Photodynamic Therapy (PDT)

2. Old and New Photosensitizers

Bhaskar G Maiya

In this article, key aspects related to the design and development of drugs for use in photodynamic therapy are considered. The chemistry, photochemistry and photobiology of porphyrinic and non-porphyrinic photosensitizers, as relevant to their application in clinical PDT are discussed in detail.

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Introduction

In the first article of this series,[1] we discussed the basic principles of photodynamic therapy (PDT) and also the relevant physical and biochemical mechanisms associated with it. PDT involves irradiation of a tissue-bound photosensitizer which goes to its triplet excited state and generates, upon energy transfer to oxygen, the cytotoxic singlet oxygen ($^1O_2$) resulting in cell necrosis. Thus, the photosensitizer plays a key role in this modern cancer management protocol. Naturally, the qualities of the photosensitizer matter most in realizing an efficient photodynamic action. In this article, we focus on the drug design strategies involved in PDT. Recall, however, that the drug in PDT is actually 'photosensitizer + light'. Therefore, besides the chemistry, photochemistry and photobiology of old and new photosensitizers, this article also discusses, albeit briefly, the 'light-related' aspects (i.e., light sources, irradiation strategies, etc.) of PDT.

Qualities of a Good Photosensitizer for PDT

By definition, photosensitizers are compounds that are capable of absorbing light of specific wavelength (chromophores) and transforming it into useful energy. In the case of PDT, this would involve the production of lethal cytotoxic agents. Going by this definition, there are hundreds of natural and synthetic

Previous part of this series
1. The Basic Principles of PDT, *Resonance*, Vol. 5, No. 4, p. 6, 2000.

For visible wavelength, hemoglobin and melanin are the primary endogenous chromophores in the skin.

dyes ranging from plant extracts to complex synthetic macrocycles that, in principle, can function as photosensitizers for PDT. However, a good PDT photosensitizer is expected to fulfill the following minimum criteria. It should

(a) have a strong absorbance with high extinction coefficient ε at longer wavelengths (600-850 nm) where tissue penetration of light is at a maximum and still energetic enough to produce $^1O_2$,

(b) have excellent photochemical reactivity, with high triplet state yields (Φ_T) and long triplet state lifetimes (Φ_T) and be able to effectively produce $^1O_2$ and other reactive oxygen species,

(c) have minimal dark toxicity and only be cytotoxic in the presence of light,

(d) be preferentially retained by the target tissue,

(e) be rapidly excreted from the body, thus inducing a low systemic toxicity, and

(f) be chemically pure and of known specific composition.

Among these six major guidelines, the first two are the most important ones and the rationale behind such stringent optical and photophysical criteria are discussed below.

For visible wavelength, hemoglobin and melanin are the primary endogenous chromophores in the skin. Hemoglobin has strong absorption bands near 418, 542 and 577 nm and melanin absorbs over a broad range with a maximum in the 300-500 nm region, but decreasing steadily in the visible range. Thus, it is necessary that the illumination wavelengths be greater than 600 nm to have significant light penetration past the dermal capillary plexi. At wavelengths longer than ~ 1000 nm, light absorption by water molecules becomes substantial. Therefore, there is a 600-1000 nm 'therapeutic window' that permits significant light penetration into the tissue as illustrated in *Figure* 1. Within this window, longer wavelengths penetrate more deeply because of decreasing tissue absorbance and decreasing light scattering. However, for wavelengths greater than 850-900 nm, the photons

