

Antimicrobial Photodynamic Therapy: A New Therapeutic Option to Combat Infections

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Abstract

Minimization or elimination of antibiotic resistance in microbial species is an urgent need of the present times. Antimicrobial photodynamic therapy is a new therapeutic option through which development of resistance in microorganisms can be avoided. Interest in the use of photodynamic therapy to combat microbial infection is not only linked to the fact that this technique prevents antibiotic resistance in microorganisms, but is also due to the broad-spectrum antimicrobial effect, selectivity, with little or no local or systemic side effects, to microorganisms, ease of application of the technique, among other beneficial features. In this review, the major aspects related to the use of antimicrobial photodynamic therapy with respect to the major photosensitizing dyes and light sources used in the therapy have been discussed. In addition, current applications, wherein the use of the technique has yielded successful results, have also been discussed along with the possibility of future applications of this new therapeutic option to combat infections.

Keywords: Antimicrobial photodynamic therapy; Dyes; LED; Laser

Introduction

The incorrect prescription and/or over-prescription of antibiotics for the treatment of every disease can be partially attributed to the excessive demand for antibiotics in the last half-century, and, as expected, antibiotic-resistant strains of bacteria developed within a year of introduction of penicillin in clinical settings [1]. It is now well known that antibiotics must be used with caution to ensure that the chemotherapeutic advantages of antibiotics against microbial infections are retained. However, to maximize these advantages, other approaches to microbial disinfection must be adopted in combination with the conventional methods [2].

Antimicrobial photodynamic therapy (APDT) is an alternative therapy indicated for the treatment of microbial infections. APDT has now been in application for over 1000 years across Egypt, India, and China; however, only few researches had been conducted on it until the 1960s. Using microbial cells, Oskar Raab and Hermann Von Tappiener made the first demonstration over 100 years ago when they noticed that cells of *Paramecium* spp. stained with acridine orange were destroyed upon exposure to bright light. Since then, APDT has primarily been developed as a treatment for cancer, ophthalmological disorders, and for use in dermatology. However, in recent years, interest in the antimicrobial effects of APDT has been revived due to the rapid emergence of antibiotic resistance among pathogenic bacteria. Moreover, APDT has been proposed as a therapy for a large variety of localized microbial infections [3-7].

APDT provides significant advantages over the existing antimicrobial therapies. It appears to be equally effective at killing both multi-drug resistant microbes as well as native bacterial strains. Furthermore, the effect of APDT on microorganisms is much more

rapid as compared to that of other antimicrobial agents, and there is no evidence of APDT resistance until date [8].

Mechanism of Action

The APDT technique uses a non-toxic compound to cause cell death, a process called lethal photosensitization, in which microbial cells are pre-impregnated with a photosensitizer dye (PS) and subsequently exposed to a specific source of light [9-12]. After sensitization, the dye deposited on the target organisms transforms molecular oxygen into reactive oxygen species (ROS), which impart cytotoxic effect on microbial cells. During this process, the components of the PS, on exposure to a specific wavelength of light, acquire an excited state through the transition of electrons to a higher energy level. In this excited state, the PS can interact with molecular oxygen to initiate the formation of ROS (process type II) or interact with other molecules as an electron acceptor to produce hydroxyl radicals and other organic radicals (process type I) [13-17]. The products generated in these reactions can cause various damages to the components of the microbial cells or can alter their metabolic activities irreversibly, thus resulting in death [18]. In general, the principle of the therapy is that the energy absorbed via intracellular photosensitization is transferred to the oxygen molecule to cause severe damage to the oxidative reaction pathways [19] in the plasma membrane and to the genetic materials of the microbial cells, without exerting any toxic effects on the host cells themselves [16].

The basic principles of APDT are relatively simple and assure reliability and efficiency. This technique can be highly effective if it is ensured that, during the execution of phototherapy, all the necessary components, i.e., the dye, oxygen, and light, are present in sufficient amounts to cause damage to the target cells [20].

