were killed in 15 minutes. Antibiotic resistance patterns of these strains were different before and after laser radiation, so that they were resistant to gentamicin 10 μg prior to the laser irradiation, and had an intermediate resistance to vancomycin. Moreover, after laser radiation, they became sensitive to these antibiotics.[31] Tang et al showed the effect of photodynamic therapy on clinical isolates of MRSA strains and *S. aureus* strain ATCC 25923 under the conditions of using TBO photosensitizer at concentrations (80 μM-400 μM) and Pl-ce6 at a concentration of (8 μM) and an optical source of laser 600 nm wavelength with doses (10-30 J/cm^2) for 30 minutes. The results of this study showed that Pl-ce6 and TBO at the concentration (8 μM, 30 J/cm^2- 80 μM, 30 J/cm^2) induced killing of MRSA (4log$_{10}$ and 3log$_{10}$) and *S. aureus* (ATCC 25923 (3log$_{10}$-2log$_{10}$)).[32] Pourhajibagher et al determined the efficacy of sub-lethal photodynamic therapy with the ability to form biofilm and the metabolic activity of *Enterococcus faecal* under in vitro conditions by using indocyanine green photosensitizer at concentrations (2 mg/mL) and TBO and MB at a concentration of 0.2 mg/mL with a diode laser as light source for TBO and MB and indocyanine green were 635 nm-200 mW, 660 nm-150 mW, 810 nm-200 mW, respectively. The findings showed that PDT-ICG and PDT-MB and TBO-PDT sub-lethal reduces 42.8%, 22.6%, and 19.5% of biofilms, the sub-lethal dose of PDT-ICG and PDT-MB and TBO PDT reduced 98%, 94%, 82% of metabolic activity, in *Enterococcus faecal*, respectively.[33] In another study, Pourhajibagher et al investigated the effect of sub-lethal photodynamic therapy on *Porphyromongus gingivalis* isolated from endodontic infections using diode laser light. The findings of this study showed that MB-sPAD (25 μg/ml-117.18 J/cm^2) and (50-100 μg/mL-117.18 J/cm^2) significantly inhibited the growth of *P. gingivalis* than the control group.[34] Boluki et al studied the effect of photodynamic therapy (TBO with 630 nm LED laser light) with colistin on *A. baumannii* pandrug resistant strains. The results of this study showed that the effect of TBO+LED significantly reduced the growth of bacterium. Although after calculating sublethal effects, the expressions of *Pmr A-Pmr B* genes were evaluated through real-time PCR method, it was indicated that rates were reduced in 6.1 to 4.1 fold.[35] The efficacy of photodynamic therapy on the formation of biofilm *P. gingivalis* was evaluated by Pourhajibagher et al using the inducianin green photosensitizer. The results showed that indosanine green at a concentration of 62.5-1000 μg/mL reduced a significant number of bacteria, which decreased compared to the control group (23.2%-93.7%).[36] Soukos et al investigated the effect of photodynamic therapy on *E. faecalis* in biofilm formed at root infections under the laboratory conditions. The findings of the study suggest that 97% of the bacteria were reduced when they were exposed to red laser light and MB at a concentration of (25 mg/mL).[37]

Application of Photodynamic Therapy In Vivo

Maisch et al investigated the intrusive and antibacterial effects of XF 73 (porphyrin cationic photosensitizer) on *S. aureus* (MRSA) in the pig's skin. They applied 2 methods for conducting the study. In the first method, they placed the bacterium in a XF73 solution, and then placed it on the pig's skin. In the second method, they placed the bacterium on the pig's skin, then placed the XF73 on the pig's skin for just 1 hour of dressing. Photoinactivation in the first method led to a 3-log$_{10}$ reduction in *S. aureus*, while in the second method *S. aureus* reduced by1-log$_{10}$.[38] Orenstein et al used a mixture of deuteroporphyrin-hemin, and successfully disinfected burns infected with *S. aureus*.[39] Fonseca et al contaminated single-root teeth with *E. faecalis* and incubated them for 48 hours at 35°C. Then the teeth placed in a TBO solution (0.0125%) for 5 minutes and for photodynamic therapy used 660 nm and 50 mW light irradiation. The reduction in the number of CFUs in the treated group was about 99.9, while in untreated group there was an increase of 2.6%.[40] Sivieri-Araujo et al in a survey examined the effectiveness of antimicrobial photodynamic upon tissue responses and cytokine production using a curcumin photosensitizer agent. The study process was conducted in which saline polyethylene tubes were introduced as control samples and sodium hypochlorite 5% and aPDT containing 500 mg/mL curcumin photosensitizer was put in the back of the connective tissue of the wistar rats. Seven, 15, 30, 60 and 90 days after planting, animals were exterminated and the tubes were removed with surrounding tissue. The results of the study indicated that the tissues reacted at days 7. Antimicrobial photodynamics increased the amount of IL-1B at all times (P <0.05). IL-6 levels were highest at a 90-day period with aPDT.[41] Jung et al investigated the effect of PDT on *Hemophilus influenza* in vivo, and *Streptococcus pneumoniae* in Meriones unguiculatus rats. Twenty microliters of the bacterial solution (10^6 CFU) injected into the bullae under sterile conditions. PDT was performed 2 days after infection by injection of 20 μL a Hematoporphyring (1 mg/mL) derivative photogeram inside the bulla and exposure to a 632 nm laser light with 90 J power. PDT was effective in eliminating *S. pneumonia* in 87%, whereas it was effective in killing of *H. influenza* in 50% of the infected bullae with otitis media with effusion.[42] In a preliminary clinical trial, 13 patients orally administered 20 mg/kg 5-aminolevulinic, and 45 minutes later, the antrum area of the stomach was irradiated by an endoscope with blue laser (50 J/cm^2-405 nm). Their results showed that in the samples that were exposed to radiation, the amount of *H. pylori* was lower, compared to the control areas.[43] Geralde et al using PDT treated