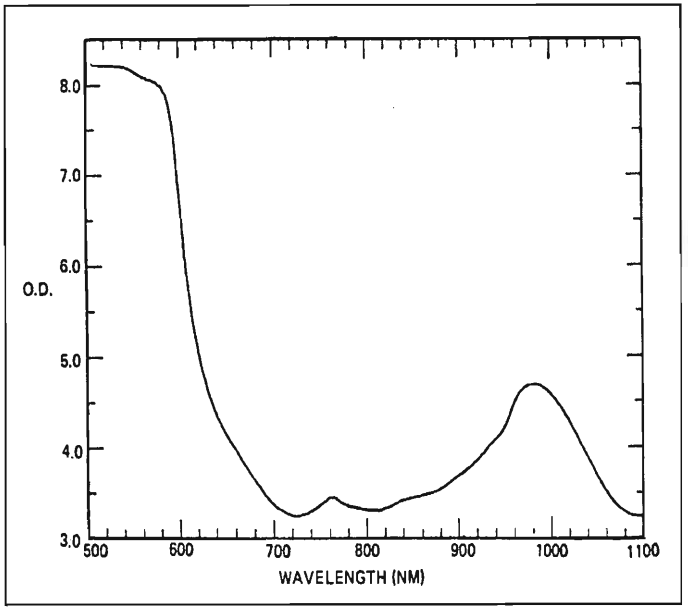


Figure 1. Illustration of the 'absorption spectrum of a human hand' that clearly shows the PDT 'therapeutic window' Tissue chromophores (viz., amino acids, nucleotides, heme, melanin, etc.) absorb light of ca.< 600 nm (UV-visible). At longer wavelengths, the all-pervading water molecules start absorbing light (infra-red). Thus, an ideal photosensitizer for PDT has to absorb light in the pure visible region of the electromagnetic spectrum, towards the red end.

may not have sufficient energy to participate in photochemical reactions. Thus, the available wavelengths for photodynamic sensitizers are 600-850 nm (red light). In general, the use of sensitizers with stronger absorption (large extinction coefficient) offers the possibility to inject smaller drug doses.

Besides being able to strongly absorb red-light, a good PDT photosensitizer should also possess favorable triplet state properties. The effectiveness of photodynamic activity has notable dependence on the triplet state quantum yield and its lifetime. A long triplet state lifetime and a high triplet quantum yield are both considered as preconditions for efficient photosensitization. This is obvious because longer life times permit diffusion of the reactants (i.e. the triplet sensitizer and molecular oxygen) to form the initial encounter complex which ultimately relaxes to the products (i.e. ground state photosensitizer and $^1O_2$). Similarly, copious amount of $^1O_2$ can be generated if the precursor triplet itself is produced in large quantities. Indeed, the quantity S_Δ, which is the ratio of the singlet oxygen yield and the triplet quantum yield ($S_\Delta = \Phi(^1O_2)/(\Phi_T)$, is a well acknowledged photophysical bench mark for a good PDT photosensitizer.

Porphyrins, by virtue of their robust aromatic 18π-electron system and their rich redox and photophysical activity as well as biocompatibility, fulfill most of the criteria for an ideal photosensitizer.

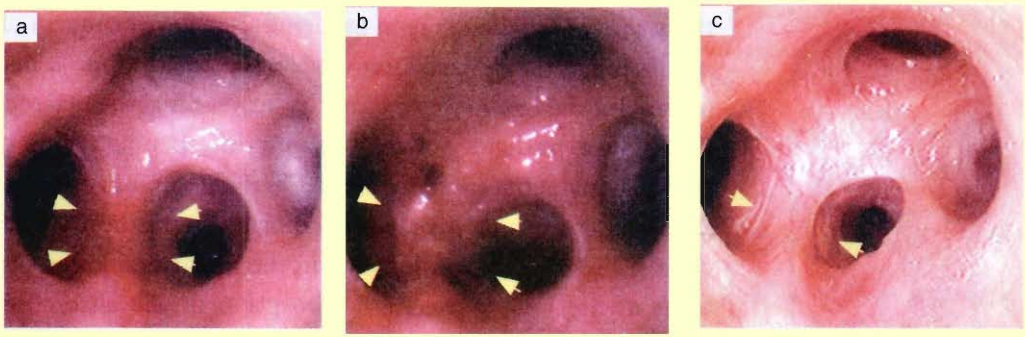
Finally, it is not only triplet lifetime and yield, but also its energy that is an important factor which enables a given sensitizer to be a good PDT agent. This is because compounds having triplet state energy which is about 8 kJ mol^{-1} lower than that of $^1O_2$ (94 $kJmol^{-1}$) are generally unable to transfer their excitation energy to molecular oxygen. Therefore, energy of the first excited triplet state of a photosensitizer should be approximately greater than 86 kJ mol^{-1} to promote a type II mechanism; the corresponding ground state absorption for such photosensitizers does not, usually, extend beyond ca.850 nm.

