A New Strategy to Destroy Antibiotic Resistant Microorganisms: Antimicrobial Photodynamic Treatment

Tim Maisch*

Clinic and Polyclinic for Dermatology, Regensburg University Hospital, Germany

Abstract: Photodynamic activity of chemical compounds towards microorganisms was first published at the turn of 20th century and it is based on the concept that a chemical compound, known as the photosensitizer, is localized preferentially in the microorganism and subsequently activated by low doses of visible light of an appropriate wavelength to generate reactive oxygen species that are toxic to the target microorganisms. Processes, in which absorption of light by a photosensitizer induces chemical changes in another molecule, are defined as photosensitizing reactions. Since the middle of the last century, antibacterial photosensitizing reactions were forgotten because of the discovery and the beginning of the *Golden Age* of antibiotics. Certainly, in the last decades the worldwide rise in antibiotic resistance has driven research to the development of new anti-microbial strategies.

Different classes of molecules including phenothiazine, porphyrines, phthalocyanines, and fullerenes have demonstrated antimicrobial efficacy against a broad spectrum of antibiotic resistant microorganisms upon illumination. Due to their extended pi-conjugated system these molecules absorb visible light, have a high triplet quantum yield and can generate reactive oxygen species upon illumination. This mini-review will focus on some major advances regarding physical and chemical properties of photosensitizers and light sources that appear to be suitable in the field of antimicrobial photodynamic therapy. Currently, topical application of a photosensitizer on infected tissues and subsequent illumination seems to be the most promising feature of antimicrobial photodynamic therapy, thereby not harming the surrounding tissue or disturbing the residual bacteria-flora of the tissue.

INTRODUCTION

The discovery of penicillin was the beginning of the *Golden Age* of antibiotics. Penicillin made its clinical debut in the 1940s; virtually all strains of *S. aureus* were susceptible. The rapid isolation of further antibiotics, like streptomycin, chloramphenicol and tetracycline soon followed, and by the 1950's, these and several other antibiotics were clinically used. Resistance to penicillin by penicillinase producing bacteria was recognized almost immediately after the first test in patients in 1944 [1]. Already in the late 1950s, 50 percent of all *S. aureus* strains were resistant against penicillin. A few years later, methicillin was released in 1960 followed rapidly by the development of resistant strains of *S. aureus* in 1961. The coagulase-positive *S. aureus* as well as both coagulase-negative *S. epidermis* and *S. hemolyticus* exhibit the capacity of developing resistance to each new generation of licensed antibiotics. Due to the resistance to all beta-lactam antibiotics, vancomycin, a glycopeptid antibiotic, remained as last line of defense against Gram-positive bacteria. In 1996, the first clinical isolate of a methicillin-resistant *S. aureus* (MRSA) with reduced susceptibility against vancomycin (MIC = 8 μg/ml; vancomycin intermediate resistance type) was reported from Japan [2]. A few years later, even clinical infections caused by vancomycin-intermediate *S. aureus* (VISA) were confirmed in the United States [3, 4]. The first documented case of infection caused by vancomycin-resistent (VRSA) *S. aureus* (MIC ≥ 32 μg/ml) was reported in July 2002 [5]. Nowadays, successful antibiotic treatments are complicated noticeably, because of the existence of community associated methicillin resistant *S. aureus* (cMRSA) strains. In these strains, development of resistance is linked genetically to the existence of virulence factors. These factors can cause skin and soft tissue infections as well as severe necrotizing infections [6, 7]. Overall, resistance to antibiotics occurs typically as a result of drug inactivation/modification, target structure alteration, and reduced accumulation owing to a decreased permeability in the bacterial cell wall/membrane areas and/or increased active efflux of the antibacterial agents from the cell [8-10]. Therefore, the worldwide rise in antibiotic resistance has driven research to the development of new antibacterial strategies, like antibacterial photodynamic therapy.

The purpose of this review is to summarize the major advances regarding physical and chemical properties of photosensitizers and light sources that appear to be suitable in the field of antimicrobial photodynamic therapy.

MECHANISM OF ACTION

Antimicrobial photodynamic therapy (aPDT) is based on the concept that a non-toxic chemical compound (showing preferentially no dark toxicity, depending on the used concentrations), known as a photosensitizer (PS), should be preferentially localized in the pathogens and not in the surrounding tissue, and subsequently activated by visible light of the appropriate wavelength to generate reactive oxygen species (ROS) that are cytotoxic to the pathogens Fig. (**1**)

*Address correspondence to this author at the Antimicrobial PDT, Clinic and Polyclinic for Dermatology, Regensburg University Hospital, Germany, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany;
E-mail: tim.maisch@klinik.uni-regensburg.de