Light Sources

In health care and management, laser light therapy is increasingly being used in routine clinical practice, mainly because of the advancement in the laser application techniques and improvement in fiber-optic technology. The technological development requires a convergence of researchers from diverse fields, including optical physics, engineering, biochemistry, pharmacology, and the different sectors of health care [21].

The first light source used in APDT was the conventional bulb. However, these bulbs did not yield good results owing to the characteristic of polychromaticity, strong thermal component, and incoherency. Various wavelengths of light are used in order to excite the photosensitizer molecules, including the most commonly used source light amplification by stimulated emission of radiation (laser) and light emitting diode (LED) [9,20,22-25].

Laser is an exceptional source of radiation that is capable of producing extremely fine spectral bands, intense, coherent electromagnetic fields extending from the near infrared to ultraviolet [26], whose light color depends on its wavelength. Shorter wavelengths emit violet light of different variations until the displayed red light is in longer wavelengths. The emitted laser light is characterized by the ever-present electromagnetic waves with the same wavelength, direction, frequency, and color that differ from the conventional light with different wavelengths in all directions, which is a result of the combination of several spectra [27].

For the purpose of eliminating the microorganisms, a laser with low power or low intensity that typically operates with a power of ≤ 100 mV and that may produce energy in the visible spectrum (400–700-nm wavelength) either in the ultraviolet (200–400 nm) or the near infrared (700–1500 nm) regions should be used. The first low-intensity lasers used, as the active medium, a gas mixture of helium and neon (HeNe laser) emitted in the red spectrum (632.8 nm). Presently, a vast majority of lasers use a crystal of semiconductor diode of gallium arsenide (GaAs) produced in the laboratory, which can be doped with various other elements, depending on the desired intensity or the intended purpose [28-30].

LEDs are another alternative for the use of APDT. This device has a semiconductor active medium that emits visible light when energized; this light is formed when a certain voltage is applied between layers of semiconductor dopants [31]. Although not monochromatic like lasers, LEDs are capable of producing a high intensity of a broad-spectral band. In the LED, light wave can be produced in various lengths and in adequate power, although laser has a greater light penetration capacity into the tissue [32]. In addition to its relatively low cost and great versatility, LEDs can be arranged in various ways and in large quantity for irradiation of large areas [33].

Photosensitizers

The APDT technique requires the presence of a PS (preferably located on the target to be treated), a light source, and tissue molecular oxygen. Although several photosensitizing molecules are naturally present as components of cells and tissues, in APDT, these molecules must first be introduced on the treatment area to bind to the target cells. An ideal PS should have low toxicity to host cells when not activated by the light. It should have a pure composition, a stable shelf-life, be soluble in water or in a mixture of non-toxic aqueous solvents,

and produce a high amount of ROS in a short period of illumination time [20,24,25].

Not all chemical compounds are photosensitizers. Most photosensitive molecules have a heterocyclic ring similar to that present in the chlorophyll and hemoglobin molecules, justifying the fact that, when these molecules are exposed to light, the light energy is captured and subsequently transferred to other molecules, resulting in the release of ROS, which in turn interact with the biological systems and cause tissue damage. In addition, not every substance that can absorb light can act as a photosensitizer, as photosensitizers also need to have other important characteristics [34].

Photosensitizers are classified according to their chemical structures into different types as discussed below:

Porphyrins

Porphyrins are the first generation of PSs consisting of a mixture of monomers, dimers, and oligomers of hematoporphyrin derivatives. Among the various porphyrins, cationic porphyrins are most promising for use as sensitizers in APDT as they absorb light between the wavelengths of 610 and 630 nm [35]. A positively charged PS tends to move across to the outer membrane via a self-promoted uptake pathway by a mechanism involving the interaction between divalent cations of the compound with the adjacent bacterial lipopolysaccharide (LPS) [36].

