Potentiation of antimicrobial photodynamic inactivation by inorganic salts

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Abstract

Introduction—Antimicrobial photodynamic inactivation (aPDI) involves the use of non-toxic dyes excited with visible light to produce reactive oxygen species (ROS) that can destroy all classes of microorganisms including bacteria, fungi, parasites, and viruses. Selectivity of killing microbes over host mammalian cells allows this approach (antimicrobial photodynamic therapy, aPDT) to be used *in vivo* as an alternative therapeutic approach for localized infections especially those that are drug-resistant.

Areas covered—We have discovered that aPDI can be potentiated (up to 6 logs of extra killing) by the addition of simple inorganic salts. The most powerful and versatile salt is potassium iodide, but potassium bromide, sodium thiocyanate, sodium azide and sodium nitrite also show potentiation. The mechanism of potentiation with iodide is likely to be singlet oxygen addition to iodide to form iodine radicals, hydrogen peroxide and molecular iodine. Another mechanism involves two-electron oxidation of iodide/bromide to form hypohalites. A third mechanism involves a one-electron oxidation of azide anion to form azide radical.

Expert commentary—The addition of iodide has been shown to improve the performance of aPDT in several animal models of localized infection. KI is non-toxic and is an approved drug for antifungal therapy, so its transition to clinical use in aPDT should be straightforward.

Keywords

Antimicrobial photodynamic inactivation; potassium iodide; methylene blue; Rose Bengal; fullerenes; titanium dioxide photocatalysis; photochemical mechanism

1. Introduction

Antimicrobial photodynamic inactivation (aPDI) was discovered in the year 1900 by a medical student called Oscar Raab who was working with Prof Hermann von Tappenier in Munich. He observed that when the single-cell microorganisms called paramecia were incubated with the fluorescent dye, acridine orange, and exposed to light, they were killed,

while those kept in the dark survived [1]. When it was soon discovered that this phenomenon required oxygen to be present, the term 'photodynamic' was coined [2]. It was realized in 1910 that this photodynamic effect could be used as a medical therapy (photodynamic therapy [PDT]) to destroy undesirable biological tissue, such as skin cancers [3]. Any idea of using PDT as a treatment for bacterial infections was forgotten when in the 1940s antibiotics such as penicillin were introduced [4]. Instead of being used as an antimicrobial, PDT was largely developed as a treatment for cancer [5]. There were a few sporadic reports of the antimicrobial effects of PDT between 1900 and the modern era that started around 1990 (see below).

The photochemical mechanism of PDT was first reported in 1977 by Weishaupt et al. [6]. They identified singlet molecular oxygen as the most important cytotoxic agent in the destruction of cancer cells incubated with hematoporphyrin and exposed to red light. It was later realized that the key feature of compounds (dyes) such as porphyrins used for PDT was the existence of a long-lived triplet state that could be formed by an 'intersystem crossing process' from the excited singlet-state porphyrin, formed when the ground-state porphyrin absorbed a photon of light [7]. Since ground-state oxygen has a triplet electronic configuration, the excited porphyrin triplet state is allowed to undergo energy transfer to produce the ground-state singlet porphyrin and the excited-state singlet oxygen ($^1O_2$). $^1O_2$ is a highly reactive molecule that can oxidize lipids, proteins, and nucleic acids, thus causing the death of any kind of cells (bacteria, fungus, cancer, normal cells, etc.). Because $^1O_2$ is so reactive, it can only damage tissue in the precise location where it is produced (i.e. where both dye and light are present at the same time).

In addition to $^1O_2$ (which became known as the Type II photochemical mechanism), it was realized that other different reactive oxygen species (ROS) were also involved in the PDT effect depending on a number of factors. These ROS (including hydroxyl radicals, hydrogen peroxide, and superoxide anion) were produced by what became known as the Type I photochemical mechanism [8]. It is thought that this initially involves a one-electron transfer from the photosensitizer (PS) triplet state to oxygen to form superoxide anion. Two additional one-electron reduction steps then transform superoxide into hydrogen peroxide and then to hydroxyl radicals [9]. The two photochemical mechanisms (Type 1 and Type 2) are schematically shown in a Jablonski diagram in Figure 1. These highly ROS $^1O_2$ and HO· can cause oxidative damage to nucleic acids, lipids, and proteins and effectively kill any type of microorganisms whether they are gram-positive bacteria, gram-negative bacteria, fungi, viruses, or parasites [10].

Around the 1990s, it was realized that there was a difference between broad-spectrum antimicrobial photosensitizers and those compounds that were efficient in killing cancer cells [11,12]. While highly active anticancer PS are mostly neutral or anionic structures with a certain degree of hydrophobicity, highly active antimicrobial PS are more likely to be water soluble and to bear a number of cationic charges, frequently organic-substituted quaternary ammonium groups [13]. Phenothiazinium dyes such as methylene blue (MB) are often used as PS for aPDI, as well as cationic tetrapyrroles such as porphyrins and phthalocyanines (see Figure 2 for the structure of MB and several other photosensitizers described in this review article).

There have been quite a few clinical applications involving aPDI for different kinds of localized infections, particularly dental infections such as periodontitis [13]. In general, the PS is topically applied into the infected areas followed after a short period of time by illumination with the correct wavelength of light. Selectivity for the microbial cells compared to the surrounding host cells is provided by choosing a short drug light interval and the cationic structure of the PS [14].

2. Effects of sodium azide

Sodium azide is frequently used as a physical quencher of singlet oxygen. Although the compound is toxic to mammalian cells, and is used as a preservative, it is not particularly toxic to bacteria. This means that it can be added to suspensions of bacteria (at least up to 50 mM) and mixed with different PS and then exposed to light in order to gain information about the photochemical mechanism involved, Type 1 or Type 2. If the PS generates mostly singlet oxygen that kill the microbial cells (Type 2), then it is expected that addition of azide will protect the cells from being killed. This is because azide is an efficient physical quencher of singlet oxygen [15]. On the other hand, if the PS mainly functions by a Type 1 mechanism (superoxide, hydrogen peroxide, and hydroxyl radicals), then it would be expected that the azide would have little to no effect in protecting the cells from being killed. We initially used azide to quench the aPDI killing mediated either by a functionalized cationic fullerene (BB6) excited by white light or by a conjugate between polyethylenimine and chlorin(e6) (PEI-ce6) excited by red light [16] (see Figure 2(b,c) for structures). These two compounds were known to bind to both gram-positive and gram-negative bacteria and to allow broad-spectrum killing. Azide quenched the killing of both gram-positive and gram-negative bacterial species mediated by both PS, but the quenching was less for BB6 (1–3 logs compared to 3–4 logs for PEI-ce6) and less for gram-positive bacteria (1–2 logs compared to 3–4 logs for gram negatives). The conclusion was that BB6 produced more Type I species (hydroxyl radicals), while PEI-ce6 produced more singlet oxygen. We then went on to test whether another PS known to work partly via Type 1 photochemistry (the phenothiazinium dye, MB; Figure 2(a)) would be quenched by azide. To our surprise, we found that aPDI killing was actually potentiated and not quenched (termed 'paradoxical potentiation') [17]. Both gram-positive and gram-negative bacteria were killed 1–2 logs more in the presence of 10 mM azide. The species responsible for this extra killing was considered likely to be azide radical ($N_3^\bullet$) as demonstrated by electron spin resonance studies using a 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) spin trap. The azide radical was generated in the absence of oxygen, suggesting that the mechanism was a one-electron transfer from azide anion to the diprotonated MB triplet state (Equation (1)).

$$^3\mathrm{MBH}^{2+} + \mathrm{N}_3^- \rightarrow \mathrm{MBH}^{\bullet+} + \mathrm{N}_3^\bullet \quad (1)$$
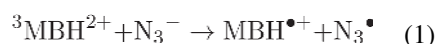
Although the pK_a for $^3\mathrm{MBH}^{2+}$ is 7.2, indicating that, under the experimental conditions used, both the protonated and unprotonated forms of the MB triplet excited state exist, we consider only the protonated form. This is because the one-electron reduction potential

of $^3MBH^{2+}/MBH^{\bullet+}$ being 1.33 V, is more favorable for oxidation of azide than that of ($^3MB^+/MB^{\bullet}$), which is 1.21 V [18].

