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REVIEW ARTICLE

Photophysics and photochemistry of photodynamic therapy: fundamental aspects

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Abstract Photodynamic therapy (PDT) is a treatment modality for cancer and various other diseases. The clinical protocol covers the illumination of target cells (or tissue), which have been loaded with a photoactive drug (photosensitizer). In this review we describe the photophysical and primary photochemical processes that occur during PDT. Interaction of light with tissue results in attenuation of the incident light energy due to reflectance, absorption, scattering, and refraction. Refraction and reflection are reduced by perpendicular light application, whereas absorption can be minimized by the choice of a photosensitizer that absorbs in the far red region of the electromagnetic spectrum. Interaction of light and the photosensitizer can result in degradation, modification or relocalization of the drug, which differently affect the effectiveness of PDT. Photodynamic therapy itself, however, employs the light-induced chemical reactions of the activated photosensitizer (triplet state), resulting in the production of various reactive oxygen species, amongst them singlet oxygen as the primary photochemical product. Based on these considerations, the properties of an ideal photosensitizer for PDT are discussed. According to the clinical experience with PDT, it is proposed that the innovative concept of PDT is most successfully implemented into the mainstream of anticancer therapies by following an application-, i.e. tumor-centered approach with a focus on the actual clinical requirements of the respective tumor type.

Keywords Photodynamic therapy · Photophysics · Photochemistry · Photosensitizer · Singlet oxygen

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Introduction

Photodynamic therapy (PDT) represents a modality for the removal of harmful or unwanted cells. The treatment protocol covers the (systemic) administration of a light-sensitive but, per se, harmless drug (photosensitizer, PS), followed by uptake of this compound and more or less selective accumulation/retention in the target cells or tissue and the subsequent irradiation of the photosensitizer with light of the appropriate wavelength. Upon absorption of quanta, reactive oxygen species (ROS) are formed, which oxidize intracellular molecules and thereby destroy cells [1–4].

By now, PDT with various photosensitizers has been clinically applied and approved for the treatment of several malignant and non-malignant diseases (see Table 1). While PDT has focused on cancer since 1975, it has become clear within the past decade that PDT is endowed with several favorable features for the treatment of microbial infections. PDT has achieved significant inactivation of gram-positive and gram-negative bacteria and is a promising tool to overcome the problem of (multi-) drug resistance of bacteria and to treat viral, fungal and parasitic infections (antimicrobial PDT has been reviewed in [5, 6]).

In clear contrast to chemotherapy, PDT appears to be free of major side-effects. After a treatment, patients have to stay in subdued light for a certain period and must avoid exposure to bright sunlight for some days thereafter. However, to avoid increased skin sensitivity and to

Table 1 Overview of photosensitizing agents currently used in clinical application

Generic name	Chemical name	Approval	(Potential) indications
Benzvix	Benzyl δ-aminolevulinic acid	No[a]	Gastrointestinal tumors
BOPP	Boronated protoporphyrin	No[a]	Brain tumors
Hexvix	Hexyl δ-aminolevulinic acid	Yes[b]	Bladder cancer
Levulan	δ-Aminolevulinic acid	Yes[c]	Actinic keratosis
		No[a]	Head and neck tumors, gynecological tumors
Lu-Tex	Lutetium texaphyrin	No[a]	Cervical cancers, prostate cancer, brain tumors
Metvix	Methyl δ-aminolevulinic acid	Yes[d]	Basal cell carcinoma
Pc-4	Phthalocyanine-4	No[a]	Cutaneous/subcutaneous lesions of diverse solid tumor origins
Photochlor	2-(1-Hexyloxyethyl)-2-devinyl pyropheo-phorbide-alpha	No[a]	Basal cell carcinoma
Photofrin, Porfimer sodium	Hematoporphyrin derivative, polyhematoporphyrin	Yes[e]	Barrett's high-grade dysplasia
		Yes[f]	Cervical dysplasia and cervical cancers
		Yes[g]	Endobroncheal cancer
		Yes[h]	Esophageal cancer
		Yes[f]	Gastric cancer
		Yes[I]	Papillary bladder cancer
		No[a]	Cholangiocarcinoma, brain tumors
Photosense	Aluminum phthalocyanine	Yes[j]	Head and neck cancer
SnET2	Tin ethyl etiopurpurin	No[a]	Breast cancer, basal cell carcinoma, Kaposi sarcoma, prostate cancer
Foscan, Temoporfin	Meso-tetrahydroxy-phenyl chlorine	Yes[k]	Palliative therapy of head and neck cancer
		No[a]	Prostate cancer, pancreas cancer, cholangiocarcinoma
Visudyne, Verteporfin	Benzoporphyrin derivative monoacid ring A	No[a]	Basal cell carcinoma

[a] Not yet approved
[b] European Union (EU)
[c] EU, USA
[d] EU, Australia
[e] Canada, EU, UK, USA
[f] Japan
[g] Canada, Denmark, Finland, France, Germany, Ireland, Japan, The Netherlands, UK, USA
[h] Canada, Denmark, Finland, France, Ireland, Japan, The Netherlands, UK, USA
[I] Canada
[j] Russia
[k] EU, Iceland, Norway

minimize other possible side effects a precise dosimetry of both drug concentration and light application is required [7, 8].