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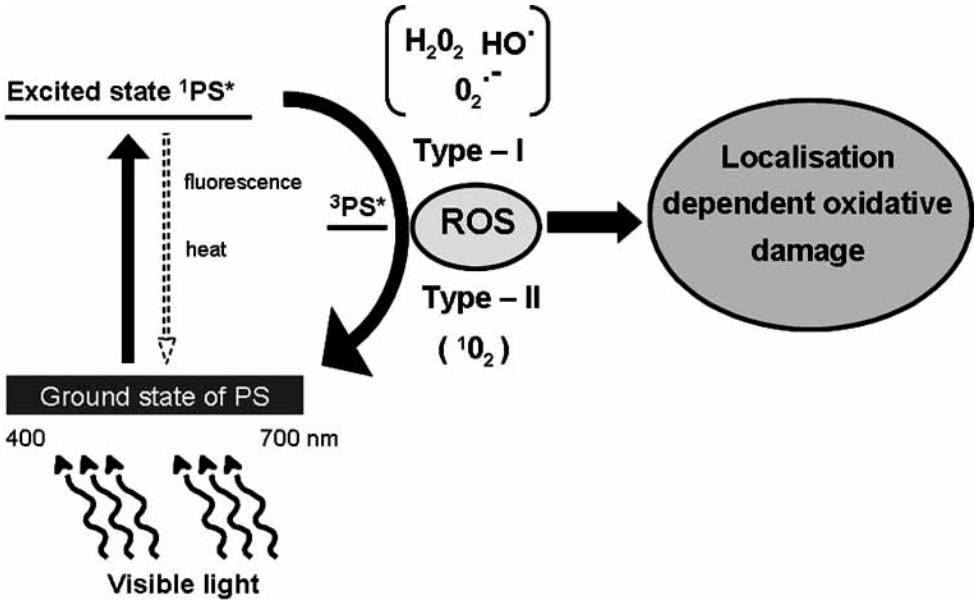


Fig. (1). Mechanism of action of aPDT.

Generation of reactive oxygen species (ROS) can follow two alternative pathways after light activating of a given photosensitizer. Upon absorption of a photon by the ground-state photosensitizer, the singlet excited state ^{1}PS* is formed. Excited ^{1}PS* state is short-lived and can undergo intersystem crossing to a long-lived triplet state, or alternatively can return to the ground state by fluorescence emission and/or heat. Generally the triplet state acts as a mediator of type-I / type-II photosensitization processes. Type-I: Generation of hydrogen peroxide (H_20_2), hydroxyl radical ($H0^{\bullet}$), and super oxide anion ($0_2^{\bullet-}$) by charge transfer from excited PS. Type-II: The triplet state of ^{3}PS*can undergo energy exchange directly with triplet ground-state oxygen, leading to the formation of singlet oxygen, $^1O_2$. The generated ROS react rapidly with their environment depending on the localisation of the excited PS: bacteria cell wall, lipid membranes, peptides, and nucleic acids.

[11, 12]. The mechanism of action is given in detail in some reviews published elsewhere [12-14]. Briefly, after light activating of the ground state of a given photosensitizer (PS), excited form of the ^{3}PS* (Triplet state) can follow two alternative kinds of pathways to generate reactive oxygen species (ROS) Fig. (**1**). A type I process involves the direct interaction of ^{3}PS* with the surrounding substrates to generate radicals or radical ions like hydroxyl radicals ($H0^{\bullet}$) and super oxide anions ($0_2^{\bullet-}$) *via* charge transfer. Whereas in a type II mechanism, generation of singlet oxygen by the excited ^{3}PS* usually takes place by direct energy transfer from the triplet state of the PS to molecular oxygen. This highly reactive singlet oxygen initiates further oxidized intermediates at the cell wall, cell membrane, on peptides and lipids depending on the localization of the photosensitizer. There is always a competition between the generation of radicals (type-I) and singlet oxygen (type-II) after exciting a photosensitizer. This is depends on the solvent [15], on monomeric or aggregated forms of the photosensitizer [16] or by the oxygen concentration [17]. It has been recently shown for exogenous photosensitizers that the quantum yield of singlet oxygen depends critically on the oxygen concentration (oxygen partial pressure) in the respective experimental setup [17, 18]. This is important when comparing experiments of *in vitro* (pO_2; 150 mmHg) and *in vivo* (e.g., skin: pO_2, 20 mmHg) conditions.

In summary, the photodynamic activity to induce cell damage or cell death is determined by six important photophysical/ photochemical properties including

(I) an overall lipophilicity of the photoreactive dye,

(II) positive charges

(III) the molecular extinction coefficient ε,

(IV) quantum yield of the triplet state formation Φ_T,

(V) redox potentials of the excited states of the PS^S_{red} or PS^T_{red}, if the reaction follows the type I mechanism or

(VI) the quantum yield of the singlet oxygen $^1O_2$ generation, if the reaction occurs by a type II photosensitization [19].

In contrast to UV radiation or γ-radiation, a damage of the genomic DNA or the plasmid-DNA seems not to be the primary mechanism for the bacterial cell death. *Deinococcus radiodurans*, which possesses a very efficient DNA repair mechanism can be destroyed very efficiently by the photodynamic reaction *via* singlet-oxygen [20], even though this bacterium is very tolerant to γ-radiation.

PHOTOSENSITIZERS

Different classes of molecules have demonstrated killing efficacy against a broad spectrum of multi-resistant Gram-positive and Gram-negative bacteria upon irradiation with visible light [21-24].

First of all the synthetic non-porphyrin compounds have shown photosensitizing ability, like the phenothiazine dyes: methylene blue and toluidine blue Fig. (**2**) [25, 26]. An improved phototoxicity was achieved after altering the structure of these phenothiazinium dyes against both the Gram-positive strains *S. aureus*, *B. cereus E. faecalis* and the Gram-negative strains *E. coli*, and *Ps. aeruginosa* [27, 28].

