

REVIEW

PHOTODYNAMIC THERAPY: NEW LIGHT IN MEDICINE WORLD

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ABSTRACT

Photodynamic therapy (PDT) is a considerably new kind of photochemotherapeutic treatment in medical field. It combines the use of three components, which are a photosensitizer, light and oxygen. Photosensitizer is a compound activated by light. The application can be oral, topical or intravenous. It usually member of porphyrin group with amphiphilic characteristics. Photosensitizer can be of generation I, II or III, each generation step develops more specificity, selectivity and deeper tissue application. This review will discuss photosensitizer development consecutively, with its benefit and lackness. The light used is usually on red region, while the oxygen is involved in reactive oxygen species generation. Its mechanism action can go through either in type I or type II reaction. This kind of therapy is usually being used in oncology, especially in superficial and in-lining cancers, dermatology and ophthalmology field. This therapy can be safely given to patients with complication and has distinct advantages compare with other treatment such as chemotherapy and surgery. It also considerably has lesser side effects and risks. Broader application is being developed through various experiments and photosensitizer modification.

Keywords: light spectrum, photoactivation, photodynamic therapy, photosensitizer

INTRODUCTION

History of PDT

The very first usage of photosensitizer drug dates back to Ancient Egypt, India and Greece. They use psoralen-containing plant extracts and light to treat psoriasis and vitiligo. The term photodynamic was raised by Von Tappeiner in 1904 to describe oxygen-dependent chemical reactions induced by photosensitization.

The German physician Friedrich Meyer-Betz carried out the first study of PDT in humans in 1913. The use of porphyrins was derived from the fact that researchers who inject themselves with porphyrins had sunburns. Meyer-Betz tested the effects of hematoporphyrin-PDT on his own skin. The tumor-accumulating property of porphyrins was revealed by Policard. He found the characteristic red fluorescence of neoplastic, embryonic and traumatized tissues after the application of hematoporphyrin. The testing then was done at the Mayo Clinic and Roswell Park Cancer Center, until it become widespread with the work of Thomas Dougherty. He initiated clinical trials and presented extensive data on the successful application of this novel technique for the treatment of cancer in 1978 [1-4]. Later, he formed the International Photodynamic Association in 1986. It was feasible in the 1980's at many universities and health centers that PDT is one of the promising new therapy [5].

Overview of PDT

Photodynamic therapy (PDT) is a special form of phototherapy. Phototherapy is all treatments which use

light to induce certain reactions in the body for the benefit of patients. Moreover, PDT is a special photochemotherapy, combining the use of light, oxygen and a certain photosensitizer drug. In photochemotherapy, the photosensitizers is become excited by the light of particular wavelength and then the excited photosensitizers, in turn, transferring its energy to oxygen and change the latter to its singlet and this activated oxygen will destroys target cells, through either the apoptotic or necrotic process [6].

COMPONENTS OF PDT

Photosensitizer

Photosensitizers are chemical compounds with special property of absorbing the energy of light of specific wavelength. This absorption of energy enables certain subsequent reaction in the body as the consequences. The absorbing specific wavelength is better from the red light, which are characteristics for the photosensitizer's absorption peak. The red light is preferable because it penetrates tissues better than blue light. Its lower energy makes it safe for the cell.

The required characteristics for photosensitizer compound are: a single compound to simplify the studies of the relationship between photosensitizer and its effect (pharmacokinetics and metabolism effect) and to the ease of clinical approval, has increased absorbance in the red region of visible light (to optimize tissue penetration) and increased molar absorption coefficients which give rise to more excited photosensitizer at deeper tissue sites and hence

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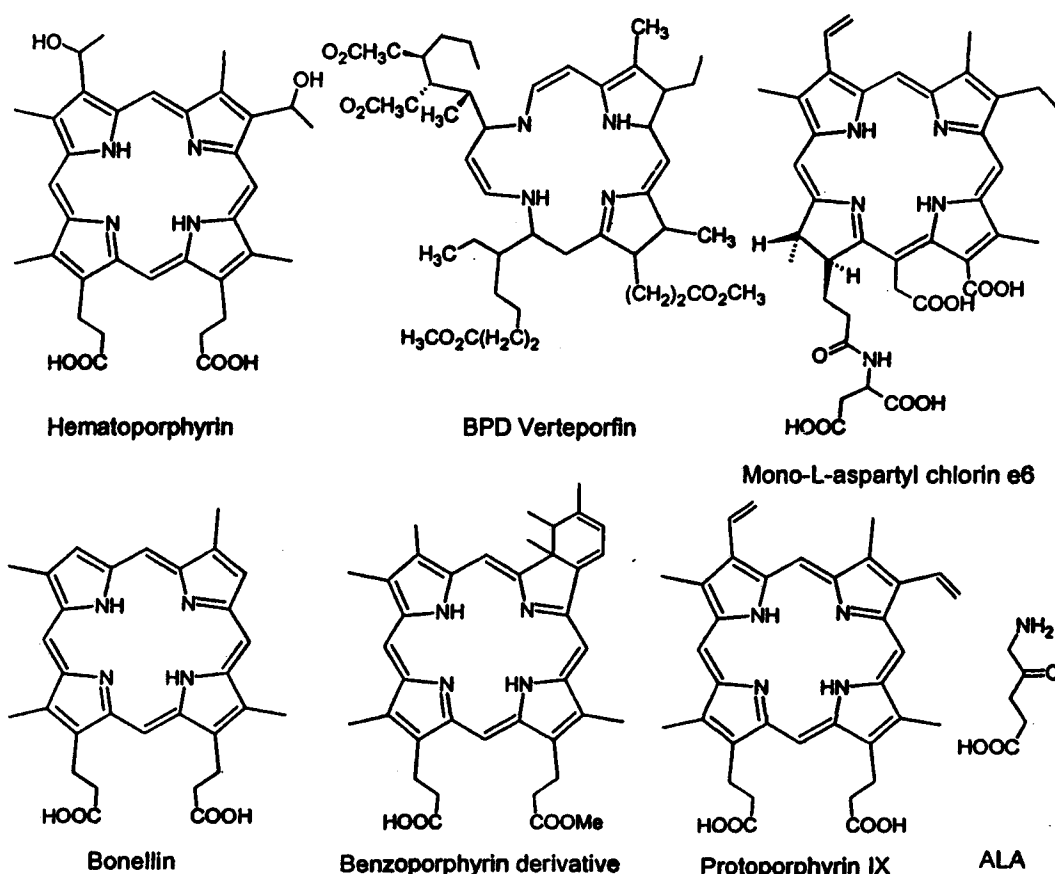


