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Photodynamic therapy and cancer: a brief sightseeing tour

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Photodynamic therapy (PDT) combines a drug (a photosensitiser or photosensitising agent) with a specific type of light to kill cancer cells. It is a minimally invasive treatment, with great potential in malignant disease and premalignant conditions. Following the administration of the photosensitiser, light of the appropriate wavelength is directed onto the abnormal tissue where the drug has preferentially accumulated. Upon light activation, the photosensitiser transfers its excess energy to molecular oxygen to produce an excited state (i.e., the highly reactive singlet oxygen) that causes oxidative damage at the site of its generation. The energy transfer occurs either directly to oxygen or through an indirect mechanism that requires the formation of intermediate radical species. Many photosensitisers have been developed, but only a few have been approved for therapy in humans. Basic research in model systems (animals, cell lines) has unravelled some fundamental cellular processes involved in the cell response to PDT. The exploitation of relevant molecular observations, the discovery and introduction of new sensitisers, the progress in the light delivery systems and light dosimetry are all concurring to the increase of PDT therapeutic efficacy. However, this field has not yet reached maturity. This review briefly analyses the relevant properties of most photosensitisers and their field of application. Special attention is dedicated to the effects observed in model cancer systems; speculation and suggestions of possible future research directions are also offered.

Keywords: cancer laser, photodynamic therapy, photosensitisers, singlet oxygen

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1. Introduction

Photodynamic therapy (PDT) is a laboriously emerging form of therapy that has great potential in the treatment of many diffused human diseases, including macular degeneration, several dermatological disorders and cancer. Despite the large number of published papers (> 10,000), very few individuals outside the specialty areas of ophthalmology and dermatology are familiar with this therapeutic approach and its fundamentals.

PDT of tumours was born ~ 100 years ago when H Von Tappeiner, in Munich, burned a neoplastic skin lesion imbibed with eosin solution using the white light of a lamp [1]. This demonstration was based on the observations of a medical student working in the same laboratory, who described the lethal effect of light on bacteria treated with acridine. The therapeutic effect on the tumour was ascribed to a photodynamic action of light on the dye adsorbed by the lesion. Although this observation opened the door to modern PDT for cancer, PDT has remained largely unexploited until recently. In 1960, Lipson and Baldes [2,3] reported the preparation of a non-toxic haematoporphyrin derivative that appeared to have a particular propensity to accumulate within neoplastic tissues. However, it has only been since 1970 that the clinical utility for tumour removal has been finally realised. Since then, as a result of continuous improvements in drug preparation

and availability of suitable light sources, PDT has found positive indications in several conditions and should be considered as a modality for the treatment of cancer in humans. At present, it is especially employed for the treatment of skin cancers (mainly non-melanoma skin cancers), some precancerous lesions (i.e., Barrett's esophagus) and some forms of advanced cancers. In advanced diseases, in which other therapies are not effective, the intrinsic advantage of PDT, besides its limited invasiveness, is the option of repeated treatments. Furthermore, it does not prevent switching to more aggressive types of cures later on, if the patient conditions become more permissive.

2. The basis of the photodynamic effect

PDT requires the selective uptake and retention of a photosensitiser in the neoplastic tissue, tracked by irradiation with a particular wavelength. As a result of the irradiation, two competing processes occur as a consequence of the excitation of the photosensitiser. The first process is fluorescence emission, resulting from the return of the excited photosensitiser to its ground state. This emission of light (of a longer wavelength) can be of great aid in localising the tumour cells. The second process regards the production of a highly toxic singlet oxygen that ultimately kills the cells. This process is easily explained by photophysics. Because of its nature and chemical structure, the photosensitiser must be a molecule with an absorption maximum at a wavelength corresponding with that of the incident light. Following adsorption, the photosensitiser is promoted to an excited state. The excited molecule can decay back to the ground state with release of fluorescence. However, if the lifetime of the excited state is long enough – and this is true for all photosensitisers used in PDT – the energy excess may be used to drive chemical reactions. In the presence of oxygen, two types of reactions, namely Type I and II (Figure 1), may occur. In a Type I reaction, the activated photosensitiser abstracts hydrogen atoms directly from molecular components of the cell, causing the formation of a radical. Such species, in turn, transfer their excessive energy to ground-state oxygen ($^3O_2$) to form toxic singlet oxygen ($^1O_2$) and other oxygenated reactive species. In Type II reaction, the energy transfer occurs directly from the excited photosensitiser to $^3O_2$, which is readily transformed to $^1O_2$. Indeed, this species is a very cytotoxic molecule, characterised by a short lifetime (< 0.04 μs) and a short radius of action [4]. Therefore, the damaged area lies essentially within the tissue exposed to light. As the light (usually a laser source) may be precisely applied through optic fibres, the effect is local, rather than systemic, even when the drug has been given to patients systemically. The local nature of PDT may be seen as an advantage in that it cures the specific pathological area. Conversely, PDT cannot be used in cases of diffuse disease. It is also clear that as photoactivation is only possible when the photosensitiser is exposed to light, non-superficial tumours are not easily accessible, unless the light used is capable of deep penetration. This is partially achieved with the second-generation photosensitisers, whose activation requires wavelengths of 650 – 800 nm. These wavelengths can penetrate up to 1 cm or more in human tissues.