To test the generality of this somewhat surprising result, we compared a set of six different phenothiazinium salts, which had all previously been used for aPDI [19]. These dyes were MB, toluidine blue O, new methylene blue, dimethylmethylene blue, azure A, and azure B. We found a wide variation between potentiation of killing (up to 3 logs) and quenching of killing (up to 3 logs). Potentiation was more likely to occur with gram-negative *Escherichia coli*, with more lipophilic dyes, and when the unbound dye was washed away from the suspended cells. Since binding between cells and dye is stronger for gram-negative bacteria (more pronounced anionic charge), for more lipophilic cationic dyes, and when washing has selected for only bound dye, the conclusion was that binding between the cells and the dye encouraged potentiation by azide. A plausible explanation for this finding is that the azide radical has a very short diffusion distance and can better damage the bacterial cells when it is generated in very close proximity to its target. Unfortunately, azide anion is toxic to mammalian cells, and it would be highly unlikely that it could be used in any clinical aPDI protocol to enhance the treatment. Therefore, we went on to look at other inorganic salts that do not suffer problems with toxicity.

3. Effects of potassium iodide

We initially assumed that if there was any potentiation of aPDI killing by potassium iodide (KI) that the mechanism would be similar to the mechanisms established for azide. This hypothesis meant that we expected that PS that operates mainly by Type 1 (electron transfer mechanisms) would be likely to allow a one-electron transfer from the PS to produce iodine radicals and PS radical anions. This could in theory take place in the absence of oxygen. In order to study what we expected would be a Type 1 aPDI process, we chose to look at titanium dioxide (TiO_2) photocatalysis. Photocatalysis described a process by which a large band-gap semiconductor such as TiO_2 (preferable in the form of nanoparticles) is excited by the correct wavelength light (often UVA light in the 360 nm range). The valence electrons are excited into the conductance band and have enough energy so they can escape completely and carry out a one-electron transfer to ambient oxygen to form super-oxide anion. On the other hand, the complete removal of an electron from the semiconductor molecule will leave behind a positively charged 'hole.' This hole is a strong oxidizing agent and can oxidize water to produce hydroxyl radicals. The super-oxide produced can also go on to form hydroxyl radicals (see Figure 3 for a schematic depiction of titania photocatalysis). The net result of photocatalysis is a robust photoproduction of ROS by a heterogeneous system, which is used to destroy organic pollutants and to kill microorganisms. One of the attractions of photocatalysis as a process is that the TiO_2 can be activated by natural sunlight, thus obviating the need for any external light source.

We studied the potentiation of TiO_2 antimicrobial photocatalysis by addition of KI to the suspension of titania nanoparticles and microbial cells [20]. The killing of gram-positive bacteria, gram-negative bacteria, and fungi was increased by up to 6 logs by addition of KI. The microbial killing depended on the concentration of TiO_2, the fluence of UVA light, and the concentration of KI (the best effect was at 100 mM). There was formation of long-lived
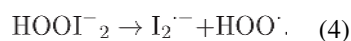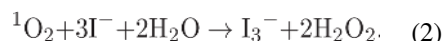
antimicrobial species (probably hypoiodite and iodine) in the reaction mixture (detected by adding bacteria after light), but short-lived antibacterial reactive species (bacteria present during light) produced more killing. Fluorescent probes for ROS (hydroxyl radical and singlet oxygen) were quenched by iodide. Tri-iodide (which has a peak at 350 nm and forms a blue product with starch) was produced by TiO_2–UVA–KI but was much reduced when methicillin-resistant Staphylococcus aureus (MRSA) cells were also present. The model tyrosine substrate N-acetyl tyrosine ethyl ester was iodinated in a light dose-dependent manner. The conclusion was that UVA-excited TiO_2 in the presence of iodide produces reactive iodine intermediates during illumination that kill microbial cells and long-lived oxidized iodine products that kill cells after light has ended.

We then proceeded to study the effect of KI on aPDI mediated by more conventional antimicrobial photosensitizers such as MB [21] excited by red light. In the first study [22], we only used concentrations of KI as high as 10 mM. We found approximately 3 logs of extra aPDI killing with gram-positive species (*Staphylococcus aureus*) and 1–2 logs of extra killing with gram-negative *E. coli*. Although we were able to show the generation of molecular iodine after in solution illumination of a mixture of MB and KI, this was insufficient to kill bacterial cells that were added after light. Interestingly, addition of KI (10 mM) produced a large potentiation (>6 logs) of killing using Fenton reaction ($FeSO_4$ + H_2O_2) or horseradish peroxidase plus H_2O_2. This finding combined with the known tendency of photoactivated MB to produce both Type 1 and Type 2 ROS encouraged us to believe that the mechanism of potentiation involved reaction of KI with hydroxyl radicals produced during aPDI.

However, we were forced to reevaluate our hypothesis after our next study. We looked at a clinically approved photosensitizer called Photofrin (PF, Lerdele Pharmaceuticals Inc., Carolina, Puerto Rico) [23] (Figure 2(d)). PF is a water-soluble derivative of hematoporphyrin that was the first compound to gain worldwide regulatory approval to treat different kinds of cancer with PDT after intravenous injection [5]. PF has been previously shown to be quite effective at killing gram-positive bacterial and fungal cells but is completely inactive when used for aPDI killing of gram-negative species [24].

Since we had found in the study using TiO_2 photocatalysis [23] that concentrations of KI of 100 mM were much better than concentrations of 10 mM, we used higher KI concentrations than we did with MB [22]. We used blue light to activate PF due to the much higher absorption coefficient at the Soret band compared to the Q-bands (200,000 compared to 3000–15,000). Microbial cells (10(8)/mL) + PF (10 μM hematoporphyrin equivalent) excited by 415 nm light (10 J/cm^2) could eradicate (>6 log killing) five different gram-negative species (*E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, and Acinetobacter baumannii*), whereas no killing was obtained with the same PF and light but without KI (Figure 4). The mechanism of action was determined to be generation of microbicidal molecular iodine (I_2/I_3^-) as shown by comparable bacterial killing when cells were added to the mixture after completion of illumination and light-dependent generation of iodine as detected by the formation of the starch complex. In the case of gram-positive MRSA which is much more sensitive to aPDI (200–500 nM PF), potentiation by KI could also be mediated by short-lived iodine reactive species. The fungal

yeast *Candida albicans* displayed intermediate sensitivity to PF–aPDI, and killing was also potentiated by KI. The reaction mechanism occurred via singlet oxygen ($^1O_2$) as shown by the finding that KI quenched $^1O_2$ luminescence (1270 nm) at a rate constant of 9.2×105 M^{-1} s^{-1}. Oxygen consumption was increased when PF was illuminated in the presence of KI. Hydrogen peroxide but not superoxide was generated from illuminated PF in the presence of KI. Sodium azide completely inhibited the killing of *E. coli* with PF/blue light + KI. The proposed equations to explain the oxidation of KI by $^1O_2$ are as follows.

$$^1O_2 + 3I^- + 2H_2O \rightarrow I_3^- + 2H_2O_2 \quad (2)$$

$$^1O_2 + 2I^- + H_2O \rightarrow HOOI^-_2 + HO^- \quad (3)$$

$$HOOI^-_2 \rightarrow I_2^{\cdot -} + HOO^{\cdot} \quad (4)$$

Therefore, both stable antibacterial species could be produced ($I_3^- + 2H_2O_2$) and reactive radicals ($I_2^{\cdot -} \cdot + HOO^{\cdot}$).

In light of the surprising findings that aPDI mediated by PF was potentiated by KI, we went on to test another PS that operates primarily by the Type 2 mechanism, namely Rose Bengal (RB) [25] (Figure 2(e)). RB was also largely inactive in killing gram-negative bacteria. However, addition of the non-toxic salt KI (100 mM) potentiated green light (540 nm)-mediated bacterial killing by up to 6 extra logs with gram-negative bacteria *E. coli* and *P. aeruginosa*, gram-positive MRSA, and fungal yeast *C. albicans*. The mechanism was proposed to be similar to that proposed for PF above, namely, singlet oxygen addition to iodide anion to form peroxyiodide, which decomposes into radicals, and finally forms hydrogen peroxide and molecular iodine. The effects of these different bactericidal species could be teased apart by comparing killing in three different scenarios: (1) cells + RB + KI are mixed together and then illuminated with green light; (2) cells + RB are centrifuged, then KI added, and then green light; (3) RB + KI + green light and then cells added after light. These studies showed that for gram-positive bacteria, the influence of short-lived radicals was most pronounced, with a small effect for *C. albicans*, while all the killing of gram-negative species was produced by stable species (iodine and hydrogen peroxide).