Phthalocyanines

Phthalocyanines are macrocyclic molecules similar to porphyrin with a high structural flexibility; they are well-known photosensitizers. These compounds are more intensely absorbed in the near infrared region as compared to the original porphyrins due to their aromatic nature. Chloro-aluminum sulfonated phthalocyanine is a phthalocyanine derivative that has an increased aromatic character and therefore lower sensitivity and toxicity to the skin when exposed to ambient light; its higher photosensitivity leads to greater toxicity in the cells on exposure to red light. The advantage of phthalocyanines is the high production of ROS [37-39].

5-aminolevulinic acid (5-ALA)

5-ALA is a hydrophilic molecule that has limited ability to penetrate through cellular membranes and into the interstitial tissue space. Its application in APDT is based on the accumulation of endogenous protoporphyrin (PpIX), which is a heme precursor that, after topical application, accumulates in tissues with a rapid turnover or in tissues with high cellular activity. The synthesis of ALA is strictly controlled by the ALA synthase enzyme. When ALA is supplied to the cell, it accumulates PpIX and is converted to heme by ferrochelatase on addition of iron to the core of PpIX, presenting a photodynamic effect on exposure to light. The advantage of ALA-induced photosensitization is related to the rapid action of ALA, which limits the risk of photosensitivity [40,41].

Chlorins

Chlorins exhibit two important properties: high quantum yield of singlet oxygen and intense absorption band at longer wavelengths compared to those by porphyrins, where the biological tissues are most transparent to light (650–660 nm). The use of chlorins in APDT has

been shown to be effective and safe, with transient skin photosensitivity reported as the only side effect [34].

Xanthenes

Xanthenes are cyclic compounds with three aromatic rings in linear arrangement with an oxygen atom in the center, and they are responsible for absorbing light in the visible spectral region. These dyes do not bind to the cell membrane and are located in the cytoplasm. The following compounds belong to this group: rose Bengal, eosin Y, fluorescein, and erythrosin B [42]. Rose Bengal is a halide derivative of fluorescein that is used in ophthalmology as a dye for diagnosis of various diseases. When used in APDT, it can kill microorganisms such as viruses, bacteria, and protozoa. Exposure to light at 532 nm activates the type II reaction, generating products containing 80% singlet oxygen and 20% superoxide anions. An advantage of xanthenes over other photosensitizers is that xanthenes can be orally administered [16,34,42].

Phenothiazines

Phenothiazines have an absorption band between 600 and 800 nm, which is the most-used wavelength range in APDT. The disadvantage of phenothiazine dyes is their inherent toxicity, which reduces their therapeutic effectiveness. Examples of phenothiazine dyes include methylene blue (MB) and ortho-toluidine blue (TBO) [16,17,22,23,43-45].

MB is a well-known blue dye that was first used as a medicine against malaria. Its mechanism of action includes the interleaving of the MB cations in the structure of the nucleic acids caused by its positive charge. However, this chromophore is easily reduced in biological systems, which reduces its antibacterial activity. The blue color of an aqueous solution of MB is due to the fact that the phenothiazinium molecule absorbs visible light strongly in the 600–700 nm range, thus allowing the remainder of the visible spectrum (350–600 nm) to be transmitted [16,17,22,23,43-46]. In brief, its mechanism of action includes the breaking of the strands in the organism's nucleic acid structures [47].

TBO, a cationic phenothiazinium dye, has been widely studied for the inactivation of pathogenic microorganisms [48,49]. The maximum absorption wavelength is in the red light spectrum of 630 nm, which is capable of inactivating both gram-positive and gram-negative bacteria. This is mainly due to its physical and chemical properties and hydrophilic features that allow its free passage across the bacterial membrane and, consequently, attraction to the negatively charged potential of the mitochondria that allows direct action on this organelle [50-52].

It has been reported earlier that TBO binds to different structural components in gram-positive and gram-negative bacteria. In gram-positive bacteria, TBO binds to the teichuronic acid residues of the outer wall [4], whereas, in gram-negative bacteria, it predominantly binds to the LPS present in the outer cell envelope [53,54].