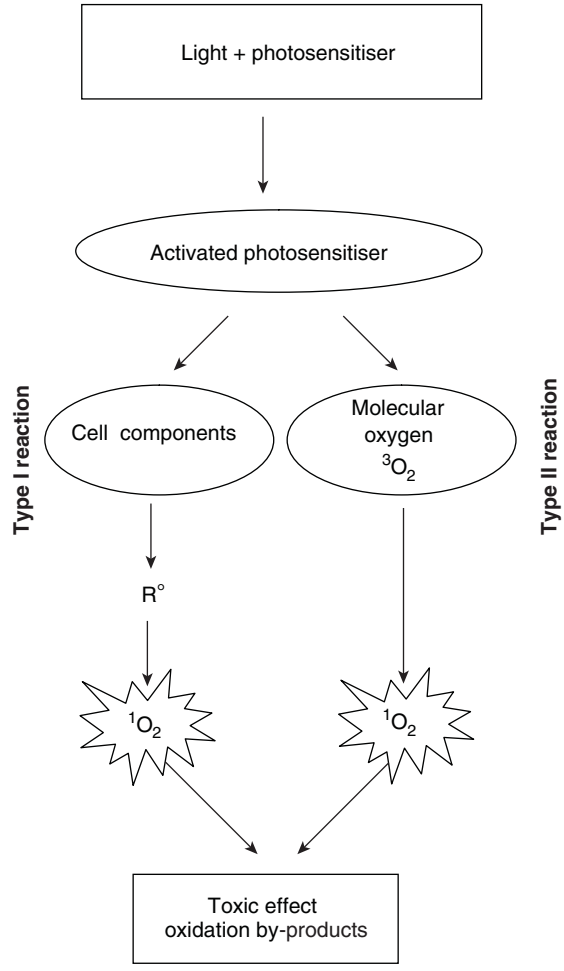

Figure 1. Photosensitisers can capture light energy. The excited photosensitiser may then decay back to ground state by using the excess energy to drive chemical reactions. Two possible pathways known as Type I (left) and Type II (right) reactions have been described.

3. Photodynamic effect and tumours

3.1 Direct effects, incomplete response and effects on vasculature

It is generally acknowledged that PDT achieves tumour damage by different mechanisms, which include rapid harm to tumour cells and delayed immune response.

Direct cell killing is the most important effect that is seen after photoactivation. Cell death is brought about by the local formation of reactive oxygen species (either oxygenated radicals or singlet oxygen). The nonhomogeneous distribution of oxygen within the tumour may be due to nonhomogeneous activation of

the photosensitiser [5] and, consequently, of incomplete therapeutic response. Another cause of incomplete response may be the asymmetrical distribution of the photosensitiser within the tumour mass and surrounding tissues, and a nonhomogeneous distribution even in the same tissue.

The localisation of photosensitisers in tumour tissues is dependent upon the chemical structure and physical nature of the photosensitiser, and the way it is administrated. Pioneering work by some groups have reported that some photosensitisers, such as porfimer sodium and $AlPcS_4$ (aluminium phthalocyanine with four sulfonate groups), preferentially locate in non-parenchymal areas, whereas others, such as $AlPcS_2$ and $AlPcS_1$ (with two and one sulfonate groups, respectively), are preferentially taken up by parenchymal cells [6,7]; since these studies, not much has been done in this regard. Indeed, the incomplete response to PDT, sometimes observed, has often been ascribed to the nonhomogeneous drug distribution within the tissues and/or lack selectivity for the target cells.