The presence of additional methyl/ethyl groups (Dimethyl methylene blue Fig. (**2B**) and New methylene blue Fig. (**2C**)), or of a nitro group (Methylene green, Fig. (**2D**)) yielded an enhanced phototoxicity compared to the parent compound methylene blue Fig. (**2A**). That means, up to 5 - 10 fold lower concentrations of Dimethyl methylene blue (DMMB) or New methylene blue are necessary to inhibit bacterial growth upon illumination compared to Methylene blue (MB) [27]. The increased efficacy in bacterial killing correlated with both the greater resistance to chemical reduction of these methylated derivatives and the increased lipophilicity. Resistance to chemical reduction is of important, because the respiratory chain of *E. coli* is located in the plasma membrane. Chemical reduction of these phenthiazinium dyes lead to the formation of colorless neutral species, which may explain the reduced non-photosensitizing activity. The lipophilic cationic dye DMMB has shown to bind to teichoic acid, which is abundant in the cell wall of Gram-positive bacteria [29]. The increased lipophilicity and the better binding to the cell wall areas may also explain the greater killing activity of DMMB than that of the more hydrophilic dye MB. In addition, the greater yield of singlet oxygen by 22% of DMMB *vs*. MB should lead to more efficient bacteria killing upon illumination [28]. Another phenothiazine dye is Toluidine Blue (TBO), which has demonstrated likewise antimicrobial activity predominantly against oral bacteria. A killing rate of >97% was achieved of oral bacteria growing in multi-species biofilms in the presence of TBO and red light [30]. O'Neill concluded that these results may be useful in the treatment of dental plaque-related diseases in the future.

Next macrocyclic molecules have shown high phototoxicity, like phthalocyanines and metal containing porphyrins as well as metal free porphyrines Fig. (**3**) [21, 23, 31-33]. However, anionic or neutral charged porphyhrin-based photosensitizers were found to bind efficiently to Gram-positive bacteria to induce growth inhibition or killing by visible light, whereas Gram-negative bacteria were not killed [34]. In this case, growth inhibition of Gram-negative *E. coli* by porphyrin-photosensitization was possible only in the presence of membrane disorganizing substances, e.g. the nonapeptide polymyxin or Tris-EDTA [32]. The addition of Tris-EDTA to Gram-negative bacteria removes divalent cations (*e.g.* Ca^{2+}, Mg^{2+} ions), which are present in large numbers at the outer membrane of bacteria to stabilize adjacent negative charged lipopolysaccharide molecules. Hence the onset of electrostatic repulsion promotes the release of up to 50% of the lipopolysaccharides into the medium, thereby allowing the penetration of molecules with molecular weights as high as 1.000–2.000 dalton to the inner cytoplasmic membrane or inner cellular compartments, which are the target areas to induce an irreversible damage [35] .

Photosensitizers like porphyrins with an overall cationic charge or meso-substituted cationic porphyrins as well as water soluble cationic zinc phthalocyanines can efficiently eradicate both Gram-negative bacteria and Gram-positive bacteria by photosensitization even in the absence of additives [21, 24, 31]. Caminos and Durantini used a set of porphyrins with three positive charges and a different pattern of meso-substitutions, which demonstrated a greater killing efficacy against *E. coli* upon irradiation as compared to a standard cationic porphyrin with four positive charges Fig. (**3A**) [36, 37]. The cationic groups of these photosensitizers were separated from the tetra-pyrol ring system by a propoxy spacer Fig. (**3B**). Thus, the charges have a high mobility and a minimal influence on the photophysical properties of the porphyrin. Therefore, the spacer (an alkyl-chain) provides a

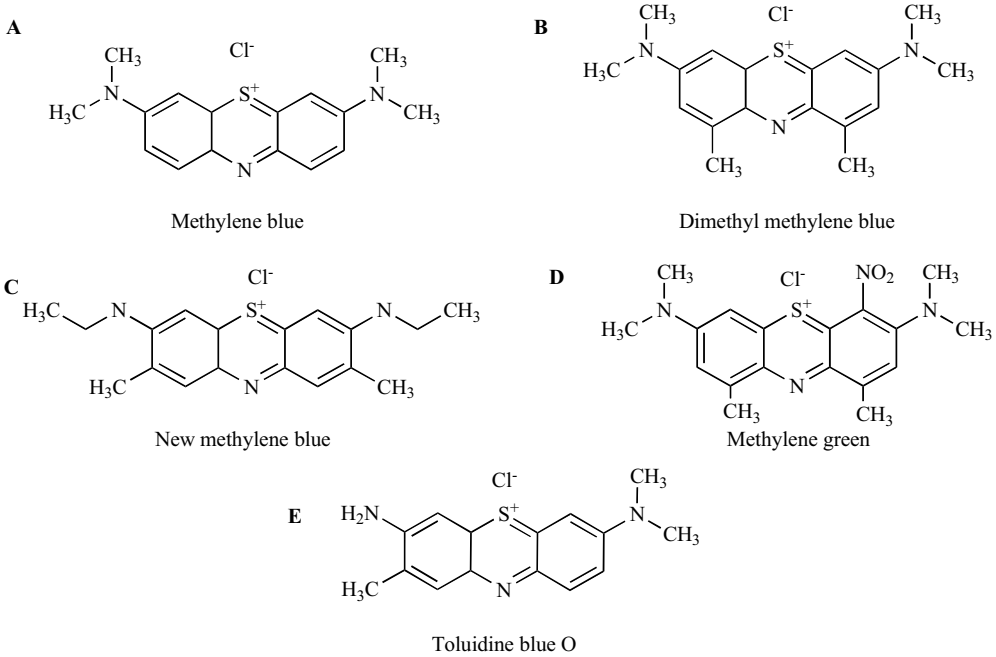


Fig. (2). Chemical structure of phenothiazine dyes.