While it is imperative that any good PDT agent should be minimally toxic in the dark and should be activated only in the presence of light (criterion c), criteria (d) to (f) are true for any drug, light activated or not.

Old and New Porphyrin-based PDT Agents

Porphyrins, by virtue of their robust aromatic 18π-electron system and their rich redox and photophysical activity as well as biocompatibility, fulfill most of the criteria for an ideal photosensitizer. Indeed, hematoporphyrin derivative (HpD or Photofrin II®, see *Figure* 3 in the first article of this series) is currently being marketed as an anti-cancer drug. HpD was originally synthesized by combining hematoporphyrin with 95% sulphuric acid in acetic acid at room temperature and neutralizing the resulting mixture with an aqueous base. This procedure led to the formation of a complex mixture of dimers and oligomers primarily involving ester and ether linkages. Partial purification of the most active of these oligomers by high performance liquid chromatography (HPLC) or size exclusion gel chromatography led to Photofrin II®, 90-95% of which is the genuinely active component. Notwithstanding this messy synthetic route and its unknown structure, Photofrin II® is approved by regulatory bodies all over the world, and is currently marketed for the treatment of early and late-stage lung cancer, superficial and advanced oesophageal cancer, bladder cancer and cervical dysplasia (a precancerous condition) with promis-

Box 1. Seeing is Believing!



A typical complete remission case of early central bronchial cancer treated by PDT.
a: squamous cell carcinoma of a 78-year-old man. b: 1 week after PDT, covered by necrosis tissue. Approximately 48 h. following intravenous injection of 2.0 mg/kg of body weight of Photofrin II®, bronchoscopic PDT was performed under topical anesthesia using light from an argon dye laser and fiber optic light-delivery device. c: 3 years after PDT, no evidence of recurrence (extracted, with permission from Yamamoto and others, *Curr. Sci.*, 77, 894, 1999).

ing clinical results. An illustration of the successful clinical use of Photofrin II® is given in *Box* 1.

Despite its apparent success, Photofrin II® (HpD) has two very important disadvantages. First of all, it is readily taken up and retained by cutaneous tissue for up to ten weeks post-injection. This is not desirable since it causes a marked skin phototoxicity that requires the patient to avoid bright sunlight for several weeks. Secondly, while there exists a number of absorption bands between 400-650 nm, the weakest absorption band of Photofrin II® at 630 nm wavelength is often used to excite this photosensitizer (remember: tissue penetration of light increases at longer wavelengths). Moreover, there is considerable uncertainty about the chemical 'purity' and also the structure of Photofrin II® resulting in 'batch to batch' variation in its photodynamic activity thus rendering the fixation of the dosage difficult. Put together, these disadvantages of Photofrin II® have necessitated the development of other photosensitizers for use in PDT. A plethora of new potential PDT agents have been reported recently. Many of these second generation photosensitizers are either porphyrins or their structural analogues.

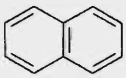
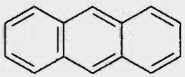
Of the six guiding principles enlisted for the design of a new photosensitizer, the one that has provided chemists with the strongest challenge is in ensuring that the porphyrin chromophore has the desired long-wavelength absorption. This has been achieved in two ways: either by 'expanding' the macrocycle to produce phthalocyanines, texaphyrins, pentaphyrins, porphyrin vinylogoues, etc. or by 'reducing' one or more of the porphyrin's pyrrole rings to give chlorins. Both these strategies cause a red shift and an increase in the molar extinction coefficient of the long wavelength absorption of the chromophore (*Box* 2).