However, the incomplete response may also have other origins. The photodynamic reaction itself consumes oxygen, which may rapidly deplete oxygen locally, and its local stock may extinguish rapidly even in sites not particularly far away from blood vessels [8]. In these conditions, the tumouricidal effect fades as the oxygen supply diminishes, because the toxic oxygenated species cannot be formed.

In addition to direct killing, other crucial elements in the action of PDT exist. One of these is the partial or even total destruction of the tumour vasculature [9]. It has been documented that PDT may induce vasculature collapse with consequent hypoxia of the neighbouring tissues [10] and rapid thrombus formation [11]. Another important indirect effect is a delayed promotion of immune response to the neoplastic tissue surviving after the photodynamic treatment [12,13].

3.2 Advantages and weakness of photodynamic therapy

PDT may provide distinct advantages over other methods for treating tumours.

The first advantage that PDT may offer over other approaches, such as surgery, is its non-invasiveness. However, the major claimed benefit of PDT originates from the selectivity that it promises in targeting cancer cells, whilst sparing healthy cells. This advantage is only in part achieved through the exploitation of the differential rate at which photosensitisers are taken up by normal and cancer cells, and by the fact that the photoactivating beam can be targeted with high precision on diseased tissue. Another concrete advantage of PDT is that repeated treatments can be performed without the restrictions that limit other cures, such as radiotherapy. Last but not least, singlet oxygen, the prime cytotoxic agent produced by photodynamic effect, has a very short lifetime and consequently harms only the cell in which it is generated. This also implies that once the light is shut off, there is no further, direct dangerous effect. In addition, the residual photosensitising drugs are essentially non-toxic.

Among the PDT drawbacks, the most important is represented by residual and prolonged photosensitivity in treated patients. Another important limitation of PDT originates from the intrinsic incapacity of light to efficiently penetrate human tissues, so that the photodynamic effect is mostly limited to the tumour surface. This aspect has been partially overcome with the introduction of second-generation sensitisers that are excited at a longer wavelength, allowing deeper tissue penetration. Finally, besides the drawbacks of inherent dark toxicity or inappropriate pharmacokinetic properties of the sensitiser, the most relevant limitation of PDT is the light dosimetry; an effective PDT treatment depends not only on the properties of the photosensitiser, but also by a suitably tailored dose of light. Unfortunately, dosimetry *in vivo* has not been developed sufficiently to guarantee the best performance and reproducibility, and remains largely empirical, despite some important recent progresses.

4. Light sources and dosimetry

The application of a proper light source for PDT is not a trivial problem, and the selection is usually a compromise. The advantages and disadvantages of the available light sources should be evaluated in individual cases. Similarly, the efficacy of PDT is dependent, among other factors, on the total light dose delivered into the target tissue

Conventional lamps, lasers and light emitting diodes are normally used in PDT [14]. Lasers may offer several advantages related to the monochromaticity and high light power (fluence). The monochromatic emission may improve efficacy if the emission wavelength coincides with the photosensitiser absorption peak. In addition, the laser's higher light fluence rate (radiant energy per second across the sectional area of irradiated spot, power/unit area, W/m^2), significantly reduces the therapeutic exposure times. However, if low power requires longer treatment times and induces undesirable biostimulation for cancer tissue, excessive power may, in turn, photobleach the photosensitiser, resulting in PDT failure. A real advantage of a laser beam is that it can be easily coupled with an optic fibre so that the light can be targeted exactly to the tumour. In the case of tumours located within body cavities (e.g., bladder, bronchial and esophageal cancers), light targeting is achieved through purposely manufactured, linear or spherical diffusive fibre tips [15,16].

In general, the ability of lasers to be coupled with optic fibres makes the task of light delivery to tumour sites more manageable, although precise dosimetry remains difficult and elusive. The effective penetration depth (δ^{eff}) of a given wavelength of light is a function of the optical properties, such as absorption and scatter at the target tissue. The fluence in a tissue is related to the depth (d), by the exponential expression: $e^{-d/\delta eff}$. Characteristically, the light penetration depth is ~ 2 – 3 mm at a wavelength 630 nm and may be significantly increased by using longer wavelengths (~ 800 nm) [17]. In general, photosensitisers with longer absorbing wavelengths and

higher molar absorption coefficients at these wavelengths are more effective photodynamic agents.

Diode lasers for PDT have been manufactured and marketed (e.g., DIOMED 630, Diomed, Inc.). They are characterised by several appealing features, such as affordable costs, high reliability, small size and portability, as well as excellent beam and spectral characteristics.