While the mode of action of several PSs has been studied intensively, dosimetry of light application is somewhat more difficult as the light passes through different media (glass fibers/air/water/tissue) with varying optical properties. Additionally, absorption of (macro-) molecules and the quantum efficiency for singlet oxygen production and/or ROS formation have to be considered for correct dosimetry [9–11].

We give an overview of the photophysical and photochemical processes involved in PDT. Light interaction with tissue and molecules, photochemical processes that lead to production of cytotoxic substances, and photodegradation of the PS molecules are discussed. Based on these considerations, the properties of an ideal photosensitizer are proposed.

Light interaction with tissue

When discussing light delivery for PDT one has to consider body tissues as a bulk medium. Light propagation through such a medium implicates processes of refraction, reflection, absorption and scattering (Fig. 1).

Reflection from the interface between two media and refraction are governed by Fresnel's law and Snell's law, respectively, and their impact on the loss of intensity are determined by the relative values of their refractive indices [11]. Since both processes are proportional to the angle of

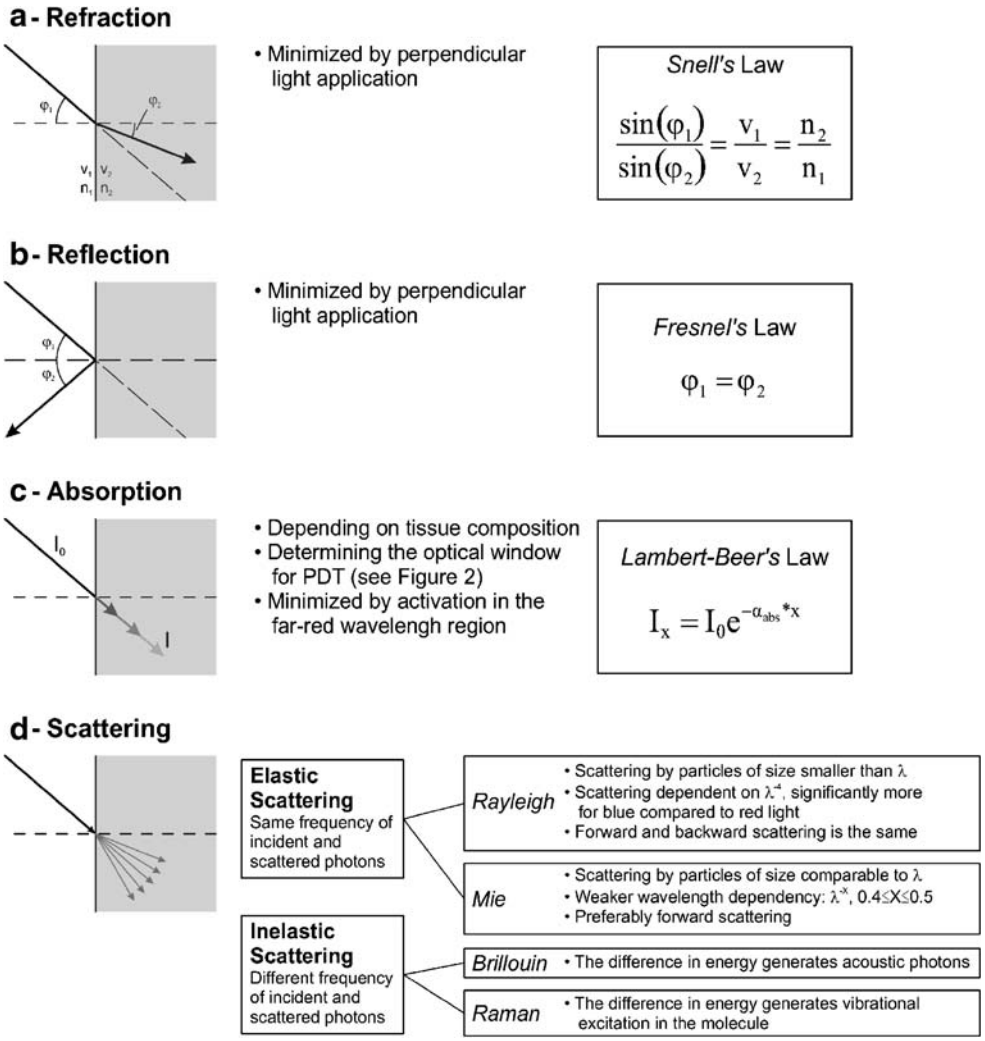

Fig. 1 Interactions between light and tissue

incidence, one can minimize them by applying the light beam perpendicular to the interface between the two media.