4. Effects of potassium bromide

Potassium bromide is a very similar salt in its overall behavior to KI, except that the bromide ion is harder to oxidize ($E^{\circ}_{ox} = -1.07$ V) than the iodide ion ($E^{\circ}_{ox} = -0.54$). We asked whether photoactivation of any of a range of antimicrobial PDT agent could be potentiated by addition of KBr. None of the traditional PS we tested (MB, RB, PF, and fullerenes) was able to be potentiated by KBr. However, interesting results were obtained using titania photocatalysis [26]. When MRSA cells (10(8) colony forming units [CFU]/mL; gram-

positive bacteria) were irradiated with UVA light in the presence of a heterogeneous suspension of TiO_2 (10 mM) with stirring, there was a light dose-dependent loss of viability reaching almost 2 logs of killing at 30 J/cm^2. However, when 10 mM KBr was added to the mixture, the antimicrobial effect was potentiated by 1–3 logs of extra killing (Figure 5(a)). When the experiment was repeated with *E. coli* (10(8) CFU/mL; gram-negative bacteria), there was a light dose-dependent killing, with TiO_2 and UVA light alone giving over 4 logs of killing at 30 J/cm^2. However, when 10 mM NaBr was added, there was an extra 1–3 logs of bacterial killing on top of that seen with photocatalysis alone (Figure 5(b)). When the experiment was repeated with 10(7) CFU/mL of *C. albicans* (fungal yeast), we found similar results (Figure 5(c)). We wished to investigate how much of the synergistic killing was due to production of a relatively long-lived stable antimicrobial species and how much was due to very short-lived radicals, so we added microbial cells at different times after completion of light delivery. When MRSA cells were added to a suspension of TiO_2 that had been treated with 40 J/cm^2 of UVA light in the presence of 10 or 100 mM bromide, there was about 1.5 logs of killing, but no killing at all without bromide. When the bacteria were added to the irradiated suspension 30 min after the end of the illumination period, there was the same degree of killing, but when 2 h was allowed to elapse after the light, there was no killing at all. Very similar results were obtained when the experiment was repeated with *E. coli*.

In order to gain some information on the identity of the long-lived antimicrobial chemical species that was produced in an irradiated suspension of TiO_2 mixed with bromide, we added 3,3′,5,5′-tetramethylbenzidine (TMB) to the suspension after the end of the illumination. TMB is a widely used chromogenic substrate for detecting oxidizing species, and it is oxidized by hypobromite and hypoiodite among other species [27]. When 10 mM bromide was added to irradiated TiO_2, there was a light dose-dependent increase in oxidized TMB with as little as 5 J/cm^2 not seen in the absence of bromide. As we wished to determine how stable this TMB-oxidizing species was (considering the loss of the antimicrobial activity over 2 h), we added TMB at different times after the end of the illumination period. After 5 min over, 50% of the TMB-oxidizing species had decayed, with over 80% gone after 30 min and 100% gone after 2 h.

We carried out bromination of N-acetyl tyrosine ethyl ester to provide chemical proof of the formation of a reactive bromine species that was produced in the presence of TiO_2, NaBr, and UVA. As recommended by other studies that have been carried out to demonstrate nitration of tyrosine derivatives [28], we employed N-acetyl tyrosine ethyl ester as the substrate for the reaction that would produce N-acetyl-3-bro-motyrosine ethyl ester. We used liquid chromatography-mass spectroscopy (LC-MS) to identify the product ($C_{13}H_{15}NBrO_4^-$ m/z = 328.02 and 330.02) and were able to construct a linear light-dose–response curve. In principle, the long-lived antimicrobial reactive species could have been bromine or hypobromite or a mixture of both. In order to try and distinguish between hypobromite and bromine (which would be present as tribromide anion in the presence of a high concentration of bromide), we used spectrophotometry at 267 nm (Br_3^- has a molar absorption coefficient of 40,900 at 267 nm) [29] and high performance liquid chromatography (HPLC) using an anion exchange column [29]. Despite numerous attempts, we were unable to detect any formation of tribromide by spectrophotometry or by HPLC.

We concluded that hypobromite was produced by TiO_2 photocatalysis in the presence of KBr and was responsible for the enhanced microbial killing.

5. Effect of thiocyanate

Thiocyanate is a natural constituent of human saliva and bronchial secretions. It can reach up to millimolar concentrations in extracellular fluids [30]. Two-electron oxidation of SCN- by H_2O_2 produces hypothiocyanite (⁻OSCN), a potent antimicrobial species. This reaction is catalyzed by a variety of peroxidases (e.g. myeloperoxidase and lactoperoxidase) that are secreted by mammalian mucosa, including the oral cavity, airway, and alimentary tract. It is considered that the SCN-/H2O2/peroxidase system forms part of the innate immune system.

We asked whether thiocyanate (SCN-) could potentiate the MB and light-mediated killing of the gram-positive *S. aureus* and the gram-negative *E. coli* [31]. SCN- enhanced PDT (10 μM MB, 5 J/cm^2 of 660 nm light) killing of *S. aureus* in a concentration-dependent manner by 2.5 log10 steps to a maximum of 4.2 log10 steps at 10 mM SCN-. SCN- increased killing of *E. coli* by 3.6 log10 steps to a maximum of 5.0 log10 steps at 10 mM. We determined that SCN- rapidly depleted the O_2 when irradiated in the presence of MB, reacting exclusively with $^1O_2$, without quenching the MB-excited triplet state. SCN-reacted with $^1O_2$, producing a sulfur trioxide radical anion (a sulfur-centered radical demonstrated by electron paramagnetic resonance (EPR) spin trapping). We found that MB-PDT of SCN- in solution produced both sulfite and cyanide anions and that addition of each of these salts separately enhanced MB-PDT killing of bacteria. We were unable to detect EPR signals of hydroxyl radicals, which, together with kinetic data, strongly suggests that MB, known to produce OH and $^1O_2$, may, under the conditions used, preferentially form $^1O_2$. The suggested reaction mechanism is shown in Figure 6. Singlet oxygen undergoes an addition reaction to thiocyanate to produce a peroxo-intermediate that decomposes to form sulfite and cyanide. Sulfite then undergoes a one-electron transfer to form sulfur trioxide radical anion.

6. *In vivo* studies

In the Hamblin laboratory, several studies have been carried out using bioluminescent bacteria and low-light imaging to monitor the results of antimicrobial PDT used to treat small animal models of localized infections [32–34]. When these bacteria are treated with PDT *in vitro*, the loss of luminescence parallels the loss of colony-forming ability. The size and intensity of the infection can be sequentially monitored in a non-invasive fashion in individual mice or rats in real time. When PS is introduced into the infected tissue followed by illumination with appropriate wavelength light, a light-dose dependent loss of luminescence is observed. If the bacterial species is invasive, the loss of luminescence correlates with increased survival of the mice, while animals in control groups die of sepsis within 5 days. Since the most powerful of the salts we have studied, and moreover one of the least toxic and inexpensive examples was KI, it made sense to test KI in our *in vivo* studies.

Initially, we studied MB + KI in a murine burn infection model using bioluminescent MRSA monitored by *in vivo* imaging [22]. The infected burn was treated with 50 μM MB, with and without the addition of 10 mM KI, and excited with 660 nm light up to 150 J/cm^2, while the

control groups were dark controls with the same amount of MB plus KI and the light-alone control received 660 nm light to 150 J/cm^2. Figure 7 shows a set of five representative bioluminescence image time courses from burns (each time course from a single mouse in each of the five groups) infected with MRSA. The bacterial bioluminescence was largely preserved in the infected burn control (Figure 7(e)) during the treatment with light alone (Figure 7(c)) and in dark controls of MB plus KI (Figure 7(d)). In contrast, PDT gave a light dose-dependent reduction of bacterial luminescence.