Non-coherent light (filament lamps) are much less expensive and can often be satisfactorily used in many situations, and especially when the lesions to be treated have a broader surface. Recently, a non-coherent PDT activation device, has been introduced (e.g., LumaCare® LC-122M; Ci-Tec Ltd), and is in use with several photosensitisers for therapy. Another widely employed device is commercialised by BioLitec; this is a 150 W quartz halogen visible light source (multispectral source) with interchangeable excitation filters and is designed for use with flexible, gooseneck fibre optics.

The light dosimetry is a more complex issue: the exposure of a tissue to a given light intensity (Watts/area) does not imply that the photons penetrate efficiently. Additional complications come from inner filter effects (photosensitiser self-shielding) and/or self-annihilation of the photosensitiser following exposure (photobleaching). Dosimetry requires the determination of the light fluence within the tissue during treatment and establishes the effective distribution of the photosensitiser. Until now, most of the photodynamic treatments are performed on empirical guidelines based on previous experiences. Suitable experimental systems and mathematical and Monte Carlo computational models that effectively optimise light dosimetry through the determination of light penetration and light threshold for the generation of the photodynamic reaction, are under investigation [18-21].

5. Photodynamic therapy, sensitisers and human cancer

It is outside of the scope of this paper to review in detail the clinical aspects of PDT and its progress. These issues have been authoritatively discussed and fully reviewed by various specialists. An articulate picture of the specific areas in which PDT is applied in human therapy may be found in several recent articles (to cite a few, see references [22-24]).

5.1 Sensitisers

In general, sensitisers used in human cancer therapy (including clinical trials) are intravenously infused. The time between the injection and exposure to light may vary from drug to drug, according to the respective pharmacokinetic properties. For external skin lesion, specific sensitisers for local administration have been developed and are satisfactorily employed.

To date, very few drugs, belonging mostly to the class of porphyrins and chlorines, have been approved to be used in selected cases. These photosensitisers include porfimer sodium, m-THPC, meta-tetrahydroxyphenylchlorin, verteporfin, benzoporphyrin derivative monoacid ring A, 5-aminolevulinic acid, methyl and benzyl aminolevulinate. This list becomes considerably longer if photosensitisers undergoing clinical trials or at the preclinical stage are also included (Table 1).

5.2 Haematoporphyrin and porfimer sodium

The very first sensitiser employed in PDT was a haematoporphyrin derivative. Haematoporphyrin derivative is typically < 50% monomeric/dimeric porphyrins and > 50% oligomeric material. The latter fraction (porfimer sodium) has been partly purified in the commercial development of Porfimer sodium (Figure 2), which has been reported to be ~ 90% oligomeric material.

To date, porfimer sodium is the most commonly used photosensitiser in Europe and North America, for the treatment of advanced and early-stage lung cancers, esophageal adenocarcinoma, superficial gastric cancer, cervical cancer, Barrett's esophagus and bladder cancer, among others (mainly in clinical trials). The drug is formulated in a water soluble preparation and should be intravenously injected well before irradiation. After 20 years of use, porfimer sodium has proved to be a non-toxic drug so that, in principle, it can be administered repeatedly, without serious consequences. However, there are documented drawbacks to treatment with this photosensitiser. As the compound is a complex mixture, there are questions concerning the identity of the active components and also the reproducibility of the synthetic process. In addition, it has a low fluorescent quantum yield and a low efficiency in generation of reactive oxygen species. Exposure to light is normally carried out 48 h after systemic administration of the drug, when its concentration within the tumour is supposed to be at the peak. Skin photosensitivity post-treatment can last for > 3 weeks. During such a time span patients have to avoid bright light and they suffer severe restrictions on everyday activities. Porfimer sodium is excited clinically at 630 nm – a wavelength that only superficially penetrates human tissues, to a depth of millimetres. For this reason, porfimer sodium is considered inappropriate for the treatment of non-superficial tumours.

Following the initial successful attempts to treat specific cancers with PDT, a number of new photosensitisers have been developed; for example, second-generation photosensitisers. In contrast to porfimer sodium and haematoporphyrin derivative, which are mixtures, these drugs are pure substances and present a higher absorbance in the red region of the spectrum. The higher molar absorption coefficient results in a more intense excitation of the photosensitiser at a wavelength that also allows a deeper penetration into tissues and consequently a more efficient therapeutic effect (provided that sufficient oxygen is available).