A) Methylene Blue [12], **B**) Dimethyl methylene blue [27]; **C**) New methylene blue [27]; **D**) Methylene Green [27]; **E**) Toluidine Blue [75].

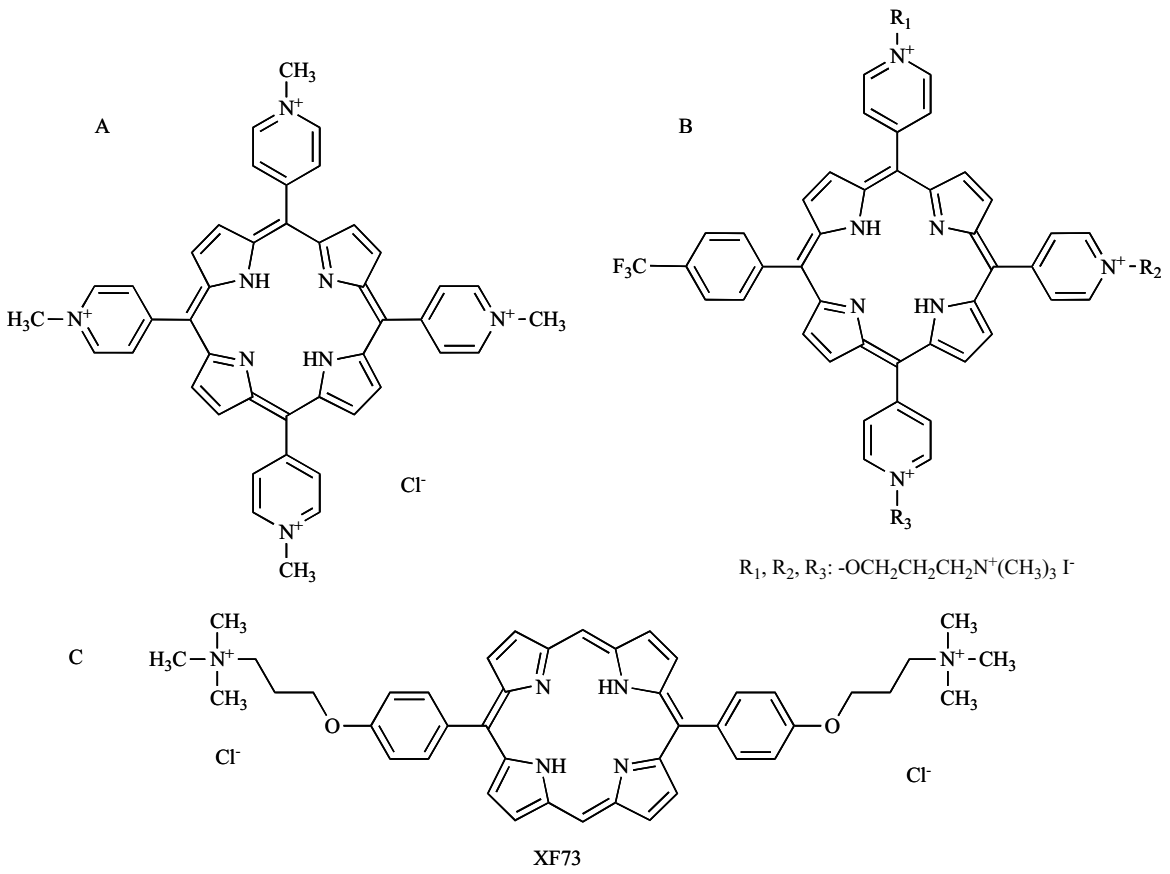


Fig. (3). Chemical structure of macrocyclic molecules.

A) 5,10,15,20-tetrakis-(4-*N*-methylpyridyl)-porphine (T_4MPyP), which is known as a standard photosensitizer to eradicate bacteria [24, 76]. **B**) 5,10,15-tris[4-(3-*N*,*N*,*N*-trimethylammoniumpropoxy)phenyl]-20-(4-tri-fluoro-methyl-phenyl) porphyrin iodide containing a "spacer" [-$(CH_2)_3$] to separate the positive charges from the tetrapyrrol-ringsystem [33], **C**) XF73 [31].

high mobility of the charge, which could facilitate the interaction with the outer membrane of the Gram-negative *E. coli*. Recently, Maisch *et al.* have shown that irradiation of methicillin-resistant *S. aureus* and methicillin-resistant *S. epidermidis* strains incubated with a novel porphyrin-based PS yielded a ≥ 99.9% decrease of viable bacteria numbers *via* reactive oxygen species without harming eukaryotic cells *in vitro* Fig. (**3C**) [31]. This dye contains two positive charges being opposite without a long alky-chain.

A new class of molecules, which can act as photosensitizers, is called "fullerenes" Fig. (**4**). Fullerenes are soccer ball-shaped all-carbon molecules and are composed of 60 carbon atoms [38]. Some biological properties have been reported for various fullerenes so far, e.g. enzyme inhibition, antiviral activity, and DNA cleavage [39, 40]. In addition, cationic fullerenes with one, two, or three pyrrolidinium groups have demonstrated a bacterial kill of more than 99.99%, after a short incubation time (10 min) followed by illumination (up to 16 J/cm^2, 200 mW/cm^2) with visible light (400-700 nm) Fig. (**4**) [41]. However, these fullerenes have performed a significantly better phototoxicity than the widely used antibacterial photosensitizer, toluidine blue (TBO). Tegos *et al.* showed that TBO produced less than 1 $\log_{10}$ reduction of bacterial killing at the same photosensitizer concentration (1–10 μM) and irradiation dose, which was used for the above indicated fullerenes [41].