Besides imparting favourable optical properties to the new PDT agents, it is important to maximize their triplet state quantum yields for an improved PDT action. This has been achieved by employing simple inorganic chemistry principles. Like heme, many photosensitizing porphyrins coordinate metals. The central metal cation coordinated by the porphyrin macrocycle is also important in determining the effectiveness of a potential PDT sensitizer. The metal cation must be chosen so that it increases the likelihood of intersystem crossing by the sensitizer to its triplet state to ensure efficient $^1O_2$ generation. This rules out paramagnetic transition metals, such as copper and iron (*Box* 3). In fact, the three metals favored so far are the diamagnetic zinc (II), aluminum(III) and tin(IV) cations, with lutetium being the next most preferred. Aluminum and tin confer a further advantage in that both these metals bind axial ligands, usually a hydroxyl group. Axial ligands inhibit aggregation by the sensitizers to a photoinactive form, thus overcoming a common problem with the dye molecules, which possess flat hydrophobic surfaces.

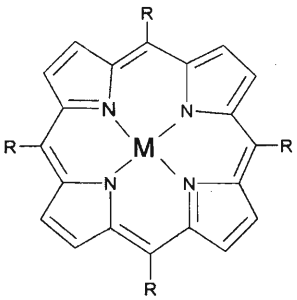
While no photosensitizer can be deemed ideal for every possible application, the salient features of a few promising new sensitizers that are being developed by various research groups are discussed below. *Figures* 2 and 3 illustrate the structures of a few new, porphyrin-based potential PDT agents.

Box 2. How to Force a Chromophore to Absorb Red-Light!

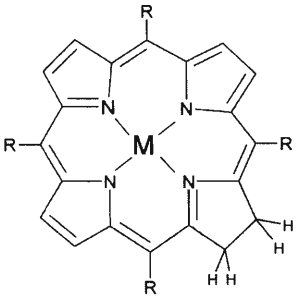
Two basic principles of electronic absorption spectroscopy are useful here. 'Expansion' of the porphyrin macrocycle by increasing the number of pyrrole rings or by linking the four pyrrole rings with additional alternating double bonds, generates systems that possess more than 18 π-electrons. The lengthened π-electron system results in greater delocalization of electrons; the energy required for the corresponding π–π^* transition is less and the probability of these transitions is higher. Thus, the most red-shifted electronic absorption band of the parent chromophore moves further to the red and gains in intensity. The variations in the spectral properties of benzene, naphthalene and anthracene provide a simple analogy.

wavelength (major $\pi - \pi^*$)	254	311	375
molar extinction coefficient ($M^{-1}cm^{-1}$)	229	301	7413



A symmetric porphyrin
point group:D_{4h}



An unsymmetric chlorin
point group: C_{2v}

Chemical reduction of a symmetric, aromatic chromophore changes its point group. For example, porphyrin, a simple, symmetrically substituted porphine has a higher symmetry (D_{4h}) than a chlorin which has one of the four pyrrole rings reduced and, formally, has C_{2v} symmetry. Application of the spectroscopic selection rules predicts that new absorption bands should appear and/or the extinction coefficient of the already allowed absorption band of the chromophore should increase in lower symmetry forms.

Box 3. Thank Goodness, Heme is Photoinactive!

It is interesting to note that need for the use of non-natural porphyrins as PDT agents would not have arisen at all had iron porphyrins themselves been good photosensitizers. The naturally occurring iron porphyrin in the blood, heme (the prosthetic group of hemoglobin and myoglobin) would have acted as an 'internal' photosensitizer. Come to think of it, if heme were indeed a good PDT agent, the following ' abnormalities' are expected: (i) cancer could have been cured at its infancy just by keeping the patient out in the sunlight relying on the heme's PDT action and (ii) noncancerous objects (i.e., healthy human beings) should have been wearing hat and gloves all through sunny periods to escape the deleterious effects of $^1O_2$ produced via heme photoexcitation. In effect, the evolutionary process would have taken a totally different course. Good for us that the oxy and de-oxy forms of heme are photochemically inert! The deoxy form of heme is a paramagnetic complex because of the presence of the central iron(II) ion in the porphyrin crevice. Paramagnetic ions enhance the rates of intersystem crossing (ISC) from the triplet state (also paramagnetic) back to the ground state. On the other hand, the oxy-form of heme, although diamagnetic contains the heavy atom iron. The triplets of chromophores that contain heavy atoms such as iron are extremely short-lived (again due to the fast ISC) and die away before they can react with the other neighbouring molecules.