Fig 1. Structures of developed photosensitizer [7]

more tumor damage, gives a high quantum yield of triplet formation and free radical generation, has good cytotoxic oxygen species generation, shows increased selectivity for malignant tissue over normal tissue, has short time interval between the administration of the drug and its maximal accumulation in hyperproliferating tissue, exhibits no dark toxicity (non toxic in dark state), has properties of aggregation, ionic charge, solubility, partition between aqueous and lipid which promote selectivity without long term retention (rapid clearance). It should be of amphiphilic molecule since the blood is a water-based system while the molecule has to travel through lipid membranes to enter the cell. It also has to be easy to administer systemically (via injection into the bloodstream) or by manual application on the skin surface.

The already known and officially approved photosensitizer for PDT is Photofrin. It has now been approved as a therapy for a limited number of applications in various parts of the world including the UK and is considered at least as good as and possibly better than alternative treatments. Development of photosensitizer is given in Fig. 1.

Haematoporphyrin and Photofrin

The first sensitizer used in clinical PDT was haematoporphyrin derivative (HpD) and its purified fraction, Photofrin. HpD was first described in 1961 and is prepared by acetylation of haematoporphyrin (Hp), followed by neutralisation prior to alkaline hydrolysis. The resulting mixture is known to contain haematoporphyrin, hydroxyethylvinyldeuteroporphyrin (HVD) and protoporphyrin (Pp), as well as a complex dimeric and oligomeric fraction containing ester, ether and carbon-carbon linkages of haematoporphyrin. HpD is typically 45% monomeric/dimeric porphyrins and 55% oligomeric material, the latter being accountable for the tumor localizing activity of HpD *in vivo*. Photofrin is composed of \pm 85% oligomeric material. The same compound was prepared in Leeds under the name Polyhaematoporphyrin or PHP.

Photofrin is manufactured by QLT Phototherapeutics. It is already in phase III clinical trials and the first PDT agent to receive regulatory approval. Photofrin has received approval in Canada for bladder carcinoma where treatment with BCG vaccine has failed. Photofrin has been approved in other countries for treatment of esophageal cancer and lung cancer.

The usage includes intravenous injection and light application within a 48–72 hour period, when the accumulation of sensitizer by tumor tissue is thought to be optimal [8].

Photofrin-mediated PDT has proved curative for a range of cancers, but because the compound is a complex mixture, there are questions concerning the identity of the active components and also the reproducibility of the synthetic process. Photofrin is excited clinically with red light at 630 nm, which can only penetrate tissue to a depth of a few mm, making Photofrin unsuitable for the treatment of deep-seated tumors. These first generation photosensitizers display prolonged and generalized photosensitivity of the skin as their primary side effect. Cutaneous photosensitivity following treatment can last for 6-8 weeks and patients are advised to avoid bright light during this period [9,10].

Benzoporphyrin derivative

A few of second generation photosensitizers (single pure compound) are now in early clinical trials, one of them is BPD verteporfin. Benzoporphyrin derivative mono-acid A (BPD) is another chlorin-type molecule, developed by QuadraLogic Technologies. It is a hydrophobic molecule with a mono-acid at either position 3 or 4 of the porphyrin ring. The absorbance peak for PDT occurs at 650 nm with an extinction coefficient of $34.000 \text{ M}^{-1}\text{cm}^{-1}$. BPD verteporfin exhibits far less photosensitization. It is already in clinical trials since 1992. Step I and II clinical trials have shown it has rapid tumor accumulation and reduced skin photosensitivity. BPD verteporfin was recently in Phase I/II clinical trials for primary skin carcinoma, cutaneous lesions where cancer has metastasized to the skin, and chronic stable plaque psoriasis [11]. The usage includes intravenous injection and irradiation within a 1.5–6 hour period. It is activated by red light (690 nm) for cutaneous cancer or ultraviolet A (290-320 nm) for psoriasis. The major side effect is generalized photosensitivity, last for 3–4 days [12].

Chlorins, Bacteriochlorins and Derivatives

In chlorins one of the exo-pyrrole double bonds of the porphyrin ring is hydrogenated, resulting in an intense absorption at $>650 \text{ nm}$. In bacteriochlorins, two of the exo-pyrrole double bonds of the porphyrin ring are hydrogenated, yielding compounds with maximum absorption at longer wavelengths, allowing treatment of much deeper tumors than HpD. Bonellin is a naturally occurring chlorin which has better photosensitising abilities than HpD. Bacteriochlorins have almost ideal optical properties in terms of tissue penetration. These compounds absorb light strongly above 740 nm, although their stability remains in some doubt. The derivatives of BChl a have absorption in therapeutic window and shorter clearance from the body. Chemical modification of BChl, by alteration of substituents on the

macrocycle, with or without replacement of the central metal with other metal may modify and enhance the solubility, selectivity and their free radical quantum yield [13-16]. One of the distinct photosensitizer in this group is Pd-Bpheide (Tookad). It is a novel water soluble derivative of BChl, with peak absorption at 763 nm. It is usually used to treat prostate cancer through the passive targeting, determined to destroy the vascularity of the tumor cell. It is now in the phase I/II clinical trial [17-19].

Meta-Tetra hydroxyphenyl chlorin

Meta-tetra hydroxyphenyl chlorin (m-THPC) or Foscan or Temoporfin, is a second generation photosensitizer (single pure compound), developed by Scotia QuantaNova. It has a hydrophobic chlorin core and hydroxyphenyl groups at the meso position to increase solubility of the photosensitizer. The first clinical study with m-THPC began in 1990 for the treatment of human mesothelioma and it is currently in clinical trials for gynecological, respiratory and head and neck cancers in USA, Europe and the UK.

