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Photodynamic Therapy for Cancer and for Infections: What Is the Difference?

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Abstract

Photodynamic therapy (PDT) was discovered over one hundred years ago when it was observed that certain dyes could kill microorganisms when exposed to light in the presence of oxygen. Since those early days, PDT has mainly been developed as a cancer therapy and as a way to destroy proliferating blood vessels. However, recently it has become apparent that PDT may also be used as an effective antimicrobial modality and a potential treatment for localized infections. This review discusses the similarities and differences between the application of PDT for the treatment of microbial infections and for cancer lesions. Type I and type II photodynamic processes are described, and the structure-function relationships of optimal anticancer and antimicrobial photosensitizers are outlined. The different targeting strategies, intracellular photosensitizer localization, and pharmacokinetic properties of photosensitizers required for these two different PDT applications are compared and contrasted. Finally, the ability of PDT to stimulate an adaptive or innate immune response against pathogens and tumors is also covered.

Keywords

antitumor agents; antimicrobial agents; cancer; photodynamic therapy; sensitizers

1. Introduction

Over one hundred years ago in 1900, in Munich, the German medical student Oscar Raab and his supervisor Prof. Hermann von Tappeiner noticed that paramecia (a type of aquatic microorganism) that had been incubated with the dye acridine orange died when exposed to sunlight from an adjacent window.[1] Shortly afterwards von Tappeiner and the Munich dermatologist Jesionek were the first to use this discovery as the basis of a therapy when they painted the xanthene dye eosin onto a basal cell carcinoma on the skin of a patient and illuminated it with light.[2] This was the first use of photodynamic therapy (PDT) to treat a disease. In 1904 these investigators established the importance of atmospheric oxygen in this phenomenon and the term photodynamic action was proposed.[3]

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In 1972, driven by the discoveries of investigators such as Lipson and Baldes in the USA, the use of hematoporphyrin derivative (HPD) combined with red light to treat bladder cancer was reported in animals[4] and in humans.[5] Since those early reports, PDT has been developed as an anticancer therapy for tumors of the skin and mucous membranes, for tumors of hollow organs accessible by endoscope, and recently for deep solid tumors where light can be delivered by interstitial fibers inserted into the tumor. The use of PDT in the eye to treat choroidal neovascularization secondary to age-related macular degeneration was approved in 2000,[6] and around the same time PDT mediated by topically applied 5-aminole-vulinic acid (ALA) became widely used in dermatology.[7]

Despite several reports describing photodynamic inactivation (PDI) of microorganisms,[8] PDT has not become established as a standard therapy for infectious disease.[9] Potential reasons for the lack of development of PDT for infections may include the ongoing debates about the properties of the optimum antimicrobial photosensitizer (PS) and their delivery routes into infected tissues, and the potential lack of PS selectivity for microbial cells and consequent collateral damage to host tissue.

Nevertheless, PDT researchers continue to provide compelling data to support the notion that PDT may become a next generation therapy for selective treatment of infectious and cancerous lesions. This review attempts to compare and contrast the different aspects of PDT as applied for cancer and for infections.

2. Photosensitizer Design

Typical chemical structures of PS that have been employed to mediate PDT of cancer and infections are shown in Table 1. Tetrapyrrole compounds such as porphyrins have traditionally been used as PS for mediating PDT of cancer. PDT for cancer was originally developed using HPD, which became clinically approved as Photofrin, while protoporphyrin IX (PpIX, the metabolic product of ALA when applied to cells and tissue) is also widely used as a PS, especially for skin cancer. HPD is a water-soluble compound, which facilitated its preparation in formulations suitable for intravenous injections. ALA is usually applied topically and the PpIX is formed within the cells of tissues to be treated. Porphyrins in general have remarkably weak absorption bands in the red region of the spectrum that is preferred for PDT due to the ability of red and far-red light to penetrate tissue better than light of other wavelengths. Many other non-porphyrin tetrapyrrole compounds have been investigated as anticancer PS, with an emphasis on the design of molecules that have much higher absorption bands in the far-red spectral region. Many of these newer tetrapyrroles can be classified as chlorins (tetrapyrroles with one double bond in a single pyrrole ring reduced), such as benzoporphyrin derivative (BPD, verteporfin),[12] *m*-tetra(hydroxyphenyl)chlorin (mTHPC, Foscan),[26] 2-(1-hex-yloxyethyl)-2-devinyl pyropheophorbide-a (HPPH),[27] and tin ethyl etiopurpurin (SnET2).[28] These compounds, however, are not usually water soluble and require a drug delivery vehicle for intravenous injection. These vehicles vary widely and include a lipid formulation (BPD),[29] PEG/ethanol solvent (mTHPC),[30] Tween 80 (HPPH),[31] and Cremophor EL (SnET2).[32] Phthalocyanines are an- other class of tetrapyrroles with strong far-red absorption bands and a silicon phthalocyanine derivative known as Pc4 has advanced to clinical trials.[33] Bacteriochlorins have two double bonds in two rings of the tetrapyrrole skeleton reduced, and this structural feature provides strong absorption bands in the near-infrared spectrum around 750 nm. The palladium bacteriopheophorbide known as TOOKAD or WST-09 has been in clinical trials for prostate cancer,[34] and a newer water-soluble derivative of the molecule called TOOKAD soluble or WST-11 has now been advanced to the clinic.[22]

It has long been known that PDT for cancer after intravenous injection of PS can have both direct tumor cell-killing effects and vascular shutdown effects.[35] The balance between these two tumor destruction mechanisms depends on the chemical structure of the PS and crucially on the drug-light interval (time between injection and illumination). PS such as Photofrin and Foscan are usually employed with a relatively long drug-light interval (24–48 hours), while compounds such as BPD and TOOKAD are used with much shorter intervals (0–60 min). The long drug-light interval was based upon the idea that the best localization of the PS in the tumor (giving the optimum tumor-to-normal-tissue ratio) occurred after a considerable delay. However, this accumulation of PS in the tumor did not necessarily lead to the best antitumor effect.[36] It appears that the pronounced vascular shutdown effects obtained with short drug-light intervals when the PS is still present in the blood vessels lead to a more efficient tumor destruction.