Texaphyrins are porphyrins featuring a penta-aza core. The group in Texas that discovered this class of molecules noted that the symmetrical structure of the expanded penta-coordinate compound resembles the five-pointed star emblem of the 'lone star state'. Moreover, the large central cavity (compared to a normal porphyrin) seems to be in keeping with the tradition that things in Texas tend to be bigger. These features prompted the research group to name the molecule texaphyrin! Under the trade name Lutrin™, a derivative of lutetium texaphyrin is undergoing Phase II clinical trials as a possible therapy for

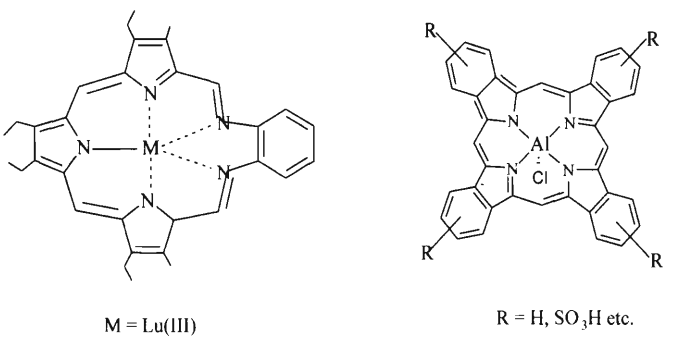
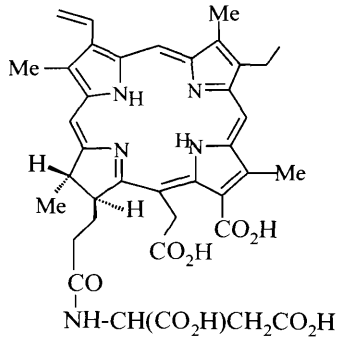
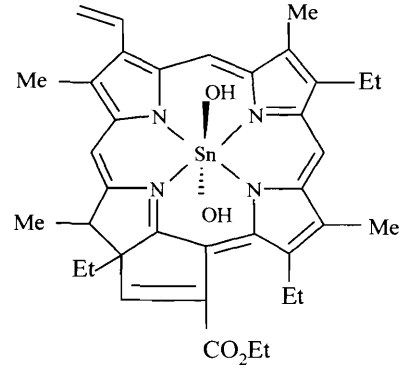


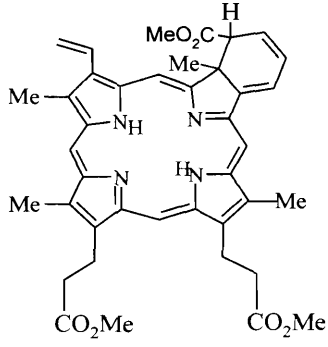
Figure 2. Texaphyrin (the 'expanded porphyrin') and phthalocyanine based PDT agents .



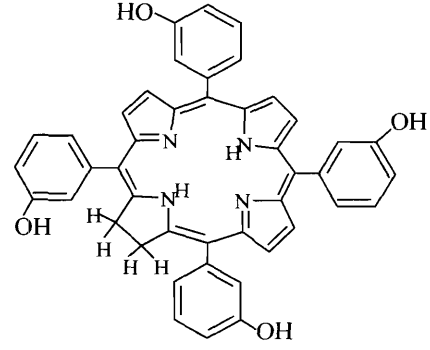
Monoaspartyl chlorin e_6 (Npe_6)



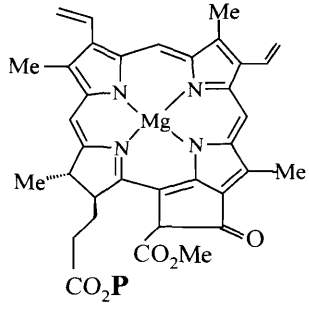
Tin etiopurpurin (SnET2)



Benzoporphyrin derivative (BPD)



tetra(m-hydroxyphenyl)chlorin (m-THPC)



Chlorophyll-a

Me = $-CH_3$

Et = $-CH_2CH_3$

P = phytyl chain

Figure 3. Chlorin-based second generation PDT agents.

breast cancer. The main advantage of using texaphyrins as PDT agents is their strong absorbency at much longer wavelengths (732 nm) so that the treatment can be carried out effectively on a larger tumor or at a greater depth.