Scattering of light in tissue has the most pronounced effect on light intensity and directionality. Scattering causes, together with refraction, a widening of the light beam, resulting in a loss of fluence rate (given as power per unit area of light in [$W.m^{-2}$]) and a change in the directionality of the light beam. Scattering in tissue is quite complex (see Fig. 1). Inelastic scattering (Brillouin scattering and Raman scattering) does not seem to play an important role in this case. For elastic scattering, neither Rayleigh scattering nor Mie scattering completely describes the effects observed in tissue; here, photons are mainly scattered in the forward direction. The experimentally observed scattering shows weaker wavelength dependence than that predicted by Rayleigh's theory, but the effect is stronger than that given by Mie scattering [10, 12].

Besides scattering, absorption of light quanta is most relevant for the loss of light intensity with the penetration depth. The reduction in intensity caused by both processes can be mathematically described by an exponential function similar to Lambert–Beer's law. The intensity at a given depth x can be calculated by equation (1), with I_x being the intensity at depth x and I_0 the intensity at the media interface. The parameters α_{abs} and α_{sca} represent the absorption and scattering coefficients, respectively.

$$I_x = I_0 e^{-(\alpha_{abs}+\alpha_{sca})x} \quad (1)$$

The most important chromophores in tissue are water, oxyhemoglobin (HbO_2) and deoxyhemoglobin, melanin and cytochromes. The absorption spectra of these molecules define the optical window for PDT in tissue (Fig. 2, [9, 11, 13]). One should keep in mind that hemoglobin (Hb) and HbO_2 show different absorption in the range of 600–800 nm, which is commonly used for PDT. In vivo, there might be a significant difference in the amounts of HbO_2 and Hb between non-tumor and tumor tissue due to a possible lower oxygenation and pH of the latter [14].

For PDT on solid tumors, the effective penetration depth is of great relevance. It is defined by the depth x, where $I_{(x)}$ decreases to 37% of I_0 [11]. For clinical treatment with

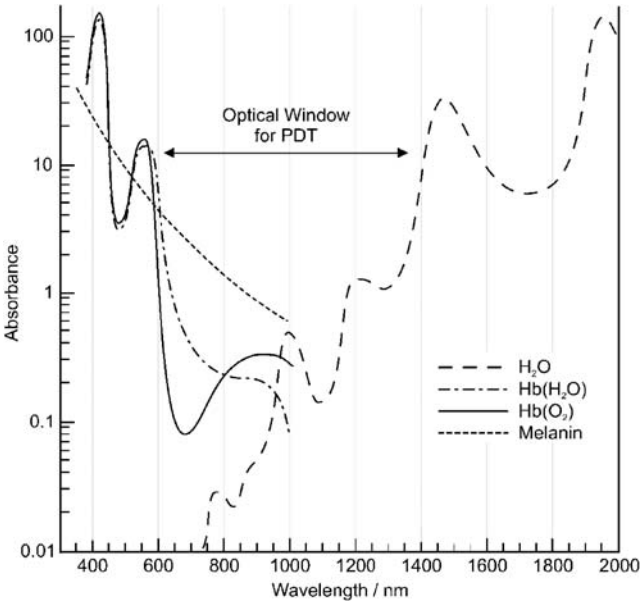

Fig. 2 The 'optical window' for PDT. Absorption of light by tissue chromophores limits the wavelength range suitable for PDT to about 650–1200 nm. This range is further reduced, since PS triplet-state molecules excited by $\lambda > 850$ nm have too low energy to produce singlet oxygen efficiently (see text)

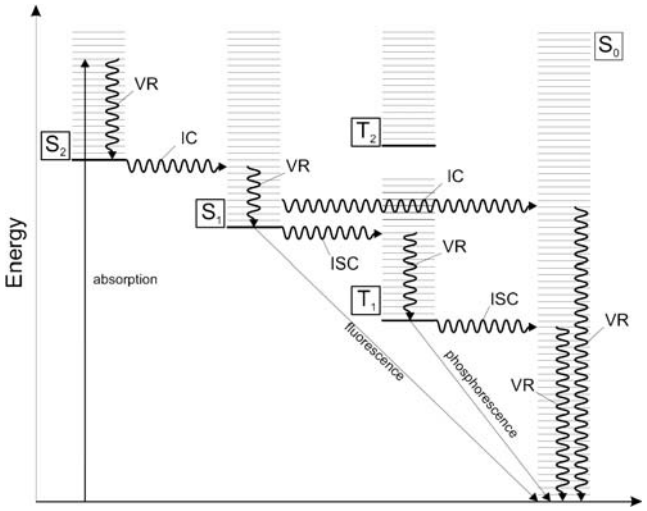

Fig. 3 Jablonski diagram of the primary photophysical processes. The *ordinate* indicates increasing energies of the electronic states; on the *abscissa* the latter are grouped by spin multiplicity. *IC* internal conversion, *ISC* intersystem crossing, S_x singlet states, T_x triplet states, *VR* vibrational relaxation

Photofrin, a hematoporphyrin derivative for excitation at 630 nm, the light penetration depth is approximately 3–5 mm, depending on the tissue [15]. The use of PSs with absorption peaks at wavelengths >700 nm (or even higher) should, at least, double the penetration depth and thus enable treatment of thicker tumors [16, 17]. However, the upper limit for the excitation wavelength is given for the minimal energy required for singlet oxygen production (see below).