Although many of the early attempts to kill microorganisms with PDT employed the same PS that were used for PDT of cancer,[37] it was later realized that these structures were not optimal. Because phenothiazinium dyes that have an intrinsic cationic charge were able to photoinactivate many classes of microorganism, it was concluded that the presence of cationic charges was crucial for broad spectrum antimicrobial effects.[38] Although neutral and anionic PS are able to kill Gram-positive bacteria, for Gram-negative bacteria one needs positive charges on the PS to bind and penetrate through the outer permeability barrier composed of the negatively charged lipopolysaccharide.[39] Some studies have shown that a high number of cationic charges make a very efficient PS against Gram-negative bacteria, but fewer cationic charges are better for killing Gram-positive bacteria and fungi.[40,41] Therefore, the best structure for a broad-spectrum antimicrobial PS will likely involve a careful balance between the cationic charges and the hydrophobic character of the molecule.[41] In addition to phenothiazinium salts (e.g., methylene blue and toluidine blue O) and related cationic dye structures,[42] many investigators have attached quaternary nitrogen groups to tetrapyrrole compounds to produce antimicrobial PS with constitutive positive charges. The resulting structures include structurally modified porphyrins,[43] phthalocyanines,[44] porphycenes,[17] bacteriochlorins,[19] and fullerenes.[23]

Another approach is to attach or encapsulate a noncationic PS molecule to a cationic delivery vehicle. These vehicles can include positively charged polymers such as polylysine,[45] polyethylenimine,[46] cationic liposomes,[47] or nanoparticles bearing positive charges.[48]

3. Photochemical Mechanisms

To commence the photodynamic process, light – traditionally in the visible and near-infrared wavelengths – must be applied to excite a PS. The ground state PS is a singlet state, whereby the PS possesses paired electrons with opposite spins in frontier molecular orbitals. Upon exposure to light of the appropriately designated wavelength, the PS absorbs the light, resulting in the excitation of a single electron from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO), while the spin remains unchanged during the process. This excited state, known as the excited singlet state, is relatively short lived, being at most a few nano-seconds. The excited singlet state PS may simply lose its excitation energy by emitting light (fluorescence) or by radiationless relaxation that internally converts the energy to heat. On the other hand, the excited electron may reverse its spin, which is a relatively slow process for most organic compounds, requiring a relatively long-lived singlet state. The triplet state is much longer lived because the excited electron possesses a parallel spin to its paired, unexcited electron. In the triplet state, the excited electron cannot easily fall back to ground level (a “spin-forbidden” process such as this would violate the Pauli exclusion principle). It is this long-lived triplet state that

permits the PS to react with oxygen and gives PDT its characteristic oxidative damage.[49] Figure 1 displays these pathways together with the ensuing photochemistry and proposed mechanisms of damage to different targets.

The excited triplet state PS may interact with oxygen in two distinctly different photochemical processes.[50] The type I pathway involves an electron transfer reaction to, from, or between the triplet state PS, producing a radical anion or a radical cation. A frequent reaction pathway involves electron transfer from $PSC^{\cdot -}$ to molecular oxygen (O_2) to generate the superoxide anion ($O_2C^{\cdot -}$). Other toxic reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\cdot}OH$) can then be formed.[22] The type II pathway involves an energy transfer reaction between the excited triplet state PS and ground triplet state O_2, yielding the transient and highly reactive singlet oxygen ($^1O_2$). This reaction is favored because the spin selection rules favor reactions which do not involve spin reversal (e.g., triplets reacting with triplets), and moreover, oxygen is unusual in that its ground state is a triplet rather than a singlet (hence the paramagnetism of oxygen). The reactive $^1O_2$ then subsequently reacts with proteins, lipids, and nucleic acids of microbial and malignant cells, resulting in spatially limited cellular inactivation and death that is selective to the vicinity of its production.

3.1. Type I Photochemical Reactions

In the type I process, ROS are generated through electron transfer reactions. H_2O_2, though relatively stable by itself, reacts with picogram concentrations of ferrous iron, cuprous copper (which is rare), or other transition metals in a Fenton-like reaction to yield a hydroxide ion and $^{\cdot}OH$ through homolytic fission of the oxygen-oxygen bond in H_2O_2.[51] H_2O_2 is capable of diffusing through cytoplasmic membranes and may then create internal havoc through the generation of $^{\cdot}OH$ in the cytoplasm, leading to the destruction of proteins necessary for homeostasis.

Superoxide anion ($O_2C^{\cdot -}$) alone is not particularly reactive when generated outside a target cell as it is largely insoluble in membranes, but may oxidize macromolecules in the aqueous cytoplasm or upon protonation, whereupon it is soluble.[52,53] $O_2C^{\cdot -}$ may also react with nitric oxide (NO^-) in a diffusion rate–controlled reaction to form the highly reactive peroxynitrite ($OONO^-$).[54] Moreover, $O_2C^{\cdot -}$ may be dismutated by superoxide dismutases (SODs), which results in the formation of H_2O_2 and O_2. $O_2C^{\cdot -}$ may also generate $^{\cdot}OH$ through facilitating the Fenton reaction by first acting as a reducing agent to convert ferric iron to ferrous iron.