We studied the aPDT effects using a C60-fullerene (LC16) bearing a decaquaternary chain and an additional chain of decatertiary-amino groups (Figure 2(e)) [35]. LC16 was excited by UVA light (360 nm). The 10 quaternary ammonium groups were designed to bind to the anionic groups of the bacteria, and the extra 10 tertiary amino groups were designed to provide a source of electrons for electron transfer reactions. Addition of KI facilitated the electron transfer reactions from the photoexcited fullerene. We used a mouse abrasion infected with *A. baumannii* and treated with PDT. The infected abrasion was treated with 200 μM of LC16, with and without the addition of 10 mM of KI, and excited with UVA light up to 20 J/cm^2, while the control groups were dark controls with the same amount of LC16 + KI and light-alone control received UVA light up to 20 J/cm^2. A complete elimination of the bioluminescence signal was observed after a UVA dose of 20 J/cm^2 was delivered in the presence of LC16 + KI. However, the LC16 + UVA group without added KI still had bioluminescence signals remaining in the wound. Both light and dark controls had no measurable diminution in bioluminescence signal during the course of the experiment.

We used a mouse model of a partial thickness skin wound (abrasion) to test the novel combination (RB + KI + 540 nm) *in vivo* [25]. We chose *P. aeruginosa* as the bacterial pathogen for the following reasons. (a) *P. aeruginosa* is a gram-negative bacterial species and would not be expected to be much affected by RB + light alone (without KI). (b) *P. aeruginosa* is sufficiently pathogenic to form a long-lasting infection with a reasonable infective dose of cells. (c) This particular strain of *P. aeruginosa* is not sufficiently virulent in this model to cause a systemic infection, which would lead to death of the mice. The results were broadly similar to those shown above (where PF and LC 15 were used as the PS). When KI was added to the RB in the *P. aeruginosa*-infected wound and green light was delivered, there was a complete loss of bioluminescence when KI was present, while with RB + green light alone, there was no detectable reductions in signal (consistent with RB being ineffective at mediating aPDI of gram-negative bacteria). Regrowth of the luminescence signal in the wound following apparently successful eradication by PDT was observed and is the chief drawback of using PDT as an antibacterial therapy *in vivo* [36,37].

We then investigated the effects of adding KI to potentiate PDT in a model of oral candidiasis developed in mice that had been immunosuppressed with corticosteroids [38]. We used a Gaussia luciferase-expressing strain of *C. albicans* that allowed noninvasive monitoring of the infection by bioluminescence imaging when coelenterazine (luciferase substrate) was introduced into the mouse mouth. The phenothiazinium salt MB combined with red light killed 1–2 more logs of Candida when KI was added ($p < 0.001$). This combination was chosen for treating the *in vivo* model of oral *Candida* infection. After 5 days of treatment, the disease was practically eradicated, especially using MB plus KI with

40 J. Pathology examination of the tongue removed from sacrificed mice showed a remarkable preservation of the normal structure of the tissue (taste buds) in mice treated with MB-PDT + KI.

7. Potential clinical applications

The best established clinical applications of aPDT lie in the general field of dentistry. Periodontitis is a chronic infection of the gums, in which bacteria such as *Porphyromonas gingivalis* occupy the dental pocket and cause inflammation leading to destruction of the periodontal ligament and loss of teeth [39]. The standard treatment is 'scaling and root planning' (SRP) which is basically a mechanical removal of bacterial plaque [40]. Periodontitis can be treated by injected a PS such as MB into the dental pocket and then introducing a narrow laser fiber to excite the dye with red light. PDT is often combined with SRP as an adjuvant therapy [41]. It would be relatively easy to introduce a small amount of KI solution into the dental pocket, just before light delivery. Chemical disinfectants such as sodium hypochlorite and hydrogen peroxide are often employed to destroy infection in root canal during endodontic treatment along with mechanical removal of bacterial biofilm [42]. aPDT is being studied as an alternative approach in which PS can be introduced into the root canal and light delivered by an appropriately designed fiber optic [43] Again, a small amount of KI solution could be introduced just before light delivery. Other dental indications to have been treated with aPDT include peri-implantitis [44] and oral candidiasis (fungal infection) [45]

Other clinical applications of aPDT have been investigated. These include treatment of nonhealing leg ulcers (vascular or diabetic) with topically applied PS and illumination with red light [46]. This could be combined with topically applied KI solution. Some other applications of aPDT such as onychomycosis and gastric *Helicobacter pylori* infection ref may have some difficulty in being combined with KI solution, but perhaps an ingenious approach can be devised.

8. Conclusions and future directions

It has taken us a considerable amount of time to even partly understand the mechanism of action of this potentiation of aPDI effect using different inorganic salts. This is partly because there are a lot of parameters involved, including the chemical structure of the PS, the type of microbial cell, the identity of the salt, the concentration of both salt and PS, and the amount of light. Moreover, it took us some time to realize that a surprisingly high concentration of salt was necessary (up to 100 mM or even higher). We have some preliminary data that yet further inorganic salts can potentiate PDI including sodium nitrite and sodium selenocyanate. Interestingly, there appears to be differences between sodium selenocyanate and sodium thiocyanate. We are actively working on both these salts.

Another intriguing possibility is whether a delivery vehicle can be prepared to contain the combination of an antimicrobial PS and a sufficient quantity of an inorganic salt (presumably KI). This could in principle be accomplished with a liposomal system with the PS encapsulated in the lipid bilayer and the salt dissolved in the aqueous interior. However,

the fact that one apparently needs about a 10,000-fold molar excess of salt to PS may make this co-encapsulation challenging.

9. Expert commentary

The key question to be answered is: will potentiation of aPDI by addition of inorganic salts (almost certainly KI) make a big difference to the extent this therapy has been adopted by the medical community at large? The question is sometimes raised as to the toxicity of these salts to mammals. The acute toxicity values (LD50 in mg/kg) are presented in Table 1. While iodide and bromide are nontoxic and thiocyanate and nitrite are of low toxicity, while azide and selenocyanate are of intermediate toxicity. We cannot deny that the overall uptake of aPDT as an anti-infective therapy has been disappointing at best. Besides a few applications in dentistry (periodontitis and endodontics), most localized infections are not treated with PDT. It is possible that addition of KI to the mix could bring about a fundamental change.

It has become apparent that the main drawback to using PDT as an antibacterial intervention in models of localized infection is the fact that after the light has been turned off, the generation of antimicrobial ROS ceases and any remaining bacteria left alive are completely free to regrow. However, in the case of added KI, it is likely that free iodine/tri-iodide is generated within the infected area by the action of photogenerated singlet oxygen on iodide anions. This free iodine/tri-iodide may remain active within the infection for a much longer time and may inhibit bacterial regrowth for some time to come.

The question may be raised 'Why not just use a topical iodine preparation such as povidone iodine or Lugol's solution?' instead of going to all the trouble of generating iodine by photodynamic action. The answer to this criticism lies in the fact that the combination of aPDI and KI provides an '*in situ*' and 'on demand' route to production of iodine together with hydrogen peroxide to kill gram-negative bacteria. It is likely that the drawbacks of using topically applied iodine preparations such as povidone-iodine as a technique for treating localized infections [47–49] consist principally in the ability of molecular iodine to damage host mammalian cells at the same time as killing bacteria. Moreover, when iodine is added to infected tissue, there is a very short time available to allow it to penetrate down to where the bacteria actually are located within the tissue. The iodine will be rapidly adsorbed by biomolecules (such as proteins) in the tissue. However, if the PS is allowed to penetrate into the tissue after topical application, and if the iodide (as a small anion) can also penetrate easily, then it may be possible to photogenerate molecular iodine and hydrogen peroxide exactly where it is needed. However, further work will be needed to examine whether the *in situ* produced iodine can cause any damage to host tissue as is seen with other iodine preparations. Zubko et al. have shown that the combination of iodine and hydrogen peroxide can have synergistic effects on killing both bacteria and yeasts [50].

Before KI can move into the clinical realm, more research will need to further identify the mechanisms involved, determine to what extent the free iodine produced during the treatment presents a hazard to the tissue, and solve the problem of bacterial regrowth, after apparently successful killing of several logs of bacterial cells. My personal interest lies in

teasing apart the details of the photochemical mechanisms displayed by the different salts and correlating the mechanism with the PS structure and the microbial physiology.

There are of course many other lines of investigation being pursued to try to find other methods of potentiating aPDI. Worthy of mentioning are the following: combination of the PS with a variety of nanoparticles [51] or other nano-delivery vehicles [52]; combination of aPDI by conjugation of PS to ligands as an active targeting strategy such as antibodies [53] and peptides [54]; combination of aPDI with antimicrobial peptides [55]; and combination of PS with cationic polymers such as chitosan [56] or polyethylenimine [57].