Light interaction with molecules

A photosensitizer molecule in its normal state is characterized by paired electrons with a total spin of S=0 and a spin multiplicity of 1. This configuration is called the singlet state and the configuration with all electrons in their energetically lowest possible orbital is called the ground state S_0. Upon absorption of a light quantum with the appropriate energy, one of these electrons is shifted to a previously unoccupied orbital of higher energy. Depending on the energy transferred, the photosensitizer undergoes a transition to an excited singlet state S_x (with x=1, 2, 3, ..., in the order of increased energetic state). Any excited state S_x is further sectioned in so-called vibrational levels with increasing energy. As any excited state is energetically less preferable than the ground state, the molecule returns to S_0 after a short period of time. This deactivation of S_x can occur by several mechanisms (see Fig. 3, [11, 18]).

An electron in a high vibrational level of an excited state (e.g., S_1) rapidly falls to the energetically lowest level of that state. This process is called vibrational relaxation (VR) and the energy is dissipated as heat. If an electron has been boosted to a higher energetic state S_x, it can, following VR, fall to the first excited singlet state S_1. Molecular relaxation to S_0 will occur by either emission of a secondary photon (i.e., fluorescence emission) or by heat dissipation. Fluorescence emission will always start from the lowest vibrational level of S_1. Therefore, the general form (not the intensity distribution) of the emission spectrum does not depend on the light wavelength used for excitation. Furthermore, the emitted quanta have lower energies (and wavelengths) than those used for excitation of the molecule [11, 18].

From S_1 the molecule may cross to an isoenergetic level of the triplet state, T_1, where two electrons are unpaired and have the same spin. This non-radiative process is called intersystem crossing (ISC). Most photosensitizers have a high quantum efficiency for that transition. The process violates the rule of no spin change during a change of an electronic state ('spin-forbidden transition'). As a consequence of spin-orbit coupling (i.e., interaction with the electron's magnetic momentum and its spin-motion magnetic momentum), such transitions take place with a certain probability. After a rapid VR within the T_1 energetic level, a radiative relaxation to S_0, a process called phosphorescence (i.e., emission of an electromagnetic quantum), may occur. Here, another switch of the electron's spin takes place. Triplet states are generally characterized by a relative long lifetime (up to seconds). Thus, as an alternative to phosphorescence, photochemical reactions may be energized from tripled-state molecules [19]. An overview of photophysical processes of excited photosensitizer molecules is given in Table 2.

Table 2 Characteristics of primary photophysical/photochemical processes during PDT (*E* energy, k_x rate constant, S_x singlet-state molecule, T_x triplet-state molecule, *[X]* concentration/chemical activity of compound X)

Process	Reaction	Timescale [s]	Constant
Excitation	$h\upsilon + S_0 \rightarrow S_1, S_2, ..., S_x$	$\sim 10^{-15}$–10^{-12}	k_{Exc}
Internal conversion	$S_x, ..., S_2 \rightarrow S_1 + \text{heat}$	$\sim 10^{-13}$–10^{-10}	$k_{IC}*[S_x]$
Internal conversion	$S_1 \rightarrow S_0 + \text{heat}$	$\sim 10^{-13}$	$k_{IC}*[S_1]$
Intersystem crossing	$S_1 \rightarrow T_1 + \text{heat}$	$\sim 10^{-7}$	$k_{ISC}*[S_1]$
Chemical reaction	$S_1 \rightarrow S_0 + \text{reaction}$		$k_{React(S)}*[S_1]$
Fluorescence	$S_1 \rightarrow S_0 + h\upsilon_{Fluor}$	$\sim 10^{-11}$–10^{-8}	$k_{Fluo}*[S_1]$
Intersystem crossing	$T_1 \rightarrow S_0 + \text{heat}$	$\sim 10^{-2}$–10^{2}	$k_{ISC(T)}*[T_1]$
Phosphorescence	$T_1 \rightarrow S_0 + h\upsilon_{Phosp}$	$>10^{-6}$	$k_{Phosp}*[T_1]$
Chemiluminescence	$E + S_0 \rightarrow S_1 \rightarrow S_0 + h\upsilon_{Chem}$	$>10^{-6}$	$k_{Chem}*[S_1]$
Chemical reaction	$T_1 \rightarrow S_0 + \text{reaction}$		$k_{React(T)}*[T_1]$