Triarylmethanes

Another substance that can be used as a photosensitizer is malachite green, a cationic dye of the triarylmethane family, including crystal violet. This dye shows strong absorption in the red region of the visible spectrum [55] and crosses the cell wall of gram-positive and gram-negative microorganisms [56].

New dyes

Currently, a new dye called curcumin is being extensively studied. Curcumin is a natural yellow pigment extracted from the rhizomes of *Curcuma longa* and presents a wide range of pharmacological effects including anti-inflammatory, anti-carcinogenic, and anti-infection effects [57,58]. Curcumin absorbs light in the blue wavelength range (300–500 nm) of the visible spectrum. Blue light has a lower penetration depth as compared with red light, due to scattering and absorption of the light by biomolecules; therefore, the use of curcumin in APDT is restricted to topical use for treating superficial wounds [59].

Several studies have reported the successful photokilling action of curcumin in APDT on activation with appropriate light sources [60,61]. In addition, affordable cost, ease of handling, and effectiveness are the major advantages of this PS. However, its solubility in water is highly limited, and it requires the use of oils and synthetic solvents to facilitate its dissolution in water [62,63].

Possible Applications

The most promising therapeutic possibilities employing APDT is the eradication of microorganisms in certain types of infections, especially in cases of localized superficial infections and those of known microorganisms as an alternative to the use of traditional antimicrobial agents in treatments [64-68].

The bactericidal action of this new therapeutic option has been evidenced in different microorganisms including *Porphyromonas gingivalis*, *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Haemophilus influenzae*, *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, *Fusobacterium nucleatum*, and *Streptococcus sanguis*, with superior results seen in black-pigmented bacteria owing to their natural chromophores [69,70].

Gram-positive bacteria are less affected than gram-negative bacteria, as the latter are significantly more resistant to several commonly used PS in APDT as well as to the action of singlet oxygen. This difference is due to the structure and components of the cellular envelope of gram-negative bacteria, which present a complex outer membrane with lipid bilayers serving as a physical and functional barrier between the cell and its environment. However, this membrane can form a chemical trap, since this layer is rich in fatty acids and proteins that can react with singlet oxygen. However, the LPS and outer membrane of gram-negative bacteria restrict the lethal effect of singlet oxygen as these cell components can be removed without causing cell death [54,71].

The interest in efficient fungicide treatments has been increasing recently due to the increase in the number of fungal pathogens implicated in the high occurrence of nosocomial infections or due to the increase in the opportunistic mycoses in immunocompromised patients. Unfortunately, insurance and fungi-specific agents are scarce, and most agents are only fungistatic, i.e., they do not cause death, but only inhibit cell multiplication. In addition, the routine use of antibiotics can lead to gradual emergence of resistant strains [72]. The main targets of antibiotics are pathogenic or potentially pathogenic fungi such as *Aspergillus fumigatus*, *Trichophyton rubrum*, and especially *C. albicans*, which are a common resident of the mouth, throat, digestive tract, and skin. In hosts with a compromised immune

system, *C. albicans* can become pathogenic; therefore, oropharyngeal candidiasis is one of the opportunistic infections that may affect acquired immunodeficiency syndrome (AIDS) patients [73]. The resistance of *C. albicans* against fluconazole, a commonly used antifungal agent for these patients, has emerged [74].

Recently, it was shown that *Helicobacter pylori* naturally accumulate sufficient porphyrin to allow photoinactivation by blue light. This pathogenic bacterium causes endemic gastroduodenal ulcerations in humans, and it is linked to the development of stomach cancer. The emergence of resistance to antibiotics in conventional therapy has been reported for *H. pylori*. In a preliminary clinical trial, when the gastric cells of 13 patients orally administered with ALA were exposed to a blue laser coupled to an endoscope, this microorganism was found to be successfully eradicated [3,34,75]. Some studies have revealed that APDT is effective in the treatment of localized viral infections. APDT has been shown to be particularly effective in the treatment of human papilloma virus (HPV) infections [76,77].