10. Five-year view

It is likely that the potentiation of aPDI by nontoxic inorganic salts will continue to be of interest in the next 5 years. aPDI has been rising in popularity lately, as the widespread international concern about antibiotic resistance shows no signs of lessening, and if anything is increasing. Points in favor of the KI combination are that (a) the effect of KI is substantial (up to 6 logs more killing) and (b) KI approved for clinical use (oral administration of saturated KI solution) is the treatment of choice for some superficial fungal infections (sporotrichosis and entomophthoromycoses) [58]. As mentioned above, it will necessary to determine the optimum procedure of application of the antimicrobial PS and the KI solution into the infected area and to carry out studies to rule out the possibility of causing any additional damage to the surrounding hoist tissue by adding KI.

Acknowledgments

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Von Tappenier H. Uber die wirkung fluoreszierender stoffe auf infusorien nach versuchen von O. Raab Muench Med Wochenschr. 1900; 47:5.

2. Von Tappeiner H, Jodlbauer A. Uber wirkung der photodynamischen (fluorieszierenden) stoffe auf protozoan und enzyme. Dtsch Arch Klin Med. 1904; 80:427–487.

3. Hausman W. Die sensibilisierende wirkung des hematoporphyrins. Biochem Z. 1911; 30:276.

4. Lobanovska M, Pilla G. Penicillin's discovery and antibiotic resistance: lessons for the future? Yale J Biol Med. 2017 Mar; 90(1):135–145. [PubMed: 28356901]

5. Agostinis P, Berg K, Cengel KA, et al. Photodynamic therapy of cancer: an update. CA Cancer J Clin. 2011 May 26; 61(4):250–281. [PubMed: 21617154]

6. Weishaupt KR, Gomer CJ, Dougherty TJ. Identification of singlet oxygen as the cytotoxic agent in photoinactivation of a murine tumor. Cancer Res. 1976 Jul; 36(7 PT 1):2326–2329. [PubMed: 1277137]

7. Ochsner M. Photophysical and photobiological processes in the photodynamic therapy of tumours. J Photochem Photobiol B. 1997 May; 39(1):1–18. [PubMed: 9210318]

8. Da Silva Baptista M, Cadet J, Di Mascio P, et al. Type I and II photosensitized oxidation reactions: guidelines and mechanistic pathways. Photochem Photobiol. 2017 Jul; 93(4):912–919. DOI: 10.1111/php.12716 [PubMed: 28084040]

9. Garcia-Diaz M, Huang YY, Hamblin MR. Use of fluorescent probes for ROS to tease apart Type I and Type II photochemical pathways in photodynamic therapy. Methods. 2016 Oct; 15(109):158–166.

10. Hamblin MR. Antimicrobial photodynamic inactivation: a bright new technique to kill resistant microbes. Curr Opin Microbiol. 2016 Oct.33:67–73. [PubMed: 27421070]

11. Malik Z, Ladan H, Nitzan Y. Photodynamic inactivation of gram-negative bacteria: problems and possible solutions. J Photochem Photobiol B. 1992 Jul 15; 14(3):262–266. [PubMed: 1432395]

12. Minnock A, Vernon DI, Schofield J, et al. Photoinactivation of bacteria. Use of a cationic water-soluble zinc phthalocyanine to photoinactivate both gram-negative and gram-positive bacteria. J Photochem Photobiol B. 1996 Feb; 32(3):159–164. [PubMed: 8622179]

13. Abrahamse H, Hamblin MR. New photosensitizers for photodynamic therapy. Biochem J. 2016 Feb 15; 473(4):347–364. [PubMed: 26862179]

14. Sharma SK, Mroz P, Dai T, et al. Photodynamic therapy for cancer and for infections: what is the difference? Isr J Chem. 2012 Sep; 52(8–9):691–705. [PubMed: 23248387]

15. Tavares A, Dias SR, Carvalho CM, et al. Mechanisms of photodynamic inactivation of a gram-negative recombinant bioluminescent bacterium by cationic porphyrins. Photochem Photobiol Sci. 2011 Oct; 10(10):1659–1669. [PubMed: 21799996]

16•. Huang L, Xuan Y, Koide Y, et al. and Type II mechanisms of antimicrobial photodynamic therapy: an in vitro study on gram-negative and gram-positive bacteria. Lasers Surg Med. 2012 Aug; 44(6):490–499. The first evidence that salts may be important in aPDI. [PubMed: 22760848]

17••. Huang L, St Denis TG, Xuan Y, et al. Paradoxical potentiation of methylene blue-mediated antimicrobial photodynamic inactivation by sodium azide: role of ambient oxygen and azide radicals. Free Radic Biol Med. 2012 Dec 1; 53(11):2062–2071. Shows that azide can actually increase aPDI killing as opposed to the expected reduction in killing due to quenching singlet oxygen. [PubMed: 23044264]

18. Ohno T, Lichtin NN. Electron transfer in the quenching of triplet methylene blue by complexes of iron(II). J Am Chem Soc. 1980; 102(14):4636–4643.

19•. Kasimova KR, Sadasivam M, Landi G, et al. Potentiation of photo-inactivation of gram-positive and gram-negative bacteria mediated by six phenothiazinium dyes by addition of azide ion. Photochem Photobiol Sci. 2014 Nov; 13(11):1541–1548. Shows that the potentiation of aPDI by azide can be generalized to other photosensitizers. [PubMed: 25177833]

20••. Huang YY, Choi H, Kushida Y, et al. Broad-spectrum antimicrobial effects of photocatalysis using titanium dioxide nanoparticles are strongly potentiated by addition of potassium iodide. Antimicrob Agents Chemother. 2016 Sep; 60(9):5445–5453. Shows a strong potentiation of antimicrobial photocatalysis by KI. [PubMed: 27381399]

21••. Wainwright M, Maisch T, Nonell S, et al. Photoantimicrobials – are we afraid of the light? Lancet Infect Dis. 2017 Feb; 17(2):e49–e55. Important review on the clinical applications of aPDT. DOI: 10.1016/S1473-3099(16)30268-7 [PubMed: 27884621]

22. Vecchio D, Gupta A, Huang L, et al. Bacterial photodynamic inactivation mediated by methylene blue and red light is enhanced by synergistic effect of potassium iodide. Antimicrob Agents Chemother. 2015 Sep; 59(9):5203–5212. [PubMed: 26077247]

23••. Huang L, Szewczyk G, Sarna T, et al. Potassium iodide potentiates broad-spectrum antimicrobial photodynamic inactivation using Photofrin. ACS Infect Dis. 2017 Apr 14; 3(4):320–328. Shows that a photosensitizer widely known to be ineffective at killing gram-negative bacteria can be made broad spectrum by KI. DOI: 10.1021/acsinfecdis.7b00004 [PubMed: 28207234]

24. Maisch T, Baier J, Franz B, et al. The role of singlet oxygen and oxygen concentration in photodynamic inactivation of bacteria. Proc Natl Acad Sci U S A. 2007 Apr 24; 104(17):7223–7228. [PubMed: 17431036]

25•. Wen X, Zhang X, Szewczyk G, et al. Potassium iodide potentiates antimicrobial photodynamic inactivation mediated by Rose Bengal: in vitro and in vivo studies. Antimicrob Agents Chemother. 2017 Jun 27.61(7) pii: e00467–17. Shows that Rose Bengal behaves in a similar manner to Potofrin in the presence of KI. doi: 10.1128/AAC.00467-17

26•. Wu X, Huang YY, Kushida Y, et al. Broad-spectrum antimicrobial photocatalysis mediated by titanium dioxide and UVA is potentiated by addition of bromide ion via formation of

hypobromite. Free Radic Biol Med. 2016 Mar; 23(95):74–81. Shows that bromide can potentiate microbial killing by TiO2 photocatalysis.

27. Li H, Cao Z, Zhang G, et al. Vascular peroxidase 1 catalyzes the formation of hypohalous acids: characterization of its substrate specificity and enzymatic properties. Free Radic Biol Med. 2012 Nov 15; 53(10):1954–1959. [PubMed: 22982576]

28. Ieda N, Nakagawa H, Peng T, et al. Photocontrollable peroxynitrite generator based on N-methyl-N-nitrosoaminophenol for cellular application. J Am Chem Soc. 2012 Feb 8; 134(5):2563–2568. [PubMed: 22239467]

29. Weinberg HS, Yamada H. Post-ion-chromatography derivatization for the determination of oxyhalides at sub-PPB levels in drinking water. Anal Chem. 1998 Jan 1; 70(1):1–6. [PubMed: 9435465]

30. Chandler JD, Day BJ. Biochemical mechanisms and therapeutic potential of pseudohalide thiocyanate in human health. Free Radic Res. 2015 Jun; 49(6):695–710. [PubMed: 25564094]

31. St Denis TG, Vecchio D, Zadlo A, et al. Thiocyanate potentiates antimicrobial photodynamic therapy: in situ generation of the sulfur trioxide radical anion by singlet oxygen. Free Radic Biol Med. 2013 Aug 19.65C:800–810.