Photochemical processes in PDT

Photosensitizer molecules have a high probability of triplet-state formation after excitation. Activated PSs in the excited triplet state can induce chemical changes in a neighboring molecule via two competing pathways, called type-I and type-II photochemical reaction (Fig. 4). Type-I photochemical reaction means the transfer of electrons (or protons) to oxygen or other adjacent molecules to form a radical anion or cation, respectively. These radicals are likely to react with molecular oxygen to produce ROS. Type-I photochemical reactions frequently result in the formation of superoxide anions by transfer of an electron from the PS to molecular oxygen [19, 20]. Superoxide anions are not very reactive in biological systems, but they can react to produce hydrogen peroxide (H_2O_2). Hydrogen peroxide can easily pass through biological membranes. Since damage set by H_2O_2 is not restricted to one cellular compartment, hydrogen peroxide is quite relevant in producing cellular damage [21, 22].

At higher concentrations, hydrogen peroxide can react with superoxide anions to form the very reactive hydroxyl radical (Haber Weiss reaction in Fig. 4). With a redox potential of E_0=1.35 V) it can attack and oxidize any molecule within a cell, and the activation energies for these reactions are very low. Hydroxyl radicals easily diffuse through membranes; thus, as for hydrogen peroxide, damage is not limited to one cellular compartment [21, 23]. In the presence of metal ions such as iron or copper, hydroxyl radicals can also be produced by the Fenton reaction; see Fig. 4 [23–25].

The transfer of energy (not electrons) to molecular oxygen has been named type-II photochemical reaction [20]. Molecular oxygen has a special attribute, since the

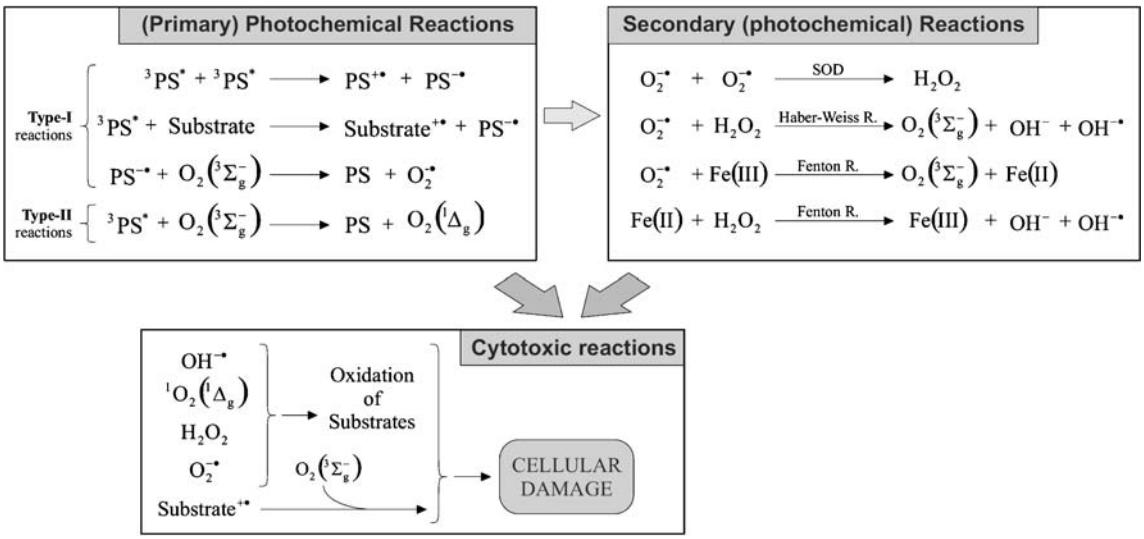

Fig. 4 Overview of photochemical reactions during PDT. Several types of primary and secondary photochemical reactions cause production of reactive oxygen species and dose-dependent cellular damage. H_2O_2, hydrogen peroxide; $O_2(^1\Delta_g)$, singlet oxygen (excited state); $O_2(^3\sum_g^-)$, triplet oxygen (ground state); $O_2^{-\bullet}$, superoxide anion; $OH^{-\bullet}$, hydroxyl radical; *SOD*, superoxide dismutase; $X^{-/+}$, anion/cation species; $X^{\bullet}$, radical species

triplet configuration ($^3O_2$) represents the ground state of the molecule. During energy transfer from a type-II photochemical reaction, the very reactive singlet oxygen ($^1O_2$) is formed (for details see next section). Both type-I and type-II photochemical reactions occur in parallel, and the ratio depends on several parameters, with the photosensitizer used and the oxygen concentration being the most important [19, 20]. For most photosensitizers employed in PDT, the type-II photochemical reaction represents the dominant process [26–28].

Singlet oxygen in PDT

In its ground state, molecular oxygen has two unpaired electrons with parallel spins in two degenerate antibonding orbitals, which gives a spin multiplicity of 3. Thus, without activation, molecular oxygen is in the triplet state. Singlet oxygen represents an excited singlet state characterized by paired electrons (with opposite spins) in the outer orbital.