Although H_2O_2 and $O_2C^{\cdot -}$ are quite toxic, the majority of type I oxidative destruction from PDT is presumed to be due to $^{\cdot}OH$. At present, it is believed that $^{\cdot}OH$ may be directly generated by both the transfer of electrons and protons from triplet state PS to O_2.[53] Much like H_2O_2, $^{\cdot}OH$ may easily pass through biological membranes. Damage by $^{\cdot}OH$ is considered diffusion rate–limited – that is, the reaction rate of $^{\cdot}OH$ is equivalent to its rate of transport through a solution. This transient species may readily combine with organic substrates or abstract electrons from them – this frequently occurs with fatty acids, cholesterols of eukaryotes, and other lipids. In both cases, $^{\cdot}OH$ favors the further formation of radical species, resulting in radical chain reactions, thus accounting for the extensive damage caused by $^{\cdot}OH$.

3.2. Type II Photochemical Reactions

Singlet oxygen ($^1O_2$) is not a radical species, as all electrons are spin paired, and thus reacts by drastically different pathways. The type II oxidation of macromolecules is the result of $^1O_2$ reactivity with double bonds or sulfur atoms. The reactions between $^1O_2$ and the

double bonds in cyclic macromolecular structures can be broken down into three primary addition reaction categories.

The first of these reactions is the ene reaction, whereby $^1O_2$ adds to a carbon of a double bond, thereby shifting the double bond to a neighboring carbon and forming a hydroperoxide.[55] Secondly, $^1O_2$ may participate in Diels-Alder cycloadditions, resulting in the formation of bridged endoperoxides.[56] The third type is the addition of $^1O_2$ to an activated carbon-carbon double bond to form an unstable 1,2-dioxetane ring system.

Additionally, $^1O_2$ may also react with an electron to form $O_2C^{\cdot -}$ The reduction potential of $^1O_2$ to form $O_2C^{\cdot -}$ is relatively low; however, this process must be facilitated by a reducing agent (biological reducing agents should presumably suffice).[57] Moreover, $^1O_2$ may lead to the formation of $^{\cdot}OH$ by reacting with unsaturated lipids to produce lipid hydroperoxides that initiate free radical chain reactions catalyzed by the presence of ferrous iron.

Consistent with the $^1O_2$ cycloadditions described above, type II mechanisms are known to target aromatic amino acid side chains, specifically tyrosine, histidine, and tryptophan. Moreover, $^1O_2$ favors reaction with the sulfur-containing amino acids cysteine and methionine to form various sulfoxides, as the sulfur moieties have relatively high electron densities.[58] $^1O_2$ can also lead to extensive protein carbonylation and is responsible for the destruction of prosthetic groups (e.g., heme in catalase).[59,60] Besides the ene-type reaction between unsaturated lipids and $^1O_2$ to form hydroperoxides, $^1O_2$ will react with cholesterols to form cholesterol hydroxyl groups and hydroperoxides.[61] $^1O_2$ will also react with components of DNA, specifically the purine nucleoside guanosine, creating unstable and consequently highly reactive endoperoxides.[62]

3.3. Photochemical Pathways Involved in Killing Cells and Microbes

Although the differences between the propensities of type I and type II ROS to kill mammalian and microbial cells is uncertain and still under investigation, we can propose some possible guidelines. Many studies have looked at the role of singlet oxygen in killing mammalian cells (frequently cancer cells), and recently Peter Ogilby has developed a “singlet oxygen microscope” for imaging this process.[63] The predominance of emphasis on the role of $^1O_2$ in anticancer PDT may reflect the type of PS commonly used in this application. Porphyrins and the many other tetrapyrroles discussed above produce high levels of $^1O_2$ and there is evidence for cellular oxidation products typical of singlet oxygen being found in PDT-treated cells. However, a notable exception to this rule is the Pd-bacteriopheophorbide TOOKAD, which appears to kill cancer cells via type I photochemistry.[22] We also found that a Pd-containing porphyrin killed cancer cells efficiently via a type I mechanism.[64]

On the other hand, there is evidence that killing of bacteria by PDT can often involve type I ROS. Martin et al. investigated a series of thiazine, xanthene, acridine, and phenazine dyes and their phototoxicities towards *Escherichia coli*.[65] Hydroxyl radical scavengers conferred dose-dependent protection against the photodynamic action of all of the representative dyes. The authors concluded that oxygen radicals were primarily responsible for the oxygen-dependent toxicity of the dyes examined.[66] However, other investigators have concentrated on the role of $^1O_2$ in bacterial killing as well.[67,68]

4. Cellular and Tissue Targeting

As PDT finds major application in anticancer therapies as well as newer applications as an antimicrobial treatment, it becomes pertinent to discuss the cellular- and tissue-targeting

strategies adopted for these two different applications. Figure 2 compares the localization of the appropriate PS in organelles of cancer cells and in cell walls and plasma membranes of Gram-positive and Gram-negative bacteria.