32. Demidova TN, Gad F, Zahra T, et al. Monitoring photodynamic therapy of localized infections by bioluminescence imaging of genetically engineered bacteria. J Photochem Photobiol B. 2005; 81:15–25. [PubMed: 16040251]

33. Hamblin MR, O'Donnell DA, Murthy N, et al. Rapid control of wound infections by targeted photodynamic therapy monitored by in vivo bioluminescence imaging. Photochem Photobiol. 2002; 75(1):51–57. [PubMed: 11837327]

34. Hamblin MR, Zahra T, Contag CH, et al. Optical monitoring and treatment of potentially lethal wound infections in vivo. JInfectDis. 2003; 187(11):1717–1726.

35•. Zhang Y, Dai T, Wang M, et al. Potentiation of antimicrobial photo-dynamic inactivation mediated by a cationic fullerene by added iodide: in vitro and in vivo studies. Nanomedicine. 2015 Mar; 10(4):603–614. Shows that iodide can also potentiate aPDI mediated by a cationic fullerene. [PubMed: 25723093]

36. Dai T, Tegos GP, Lu Z, et al. Photodynamic therapy for *Acinetobacter baumannii* burn infections in mice. Antimicrob Agents Chemother. 2009 Sep; 53(9):3929–3934. [PubMed: 19564369]

37. Dai T, Tegos GP, Zhiyentayev T, et al. Photodynamic therapy for methicillin-resistant *Staphylococcus aureus* infection in a mouse skin abrasion model. Lasers Surg Med. 2010 Jan; 42(1):38–44. [PubMed: 20077489]

38. Freire F, Ferraresi C, Jorge AO, et al. Photodynamic therapy of oral *Candida* infection in a mouse model. J Photochem Photobiol B. 2016 Apr; 1(159):161–168.

39. How KY, Song KP, Chan KG. *Porphyromonas gingivalis*: an overview of periodontopathic pathogen below the gum line. Front Microbiol. 2016; 7:53. [PubMed: 26903954]

40. Angst PDM, Stadler AF, Oppermann RV, et al. Microbiological outcomes from different periodontal maintenance interventions: a systematic review. Braz Oral Res. 2017 May.04(31):e33.

41. Ge L, Shu R, Li Y, et al. Adjunctive effect of photodynamic therapy to scaling and root planing in the treatment of chronic periodontitis. Photomed Laser Surg. 2011 Jan; 29(1):33–37. [PubMed: 21166588]

42. Good M, El KI, Hussey DL. Endodontic 'solutions' part 1: a literature review on the use of endodontic lubricants, irrigants and medicaments. Dent Update. 2012 May; 39(4):239–40. 42–4, 46. [PubMed: 22774686]

43. Sabino CP, Garcez AS, Nunez SC, et al. Real-time evaluation of two light delivery systems for photodynamic disinfection of *Candida albicans* biofilm in curved root canals. Lasers Med Sci. 2015 Aug; 30(6):1657–1665. DOI: 10.1007/s10103-014-1629-x [PubMed: 25060900]

44. Vohra F, Al-Rifaiy MQ, Lillywhite G, et al. Efficacy of mechanical debridement with adjunct antimicrobial photodynamic therapy for the management of peri-implant diseases: a systematic review. Photochem Photobiol Sci. 2014 Aug; 13(8):1160–1168. [PubMed: 24924586]

45. Javed F, Samaranayake LP, Romanos GE. Treatment of oral fungal infections using antimicrobial photodynamic therapy: a systematic review of currently available evidence. Photochem Photobiol Sci. 2014 May; 13(5):726–734. [PubMed: 24686309]

46. Morley S, Griffiths J, Philips G, et al. Phase IIa randomized, placebo-controlled study of antimicrobial photodynamic therapy in bacterially colonized, chronic leg ulcers and diabetic foot ulcers: a new approach to antimicrobial therapy. Br J Dermatol. 2013 Mar; 168(3):617–624. [PubMed: 23066973]

47. Burks RI. Povidone-iodine solution in wound treatment. Phys Ther. 1998 Feb; 78(2):212–218. [PubMed: 9474112]

48. Goldenheim PD. An appraisal of povidone-iodine and wound healing. Postgrad Med J. 1993; 69(Suppl 3):S97–105.

49. Khan MN, Naqvi AH. Antiseptics, iodine, povidone iodine and traumatic wound cleansing. J Tissue Viability. 2006 Nov; 16(4):6–10.

50. Zubko EI, Zubko MK. Co-operative inhibitory effects of hydrogen peroxide and iodine against bacterial and yeast species. BMC Res Notes. 2013 Jul.15(6):272.

51. Perni S, Prokopovich P, Pratten J, et al. Nanoparticles: their potential use in antibacterial photodynamic therapy. Photochem Photobiol Sci. 2011 May; 10(5):712–720. [PubMed: 21380441]

52. Kashef N, Hamblin MR. Advances in antimicrobial photodynamic inactivation at the nanoscale. Nanophotonics. 2017 in press.

53. Embleton ML, Nair SP, Cookson BD, et al. Antibody-directed photo-dynamic therapy of methicillin resistant *Staphylococcus aureus*. Microb Drug Resist. Summer. 2004; 10(2):92–97.

54. Bourre L, Giuntini F, Eggleston IM, et al. Effective photoinactivation of gram-positive and gram-negative bacterial strains using an HIV-1 Tat peptide-porphyrin conjugate. Photochem Photobiol Sci. 2010 Dec; 9(12):1613–1620. [PubMed: 20931134]

55. Johnson GA, Ellis EA, Kim H, et al. Photoinduced membrane damage of *E. coli* and *S. aureus* by the photosensitizer-antimicrobial peptide conjugate eosin-(KLAKLAK)2. PLoS One. 2014; 9(3):e91220. [PubMed: 24608860]

56. Choi SS, Lee HK, Chae HS. Synergistic in vitro photodynamic antimicrobial activity of methylene blue and chitosan against *Helicobacter pylori* 26695. Photodiagnosis Photodyn Ther. 2014 Dec; 11(4):526–532. [PubMed: 25174558]

57. Tegos GP, Anbe M, Yang C, et al. Protease-stable polycationic photosensitizer conjugates between polyethyleneimine and chlorin(e6) for broad-spectrum antimicrobial photoinactivation. Antimicrob Agents Chemother. 2006 Apr; 50(4):1402–1410. [PubMed: 16569858]

58. Costa RO, Macedo PM, Carvalhal A, et al. Use of potassium iodide in dermatology: updates on an old drug. An Bras Dermatol. 2013 May-Jun;88(3):396–402. [PubMed: 23793210]

Key issues

- Antimicrobial photodynamic inactivation employs a photosensitizer excited by visible light to produce reactive oxygen species and kill microbial cells.

- There are two broad photochemical mechanisms: Type 1 involving hydroxyl radicals and Type 2 involving singlet oxygen.

- Sodium azide can be used to distinguish between the mechanisms displayed by methylene blue, because it quenches Type 2, but paradoxically potentiates Type 1.

- MB is potentiated by addition of KI, and compounds such as Photofrin and Rose Bengal which are inactive against Gram-negative bacteria, can be turned into broad-spectrum photosensitzers.

- Titania photocatalysis can be potentiated by iodide and bromide and forms hypohalites that kill microbial cells and halogenate tyrosine.

- Thiocyanate potentiates aPDI via sulfur trioxide radical anion.