The second-generation of photosensitisers includes many families of molecules, such as modified porphyrins, chlorines, bacteriochlorins, phthalocyanines, naphthalocyanines, pheophorbides and purpurins.

Table 1. An incomplete list of photosensitisers presently used in clinical and preclinical studies.

Phtosensitiser	Major reported uses	Wavelength (nm)
Photosensitisers used in clinical and preclinical cancer studies.		
Porfimer sodium	Cervix, advanced and early lung, esophageal, bladder, superficial gastric, brain cancers	~ 630
m-THPC	Head and neck cancer (selected cases of prostate and pancreatic cancer)	~ 650
BPD-MA	Basal-cell carcinoma	~ 690
5-ALA	Basal-cell carcinoma, head and neck, gynaecological	635
5-ALA methyl ester	Basal-cell carcinoma	635
5-ALA benzyl ester	Gastrointestinal cancers	
5-ALA hexyl ester	Cancer diagnosis	370 – 400
Photosensitisers used in preclinical cancer studies and/or clinical trials		
Mono-L-aspartyl chlorin e6	Early endobronchial carcinoma, preclinical studies	664
Disulphonate-Al-phtalo-cyanine	Head and neck cancers, preclinical studies	650 – 800
[5,10,15,20]-tetrakis-m-hydroxy phenyl-chlorin	Liver metastasis, preclinical studies	740
Lu(III)-texaphyrin or Motexafin-Lu (III)	Prostate cancer, preclinical studies	732
Pd-bacteria-pheophorbide	Prostate cancer (after radiation failure), preclinical studies	763
2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide	Esophageal cancers	665
Photosensitisers used in preclinical cancer studies		
Rostaporfin	Recurrent skin metastatic breast cancer, cell lines	~ 660
Sulphonated aluminum phthalocyanines	Animal studies, cells	650 – 700
Hypericin	Cell lines	550 – 590
9-acetoxy-2,7,12,17-tetrakis-[beta-methoxy -ethyl]-porphycene	Animal studies, cells	~ 640
Indocyanine green	Cell	790
Sulfonated meso-tetraphenylporphines	Animal studies, cells	~ 630

5-ALA: Aminolaevulinic acid; BPD-MA: Benzoporphyrin derivative monacid ring A; m-THPC: Meta-tetrahydroxyphenyl chlorine.

5.3 Chlorins and bacteriochlorins

Chlorins and bacteriochlorins are derived from hydrogenation of one or two of the exo-pyrrole double bonds of the porphyrin. These compounds, having a maximum absorption well above 630 nm (≤ 650), seem particularly suitable for PDT and are under intense investigation.

The meta-tetrahydroxyphenyl chlorin (m-THPC; Figure 3) is a second-generation photosensitiser, developed for clinical use by Scotia QuantaNova (SQN). This molecule was introduced in clinical trials > 15 years ago for the treatment of human mesothelioma. Now it is widely used in many countries to treat respiratory, gynaecological and, particularly, head and neck cancers [24]. The chlorin derivative, m-THPC, is a pure compound, whose effectiveness in PDT has been reported as ~ 200-fold higher than porfimer sodium [25]. In comparison with this photosensitiser, m-THPC is excited at a longer wavelength and with a molar absorbance coefficient of ~ 15-fold higher. In addition, its longer half-life in the excited state (triplet) causes the production of higher amounts of cytotoxic oxygen species, and its higher hydrophobicity facilitates cellular uptake. Indeed, the physicochemical properties of m-THPC require its dissolution in polyethylene glycol or covalent binding to some types of PEGs. The formulation known as Foscan® 2 (QuantaNova) is a predissolved preparation of m-THPC. However, the residual photosensitivity with m-THPC is probably not much lower than that with porfimer sodium, which it was originally claimed to be.