4.1. Cellular Localization of PS

The intracellular localization of PS is very different between anticancer and antimicrobial PDT (see Figure 2 for a schematic illustration). There have been a large number of reports that studied the subcellular localization of PS in cancer cells.[69,70] Mitochondria are thought to be an important site of action for many powerful PS and this is considered to be related to the known role of mitochondrial damage in initiating apoptosis.[71] The anti-apoptotic protein Bcl-2 located in the mitochondrial outer membrane has been shown to be damaged by PDT, and this damage leads to cytochrome *c* release from the mitochondria and subsequent activation of caspase 3.[72] The endoplasmic reticulum (ER) is also considered to be a highly sensitive location for PS.[73] The increasing amount of knowledge about the role of ER stress and the unfolded protein response in cell death pathways suggests that PDT-induced oxidative damage to cell proteins can exert an intolerable effect on cell viability.[74] Interestingly, a recent paper showed that a proteasomal inhibitor potentiated PDT-mediated cell killing,[75] presumably by increasing the accumulation of PDT-damaged proteins in the ER. Many PS accumulate in the lysosomes and it has been shown that light delivery leads to lysosomal rupture.[76] It is thought that enzymes such as cathepsins released from PDT-ruptured lysosomes mediate cleavage of BH3-interacting domain death agonist (BID), which can translocate to mitochondria and initiate apoptosis.[63]

Unlike the extensive body of knowledge that has been accumulated about the subcellular localization of PS in cancer cells, much less is known about their localization in microbial cells. It is known that there is a basic difference in susceptibility to PDT between Gram-negative and Gram-positive bacteria.[77] Gram-positive bacteria are more susceptible to PDT and can be killed by neutral, anionic, or cationic PS molecules, whereas only cationic PS or methods that allow noncationic PS to breach the Gram-negative permeability wall are able to kill up to six logs (99.9999%) of Gram-negative species. This difference in susceptibility to PDT is due to differences in the cell membrane architecture. Gram-positive species have a cytoplasmic membrane bordered by a comparatively permeable cell wall composed of peptidoglycan and lipo-teichoic acid that permits the PS to traverse through it. Gram-negative bacteria have an inner cytoplasmic membrane and an outer membrane between which lies the peptidoglycan-containing periplasm (Figure 2). This outer membrane of Gram-negative bacteria forms a strong permeability barrier between the cell and its environment that leaves these bacteria less susceptible to PDT by restricting the binding and penetration of many PS.[78] Fungal cell walls have a moderately thick layer of chitin and β-glucan that results in a permeability barrier that is less than the Gram-negative but more than Gram-positive bacteria.

In general, it is thought that the positively charged PS bind to the negatively charged residues on the outside of microbial cells. For Gram-negative bacteria these negative charges are on the lipopolysaccharide (LPS) that forms the major structural barrier of the outer membrane. This binding between bacterial cells and PS also involves the displacement by the PS of the divalent cations that normally maintain the LPS structure. The PS molecule, however, is bigger than the calcium and magnesium ions and the LPS structure is consequently disrupted, which allows even more PS to bind and penetrate the outer membrane. This is an example of the “self-promoted uptake pathway” first described by Hancock.[79] Gram-positive bacteria lack an outer membrane and have a much more porous cell wall, which allows PS to penetrate into the plasma membrane. In both cases it is considered that PDT damages the plasma membrane, which permits cellular constituents to leak out with fatal consequences.

4.2. Cancer Targeting of PS

In the case of anticancer PDT the PS is generally delivered systemically, whereupon it travels through the bloodstream to reach the tumor tissue. It is known that lipoproteins are major carriers of lipophilic porphyrins in the bloodstream.[80] The tumor microenvironment has particular characteristics that distinguish it from the surrounding normal tissue and which also account for the varying degrees of selectivity of the PS for the tumor tissue over the normal tissue. These tumor characteristics include the high proliferation rate of cancer cells, upregulated expression of the low-density lipoprotein (LDL) receptors,[81] decreased intratumoral pH,[82] the presence of tumor-associated macrophages,[83] and the presence of large interstitial spaces and poor lymphatic drainage.[84] Many of these tumor tissue characteristics are innately responsible for the selectivity of the most commonly used PS. The tumor cellular markers that have been investigated for active photodynamic targeting are primarily surface-based markers, including growth factor receptors, LDL receptors, transferrin receptors, folic acid receptors, glucose transporters, integrins, and insulin receptors. Intracellular targeting is difficult due to the trouble of achieving adequate penetration into the target cell, but considerable progress has been made in this field. Some studies have been performed to actively target tumor endothelial markers, such as VEGF receptor-2, fibronectin, and neuropilin-1.[85,86]

Although PDT for cancer is an inherently selective modality, selectivity can be further enhanced by combining other targeted therapeutic strategies. One such approach is targeting the PS to overexpressed molecules on the cancer cells.[87] This molecular targeting of receptors, anti-gens and enzymes has given new directions for the development of effective antibody, immuno-, and ligand-targeted therapies for cancer patients. Another approach is the use of synthetic peptides and nanoparticles for selective delivery of PS. The other prospective strategy is the application of targeted therapeutics that take advantage of the many significant pathways that are involved in the processes of tumorigenesis and metastasis. As the tumor is characterized by the presence of enhanced neovascularization, it has been shown that a combination of PDT targeting tumor vasculature and treatment with anti-angiogenic agents leads to improved therapeutic outcome. The PS for this approach should have certain characteristics such as high molecular weight and plasma solubility, which would allow the PS to be in the vasculature for longer periods and also reduce aggregation during administration.[88] Depending on the choice of PS and manipulation of the drug-light interval, preferential damage to the vasculature versus the cancer cells can be achieved. This is due to the relative distribution of the PS in each of these compartments according to their pharmacokinetic properties. The PS that are used with a short drug-light interval are generally confined to the blood vessels and damage the tumor vasculature.[88,89] In contrast, PS that are employed with longer drug-light intervals are known to damage the tumor cells directly, as they tend to localize in the cellular compartments. This passive targeting mechanism is governed by the inherent PS physicochemical properties.