The human herpes simplex virus (HSV) is among the most common agents responsible for viral infections in humans and is often associated with serious clinical symptoms, especially in immunocompromised patients, pregnant women, and newborns. The emerging resistance of the virus to the conventional antiviral drugs and the side effects of these drugs are the main reasons for the search of effective and less aggressive novel therapeutic alternatives [78,79].

Human pathogenic parasites can also be killed by the combination of PS and light. *Plasmodium falciparum*, which is responsible for causing malaria, can be killed by APDT with a N-(4-butanol) pheophorbide derivative and silicon phthalocyanines such as Pc4 [80]. The parasite responsible for Chagas disease, *Trypanosoma cruzi*, can also be killed by using a combination of light and photosensitizers such as hematoporphyrin and phthalocyanine [81]. In addition, human helminth eggs in sewage can be inactivated by a meso-substituted cationic porphyrin and light [82].

It should be emphasized that this approach may not be amenable to the treatment of localized infection in few areas of the body. While the actual range of suitable presentations can be appreciated only through increased use and concomitant development of clinical APDT, it can be assumed that this technique could have positive outcomes via local application of both the photosensitizer and illumination through procedures such as endoscopy and use of fiber optics. While it has often been assumed that only topical (i.e., external) application is effective, the phototreatment against infections in the nares, paranasal sinuses, lungs, and gastrointestinal tract has also been reported [83,84].

Future Prospects

So far, there exists no evidence of resistance to APDT. Therefore, this technique is expected to become extremely important in the near future, that is, after the “antibiotic era” comes to an end [3,34]. Caminos e Durantini [13] reported another advantage of APDT-the PSs used can be made selective for a target microorganism by using a combination of a specific drug and low doses of light, and the same effect can be exerted on animal cells by using higher doses of the drug and different concentrations of the PS. The author reported that none of the PSs tested so far have resulted in mutagenic activity or development of drug-resistant strains.

With respect to the potential clinical use of APDT, Bisland et al. [85] reported that its versatility, optimized drug-light regimens, state-of-the-art light sources, and interstitial placement of optical fibers for light delivery might allow the development of a therapy superior to that offered by antibiotics. APDT is applied specifically at the site of infection, thus minimizing the risk of collateral damage to the “friendly” host flora, which can occur with the systemic use of antibiotics. The technique can also be easily customized in real time according to the specific stage and severity of the infection.

However, APDT technique has also been applied to several nonconventional purposes. Blood decontamination by APDT is currently being studied as an effective method of sterilization for the preparation of red blood cell concentrates. On the other hand, the photodynamic treatment of suspensions of red blood cells may result in undesirable hemolysis during extended periods of storage at 1–6°C [86,87].

The purification of contaminated water using solar radiation in the presence of PS and dissolved oxygen can lead to degradation of pesticides as well as the death of microorganisms. However, a limitation of this treatment system is the need to remove the PS dissolved in water after the treatment [88,89].

Antimicrobial treatment of food with APDT is being explored considering that the latest methods used for bacterial control in food are not always sufficiently efficient and entirely risk-free for humans and the environment. Non-thermal technologies can alter the structure of proteins and polysaccharides, causing changes in the texture, physical appearance, and functionality of the treated food. APDT has been proposed as a method of inactivation of microorganisms associated with food-borne illnesses; moreover, it is economical and environmentally friendly owing to the fact that it involves the use of non-toxic elements and induces complete destruction of the target microorganisms [90,91].

Other possible future application of APDT is in the production of anti-infective medical devices and surfaces [92-95] and pathogen inactivation in fish farming [96].

Conclusions

Based on the studies conducted so far, it can be inferred that APDT gives significant results in the elimination of microorganisms in localized infections in different diseases. However, considering the lack of consensus among researchers regarding the concentration of PS and the ideal light dose to be used for safe and effective clinical application of APDT, further studies are required to establish an optimal protocol for implementing this new therapy. Undoubtedly, APDT has great importance in the near future not only for the treatment of infections but also in non-clinical applications in diverse fields.

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