M-THPC is approximately 200 times more effective than Photofrin. Lower photosensitizer dose and shorter illumination times are required to achieve similar results. It is excited at a longer wavelength and the molar absorbance coefficient for m-THPC is much higher than that of Photofrin, $22.400 \text{ M}^{-1}\text{cm}^{-1}$ at 652 nm and $1.170 \text{ M}^{-1}\text{cm}^{-1}$ at 630 nm (in methanol). Furthermore, m-THPC has a longer half life in the triplet state and is said to have higher tissue selectivity. M-THPC is more hydrophobic than Photofrin. However, the skin photosensitivity caused by m-THPC is only slightly less than that of Photofrin.

M-THPC is dissolved in polyethylene glycol 400 (PEG): ethanol: water, (3: 2: 5 v/v/v) for clinical use as recommended by Scotia QuantaNova. More recently a number of new formulations have been developed. Foscan 2 is a pre-dissolved preparation of m-THPC using propylene glycol: ethanol, (6:4 v/v). The difference in the two solvents lies in the chain length for PEG: $\text{H}(\text{OCH}_2\text{CH}_2)_n\text{OH}$ ($n = 8.2-9.1$) whilst propylene glycol is $\text{CH}_3\text{CHOHCH}_2\text{OH}$.

Mono-L-aspartyl chlorin e6

Mono-L-aspartyl chlorin e6 (NPe6 or MACE) is a highly water soluble chlorin-type photosensitizer. It has an absorbance peak at 654 nm with extinction coefficient of $40.000 \text{ M}^{-1}\text{cm}^{-1}$ and is effective *in vitro* and *in vivo*, shown by retention in the tumor, efficient photodynamic damage with rapid removal [20].

Phthalocyanines [21]

The pyrrole groups in phthalocyanines are conjugated to benzene rings and have nitrogen bridges. This causes the absorption spectrum to shift to longer wavelengths and the Q bands to become more

intense than the Soret peak. The bathochromic shift of the absorption peak up to 680 nm increases tissue penetration.

A long-life triplet state may be fulfilled by the incorporation of Zn or Al into the phthalocyanine macrocycle. Metal-free compounds and phthalocyanines containing Cu, Co and Fe have a much shorter triplet lifetime and display minimal phototoxicity.

Phthalocyanines are generally hydrophobic compounds although water-soluble derivatives can be readily synthesized through substitution of the ring with moieties such as sulphonic acid, carboxylic acid and amino groups. The sulphonated compounds, and in particular chloro aluminium sulphonated phthalocyanine (AIPcS) have high photodynamic efficacy. Purification of these derivatives is difficult and the final product is still a mixture of mono- di- tri- and tetrasulphonated derivatives. Furthermore, these compounds aggregate at relatively low concentrations in aqueous media which results in loss of photochemical activity. AIPcS exhibits selective retention in some tumors. This characteristic coupled with negligible dark-toxicity, minimal cutaneous photosensitivity, and excellent photodynamic activity at increased wavelengths has led to the clinical evaluation of AIPcS for PDT.

5-Aminolaevulinic acid (ALA)

Beside the administration of exogenous photosensitizer, there is an alternative way to stimulate the cellular synthesis of endogenous photosensitizers. 5-Aminolaevulinic acid (ALA) is a precursor of heme. Before the formation of heme, the ALA is converted to protoporphyrin IX (PpIX) which is a natural photosensitizer [22]. The rate of formation of PpIX is dependent on the rate of synthesis of ALA from glycine and succinyl CoA, and regulates by a negative-feedback manner of the concentration of free heme. Since the conversion of PpIX to heme is relatively slow, administration of exogenous ALA cause the build-up of phototoxic levels of PpIX [23-26].

ALA-induced PpIX has several advantages over hematoporphyrin derivative and Photofrin for the use in PDT. The optimum therapeutic ratio is reached 2-4 h following ALA administration and there is rapid systemic clearance of ALA-induced PpIX within 24 h. This rapid clearance eliminates prolonged cutaneous photosensitivity and allows repeated treatment as frequently as every 48 h without the risk of damage to normal tissue. The photosensitizing effect is due almost exclusively to PpIX, enables fluorescence-*in situ* monitoring of the sensitizer levels. PpIX rapidly undergoes photobleaching; therefore the PDT effect is determined by the concentration of sensitizers in the tissue. ALA can be administered systemically or topically depend on the kinds of lesions [27, 28]. Topically applied ALA is safe for healthy skin because it can not readily penetrate the keratinous layer of normal skin but can

penetrate malignant lesions [29]. Moreover, certain types of tumor tissue exhibit increased accumulation of ALA-induced PpIX. Some tumors may have lower activity of the enzyme ferrochelatase, which catalyses the incorporation of iron into the porphyrin ring, causes slower conversion to heme, which results in prolonged elevation of PpIX levels [30].

ALA-induced PpIX photosensitization has some drawbacks associated with the treatment. The excitation of PpIX occurs at 630 nm, offering no advantage over HpD in the depth of tissue penetration. The hydrophilic nature of ALA restricts drug penetration layer. This problem may be encountered by the use of lipophilic ALA esters which penetrate cells more easily [31, 32].

Third-generation photosensitizers

Third generation photosensitizers are second generation photosensitizers bound to carriers for selective accumulation in tumor. This kind of photosensitizers bound through conjugation with biomolecules, such as monoclonal antibodies (mAb) or liposomes [33]. The cell surface antigens in tumor cells will enable binding site for the mAb and hence, the photosensitizer accumulation.

The use of liposomes as carrier involves more sophisticated strategies [34]. First, there are conventional liposomes. Conventional or unmodified liposomes are multilamellar or unilamellar vesicles composed mostly of phospholipids. This kind of liposomes gives better results compare with usual treatment. However, this conventional liposomes exhibit a plasma half-life which is too short for efficient tumor uptake to take place and overall they did not emerge as the ultimate tools to target photosensitizer tumor selectively. The research continued with passively targeted liposomes. Many approaches based on surface modifications were explored to produce long-circulating liposomes featuring substantially enhanced plasma stability. This prolonged circulation times is due to modification with glycolipids or PEG-ylated lipids, which stabilize the molecule sterically. Unfortunately, long-circulating liposomes, with their hydrophilic surface, became too stable and hence can not interact effectively with cells and perhaps unable to release the photosensitizer content. Therefore, to what extent these extravasated liposomes accumulating in the tumor interstitial are able to transfer their photosensitizer content to tumor cells could not be predicted.