4.3. Infection Targeting of PS

PDT for infections is generally thought to be applicable for localized disease rather than systemic infections, hence the PS used for antimicrobial PDT are delivered to the infected area by topical application, instillation, or interstitial injection, or through an aerosol for airway-based infections. An important factor that should be taken into consideration for an effective therapeutic outcome is the nature of the surrounding species at the site of infection. In the case of topical infections these are mainly proteins, cells, blood, and host tissue. It has been calculated that the mass of bacteria in infected tissue can be dramatically outweighed by the mass of host tissue, with a thousand to a million times less bacteria than host tissue depending on infection severity.[90] Therefore, it is a formidable challenge to develop highly selective PDT for infection in vivo. Some in vitro studies have been designed to carry out

antibacterial PDT in the presence of host cells or tissues.[91,92] Another important issue to be addressed is that the chosen PS should exhibit enough selectivity for the microbes over mammalian cells to kill sufficient numbers of the disease-causing pathogens and at the same time avoid an unacceptable amount of PDT damage to the surrounding tissue. There is some evidence that lack of selectivity can result in more bacterial regrowth, as was found in a study that compared a bacteria-targeted PS (polylysine-ce6 conjugate) with nontargeted free ce6 in a mouse abscess model.[93]

One of the major issues to be addressed in the clinical application of antimicrobial PDT is that it is not always clear whether the overall cause for eradication of the local infection is due to the actual killing of pathogens or the PDT-induced destruction of the host cells, or indeed to healing of the tissue due to the PDT-triggered immune response, or a combination of these factors. The most common example is the case of ALA-PDT for acne, where the biological mechanisms may be the direct destruction of sebaceous glands by ROS, reduction of follicular obstruction and hyperkeratosis, and immunological changes,[94] rather than PDT-mediated killing of the *Pro-pionibacterium acnes* bacteria. Similar considerations may apply in the treatment of cutaneous leishmaniasis by ALA-PDT,[95,96] since it is known that the *Leishmania* parasites are unable to synthesize protoporphyrin IX from ALA.[97] Indeed, the marked success of PDT for periodontitis[98] may also be partly due to PDT-induced healing in the periodontal pocket as well as killing of the bacteria initially responsible for the disease.[99]

5. Drug Delivery and Route of Administration

Drug delivery is of one of the major challenges in the application of both anticancer and antimicrobial PDT. The drug delivery vehicle chosen should enable selective accumulation of the PS within the target lesion in therapeutic concentrations with minimum or no uptake by nontarget cells.[100] Figure 3 shows that PDT for cancer usually employs intravenous injection of PS followed by laser illumination, while PDT for infections is likely to use topical PS application followed perhaps by light delivery from a light emitting bandage.

5.1. Drug Delivery in Anticancer PDT

To achieve the selective accumulation of PS, anticancer PS are generally delivered systemically by intravenous injection. Preformed, lipophilic sensitizers, such as porphyrins (HPD), chlorins (BPD, SnET2, mTHPC), bacterio-chlorins (TOOKAD), phthalocyanines (Pc4), and texaphyrins (Lutex), when administered intravenously, are believed to be transported in the bloodstream bound to lipoproteins such as LDL.[101] Even small changes in the PS structure can have large effects on the binding to LDL.[102] Tumor cell membranes are known to possess disproportionately high numbers of LDL receptors,[101] leading to active accumulation of PS molecules in close proximity to tumor cells. PS may also accumulate in tumors due to abnormalities in the local microvasculature, including a disordered blood supply and enhanced vascular permeability.[101]

In addition to systemic administration, PS are also topically delivered in the treatment of superficial malignant tumors.[103] ALA and its simple derivatives are the main compounds used as PS in these applications. ALA is a naturally occurring δ-amino acid that is ultimately converted into PpIX, the immediate precursor of heme. The accumulation of PpIX following administration of ALA is more pronounced in malignant cells as compared to their normal counterparts in vitro and in vivo.[103] The reason for this phenomenon has been the subject of profound debate. Some experimental evidence suggests that the activity of the rate-limiting enzyme porphobilinogen deaminase is increased and the activity of ferrochelatase is decreased in neoplastic cells. The latter enzyme catalyzes the insertion of ferrous iron into PpIX to finally form non–photodynamically active heme.[103]

5.2. Drug Delivery in Antimicrobial PDT

Targeting of the PS to wound infections using systemic administration is not considered possible because of damage to the blood vessels and host cells, so antimicrobial PS are generally introduced into the infected tissue by local administration such as topical application, interstitial injection, or aerosol delivery.[104] One feature that differentiates bacterial cells from cancer cells (and particularly normal cells) is that the cell membranes of bacteria are more negatively charged. As a result, an approach for achieving selective antimicrobial PS accumulation after local delivery is to use PS molecules with an intrinsic positive charge, which renders possible the selective binding of PS to bacterial cells over the surrounding mammalian cells.[8] Furthermore, it is known that the binding of PS to bacteria is a rapid process (a few minutes), while the uptake of cationic PS into mammalian cells is slow because the uptake mechanisms involve endocytosis rather than diffusion through the plasma membrane. Therefore, antimicrobial PDT is generally used with a short drug-light interval after topical application.

5.3. Topical Drug Delivery Strategies

Topical delivery of PS has been widely used in PDT of superficial infections including acne,[105–110] warts,[111–113] wound infections,[114,115] and nail infections.[116] In the treatment of acne, PDT is usually mediated by ALA formulated in an oil-in-water cream at a loading of 20% w/w. ALA creams are made up immediately before use, as the stability of ALA in aqueous formulations with pH>6 is poor.[105] For PDT of warts, the PS used include ALA, methylene blue, proflavine, and neutral red. Topical creams and solutions are again the preferred drug delivery methods.[105] For PS delivery in PDT of fungal infections, Donnelly et al. developed an ALA-containing bioadhesive patch (50 mg/cm^2).[116] Application of the patch for 24 hours allowed an ALA concentration of 2.8 mM to be achieved on the ventral side of excised human nail. Application for 48 hours delivered a concentration of 6.9 mM.