Phthalocyanines are tetrapyrrolic macrocycles, which unlike the porphyrins have nitrogen atoms linking the individual pyrrole units instead of methine bridges. The periphery of the macrocycle is extended by benzene rings, which strengthen the absorption at longer wavelengths compared to porphyrins. Phthalocyanines have long been used as dyes and functional liquid crystals [2] and are recently being used as photoconducting agents in photocopying machines. They have been extensively studied as PDT agents not only because of their favourable photophysical properties but also due to the ease with which their redox potential, solubility, etc., can be altered through substitution at their periphery. A liposomal preparation of zinc phthalocyanine has been in Type I/II clinical trials for patients with squamous cell carcinomas of the upper aerodigestive tract. Trials using a mixture of sulfonated aluminum phthalocyanine against malignancies such as skin, breast, lung and gastrointestinal cancer are also under way. The addition of sulfonate groups to the periphery of the phthalocyanine greatly increases the solubility of these compounds, removing the need for liposomal delivery vehicles. Addition of a second benzene ring to the periphery of phthalocyanine produces naphthalocyanines that absorb at 770 nm (compared to 680 nm of phthalocyanines), thus increasing the therapeutic depth and rendering them potential photosensitizers for highly pigmented tumors such as melanomas.

[2] See T P Radhakrishnan, *Resonance*, 5, 6, 1998.

Phthalocyanines have long been used as dyes and functional liquid crystals [2] and are recently being used as photoconducting agents in photocopying machines.

N-aspartylchlorin e6 (Npe6), as the name suggests is a chlorin (i.e., ring reduced porphyrin, see *Box 2*) and porphycene is an isomer of porphyrin. Both absorb strongly at wavelengths above 700 nm. While Npe6 is being studied as a possible photosensitizer against cutaneous malignancies, endobronchial lung cancer and skin cancers, porphycene can be applied topically, making it useful for dermal applications. Tin etiopurpurin (SnET2,

Purytin™) is in Phase I clinical trials against prostatic cancer and in Phase II trials for cutaneous metastatic breast cancer and Kaposi's sarcoma in patients with AIDS. Preclinical work with SnET2 has included extensive examination of its effects on other malignancies such as brain, lung, skin, etc.

Verteporfin (chemically, benzoporphyrin derivative monoacid ring A, BDA) is in Phase III clinical trials for non-melanoma skin cancer and Phase I/II trials against other non-melanoma skin cancer and psoriasis. This porphyrin derivative has a much stronger absorbance at a longer wavelength (690 nm) where tissue penetration of light is 50% greater than that of Photofrin II® at 630 nm. In addition, verteporfin is rapidly absorbed by the tumor, reaching an optimal tumor-normal tissue ratio in 30-150 minutes after intravenous injection, and is rapidly cleared from the body so that skin photosensitivity lasts only for a few days.

Another chlorin that is currently in the news is Temoporfin (chemically, tetra(m-hydroxyphenyl)chlorin, mTHPC; tradename: Foscan™). It is a new second-generation photosensitizer for PDT; phase III clinical trials have begun for head and neck cancers. Temoporfin could be one of the most phototoxic of all the second-generation PDT agents. It requires very low doses (as little as 0.1 mg kg^{-1}), making it 100 fold more photoactive than Photofrin II® (drug doses: 2-5 mg kg^{-1}) and light doses of 100-200 J cm^{-2}. While improved optical properties and singlet oxygen quantum yields can partially rationalize this increased phototoxicity, it appears that the explanation resides in the subtumoral and subcellular localization of the compound.