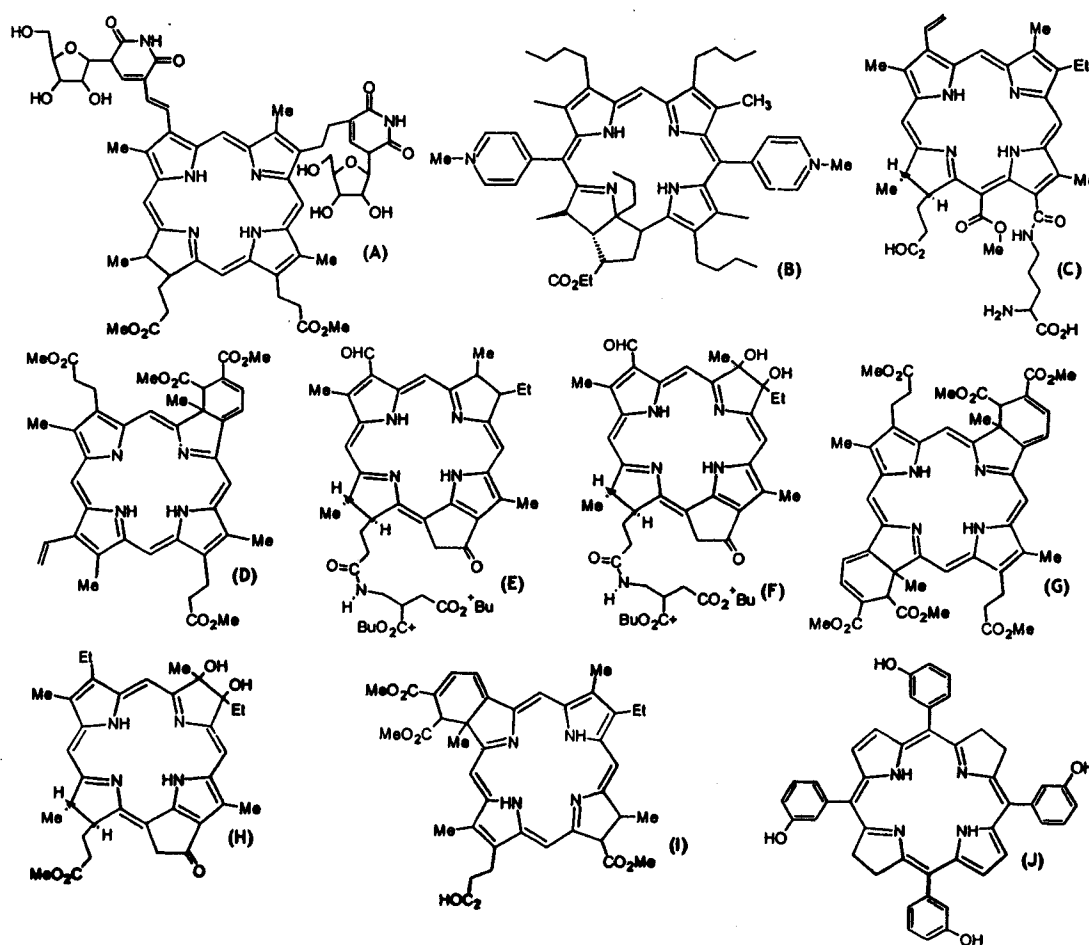
More development then called actively targeted liposomes. The objective of active targeting is the enhancement of tumor-selective accumulation by side-directed retention through target binding and a possible increase in photodynamic effect through cellular internalization of the liposomes-bound photosensitizer. However, active-targeting liposomes usually bound to

peripheral of tumor and therefore, have lesser penetrability than conventional liposomes. Moreover, there are liposomes with triggered release mechanism. This development's aim is to shorten the time period between photosensitizer application and irradiation. Among this liposomes are thermo-sensitive liposomes, fusogenic liposomes, pH-sensitive liposomes, light-sensitive liposomes and target-sensitive liposomes. These uses of liposomes are still mainly only in vitro step, based on cell culture works [34].

Other potential photosensitizer

There are other synthetic photosensitizers which have been developed with improved photophysical properties or tumor selectivity. The research to find

superior photosensitizer keeps continue. These include: purpurins, porphycenes, pheophorbides and verdins (Fig. 2). Purpurins are a class of porphyrin macrocycle with an absorption band at 630 nm to 715 nm, typified by tin etiopurpurin (SnET2) which has an extinction coefficient of $40.000 \text{ M}^{-1}\text{cm}^{-1}$ at 700 nm. Porphycenes, in spite of having activated with considerably lower wavelengths than other new photosensitizer (635nm), show higher fluorescence yields than HpD and therefore are potentially useful. Verdins contain a cyclohexanone ring fused to one of the pyrroles of the porphyrin ring and produce similar responses to HpD and purpurins. Pheophorbides are derived from chlorophylls and 20 times more effective than HpD.



- | | |
|------------------------------|----------------------------------|
| A. Porphyrin dinucleoside | F. Bacteriochlorin |
| B. Purpurin | G. Bacteriochlorin |
| C. Chlorin amino acid | H. Bacteriochlorin |
| D. Benzoporphyn (a chlorine) | I. Benzoporphyn |
| E. Chlorin amino acid | J. M-tetra hydroxyphenyl chlorin |

Figure 2. Structures of porphyrins derivatives [7]

Table 1. Comparison of the photophysical properties of the first and second generation of photosensitizers [38, 39]

| Photosensitizer | $\epsilon(M^{-1}cm^{-1})$ | $\lambda_{max}(nm)$ | Φ_T | Φ_Δ |
|----------------------------------------------------|---------------------------|---------------------|----------|---------------|
| Hematoporphyrin ^a | 3500 | 630 | 0.83 | 0.65 |
| Photofrin II ^a | ~3000 | ~630 | | ~0.2 |
| Zn phthalocyanine ^a | 150 | 675 | 0.6 | 0.59 |
| Al- phthalocyanine-tetrasulfonic acid ^b | 105 | 675 | 0.38 | 0.38 |
| Zn naphthalocyanine ^a | 160 | 764 | | 0.45 |
| Benzoporphyrin ^a | 118 | 685 | | 0.6 |
| Bacteriochlorin ^a | 150 | 785 | 0.32 | 0.32 |
| Zn etiopurin ^a | ~70.000 | ~690 | 0.83 | 0.57 |
| Porphycene ^a | 52 | 630 | 0.42 | 0.30 |
| Tetraphenylporphyrin | | 652 | 0.67 | 0.63 |
| Pheophorbide a | | 667 | 0.75 | 0.52 |
| Chlorophyll II | | 646 | 0.69 | 0.58 |
| Octaethylpurpurin | | 700 | 0.81 | 0.65 |