Gad et al. reported on the use of PDT to treat an established soft tissue infection in mice.[93] In this study, mice were injected with a poly-l-lysine chlorin e6 conjugate directly into the infected area, at a dose of 50 μL of a 1 mM ce6 equivalent solution. In an in vitro study carried out by Donnelly et al. to investigate the potential of using PDT for lung infections, delivery of the PS toluidine blue and *meso*-tetra(*N*-methyl-4-pyridyl)-porphine tetratosylate across artificial cystic fibrosis (CF) mucus was successfully achieved.[117] For PS diffusion studies, 0.1 mL solutions of PS (5–15 mg/mL) dissolved in phosphate-buffered saline were placed on top of 0.34 mL of synthetic CF mucus in an specially designed glass washer (3.0 mm thickness, 12.0 mm inside circular diameter) in the donor compartment of a modified Franz cell. Receiver compartment concentrations of both drugs after six hours were in the same range as those required to achieve high kill rates (>99%) of Pseudomonas aeruginosa isolates growing both planktonically and in biofilms.

5.4. The Use of Nanoparticles to Improve Drug Delivery in PDT

Recently, an increasing number of studies have been carried out on the use of polymer-based nanoparticles to improve PS delivery and release in both anticancer and antimicrobial PDT. For anticancer PDT, PS-containing nanoparticles have many advantages over free PS molecules.[118] These nanoparticles can be synthesized by various strategies (e.g., adsorption, covalent bonding) and their hydrophilicity and appropriate size allows for passive targeting to tumor tissues by the enhanced permeability and retention (EPR) effect. Selective accumulation can be enhanced by using surface modification to bind monoclonal antibodies or specific tumor-seeking molecules to PS-loaded nanoparticles. Engineered biodegradable polymeric nanoparticles made of poly(lactic-*co*-glycolic acid) (PLGA) are used as a drug delivery system for PS in anticancer PDT.[118]

For antimicrobial PDT, the advantages of using PS-containing nanoparticles include: (1) limiting the ability of the target cell to pump the drug molecule back out, thus reducing the possibility of drug resistance; (2) improved treatment selectivity by localized delivery agents, which can be achieved by either passive targeting or active targeting via the charged surface of the nanoparticle; and (3) the nanoparticle matrix is nonimmunogenic.[119,120] The nanoparticles that have been proposed for antimicrobial PDT include biocompatible and biodegradable matrices such as liposomes, PLGA, and cyclodextrins.[48]

6. Stimulation of Adaptive and Innate Immune Responses

The immune system consists of innate and adaptive arms, which work together to guard the host organism against both cancer and infection.[121] The innate immune system consists of all the defenses that lack antigen specificity and immunological memory. Thus, a characteristic of innate responses is that they remain unchanged however often the insult occurs. The major players in the innate immune response include cells such as neutrophils and macrophages, as well as mechanical barriers such as skin.[122]

The adaptive immune response is mediated by two types of antigen-specific cells: T cell and B cell lymphocytes. T cells are involved in the defense against cancer as well as against viral infections, while B cells produce antibodies that help to opsonize and destroy both viruses and bacteria. Figure 4 shows a comparison of the immune cells that may be involved in the stimulation of immune response after PDT for cancer and for infections.

6.1. Immune Response after PDT for Cancer

There have been several reports showing that PDT can effectively engage innate immune responses in the host s inflammatory response to cancer.[123,124] PDT of tumors leads to the expression and production of several pro-inflammatory mediators at the PDT-treated site. Among other effects, PDT effectively triggers activation of the complement system, expression of heat shock proteins, production of arachidonic acid derivatives, and secretion of chemokines and cytokines.[125] The mechanism for the development of the PDT-induced local inflammation is thought to be ROS production, which leads to concentrated damage of cellular membranes and cytoplasmic organelles in endothelial cells of tumor vessels and tumor cells themselves.[126] This local trauma threatens the integrity of the treated area and leads to a subsequent release of SOS signals and pro-inflammatory mediators to maintain the homeostasis.[127] Therefore, PDT of tumors stimulates a potent acute inflammatory response that in turn attracts neutrophils and other inflammatory cells to the treated site.[128–134] Furthermore, PDT can stimulate a powerful systemic acute phase response,[129] which leads to significant increases in serum levels of established acute phase reactants such as serum amyloid P component (SAP), mannose-binding lectin A (MBL-A), and C-reactive protein (CRP).[134,135] These local and systemic inflammatory responses can in turn be translated into the adaptive response that will develop to protect the host organism in an antigen-specific manner.[136]

In the case of cancer there are several reports describing the involvement of various types of T cells in PDT-mediated immunity. Among the populations studied, both CD4+ and CD8+ T cells have been shown to be involved. Our group was the first to define the role of T regulatory cells in the CD4+ population,[137] and recently we were also the first to define the population of epitope-specific CD8+ cells that are capable of recognizing epitopes derived from tumor antigens.[138] Unfortunately, at present the contribution of B cells remains unknown in post-PDT antitumor immune response.