Another group of dyes belongs to furanocoumarins (e.g. psoralen), a class of organic chemical compounds produced by a variety of plants. Psoralen occurs naturally in the seeds of *Psoralea corylifolia,* which originally acts in plants as a chemical defense substance against microbial or eukaryotic pathogens Fig. (**5**). In fungi, furanocoumarins normally facilitate the parasitization of plants [42]. The use of psoralens differs from the other photosensitizers discussed above in fact that psoralens intercalate in DNA rather than in sites like cytoplasma-membrane or cell wall areas of bacteria and also that these dyes predominantly absorb UV light (normally UVA, 320–400 nm) and not visible light, which is used for aPDT [43]. In terms of collateral damage of pathogens at the infection sites, there is, of course, a higher degree of risk to surrounding healthy cells or tissue (particularly by nucleic acid photodamage) upon exposure to UV light. It is questionable if this can be emended using longer wavelengths of UVA (380 – 400nm). However, typical disinfection conditions, e.g. for aminomethyltrimethylpsoralen, are 50 μg/mL of agent and a UVA dose of 38 J/cm^2 [44].

Generally, the observed resistance of Gram-negative bacteria against efficient killing by aPDT is due to the different

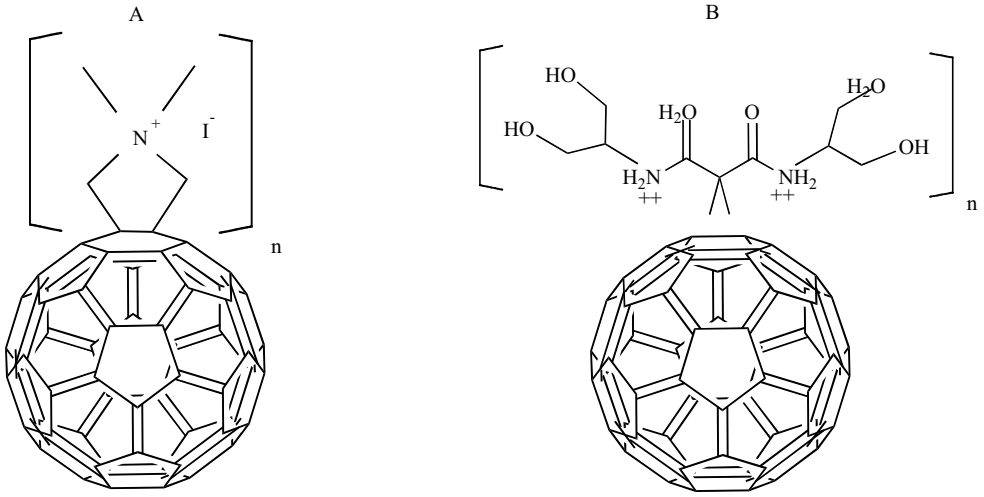


Fig. (4). Chemical structure of a cationic fullerene.

A) C_{60} fullerene with one, two, or three quarternary pyrrolidinium groups (n = 1-3); **B**) C_{60} fullerene with one, two, or three polar diserinol groups (n=1-3) [41].

outer membrane structure of Gram-positive and Gram-negative bacteria, which is discussed in details elsewhere [14].

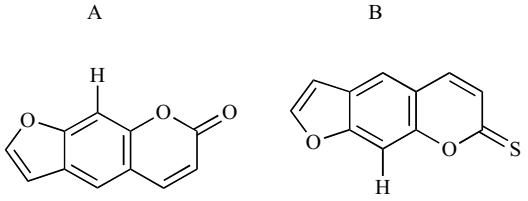


Fig. (5). Chemical structure of naturally occurring photosensitizers.

A) Psoralen, B) 2-thiofuranocoumarin.

OPTIMISATION OF PHOTOSENSITIZER UPTAKE BY BACTERIA

However, the specific uptake mechanism of photosensitizer by bacteria is not yet fully understood. One important step for a successful inactivation is a sufficient binding of the photosensitizer to bacteria, especially for Gram-negative bacteria. Therefore, a positive charge of a given photosensitizer appears to promote a tight electrostatic interaction with the negatively charged sites of lipopolysaccharides at the outer surface of Gram-negative bacteria. It is known that meso-substituted cationic porphyrines can efficiently inactivate bacteria independently of the number of one, two, three or four positive charges [21]. That means, that other factors must be prevalent than the number of positive charges. Recently, Reddi and colleagues demonstrated that alky-chain derivatives of 5,10,15,20-tetrakis-(4-*N*-methylpyridyl)-porphine (T_4MPyP) enhanced the efficiency of accumulation by both Gram-positive and Gram-negative bacteria Fig. (**3A**) and Fig. (**7**) [24]. Increasing the length of the *N*-alky substituent from one to 14 carbon atoms promotes better binding to bacteria. However, a further elongation of the alky-chain to C18 and C_{22} did not further increase the amount of bacteria bound to T_4MPyP. In this study, the amount of T_4PmPy-$CH_2(Ch_2)_8CH_3$ (C10) or T_4PmPy-$CH_2(Ch_2)_{12}CH_3$ (C14) molecules tightly associated to bacteria was ~ 50 times higher that obtained in the case of T_4MPyP Fig. (**7**) [24]. Growth inhibition of *S. aureus* and *E. coli* was in accordance with the results of the binding study. Increasing the length of the alky-chain up to C14 caused a complete growth inhibition upon irradiation.