pneumonia caused by *S. pneumoniae*. Their hairless mice with *S. pneumoniae* were infected. After 2 days with antimicrobial photodynamics, infected mice were treated. In the control group, between 10^3-10^4 CFU/mL of bacteria in mice was grown. However, in the treatment group with antimicrobial photodynamic, bacteria did not grow in 80% of the infected animals. Based on the results, the animals after 50 days were re-examined. In the PDT group, no death was observed, but in the control group, 60% of the infected mice were died.[1] Ahangari et al compared the effects of antibacterial calcium hydroxide and photodynamic therapy on *E. faecalis* in teeth with periapical lesions under in vivo conditions. This study was performed on 20 patients, and did not succeed in treating endodontic. In this study, group 1 (n=10) was treated with antimicrobial photodynamic (880 nm diode laser with 50 mg/mL MB), while calcium hydroxide was used in group 2 for 1 week. Control samples were taken with sterile paper points and rotary F3 pro taper. The findings of this survey showed that the number of bacteria were declined in both groups after intervention ($P < 0.001$). However, there was no important difference in the number of colonies among the two groups.[44] The study by Wu et al evaluated the effect of photodynamic therapy via using chlorin e6 against *P. aeruginosa* keratitis in mice under in vivo conditions. With the scratch on the mouse corneal epithelial tissue, the mouse corneal was infected with *P. aeruginosa* (strain PA54). After 24 hours, the chlorine (e6) at concentration 0% (control group), 0.01%, 0.05%, 0.1% with red light compound (PDI) were used. After 2 days, the eyes of the mice were picked up for counting the bacteria. The results showed that the number of bacteria in the group treated with PDI significantly decreased.[45] de Almeida et al investigated the therapeutic impact of photodynamic therapy in none diabetic (n=120) and diabetic (n=120) rats with the experimental periodontal disease. Then, infected rats were divided into saline washing groups with saline solution, washing with TBO, laser radiation (660 nm, 24 J) and PDT (toluiden blue + laser radiation). After that, 10 animals from the experimental groups and the subgroups were evaluated in days 7, 15 and 30. The results of this study showed that less bone loss (0.33 ± 0.05 mm^2, 0.35 ±0.06 mm^2, 0.27 ± 0.07 mm^2) and SRP (1.11 ± 0.11 mm^2, 0.84 ± 0.12 mm^2, 0.97 ± 0.13 mm^2) was observed in days 7, 15, and 30, respectively ($P < 0.05$). In the TBO group bone loss was (0.51 ± 0.12 mm^2, 0.70 ± 0.13 mm^2, 0.64 ± 0.08 mm^2) in days 7, 15 and 30, respectively. In the laser group bone loss was (0.59 ± 0.03 mm^2, 0.61 ± 0.04 mm^2, 0.61 ± 0.03 mm^2) in days 7, 15, and 30 of evaluation. Finally, the group D, which was treated with PDT, showed less bone loss (0.27 ± 0.06 mm^2, 0.24 ± 0.02 mm^2, 0.29 ± 0.03 mm^2) in days 7, 15 and 30, respectively. Therefore it was much less than other groups. Findings of this study confirmed that, PDT can be utilized as a supplementary to periodontal treatment.[46]

Conclusion

The data obtained of this study demonstrated that bacteria were susceptible to photosensitizer (MB, TBO, indocyanine green) in the presence of light diode laser. This review suggests that the PDT may useful for treatment of infection diseases. PDT is a technique that has been shown to be effective in vivo and invitro against pathogen bacteria. Doubtless, aPDT has noticeable in the nearby prospect not only for the treatment of infections, but also in non-clinical utilization in different fields.

Ethical Considerations

This report does not include any investigations with human participants or animals conducted by any of the authors.

Funding

No funding was obtained.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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