6.2. Immune Response after PDT for Infection

In the case of PDT for bacterial infections, the issue of PDT-mediated immunity is an uncharted area. A recent study showed that PDT of bacterial arthritis (*Staphylococcus aureus-infected* mouse knee joint) demonstrated a pronounced biphasic dose response.[139] Too little or too much PDT was less effective than an intermediate PDT dose. This observation led our group (Tanaka et al., manuscript in preparation) to design a study that showed for the first time that in vivo PDT could stimulate an immune response against the *S. aureus*–infected knee joint mediated by a strong infiltration of the area by neutrophils. Furthermore, the beneficial effect of PDT was strongly abrogated by anti-Gr1 antibody as well as by antibodies to several pro-inflammatory mediators. Additionally, in a preventative model where PDT treatment was delivered prior to bacterial inoculation into the knee, we were able to show that PDT-mediated infiltration of neutrophils could prevent the bacteria from establishing the infection. This effect was again abrogated by antibodies against Gr1 and several pro-inflammatory mediators. To the best of our knowledge, this is the first time that the immunological effects of PDT against bacterial infections have been convincingly demonstrated.

At present, when comparing the immune responses after PDT for cancer and bacterial infections, one can only humbly speculate about the differences and similarities (Figure 4). The data available in the literature suggest that PDT of cancer leads to activation of both arms of the immune system, namely the innate and adaptive systems,[140] while the early results from bacterial infection models lead us to believe that PDT is at least capable of stimulating beneficial activation of the innate immune system. The biggest difference, however, may lie in the activation of the adaptive immune response. While PDT is quite efficient in activating T cell–mediated immune responses toward treated tumors, the involvement of B cells remains underinvestigated. However, it is B cells that may actually play a major role in post-PDT adaptive immune response towards bacteria, as the antibodies produced by B cells are the major effective component of any immune response against bacterial infection.[141] However, it remains to be seen whether this missing element in our understanding of PDT-mediated immune response towards tumors and bacteria will prove to be a significant discovery.

7. Summary and Outlook

As PDT moves into its second century of development, rapidly increasing knowledge is leading to a much more rational approach to the design of many parameters than was previously possible. PDT for cancer is taking advantage of a range of much more powerful PS that lack the long-lasting photosensitivity that was a distressing side effect of previous regimens. Research into cell signaling pathways and molecular signatures of different cancer cells may bring the era of personalized medicine into PDT as well. Advances in knowledge of the photochemical mechanisms operating in PDT may allow further optimization of PS structures. Targeting strategies and drug delivery vehicles including nanoparticles are being actively studied in the PDT arena. The relatively new field of PDT for infectious disease has been given a boost by increasing concern about the inexorable worldwide spread of antibiotic resistance amongst bacteria and other pathogens. Advances in light delivery and dosimetry will further widen the range of diseases that can be effectively treated with PDT. The particular effects of PDT in stimulating both the innate and adaptive arms of the immune system will be of crucial importance in the future.

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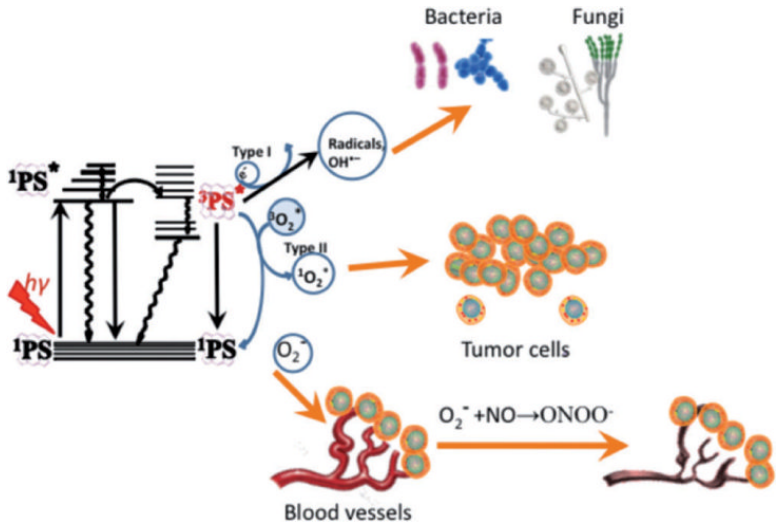


Figure 1.

Jablonski diagram showing the photochemistry arising from the PS triplet state. Type I photochemistry can produce hydroxyl radicals that may be efficient in destroying microbial cells. Type II produces singlet oxygen that may be efficient in destroying cancer cells, while peroxynitrite (formed from superoxide and nitric oxide) may be efficient in destroying tumor blood vessels.

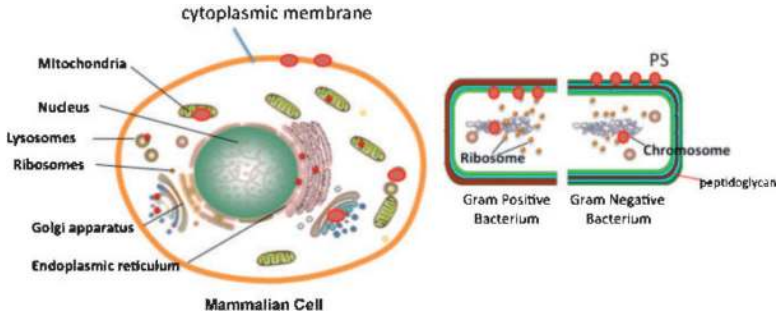


Figure 2.

Comparison of PS localization in cancer cells and bacteria. In mammalian cancer cells, PS (red dots) localize in various intracellular organelles such as lysosomes, mitochondria, the endoplasmic reticulum, Golgi apparatus, and plasma membrane, depending on the precise chemical structure and the incubation time. In Gram-positive bacteria, PS can penetrate through the cell wall to the plasma membrane and even get inside and bind to chromosomal DNA, while in Gram-negative cells penetration of the PS through the outer cell wall is more difficult.