- The low toxicity and regulatory approval of iodide encourages early translation to clinical studies.
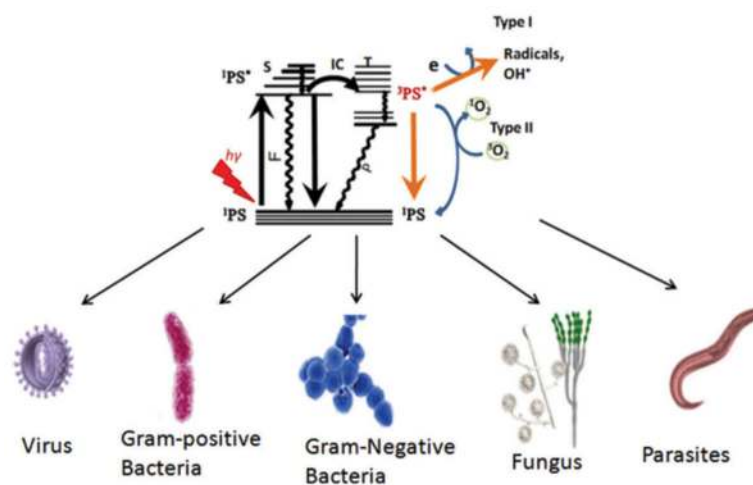
Figure 1. Jablonski diagram
A photosensitizer (e.g. tetra-N-methylpyridinium porphyrin) absorbs a photon, transitions to the short-lived excited singlet state (S^1) that can lose energy by fluorescence, internal conversion to heat, or else can undergo intersystem crossing to the long-lived excited triplet state (T^1). The triplet PS can undergo energy transfer with ground state triplet oxygen to form reactive singlet oxygen ($^1O_2$, Type 2) or else can undergo an electron transfer reaction to form hydroxyl radicals (HO·, Type 1). Both these ROS can efficiently kill all known microbial cells.
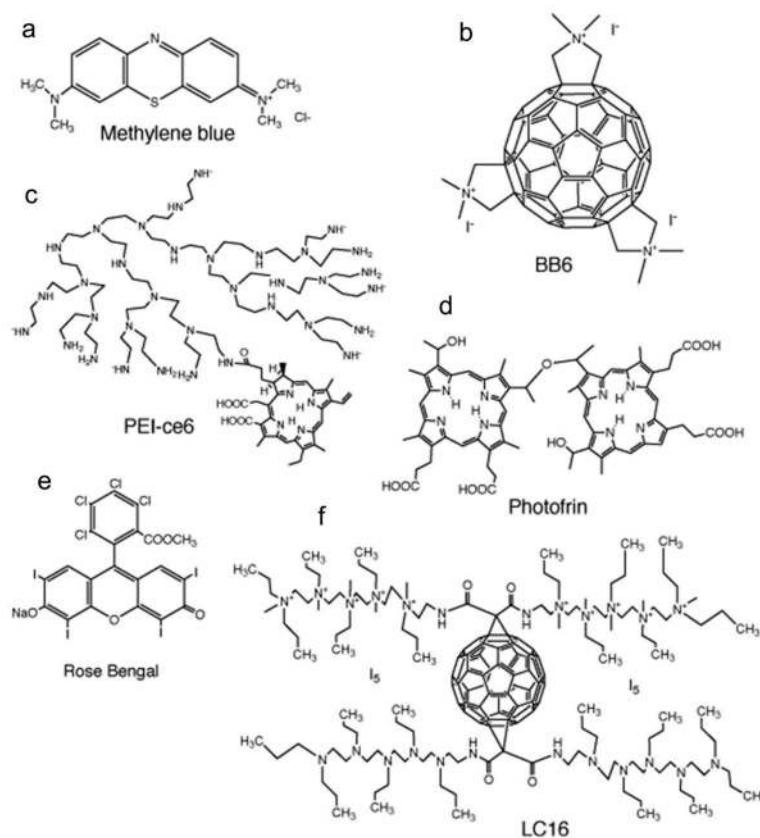
Figure 2. Photosensitizer structures

(a) Phenothiazinium salt, methylene blue, MB; (b) Tricationic C60 fullerene, BB6; (c) Conjugate between branched polyethylenimine and chlorin(e6), PEI-ce6; (d) Photofrin, PF; (e) Rose Bengal, RB; (f) C60-fullerene with a decaquaternary chain and an additional chain of deca-tertiary-amino groups (LC16).
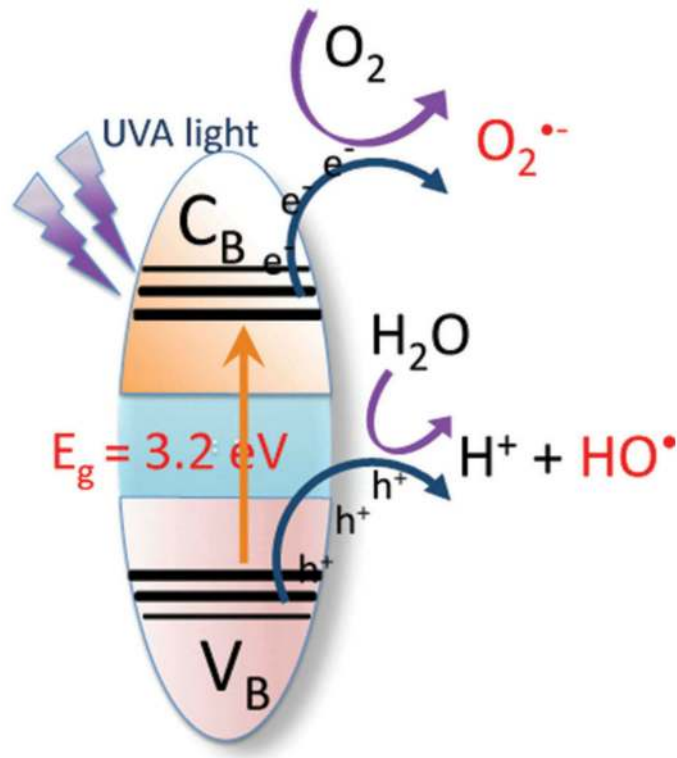
Figure 3. Titania photocatalysis

Schematic illustration of main processes in the photocatalytic reaction of TiO_2. Nanoparticles have a sufficiently large surface area to allow this process to be efficient. Electrons are excited by UVA light from the semiconductor valence band (V_B) to the conductance band (C_B). The electrons in the conductance band undergo electron transfer to oxygen to form superoxide, and the holes (h+) in the valence band react with water to form hydroxyl radicals. The ROS produced ($O_2^{\cdot-}$ and $HO^\cdot$) can kill microorganisms.
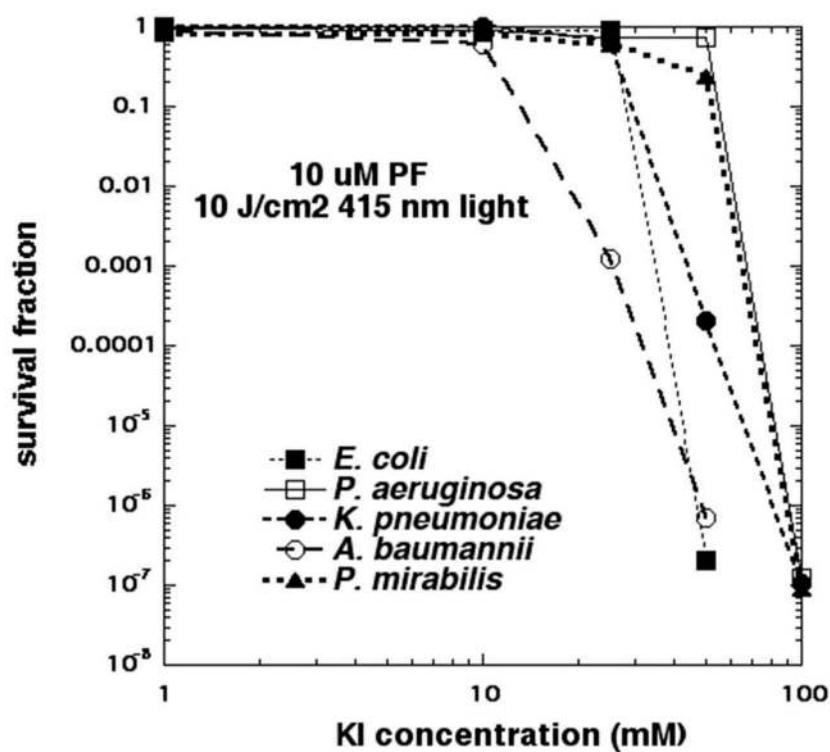
Figure 4. Photofrin, KI and light to kill Gram-negative bacteria
Five different strains of bacteria (10(8) cells/mL) were incubated with 10 *mu;M PF and
exposed to 10J/cm^2 blue light in the presence of different concentrations of KI. Reprinted
with permission from Huang L, Szewczyk G, Sarna T, Hamblin MR. Potassium iodide
potentiates broad-spectrum antimicrobial photodynamic inactivation using Photofrin.
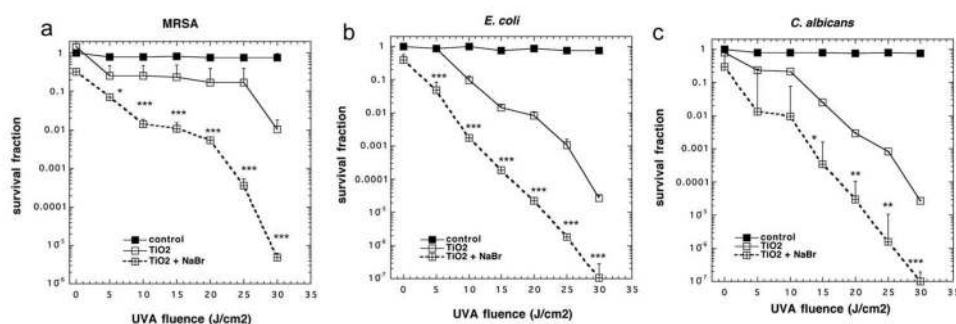American Chemical Society, 2017.