Φ_T Quantum yield of triplet formation Φ_Δ Quantum yield of singlet oxygen formation
 ϵ Extinction coefficient a determined in an organic solvent b determined in water

Table 2. Regulation status of some photosensitizer [40]

| Photosensitizer | Abbreviation | Generic name | Manufacturer |
|--------------------------------------------------|---------------------|---------------------------|------------------------------------------------|
| Approved | | | |
| Polyhematoporphyrin ether/ester | Porfimer sodium | Photofrin | Axcan Pharma, Inc. |
| Hematoporphyrin derivatives | HpD | Photogem | Moscow Institute of High Chemical Technologies |
| Hematoporphyrin derivatives | HpD | Photosan | SeeLab F&E GmbH |
| Hematoporphyrin derivatives | HiPorfin | Hematoporphyrin Injection | Chongqing Huading Modern Biopharmaceutics |
| Benzoporphyrin derivative monoacid ring A | BPD-MA, verteporfin | Visudyne | Novartis Pharmaceuticals |
| 5-aminolevulinic acid | ALA | Levulan | DUSA Pharmaceuticals |
| Methyl aminolevulinatate | MLA | Metvix | PhotoCure ASA |
| Meta-tetrahydroxyphenylchlorin | mTHPC, temoporfin | Foscan | Biolitec AG |
| Mono-L-aspartyl chlorin e6 or talaporfin sodium | NPe6, ME2906 | Laserphyrin | Meiji Seika Kaisha, Ltd. |
| Sulfonated aluminum phthalocyanine | AlPcS2-4 | Photosens | General Physics Institute |
| Tolonium chloride or Toluidine Blue O | TBO | SaveDent PAD | Denfotex Ltd. |
| Under clinical trial | | | |
| Lutetium(III) texaphyrin | Lutex | Antrin | Pharmacyclics Inc. |
| Tin ethyl etiopurpurin | SnET2, purlytin | Photrex | Miravant Medical Technologies |
| Hematoporphyrin monomethyl ether | HMME | Hemporfin | Fudanzhangjiang BioPharmaceutical |
| Deuteroporphyrins | DpD | Duetpofin | Fudanzhangjiang BioPharmaceutical |
| 2-[1-Hexyloxyethyl]-2-devinyl pyropheophorbide-a | HPPH | Photochlor | Roswell Park Cancer Institute |
| Pd-bacteriopheophorbide | WST09 | Tookad | Negma-Lerads and Steba Lab.Ltd. |

Anthracycline compounds are quite selective and members of this group such as Doxorubicin is used in chemotherapy, although there is some side effects occurrence. Some of these compounds have additional phototoxicity, enabling the potential of combination therapy using lower drug dose with comparable antitumor activity.

Psoralens and their derivatives which have been used for over 3000 years in the treatment of skin disorders are still in use today. The cytotoxic action of these compounds derives from their ability to cross-link biomolecules, such as DNA, after activation by UV.

Beside porphyrin, there are many synthetic compounds with photosensitizing ability. These include: phenothiazinium compounds such as methylene blue, toluidine blue, cyanines such as Merocyanine 540; acridine dyes as demonstrated by Raab in 1900; derivatives of the tumor marker, Nile blue; and rhodamines such as the mitochondria-specific Rhodamine 123 [35].

As photodynamic develops, there is a strong demand to introduce new and improved photosensitizers, which have specific properties of light absorption (good absorption of red light) and tissue distribution. The Leeds Centre is actively involved in the production of novel photosensitizers and developing them for specific uses as drugs and sterilizing agents. However, the use of photosensitizer for the therapy needs to be approved by the authorities after undergone required clinical trial [36, 37]. Comparison of the photophysical properties of some generation of photosensitizers and their regulation status are given in Table 1 and 2.

Light

The succesful PDT required the photoactivation of the photosensitizer by light. The transmission of light through tissue is low at 400 nm because of scattering and absorption by natural chromophores, but increases together with wavelength up to 800 nm [37, 41]. Each photosensitizer has their particular wavelength of light needed to maximize penetration and excitation. The wavelength is considered appropriate within the therapeutic window (700-800 nm). The light penetration is limited by optical scattering within the tissue, the absorption by endogenous chromophores, and the absorption of light by the sensitizing drug (self-shielding).

The sensitizer activated by any wavelength above 800 nm is not effective to yield singlet oxygen, because the triplet state of the sensitizer is below the energy level of singlet oxygen.

Photosensitization had been performed initially with conventional gas discharge lamps. It is also possible to use metal halogen lamp, emitting 600-800 nm radiation at high power density and short-arc xenon lamp which is tunable over a bandwidth between 400-1200 nm. The broad light beam produced by incoherent lamps is useful for the treatment of large lesions. Endoscopes and several optical fibers are developed further for interstitial therapy of larger tumors. The light delivery system has been greatly improved in the last 20 years.

Lasers has become the primary light source for activation because laser light is monochromatic (provide the exact selection of wavelengths), coherent (light waves are parallel permitting precise focusing), precise and intense (allowing for shorter treatment times). Pulsed lasers, like the gold vapor laser (GVL) and the copper vapor laser-pumped dye laser (GVDL), produce brief pulses in duration of millisecond to nanosecond. There is no difference between the result of continuous wave and pulsed lasers. Tunable solid-state lasers, such as the neodymium: YAG laser, are particularly useful for PDT, while tunable dye lasers are frequently used in investigative studies because they allow maximum flexibility. The disadvantages of the above lasers are expensive, relatively immobile, and require frequent repair. Semiconductor diode lasers were developed to overcome these obstacles. Portable diode lasers, such as the gallium-aluminum-arsenide lasers, produce light in the range of 770-850 nm, where usually the absorption peak of many photosensitizers is found [4]. Light emitting diode array lasers are other optional lasers and more convenient to use in clinical situations. Light Emitting Diodes (LEDs) and florescent light sources are now being used as alternative light sources as more convenient than lasers and have longer treatment times. The delivery modes of light depend on the lesion to be treated, as shown in Table. 3.