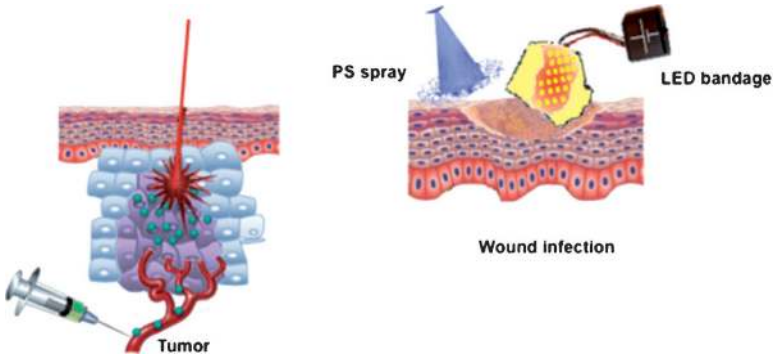


Figure 3.
Comparison of PS and light delivery routes between PDT for cancer and PDT for infections. PDT for cancer usually employs intravenous injection of the PS followed by laser illumination, while PDT for infections is likely to use topical PS application followed perhaps by light delivery from a light-emitting bandage.

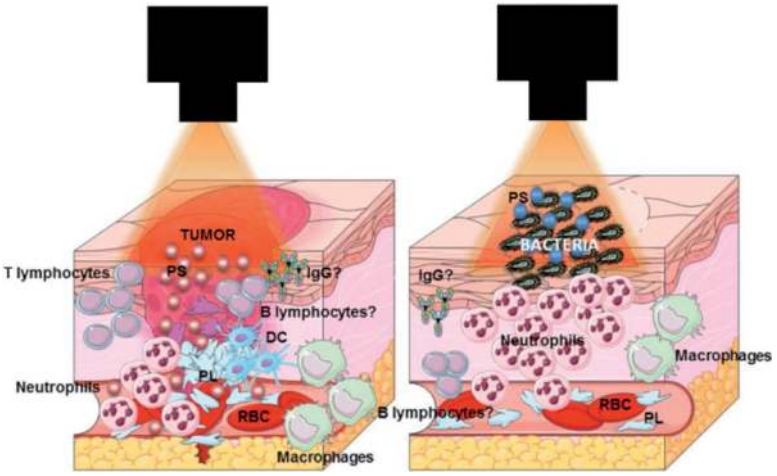
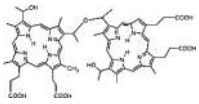
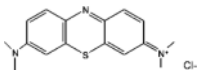
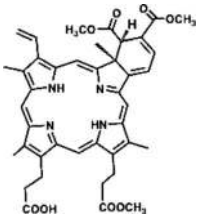
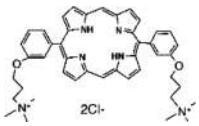
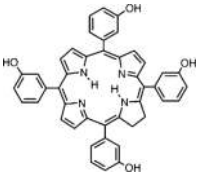
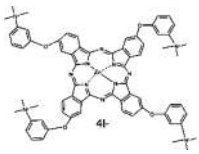
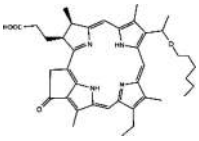
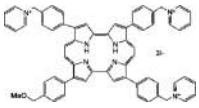
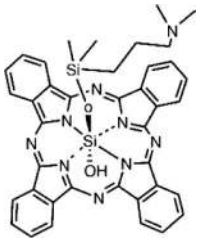
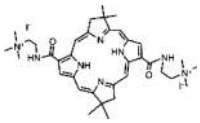
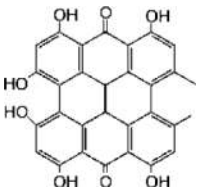
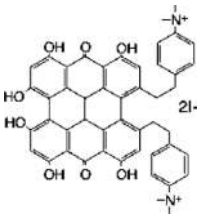


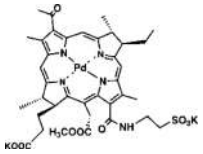
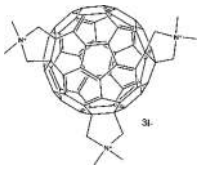
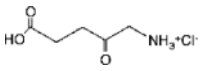
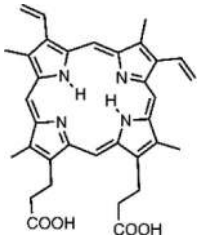
Figure 4.

Comparison of the immune cells that may be involved in the stimulation of immune response after PDT for cancer and PDT for infections. In the case of cancer, antigen-presenting dendritic cells (DC) are crucial and antigen-specific T lymphocytes play a major role in tumor destruction. For infections, it is likely that innate immune cells such as macrophages and especially neutrophils play a major role in the effect of PDT in preventing bacterial infection.

Table 1

Chemical structures of typical PS that have been used for anticancer and antimicrobial applications.

Anticancer PS	Antimicrobial PS
	
Hematoporphyrin derivative (HPD)[10]	Methylene blue[11]
	
Benzoporphyrin derivative (BPD)[12]	Cationic porphyrin (XF70)[13]
	
m-Tetra(hydroxyphenyl)chlorin (mTHPC)[14]	Cationic phthalocyanine (RLP068)[15]
	
2-(l-hexyloxyethyl)-2-devinyl pyropheophorbide-a (HPPH)[16]	Cationic porphycene[17]
	
Silicon phthalocyanine (Pc4)[18]	Cationic bacteriochlorin[19]
	

Anticancer PS	Antimicrobial PS
Hypericin[20]	Cationic hypericin derivative[21]
	
Pd-bacteriopheophorbide derivative (TOOKAD soluble)[22]	Cationic fullerene[23]
	
5-aminolevulinic acid (ALA)	Protoporphyrin IX (PpIX)

ALA-induced PpIX is widely used both for anticancer[24] and antimicrobial[25] PDT applications