5.4 Mono-L-aspartyl chlorin e6

The second-generation photosensitiser, mono-L-aspartyl chlorin e6 (NPe6; Figure 4), was reported to have significant efficacy in killing cancer cells *in vitro* and *in vivo*. This photosensitiser has many positive features, including water solubility and an

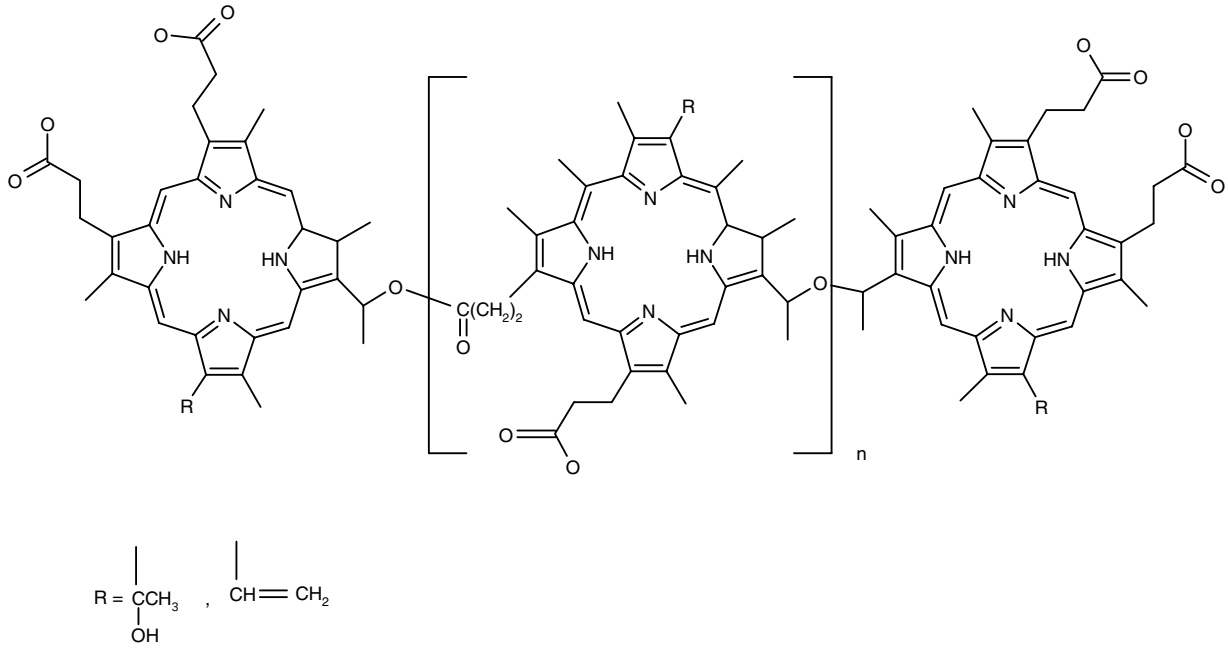

Figure 2. Porfimer sodium is a complex mixture of monomers and oligomers with n being 2 – 6.

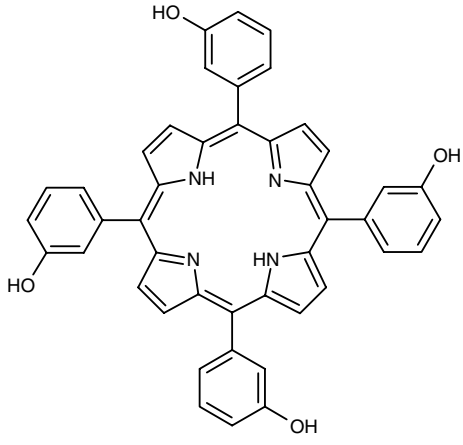

Figure 3. Meta-tetrahydroxyphenyl chlorine.

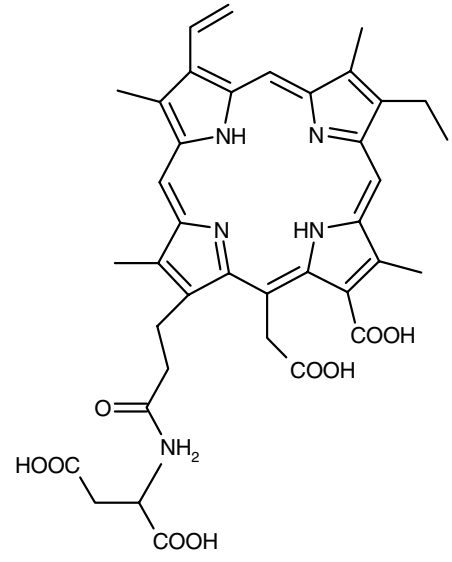

Figure 4. Mono-aspartyl chlorin e6.

absorbance peak at a longer wavelength (i.e., 654 nm), with an extinction coefficient of 40,000 $M^{-1}cm^{-1}$ and, very importantly, it is rapidly cleared from the body. This compound has been reported to induce sufficient phototherapeutic damage accompanied by mild and transient skin phototoxicity. Nevertheless, the best tumour response is obtained at higher photosensitiser concentrations, unfortunately at the expense of tissue selectivity. Until now, the use of NPe6 in humans in Western countries is limited to clinical trials [26-30].