Treatment of internal organs may be achieved through the use of endoscopes and fiber optic catheters to deliver light and intravenously-administered photosensitizers. A great deal of research

Table 3. The kinds of light delivery modes [40]

| Light delivery modes | Description | Example |
|--------------------------------------|-------------------------------------------------------------------------------------------------|-----------------|
| Front superficial irradiation | A uniform irradiance incident beam delivered to a front surface by a microlens fiber externally | Skin PDT |
| Cavity superficial irradiation | An isotropic source centered in a spherical cavity and delivering light to the cavity surface | Brain tumor PDT |
| Cylindrical superficial irradiation | A cylindrical diffuser source centered in a cylindrical lumen | Esophageal PDT |
| Cylindrical interstitial irradiation | A cylindrical diffuser source embedded in the target tissue | Solid tumor PDT |

and clinical study is now underway to determine optimal combinations of photosensitizers, light sources, and treatment parameters for a wide variety of different cancers. The kinds of light delivery modes are given in Table 3.

PROCEDURE, USAGE AND ACTION MECHANISM OF PDT

Treatment Procedures

There are many variations in PDT treatment procedures concerning the photosensitizer dose, compound type and form. It also varies in photosensitizer application ways, either by intravenous injection or topically on skin, according to the cancer or lesion type. The waiting period varies according to the type of photosensitizer and cancer/lesion. The number of repeated treatment needed and the interval time between treatments are also varying.

A typical PDT session consists of intravenous injection (I.V.) or topical application of a photosensitizer. The photosensitizer alone is harmless and has no effect on body's tissue. The drug administration is followed by a waiting period to permit time for the photosensitizer to be cleared from normal tissues and be preferentially retained by rapidly growing tissues or for topical photosensitizer to be absorbed by the skin. The period ranged between 4-72 hours. The next step is application of light directly on to the cancer (may require endoscope) to activate the drug. The irradiation may last 20-45 minutes. Tissue damage usually resulting from vasculature impairment at target area.

For skin treatment, within a few days, the exposed skin and carcinoma will scabs over and flakes away. In a few weeks, the treated area healed, leaving healthy skin behind. For extensive malignancies, repeat treatments may be required. It is also common to experience pain from the area treated. After the treatment the patient will need to avoid excessive exposure to sunlight for a period of time [42].

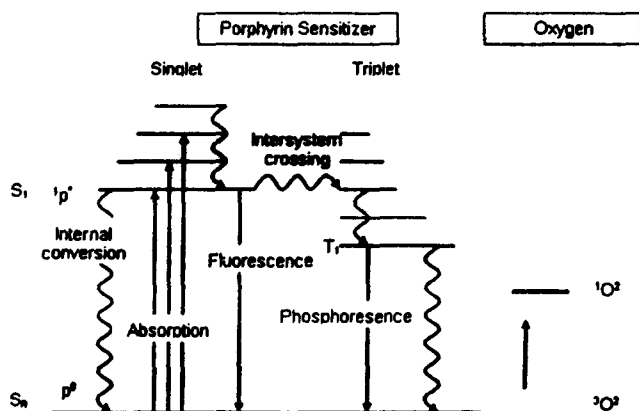


Fig 3. Photophysics of PDT sensitization [44]

Mechanism of Action

Photosensitizer excitation

Photodynamic therapy begins with the administration of a photosensitizer or the metabolic precursor of one. The tissue to be treated is exposed to light suitable for the photosensitizer. This irradiation starts a series of photophysics reaction.

Usually, the photosensitizer is excited from a stable electronic ground singlet state to an excited singlet state ($^1P^*$), with lifetime 10^{-8} - 10^{-9} s. It then can decay back to the ground state with release of energy in the form of fluorescence (photons emitting) or undergoes intersystem crossing to a longer-lived excited triplet state ($^3P^*$) (10^{-3} s). This triplet state has better possibility to meet oxygen, as one of the few chemical species present in the tissue with a ground triplet state (3O_2). When they are in proximity, the photosensitizer transfer its energy to oxygen, provided the energy of the $^3P^*$ molecule is higher than that of the product 1O_2 [9, 43]. This transfer allows the photosensitizer to relax to its ground singlet state, and create an excited singlet state oxygen molecule (1O_2) (Fig. 3). Singlet oxygen is a very aggressive compound and will very rapidly react with any nearby biomolecules. The quantum yield of free radical formation depends heavily on the photosensitizer chosen. Ultimately, these destructive reactions will result in cell killing through apoptosis or necrosis [45, 46]. The relaxed photosensitizer is able to repeat the process of energy transfer to oxygen many times [9]. The energy transfer from triplet state photosensitizer to other biomolecules occurred through either Type I or Type II reaction mechanism.

Type I and II reaction mechanisms [47]

Type I reaction involves electron/hydrogen transfer directly from the photosensitizer, producing ions, or electron/hydrogen abstraction from a substrate

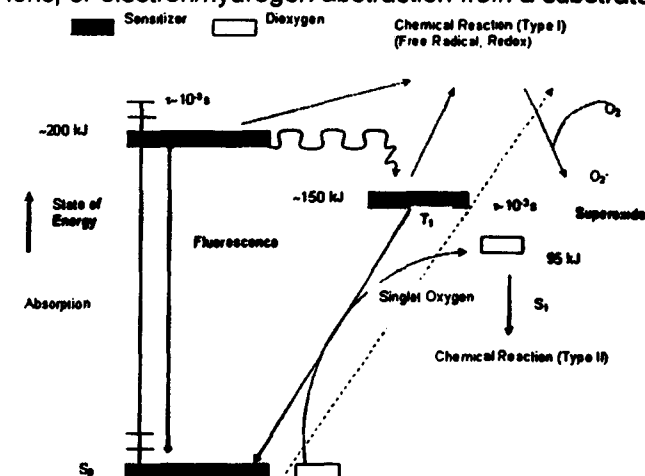


Fig 4. Generation of excited photosensitizer states and reactive dioxygen species [38]

molecule to form free radicals. These radicals then react rapidly, usually with oxygen, producing highly reactive oxygen species, such as superoxide and peroxide anions. Type II reactions produce singlet oxygen through direct interaction of the triplet state photosensitizer with oxygen (Fig. 4).