Chlorophylls – the light harvesting pigments found in plants, algae and some bacteria – are also 'formally' chlorins. In addition, it is well known that to perform the photosynthetic function, the chlorophyll molecule already has been designed by nature to form charged radicals once excited by light. In photosynthetic organisms, these radicals are quickly quenched as their energy is converted to chemical potential (formation of

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adenosine triphosphate/O_2 and carbohydrates). In mammalian cells, on the other hand, these radicals are bound to be destructive because such cells are not equipped with a similar quenching mechanism. With these ideas in mind, an Israeli group has synthesized several water-soluble chlorophyll derivatives conjugated to amino acids, peptides or even proteins. Such molecules, when excited by light (~800 nm) are shown to be photodynamically active via the radical-based Type I mechanism. The 'killing agent' in this case has been proposed to be the hydroxyl radical (·OH) rather than $^1O_2$. One particular advantage of this class of photosensitizers is that they appear to be effective even when oxygen is limited. Other photosensitizers require adequate levels of oxygen and cease to work under the so called 'hypoxic' conditions.

Non-Porphyrinic Photosensitizers

In recent years a great variety of non-porphyrinic sensitizers are being developed for use in PDT. Three such new sensitizers are discussed here (*Figure* 4). Methylene blue, a red-light absorbing phenothiaxinium dye, has previously been used extensively as a biological assay stain and can be used in the clinical diagnosis of a variety of diseases and as a tumor marker in surgery. This has prompted extensive studies with this dye in PDT research.

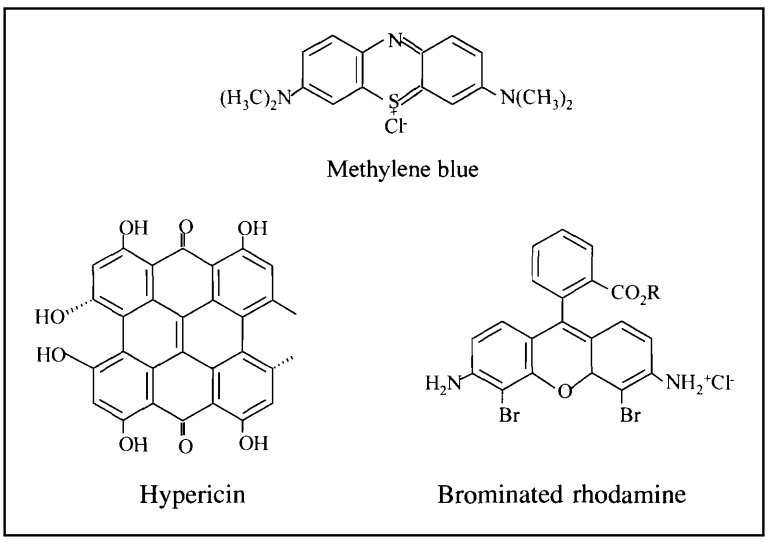

Figure 4. Non-porphyrinic PDT agents (rhodamine).

However, its use as an in vivo photosensitizer is limited by its reduction by ubiquitous cellular enzymes to the colorless form (recall that the blue methylene blue becomes colorless during redox titrations), which is photodynamically inactive. On the other hand, this dye is used by Swiss and German Red Cross Societies for the decontamination of freshly frozen plasma units. This aspect will be taken up again in a later article where we discuss non-cancer applications of PDT.

Rhodamine is an important laser dye and is being used in red-light emitting laser dyes for a long time now. Because of their specific uptake by mitochondria and their known use as a biochemical fluorescent probe, rhodamine class of molecules are being used as sensitizers in the treatment of malignant tumors. The readily available commercial dye, rhodamine 123 is a poor phototoxin because of its high fluorescence quantum yield (which leads to a low triplet quantum yield). On the other hand, chlorine/bromine substituted rhodamines show weak fluorescence and high triplet quantum yields. Due to the heavy atom effect (similar to the one described above in the Zn(II), Al(III) and Sn(IV) porphyrins) exerted by chlorine/bromine substituents, the intersystem crossing from the singlet to the triplet is increased in these chromophores thus enhancing their triplet state quantum yields. Phase I clinical trials have begun using brominated rhodamine (named TH 9402) for the treatment of chronic myeloid leukemia.