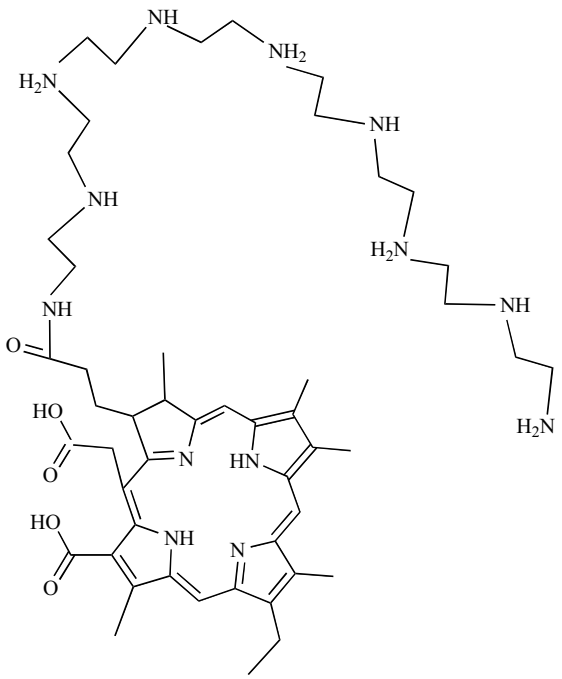
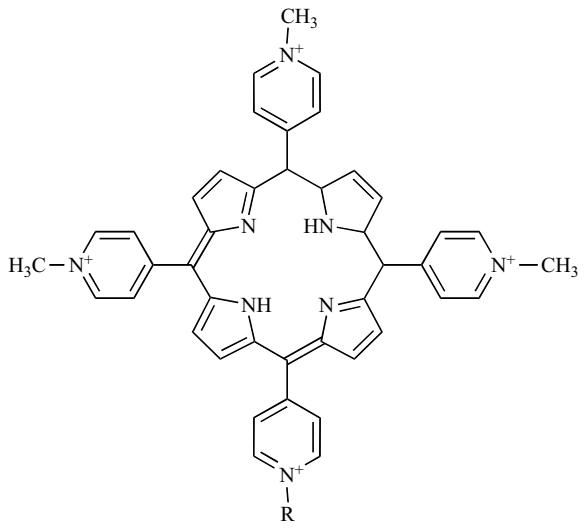


Fig. (6). Chemical structure of a protease-stable polycationic photosensitizer.

Representation of the chemical structure of a novel second-generation polycationic conjugate between chlorine(e6) and a linear form of polyethylenimine [48].This molecule does not contain any peptide bonds and therefore should be resistant to protease degradation.



R = $-CH_2(CH_2)_4CH_3$ N-alkyl-chain C_6

$-CH_2(CH_2)_8CH_3$ N-alkyl-chain C_{10}

$-CH_2(CH_2)_{12}CH_3$ N-alkyl-chain C_{14}

$-CH_2(CH_2)_{16}CH_3$ N-alkyl-chain C_{18}

$-CH_2(CH_2)_{20}CH_3$ N-alkyl-chain C_{22}

Fig. (7). Chemical structure of T_4MPyP n-alky chain derivatives.

5,10,15,20-tetrakis-(4-*N*-methylpyridyl)-porphine derivatives containing one *N*-alky substituent (R4) with increasing number of carbon atoms (C6, C10, C14, C18 and C20 [24]. Chemical structure of T_4MPyP is shown in figure 3A.

aPDT *IN VIVO* STUDIES – ANIMAL MODELS

There have been encouraging reports of the use of aPDT to treat infections in selected animal models [45-47]. Hamblin and colleagues showed that following topical application of a chlorine(e6) photosensitizer conjugated with poly-L-lysine, the Gram-negative strain *E. coli* was rapidly killed upon exposure to 660 nm laser light, using a mouse model [46]. In a subsequent report Hamblin and colleagues showed that protease-stable polycationic polyethyleneimine photosensitizer conjugates may be superior to polylysine-PS conjugates for photodynamic therapy of localized infections Fig. (**6**) [48]. These Polyethyleneimine conjugates were able to kill a panel of Gram-positive and Gram-negative bacteria and the yeast *candida albicans* after exposure to low levels (up to 16 J/cm^2) of red light (665 nm, 50 mW/cm^2). The advantage of these second generation polycationic conjugates is their resistance to degradation by proteases, such as trypsin, that hydrolyze lysine-lysine peptide bonds, because this molecule does not contain any peptide bonds, and therefore, should be resistant to protease degradation by the bacteria or eukaryotic cells [48]. Furthermore, oral candidiasis was successfully treated with Methylene Blue mediated photodynamic therapy using an immune-suppressed murine model, mimicking what is found in human patients [47].