5.5 Bacteriochlorins

A number of centres and companies are developing bacteriochlorins, which have almost ideal optical properties, in terms of tissue penetration. These compounds, which absorb light strongly at > 740 nm, show considerable promise as new PDT agents, although their stability remains in some doubt. The SQN400 agent, is activated by near-infrared light at a wavelength of 740 nm. SQN400 is the trade name of a chemical called meta-tetra(hydroxyphenyl) bacteriochlorin (mTHPBC; Figure 5). This compound has been recently used in some clinical Phase I studies [31,32].

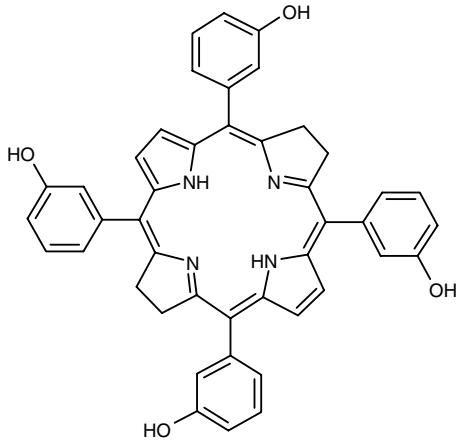

Figure 5. Meta-tetra(hydroxyphenyl) bacteriochlorin.

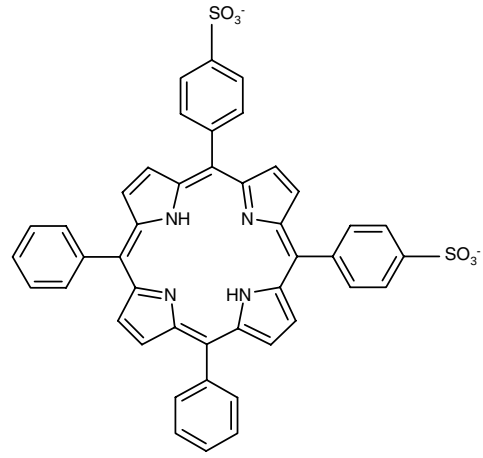

Figure 6. Disulfonated tetraphenylporphine.

5.6 Phthalocyanines

Recently, phthalocyanines have been developed as photosensitising agents for PDT. Phthalocyanines are generally hydrophobic compounds, although water-soluble derivatives can be readily synthesised through substitution of the ring with moieties, such as sulphonic acid, carboxylic acid and amino groups. A long-life triplet state is required for efficient photosensitisation, and this criterion may be fulfilled by the incorporation of a diamagnetic metal, such as Zn or Al, into the phthalocyanine macrocycle. The sulphonated compounds, and in particular disulfonated tetraphenylporphine (Figure 6) and chloroaluminium sulphonated phthalocyanines ($AlPcS_n$; Figure 7), have received the most attention with regard to photodynamic efficacy; $AlPcS_2$ is the polar molecule chloroaluminum disulfonated phthalocyanine that has been used in laboratory research since 1985 [33]. The advantages of these first-generation photosensitisers are the higher chemical stability, a superior, direct tumour cell phototoxicity, and a strong absorption peak in the red spectrum (650 – 700 nm), which permits the use of a wavelength that penetrates deeper into the tissue. The phthalocyanine $AlPcS_2$ exhibits a remarkable property in that it is selectively retained in some tumours. This property, coupled with negligible dark toxicity, minimal cutaneous photosensitivity and excellent photodynamic activity at increased wavelengths has led to the clinical evaluation of $AlPcS_2$ for PDT [34].

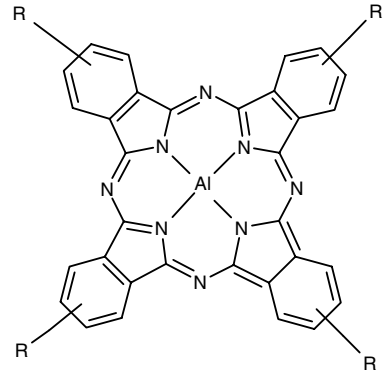

Figure 7. $AlPcS_{1\text{-}4}$. This family of molecules comprises members having 1, 2, 3 or 4 sulphonated groups.