In the sequence of PDT-mediated generation of singlet oxygen, a PS is excited to a singlet state (S_1) with an energy of approximately 170–190 kJ.mol^{-1}. This energy corresponds to a wavelength of 620–690 nm. After ISC, a triplet-state PS with an energy of about 110–130 kJ.mol^{-1} is created. This molecule transfers energy to oxygen molecules, causing the excitation of triplet-state oxygen to singlet oxygen [29] with an energy of approximately 94.5 kJ.mol^{-1} above the ground state, which corresponds to 'quantum equivalents' of about 1,270 nm [30–32]. In any case, the triplet-state photosensitizer must exceed this energy; it has been published that, due to thermal losses within the photophysical sequence, the upper wavelength limit for PDT efficiently producing $^1O_2$ is 850 nm [14].

Nearly all PSs employed in PDT give high quantum yields (ϕ) for singlet oxygen formation (~0.3 to 0.5) [14]. The quantum yield for a pure type-II photochemical reaction can be calculated by equation 2 (for abbreviations see Table 2). As can bee seen from this formula, the singlet oxygen yield is dependent on the rate of chemical reaction of the triplet-state PS and is reduced by processes such as phosphorescence, ISC from triplet to singlet states, or chemical reactions of the excited singlet-state PS.

$$\Phi = \frac{k_{\mathrm{React(T)}}[\mathrm{T_1}][\mathrm{S}]}{k_{\mathrm{Phosp}}[\mathrm{T_1}] + k_{\mathrm{ISC(T)}}[\mathrm{T_1}] + k_{\mathrm{React(T)}}[\mathrm{T_1}][\mathrm{S}]} \quad (2)$$

Singlet oxygen can, as an uncharged molecule, diffuse through the cytoplasm and biological membranes. The lifetime of singlet oxygen (τ) in pure water has been estimated to be 3×10^{-6} s [14, 22]. In the cytoplasm the lifetime is reduced by more than one order of magnitude, as a result of the presence of reacting molecules, to a maximum lifetime of 1×10^{-7} s [33–37]. As the diffusion constant D for singlet oxygen in cells is 1.4×10^{-5} cm^2.s^{-1} [38], the maximum radius of reactive action is 30 nm (see Fig. 5), as calculated by equation 3 [35]:

$$\delta = \sqrt{6\mathrm{D}\tau} \quad (3)$$

As a consequence, the intracellular localization of the PS greatly determines the site of cellular damage set by PDT.

Photodegradation, photomodification and photorelocalization of photosensitizers

Upon illumination, all photosensitizers are chemically modified or even degraded [39–41]. This results from a direct attack of ROS or singlet oxygen on the PS molecules, and these processes have a high probability of taking place, since the PS, of course, is, in the biological environment, in close proximity to the reactive molecules. In experimental spectroscopy, photodegradation and photomodification can be identified by a lowering of absorbance or fluorescence emission [42].

Photodegradation is the chemical destruction of a PS molecule that results in the splitting of the PS into small fragments, which do not absorb in the visible spectral region. As a consequence, photodegraded PS molecules lose their function within PDT [36, 39, 41]. In contrast, after photomodification (also referred to as photoproduct formation), the chemical change is less profound. Here, radical attack leads to modification of side groups and/or the molecular skeleton of the PS. Photomodification of

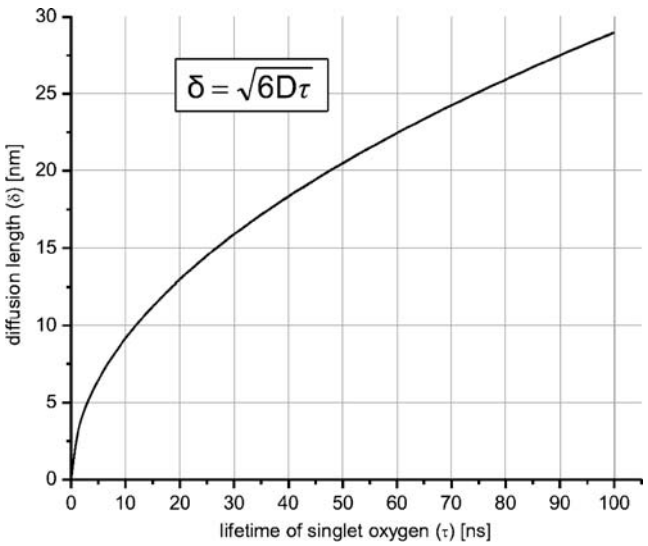

Fig. 5 Diffusion length of singlet oxygen. Based on the estimated values for the singlet oxygen lifetime (τ<100 ns) and its diffusion constant (D=1.4×10^{-5} cm^2.s^{-1}), the intracellular diffusion length (δ) of singlet oxygen is estimated

porphyrins such as protoporphyrin IX (PPIX) typically creates chlorines, accompanied by a red shift of fluorescence. For PPIX, it has been published that the photoproducts are more effective photosensitizers than their originating molecules [41, 43–46].