In PDT, it is difficult to distinguish the type of mechanism occurred. There is possibility that both of the reaction type play a role, indicating the mechanism of damage is dependent on oxygen tension and photosensitizer concentration. Type II process is considered to be more important in PDT, although cytotoxic species generated by the Type I reaction process also act in a site-specific manner.

PDT procedures produce cytotoxic effects through photodamage to organelles and biomolecules. These sites of photodamage may reflect the localization of the photosensitizer in the cell. Many cellular components such as amino acids (particularly cysteine, histidine, tryptophan, tyrosine and methionine), nucleosides (mainly guanine) and unsaturated lipids can react with singlet oxygen. The diffusion distance of singlet oxygen is relatively short (about 0.1 μM); therefore the photosensitizer must associate intimately with the substrate for efficient photosensitization [48].

Targeting and Localization

Localization of photosensitizers is influenced by many factors, such as the incubation parameters, mode of delivery and the characteristic nature of the photosensitizer [49]. The targets for photodamage may vary as the result. In cell culture studies with porphyrin-based photosensitizers, short incubation times (up to 1 h) prior to illumination leads primarily to membrane damage, while longer period resulted in the damage of cellular organelles and macromolecules [50].

Initially, photosensitizer are taken up by both normal and hyperproliferating cells, but retained longer in the latter. The mechanisms are not understood in detail. Increased blood vessel permeability and poor lymphatic drainage in neoplastic tissues may contribute to the retention in neoplastic lesions. The factors determining the specific localization of photosensitizer in the cells are sensitizer's lipophilicity, while aggregation degrees mostly determine the accumulating efficiency and localization specificity in the tumor cells [51].

The physico-chemical properties of the photosensitizer determine the efficacy of photosensitization. The tumor selectivity increases to some extent with the lipophilic character of the photosensitizer. Hydrophobic sensitizers strongly bound to lipoproteins (high density lipoproteins (HDLs) and low density lipoproteins (LDLs), distributed within the blood system and transported to the malignant tissue with a distinct selectivity, due to particularly large number of LDL membrane receptors of neoplastic cells. After endocytosis induced by the receptors, the sensitizer

molecules preferentially accumulate in the lipophilic compartments of tumor cells, including plasma, mitochondrial, endoplasmic reticulum, nuclear and lysosomal membranes. Lower tumor pH also enhance through uptake of photosensitizers. The lower pH is related to their poor oxygen supply and high glycolytic activity. Hydrophobic compounds preferentially bind membranes and will target structures such as the plasma membrane, mitochondria, lysosome, endoplasmic reticulum, and the nucleus. Oxidative degradation of membrane lipids can cause the loss of membrane integrity, resulting in impaired membrane transport mechanisms and increased permeability and ruptures. Cross-linking of membrane associated polypeptides may result in the inactivation of enzymes, receptors and ion channels [52].

Hydrophilic photosensitizers are largely carried by albumin and other serum proteins. These sensitizers preferred to localize within the interstitial space and the vascular stroma of the tumor tissues. There is only small tendency to diffuse across the plasma membrane into the cytoplasm [53].

Mitochondrial localization

Much work has focused on photosensitization of mitochondria because these organelles perform vital functions in the cell, like ATP formation for replication, protein synthesis, DNA synthesis and transport. Mitochondrial photosensitization may cause the uncoupling of respiration due to photosensitivity of several mitochondrial enzymes and carriers, resulting in the impairment of ATP synthesis and subsequent loss of cellular function. Lipophilic porphyrins have intimate intracellular association with mitochondrial membranes, whilst cationic compounds such as rhodamines and cyanines may accumulate in these organelles due to mitochondrial membrane potential. The loss of mitochondrial integrity after PDT treatment occurred before the loss of plasma membrane integrity. Mitochondrial damage can also induce nuclear chromatin condensation and subsequent apoptosis [52].

Lysosomal localization

Lysosomal localization has been observed for a number of photosensitizers, although redistributed to other cellular sites upon light exposure. Initially, membrane photodamage of lysosome causes the release of enzymes. These enzymes are thought to induce cell death, however cell survival has since been observed to 80% of cellular lysosomes.

Nuclear localization

Nucleus photosensitization has been shown to cause single/double stranded breaks and alkali-labile sites in DNA, sister chromatid exchanges and chromosomal aberrations. However, nuclear damage

and/or repair seem not to be a dominant factor in PDT mediated cytotoxicity [53].

Photosensitization Effect

In general, hydrophobic drugs attack the tumor cells mainly by direct interactions, whereby tumor cells are damaged by the direct effect of photosensitization as consequence of injure of tumor cell. In contrast, water-soluble sensitizer kill tumor cells indirectly by damaging blood vessels, destruction of the vascular system (endothelium and other components of cellular wall), destruction of intercellular matrix and interrupting the supply of oxygen and other essential nutrients. This vascular damage induced hypoxia and finally resulted in death of the neoplastic cells in tumor and necrosis. During PDT the oxygen concentration in the tumor may be further reduced by the conversion to singlet oxygen and its irreversible reactions with biomolecules and damage of supplier vessel. Tumor destruction is most efficient using compounds with a long triplet half life and a high quantum yield for the triplet excited state.

The damage of plasma membrane can be observed within a few minutes after irradiation. Manifestation of this type of damage are swelling and blebbing, shedding of vesicles containing plasma membrane marker enzymes, cytosolic and lysosomal enzymes, reductions of active transport, depolarization of plasma membrane, inhibition of activities of plasma membrane enzymes such as $\text{Na}^+ \text{K}^+ - \text{ATPase}$, a rise in Ca^{2+} , up and down regulation of surface antigens, etc. These effects will eventually induced cell death pathway, through necrosis or apoptotic process [4].

In apoptosis, the cell is actively participated in its self-annihilation. The cell mobilizes a cascade of events that leads to disintegration and the formation of apoptotic bodies. The cell will subsequently phagocytized by the neighboring cells without involving inflammation. Increased cytoplasmic Ca^{2+} concentration, cell dehydration, chromatin condensation, activation of endonuclease which has preference to DNA at the internucleosomal sections, proteolysis, fragmentation of the nucleus and cell are the most characteristic events of apoptosis. Reduction of the expression of nuclear factor kappa-B as the survival-promoting factor and inducer of caspase (main effector of apoptosis) suppression would be useful to increase therapeutic efficiency.