The fact that a naturally occurring dye such as porphyrin is the drug of choice in PDT has prompted the search for other well-known natural dyes. Hypercin is well documented as having photodynamic activity as it causes hypercism or photopoisoning in grazing animals that consume large quantities of plants containing this compound leading to skin irritation, fever and even death. This knowledge has prompted the use of hypercin class of molecules in PDT. This multicyclic quinone dye, which absorbs light of 590 nm is being investigated in Phase I clinical trials for the treatment of psoriasis and skin cancer.

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PDT Light Sources and Irradiation Protocol

Having a good photosensitizer in hand is not enough to achieve the desired target in PDT. It forms only 50% of the 'drug', the other 50% being irradiation of light of desired wavelength and intensity at the clinical level. PDT used in deep-seated cancers is fast becoming a specialized job involving medical/laser physicists. However, it is interesting that, in the early days, even the traditional 'gaz' lamps have been used to excite the photosensitizers, particularly to treat skin cancers. The light emitted by these lamps is filtered to select the photoactivating wavelength and to eliminate infrared components that can cause significant heating of tissue (remember: infrared heaters being used in hospitals as 'pain killers'). However, these lamps are limited in the way light can be delivered and in the intensity they produce. Moreover, they cannot be used for 'internal' treatment.

The potential of PDT has been greatly increased by the introduction of a combination of laser light and optical fibers. Lasers have the advantage of a narrow emission spectrum and high output power in the required spectral range, while the optical fiber allows the light to be delivered, either interstitially or endoscopically to practically any site in the human body. The lasers more frequently used in PDT are argon dye lasers, YAG dye lasers and increasingly, diode lasers. Until recently, the power produced by red emitting diode lasers has been too low for them to be used with photosensitizers such as Photofrin II®. However, the wavelength range generated by the diode lasers is suitable for use with products such as mTHPC, BPD and SnET2 and these lasers also have the advantage of low cost, high reliability, small size and portability.

The light delivery systems used depend on the location of the tumor. To reach tumors in body cavities, laser light is passed through endoscopically placed optic diffusers. For the treatment of skin or oral cavity tumors, a simple lens that produces a uniform illumination may be used (although use of optical fibers is trendy and permits more flexibility). For solid or deep-

seated cancers, the fiber optic is usually placed interstitially into the tumor through a biopsy needle. For intraluminal or intracavity illumination, a flexible fiber can be placed within the instrument channel or a standard endoscope. The spatial distribution of the light can be controlled by modifying the fiber end or placing the fiber in a light-diffusing medium.

Factors such as type of the photosensitizer, drug dose, location and size of the tumor, irradiation wavelength and intensity, severity of the disease will have to be considered in deciding the length of irradiation and how frequently it is done. It is now more of a 'clinical art' than pure science. For example, in a typical treatment with Photofrin II®, the patient receives an intravenous injection of the photosensitizer as an outpatient and then waits for 24-48 hours for drug accumulation in the tumor. The patient returns to the clinic after the waiting period and the tumor is illuminated for 10-30 min. The protocol is not the same with other photosensitizers.

Concluding Remarks

This article provides an overview of the important aspects related to PDT drug design and development. The chemistry, photochemistry and photobiology of porphyrinic and non-porphyrinic photosensitizers, as relevant to their application in clinical PDT are summarized here. Certain key issues related to light sources and irradiation procedures involved in clinical PDT are also highlighted. Unlike the case with conventional chemo/radiation therapy, the 'drug' design in PDT demands involvement of both organic chemistry (design and synthesis of new molecules) and optical physics (appropriate laser light irradiation). The outcome of this fusion will be clear only upon carrying out molecular oncological and subsequently, clinical studies. Thus, a practitioner of PDT needs to know how to 'bat, bowl and field', knowing a bit of everything: chemistry, biology, and physics. And that makes this research area quite fascinating!

Suggested Reading

[1] H Yamamoto, T Okunaka, K Furukawa, T Hiyoshi, C Konaka, H Kato, *Curr. Sci.*, Vol. 77, p. 894, 1999.

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