Figure 5. TiO2 photocatalysis with KBr to kill microbial cells

Cells were stirred in the presence of TiO_2 (0 or 10 mM) and NaBr (0 or 10 mM) while being exposed to increasing fluences of UVA light. (a) MRSA (10(8) cells/mL); (b) E. coli (10(8) cells/mL); (c) C. albicans (10(7) cells/mL). Values are means of 3 repetitions and bars are SD. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ for TiO2 + NaBr vs TiO_2 alone. Reprinted from Wu X, Huang YY, Kushida Y, Bhayana B, Hamblin MR, Broad-spectrum antimicrobial photocatalysis mediated by titanium dioxide and UVA is potentiated by addition of bromide ion via formation of hypobromite. Free Radic Biol Med, Vol 95, 74–81, 2016, with permission from Elsevier.
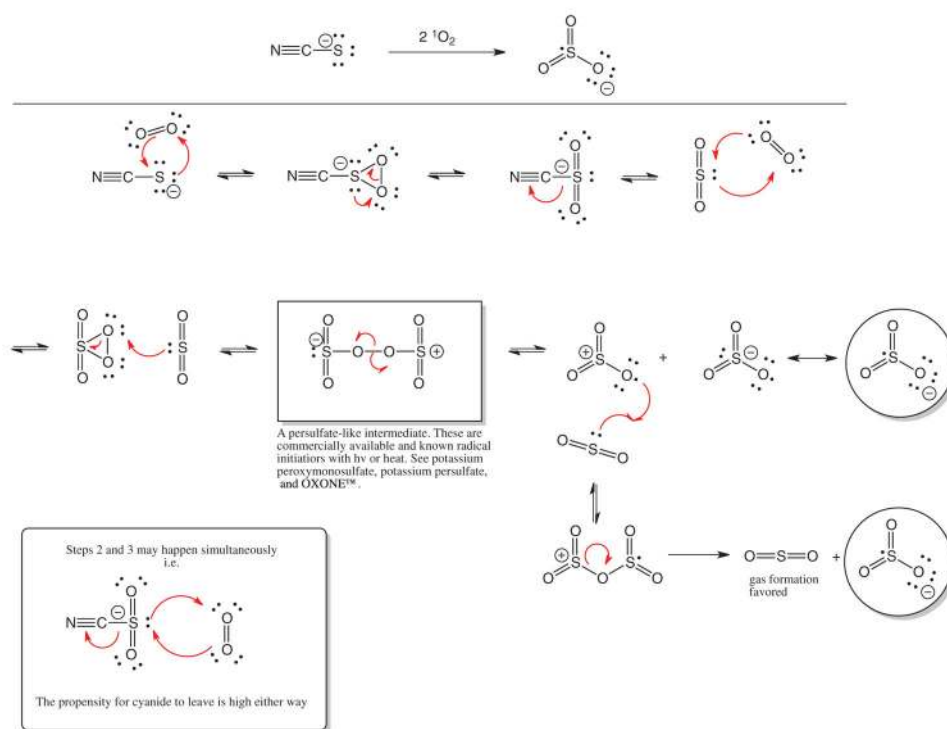
Figure 6. Proposed reaction mechanism for thiocyanate and singlet oxygen
1, SCN$^-$; 2, ^{1}O$_2$; 3, cyanosulfonyl-intermediate; 4, bisulfite, 5, sulfur trioxide radical anion SO$_3$.$^-$.
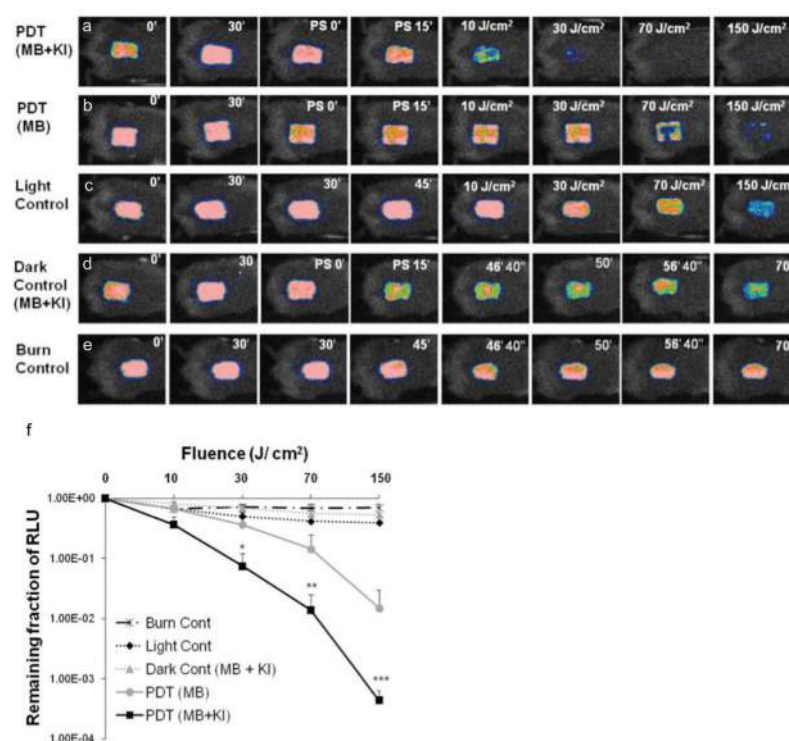
Figure 7. MB + KI excited with red light *in vivo*

(a–e) Successive bacterial bioluminescence images of representative mouse burns infected 10(8) CFU of luminescent MRSA (USA 300) treated with: (a) PDT using mixture of MB (50 μM) + KI (10 mM) or (b) PDT using MB (50 μM) at 30 min after bacterial inoculation + 15 min from PS application. PDT was carried out with a combination of 50 μL of a mixture containing MB + KI or MB alone and 150 J/cm^2 red light (660 ± 15 nm, 100 mW/cm^2). (c) Light alone; (d) applied with mixture of MB + KI, but without red light illumination (dark control); (e) burn control without any treatment. (f). Dose-response of mean bacterial bioluminescence of mouse burns infected with MRSA (USA 300) after treatment with: light alone, mixture of MB (50 μM) + KI (10 mM) (dark control), PDT using MB (50 μM) alone or mixture of MB (50 μM) + KI (10 mM). Reprinted with permission from Vecchio D, Gupta A, Huang L, Landi G, Avci P, Rodas A, et al, Bacterial photodynamic inactivation mediated by methylene blue and red light is enhanced by synergistic effect of potassium iodide. Antimicrob Agents Chemother, Vol 61, 5203–12, 2015 American Society of Microbiology.

Table 1

Acute toxicity values of some inorganic salts.

Salt	Model/route	LD50 (mg/kg)
Potassium iodide	Rat/oral	>3000
Potassium bromide	Rat/oral	3070
Sodium thiocyanate	Rat/oral	764
Sodium nitrite	Rat/oral	180
Sodium azide	Rat/oral	27
Potassium selenocyanate	Mouse/oral	25

Data was compiled from the various material safety data sheets available publicly for the salts listed.