The necrosis is an alternative to the apoptotic mechanism. Necrosis is a passive and degenerative process, usually induced by an overdose of cytotoxic agents. Necrosis triggers the inflammatory response in the tissue. The early event of necrosis is swelling of cell, followed by rupture of the plasma membrane and release of cytoplasmic contents.

Treatment Usage and Ways to Improve Efficiency

There are many studies concerning the use of PDT to treat malignant and non-malignant diseases.

Malignant diseases include: skin premalignant (squamous cell carcinomas (SCCs), basal cell carcinomas (BCCs) and malignant melanomas, and the secondary cancers originating from breast cancer, colon cancer and endometrium cancer), ophthalmic tumor (choroidal haemangioma), head and neck cancer (oral mucosa, particularly multi-focal squamous cell carcinoma), brain tumor (irradiate the cavity following surgical resection), pulmonary and pleural mesothelial cancer (non-small cell lung cancer (NSCLC), malignant pleural mesothelioma), breast cancer, gastroenterological cancer (esophageal cancer, early-stage esophageal cancer, Barrett's esophagus, Barrett's mucosa, cholangiocarcinoma), urological cancer (prostate cancer), gynecological cancer (vulvar and vaginal intraepithelial neoplasia (VIN, VAIN)). Non malignant diseases include: dermatological diseases (acne vulgaris, psoriasis, viral warts, hair removal, rosacea, port-wine stain (PWS)), ophthalmic diseases (age-related macular degeneration (AMD), subfoveal choroidal neovascularisation (CNV), pathological myopia or presumed ocular histoplasmosis syndrome), cardiovascular diseases (intimal hyperplasia, atherosclerosis or vulnerable plaque, and prevention of restenosis after coronary-stent placement), dental (dental caries and periodontal diseases), urological diseases (Benign prostatic hyperplasia (BPH)) [40, 54-57]. It is also being used for wet macular degeneration and cancer that are on, or near, the lining of internal organs, such as cancers of the head and neck area, the lining of the mouth, lung, gullet (esophagus), stomach, bladder, and bile vessel. The usage of this treatment is still limited due to limited thickness of light penetration to cell.

The combination of PDT with ionizing radiation might improve the limited depth of target tissue damage, induced after PDT. Some additivity is expected in damaging cell key-targets, inactivation of repair systems, induction of apoptosis, etc. The combination becomes possible if the sensitizers can act as radiosensitizer. The efficiency of the treatment would be significantly increased and the cost of the treatment would reduce. Response to ionizing radiation depends heavily on three factors: porphyrin dose, porphyrin type and tissue type.

ADVANTAGES, LIMITATIONS AND SIDE EFFECTS OF PDT

Advantages

The main advantages of PDT over other therapies include rather significant degree of selectivity of drug accumulation in the tumor tissue, the absence of systemic toxicity of the drug alone, the ability to irradiate only tumor, the possibility of treating multiple lesions simultaneously, the ability to retreat a tumor in order to improve the response, and compatibility with

Table 4. Overview of 1st and 2nd generation photosensitizer [39]

| Brand | Ingredients | Indication | Advantages | Shortcomings |
|------------------------|-------------------------|--------------------------------------------------|------------------------------------------------------------------------------------|------------------------------------------------|
| Photofrin ¹ | Hematoporphyrin Mixture | esophageal cancer, endobronchial, Bladder cancer | easy synthesis easy formulation worldwide approval | weak absorption in red skin photosensitization |
| Foscan ² | Temoporfin | Head and neck cancer | Absorption beyond | lack of specific targetting |
| Visudyne ² | Verteporfin | AMD | absorption specific of blood, low dosage good solubility higher tolerability | |
| Metvix ² | Methyl-ALA | Actinic keratosis | Stimulates intracellular production of porphyrins | skin photosensitization pain |

other cancer treatments such as surgery, radiotherapy and chemotherapy.

Specificity of treatment is achieved in three ways. First, light can be exposed to specific area (Table 3). In the absence of light, there is no activation of the photosensitizer and no cell killing. Second, photosensitizers may be administered in ways that restrict their mobility. Finally, photosensitizers chosen may have greater selectivity to the targeted cells. Some of the drugs (Table 4) being developed also have the desirable property of concentrating in tumors (and certain other kinds of proliferating tissue) relative to the surrounding healthy tissue, which also helps in targeting [58]. ALA is taken up much more rapidly by metabolically active cells. Since malignant cells tend to be growing and dividing much faster than healthy cells, ALA targets the unhealthy cells.

Limitation

A major disadvantage of PDT is that the activation light of most photosensitizer can not penetrate through more than one third of an inch (1 cm) of tissue. Thus the application of PDT is limited to the treatment of tumors on or under the skin, or on the lining of some internal organs, but less effective in treatment of large tumors and metastasis.

Side Effects

As with all kinds of treatment, the experience of PDT can vary from person to person. How the treatment is given and the side effects that it may cause vary, according to the area of the body affected by the cancer, the type of photosensitizing drug given, the time between giving the drug and applying the light and the amount of skin sensitivity to light following treatment.

When PDT is used to treat skin cancer, its side effects are different to when PDT is used for a cancer elsewhere in the body. For skin cancer, the possible side effects are pain, sensitivity to light, and scab on the treated area which will fall off after about three weeks. As with other cancers, the possible side effects are photosensitivity, pain in the tumor area, swelling, inflammation, constipation, nausea, and scar.

CONCLUSION

The discussion above has shown that PDT is a useful new modality for the treatment of many disorders. It has comparable and even better effect on patients than other mature treatment. Eventhough, still there are many completion needed. The search for the best fitted photosensitizer for each ailment is still on quest. The dosimetry has not been fixed for every treatment. Many of the photosensitizers had not been undergone descent clinical trial and has not been approved. The kinds of disorders to be treated are still limited and confusing. There are still many spaces left for improvement of the efficiency, efficacy and effectiveness of the treatment, like combination with other treatment or manipulation of intrinsic factor. The treatment has not been worldwide and the knowledge of this treatment is still limited besides the many meeting held. There is a long way for this treatment to become patented, trustworthy treatment and therefore, further investigation and promotion are highly needed.

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