Local administration of both PS and light is relatively straightforward e.g. in the oral cavity. Bacteria that grow in biofilms (*Porphyromonas gingivalis*, *Streptococcus mutans*), implicated in diseases such as periodontitis, have been shown to be susceptible to aPDT with PS, such as Methylene Blue, Toluidine Blue and phthalocyanine [49-51]. However, the results of these studies highlight the phenomenon that phototoxicity efficacy against oral bacteria is depending on the age of the biofilms. "Young" biofilms are less susceptible than "older" biofilms [52, 53]. Younger biofilms (a few days old) are more metabolically active and showed differences in extracellular matrix composition as compared to 9 days old biofilms [54, 55]. Further work is needed to study this phenomenon, because an oppositional study has reported that "younger" biofilms of *S. mutans* are more susceptible to aPDT using toluidine blue as the given PS [56].

There have been only a few clinical trials or animal models of oral infections investigating the efficacy of antimicrobial photodynamic inactivation *in vivo* (summary in:[57]). A preliminary randomized controlled clinical study on antimicrobial photodynamic therapy in non-surgical treatment of aggressive periodontitis have shown that photodynamic treatment *vs*. scaling and root planning with hand instruments showed similar clinical results regarding plaque index, gingival index, probing depth, bleeding on probing, gingival recession and relative clinical attachment levels at a 3 months follow up by ten patients [58]. There were no statistically significant differences in any of the clinical parameters investigated, thus indicating similar results of the two treatments in the non-surgical treatment of aggressive periodontitis.

In another study, it was suggested that aPDT may still bear some possible benefits to treat localized brain abscesses [59]. In this trial, the abscesses were drained, and a hematoporphyrin solution was injected in the cavity for 5 min. The areas were illuminated with an argon laser. Satisfactory sterilization of the cavity was obtained, as verified by appropriate culture techniques.

On the other side, photodynamic treatment of acne vulgaris with topical 5-aminolaevulinic acid showed an apparent improvement of facial appearance, a reduction in the development of new acne lesions and antibacterial effects [60-62]. It shouldn't be forgotten here that 5-Ala must be metabolized to the active photosensitizer Protoporphyrin IX in bacteria, which is a time consuming process. Overall PDT could be beneficial in the treatment of acne not only by cytotoxic/modulating effects of the skin but perhaps photodynamic treatment also has antibacterial effects against *Propionibacterium ssp.*

NOVEL TARGETING SYSTEM FOR LETHAL PHOTOSENSITIZATION OF BACTERIA

The challenge in antimicrobial PDT is to find a therapeutic window, in which bacteria are efficiently killed (> 99.99% reduction of viable bacteria) without harming the surrounding tissue at a given concentration and light dose. A new approach to enhance selectivity of aPDT is based on the concept to link the photosensitizer dye to an antibody against the surface of the target organism. Embleton *et al*. reported a lethal photosensitization of methicillin-resistant *S. aureus* using an immunoglobulin G-tin(IV)chlorine e6 conjugate as the respective photosensitizer [63]. Many isotypes of immunoglobulin G bind through the Fc region to Protein A, which is expressed and localized as a typical cell wall protein by

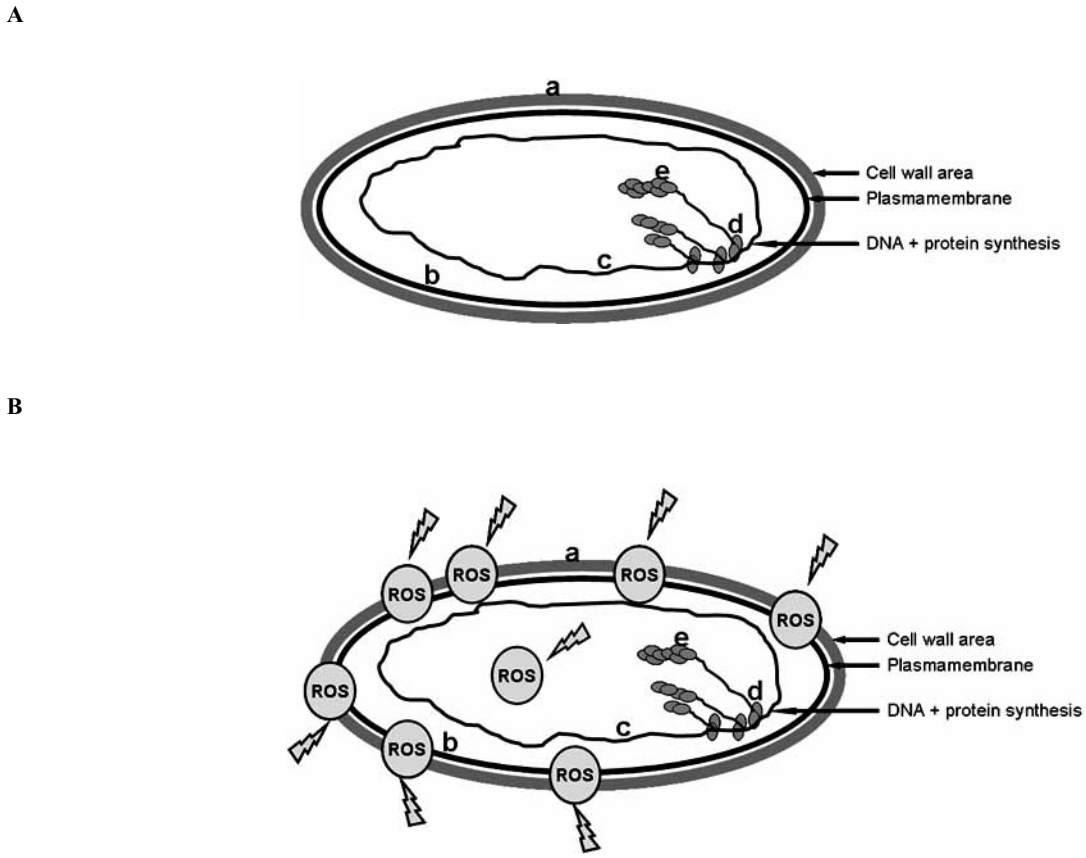


Fig. (8). Attack points of antibiotics and antibacterial PDT on bacteria.