Light exposure can also change the intracellular localization of a PS molecule, a process termed photorelocalization [47–50]. It is significant after rather low exposures, or multiple exposures, where the first illuminations might contribute to a change in the mobility of the PS within the cell [48, 51]. For some hydrophilic sensitizers, photorelocalization from lysosomes to the nucleus occurs. The resulting damage to the DNA increases the quantum yield of cell destruction of these PSs [48, 50–53].

Properties of the ideal photosensitizer

The theoretical considerations outlined above allow one to define the ideal photosensitizing agent. For this, not only the chemical, physical and 'biological' properties, but also characteristics that meet the patient's needs, have to be taken into account. The substance actually employed might not fulfill all properties mentioned in the following. For that, the clinician has to select a PS that seems most suitable for the patient's needs and with the best clinical features, albeit the particular PS may suffer from several disadvantages.

Photophysical and chemical features

Among the photophysical properties, the wavelength for activation and the quantum efficiency for the triplet state are most important. The maximum absorption of the PS should correspond to the window between 650 nm and 850 nm, where, on one hand, tissue penetration is quite high, and, on the other hand, the energy of the triplet state is sufficient for singlet oxygen production. A high quantum efficiency for ISC, and a long-lasting triplet state, is most likely to result in a high efficiency for formation of cytotoxic products (see above and [11]). However, for diagnosis of tumor tissue by fluorescence or by fluorescence-guided adjustment of the irradiation area [54], dyes have to exhibit a certain degree of fluorescence emission [55, 56]. The same is valid for evaluation of the treatment's success by comparison of the differences in fluorescence before and after PDT [57].

Photostable dyes, i.e., photosensitizers with low photodegradation, are, on one hand, optimal for extended irradiation times. On the other hand, depending on the biological clearance of the PS from cells/tissue, low photobleaching may cause photosensitivity of the patient for an extended period of time [58, 59].

For the administration of photosensitizers, the solubility is of great importance. Amphiphilicity ensures both transportation in the blood, without precipitation or aggregate formation, and effective penetration through the lipid layer of the cell membrane. Furthermore, PS molecules should not stack, i.e., form aggregates, as this can reduce their ability to absorb light and decrease the lifetime and quantum yield of the excited triplet state [11].

Pharmacokinetics and toxicity

The most important feature of a PS in this context is selectivity, which means the capability of the agent to accumulate in the target (tumor) tissue but not in normal (healthy) cells, thus restricting photo-induced damage to surrounding tissue [11, 58]. Depending on the model system, this phenomenon has been described to be caused by one of the following: (1) high vascular permeability of the PS in tumors, (2) low pH in the tumor's extracellular compartment, (3) high affinity of the PS for proliferating endothelial/cancer cells (i.e., increased low-density lipoprotein receptor expression), (4) reduced lymphatic drainage in tumors, or (5) a high number of tumor-associated macrophages that exert phagocytosis and monomerization of aggregated PS [14, 60]. Coupling of photosensitizers to tumor-specific antibodies or other carriers can enhance the selectivity of a photosensitizing agent [61, 62].

After PDT, the (biological) elimination by photodegradation, metabolization or excretion of the PS should be as rapid as possible to minimize the period of general photosensitivity. However, for some indications (for example, multiple photodynamic treatments), the retention of PS molecules in the target tissue might be necessary to avoid re-administration of the drug [11, 58, 59].

An ideal PS should not be toxic per se (i.e., without irradiation) in the concentration range applied in PDT. Neither photodegradation nor cellular metabolization of the PS should generate toxic degradation products. Most importantly, the PS should not cause mutagenic effects, irrespective of being irradiated or not. This depends in turn on a lack of tendency to (re-)localize in the nucleus [58].

Patient-oriented and clinical characteristics

Among these characteristics, minimal side effects and safety of the PDT protocol employed for a specific PS are stated [58]. Administration of the drug must not cause any toxic effect, allergic reaction or other side-effect. The overall PDT protocol should not cause serious life-threatening complications. Pain during irradiation, as observed when 5-aminolevulinic acid (ALA)-induced PPIX is used for PDT, should be avoided or counteracted if possible [63].

Once the diagnosis of a certain tumor type is assured, the PDT protocol employed for a PS must be reliable. This includes that the respective PS must be commercially available in a certified quality and composition. Last, but not least, the ideal PS should not prevent the use of other supplementary forms of treatment, e.g., surgery, radiation therapy or chemotherapy [58].

Future directions

It is more than 40 years since PDT was first used in oncology. Nevertheless, today, the potential of PDT, except in some specialties of medicine, e.g., dermatological oncology and ophthalmology, is neither widely known nor generally accepted as a powerful alternative, not to mention that PDT is far from representing a mainstream technology in oncology [64]. In the following, some obstacles to the widespread use and acceptance of PDT in the clinic are highlighted.