Mechanism of action of a given antibiotic is specific. **A**) Each individual antibiotic effect at the bacterium on specific sites: (**a**) outer cell wall areas/synthesis, (**b**) cytoplasm membrane, (**c**) DNA replication, (**d**) transcription and (**e**) translation of proteins. **B**) Photodestructive oxidation of bacteria components by ROS is unspecific during illumination, depending on the attachment or uptake of the PS only.

many MRSA strains. The amount of Protein A embedded in the cell wall areas can vary among these strains. A close relationship between Protein A amount and killing efficacy was observed in the use of the immunoglobulin G-tin(IV)chlorin e6 conjugate [64]. Furthermore, Berthiaume *et al*. evaluated the efficacy of antibody-targeted photolysis to kill P*seudomonas aeruginosa in vivo* using photosensitizer immunoconjugates [45]. Infected dorsal skin areas of mice with bacteria were injected with conjugates of tin(IV) chlorine e6-monoclonal antibodies and illuminated with 160 J/cm^2 at a wavelength of 630 nm. Irradiation resulted in a greater than 75% decrease in the number of viable bacteria at sites treated with the specific conjugate, whereas normal bacterial growth was observed in animals that were untreated or treated with a nonspecific conjugate.

Another possible approach of delivering a photosensitizer to *S. aureus* is to use a bacteriophage. Bacteriophages are viruses that infect bacteria specifically. A tin (IV) chlorine e6 photosensitizer was conjugated to bacteriophages using a zero-length crosslinker [65]. In this study, it was clearly demonstrated that bacteriophages can be used to deliver a photosensitizer to a target organism, resulting in enhanced and selective killing of multi-resistant bacteria. However, the use of a bacteriophage to deliver a photosensitizer to bacteria could have two important benefits. First a more selective killing, since binding of the photosensitizer-phage conjugate to bacteria would help to reduce collateral damage to host cells and the residual bacteria flora at the infectious target area. Secondly, a more effective killing, since the reactive oxygen species would be acting directly at the bacterial cell wall to induce irreversible damage, which is produced during illumination.

APPROPRIATE LIGHT WAVELENGTHS FOR aPDT

A sufficient light intensity must achieve the photosensitizer-loaded pathogens in infected tissue, like skin, to generate ROS. In general, light intensity decreases with the penetration depth through various skin layers due to combined effects of scattering and absorption. The skin is irregularly shaped, inhomogeneous, multilayered, and contains hair follicles and glands [66]. The penetration of light into most biological tissues increases upon increasing the wavelength, at least in the 400-700 nm range. Therefore, a compromise must be found regarding the penetration depth of light and the absorption spectrum of the sensitizer used as well as the localization sites of the pathogens. With respect to phenothiazines (methylene blue, toluidine blue) or porphyrins there is a still effective absorption of light for wavelengths above 600 nm. In addition, shorter wavelengths in the area of 400 nm (soret-band) are also suitable to excite photosensitizers,

particularly in those situations in which red light activation is not important for the light to penetrate deep into tissue (e.g. surface disinfection). At the moment, different laser systems and incoherent light sources are used in PDT, which is discussed elsewhere [67].

DISCUSSION

Whether bacteria could develop resistance to reactive oxygen species, e.g. singlet oxygen is questionable. Up to now, there exists no report concerning a potential specific resistance mechanism against reactive oxygen species (ROS). Furthermore, the mechanism of action of ROS is more or less unspecific in terms of oxidative damage of bacteria in contrast to the usage of current available antibiotics Fig. (**8**).

Overall, antimicrobial photodynamic inactivation can be repeated multiple times, without apparent induction of resistance (presumably, since DNA is not the prime target of ROS because *Deinococcus radiocurans*, which possesses a very efficient DNA repair mechanism, can be destroyed very efficiently by photodynamic generated singlet-oxygen. Efflux mechanisms of many classes of antibiotics are one of the "classic" strategies of bacteria to receive antibiotic resistance [68]. Tegos *et al*. could demonstrate differences in uptake of phenothiazinium photosensitizers by bacteria depending on the level of expression of efflux pump proteins [69]. Therefore, active efflux mechanisms might be one important step to receive resistance against the antimicrobial photodestructive efficiency of ROS generated by the photosensitizer and oxygen during illumination.

CONCLUSION

At this time, there is no routine clinical application of antimicrobial photodynamic treatment of localized skin infections, super-infected wounds or in infected periodontal pockets. Until now, the most promising *in vivo* studies of aPDT in dentistry have demonstrated that the oral cavity is especially suitable for antimicrobial photodynamic treatment, because it is relatively accessible for illumination [70-74].

Overall, if the resistance against antimicrobial agents becomes worse, antimicrobial photodynamic treatment seems to be an alternative therapy option in the clinic.

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