Regarding the technique itself, it seems probable that optimization of the various parameters which influence the outcome and success of the treatment, such as drug dose, drug-to-light interval, light dose, dosimetry, and choice of photosensitizer, requires extensive efforts. Effectively, this is only achieved in specialized clinical centers; otherwise, clinicians have to consult the basic scientific literature, since only a small number of photosensitizing agents have been approved for clinical use and are accompanied with detailed protocols for their efficient application [64].

The most serious drawback of PDT as a frontline anti-cancer therapy lies in the fact that large, controlled, comparative, randomized clinical trials either have not been undertaken yet [59] or could not prove a significant advantage over conventional approaches [64]. The latter result might correspond to the fact that PDT has been tested mostly on patients with advanced cancers that are refractory to other therapies. This implies that possible positive effects of PDT on a local level cannot be honored adequately, since cancer as a systemic disease is probably not affected to a significant extent [1]. Based on this situation, efforts to establish the potential of PDT in the clinical application might consider the arguments outlined in the following:

(1) Careful attention to physics and dosimetry of PDT will help to minimize general toxicity and side-effects [59]. The lack of dosimetric control has significant clinical ramifications, since, without proper dosimetry, it is not possible to explain treatment failures due to a shortage of PS, light or oxygen; in other words, we will not tap the full potential of even the most powerful and promising PS until accurate and reliable real-time dosimetry is available [58].

(2) Since the choice of drug type and dose, activation wavelength, and the drug-to-light interval can improve effectiveness and reduce side-effects, the treatment protocols have to be optimized and standardized [59]. Accordingly, the development of new sensitizers or protocols should start with consideration of the requirements of the actual clinical situation and should seek for optimization of each component with regard to the needs of the intended goal, i.e., the particular type of tumor. In other words, the research has to be shifted from a sensitizer-centered approach to a tumor type-centered one.

(3) PDT research should not only be focused on development and patents of new photosensitizers. Rather, focus might be placed on the development and promotion of complete packages consisting of a drug, a light source, and a specific protocol optimized for a particular application.

Careful evaluation of the potential clinical fields for PDT and according optimization and standardization with respect to the above-mentioned arguments will contribute to the establishment of PDT, pursuant to the statement that "PDT is not only a class of drugs, it is a fascinating innovative concept." [65]

Conclusion

– For PDT, dosimetry of both the photoactive drug and the irradiation is required to obtain the optimal clinical outcome and to avoid side-effects. With spectroscopic analysis, the amount of a PS in the target can be determined. The dosimetry of light used for illumination is somewhat more difficult, since several factors determine the light intensity within tissue that have to be considered, among them reflection, refraction and absorption due to tissue chromophores and other (macro-) molecules. For absorption within tissue, the spectra of hemoglobin, melanin and water are most relevant and determine the optical window for PDT. To achieve deep penetration into tissue, dyes absorbing wavelengths >650 nm are optimal. However, the energy required for effective singlet oxygen production (corresponding to PS triplet states following illumination with wavelengths of approximately 850 nm) represents the upper wavelength limit for PDT irradiation (at least for most PSs, which effectively induce a type-II photochemical reaction).
– Among the cytotoxic substances produced by triplet state PSs, singlet oxygen (from type-II photochemical reactions) represents the most important effector molecule in PDT. As diffusion lengths of $^1O_2$ are rather

short (maximum 30 nm), the intracellular localization of the PS determines the initial site of damage. The process of photorelocalization, i.e., a change in the intracellular localization during illumination, has to be considered.

– Radicals produced by PDT can attack the PS and thus lead to photodegradation and photomodification of the dye. The first leads to inactivation of the specific molecule. Photomodification may induce secondary photoproducts which are, in some cases, better PSs than the molecules they originated from.
– The ideal photosensitizer is characterized by (1) high light absorption within the optimal window for PDT (approximately 650 nm to 850 nm), (2) a high quantum yield for the triplet state, (3) a rather high photostability, (4) amphiphilicity, (5) high selectivity for the target tissue, (6) a low tendency to form aggregates, (7) no dark toxicity and mutagenicity, (8) no side-effects, and rapid clearing from the body after PDT, and, (9) a safe and reproducible PDT protocol. An optimal PS for all PDT applications does not exist. Therefore, the clinician has to evaluate the properties of the available photosensitizing agents and choose the substance for the specific indication according to those characteristics in order to provide the most advantageous clinical features.
– PDT acceptance and propagation ultimately requires (1) proper dosimetry of the photosensitizing agent, light fluence and oxygen supply in the tumor, (2) a shift from a sensitizer-centered approach to a tumor type-centered one, and, (3) development and promotion of complete treatment packages consisting of a drug, a light source, and a specific protocol suitable for a particular application.

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