5.7 Benzoporphyrin derivative

Benzoporphyrin derivative monacid ring A (BPD-MA; Figure 8) was developed by Quadra Logic Technologies (Vancouver, British Columbia, Canada), and has important applications in the treatment of choroidal neovascularisation in age-related, macular degeneration. BPD-MA is a hydrophobic molecule that is distinguished by the presence of a monoacid at either position three or four of the porphyrin ring. This molecule has been associated with lipocomplexes to reduce the photosensitiser dose, ameliorate its absorption and increase tumour specificity. BPDs, especially in conjunction with the HDL fraction of plasma lipoproteins, has been shown to be particularly effective in this context. The absorbance peak for PDT occurs at 650 nm, with an extinction coefficient of 34,000 $M^{-1}cm^{-1}$. With regards to cancer, BPD has found potential applications in dermatology. Indeed, skin cancer is one of the most widespread forms of cancer, accounting for almost 50% of all malignancies. The majority of cases are classified as non-melanoma, and include both basal cell and squamous cell carcinomas. Recent studies (Phase III clinical trials [35]) have shown that injectable BPD-MA possesses

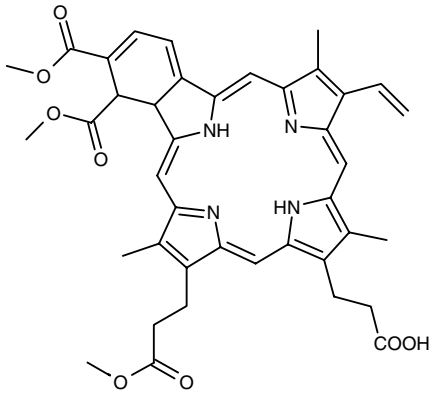

Figure 8. Benzoporphyrin derivative monoacid ring A.

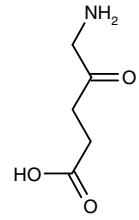

Figure 9. Aminolaevulinic acid. Methyl, hexyl and benzyl esters of this acid are other members of this family.

various, good properties, including a significant and rapid accumulation in the tumour and a low residual skin photosensitivity, and the overall cosmetic effect is acceptable. At variance with this treatment, traditional surgical procedures result in significant scarring and tissue loss for the patient, as well as being time consuming and very expensive.

6. 5-Aminolaevulinic acid and -esters

Another method to administer a photosensitising compound is to stimulate the synthesis of the active photosensitisers within the cell. Indeed, most cells contain the necessary equipment to synthesise haem. The biosynthetic pathway requires 5-aminolaevulinic acid (ALA, Figure 9) as a precursor, and involves the formation of various intermediates, including protoporphyrin IX, which is the last metabolite formed before haem synthesis. Protoporphyrin IX is an effective and potent photosensitiser. The rate of formation of protoporphyrin IX is dependent on the rate of synthesis of ALA from glycine and succinyl CoA which, in turn, is regulated in a negative feedback manner by the concentration of the final product concentration (haem). However, as the conversion of protoporphyrin IX to haem is rather slow, administration of exogenous ALA can circumvent the negative-feedback mechanism and cause accrual of significant levels of protoporphyrin IX. Furthermore, several investigations have indicated that certain types of tumour tissue exhibit increased accumulation of ALA-induced protoporphyrin IX [36]. A partial drawback of ALA as a presensitiser is its hydrophilic nature, which limits penetration into tissues. This problem has been in part reduceded by the use of lipophilic ALA esters, which can penetrate cells more easily.

ALA and its derivatives have been in use for some time; 5-aminolevulinic acid is approved for the treatment of precancerous actinic keratoses, and is being developed for the treatment of acne and other indications. An antineoplastic agent containing methyl-5-aminolevulinate is indicated for the treatment of basal cell carcinoma, as part of PDT, when other forms of therapy are not considered appropriate. Hexaminolevulinate's use in cancer therapy is confined to diagnostic purposes (bladder), by inducing *in situ* tumour fluorescence.

7. Hypericin and other potential photosensitisers

Hypericin (Figure 10), a naturally occurring substance found in plants (*Hypericum perforatum*), has been known in popular medicine for some time. However, its apparent antidepressant, antineoplastic, antitumour and antiviral (HIV and hepatitis C virus) activity has been, and still is, the object of extensive investigations, and these properties remain controversial and unexplained [37]. More recently, hypericin has been indicated as a promising PDT agent for the treatment of cancer, as it has excellent photosensitising properties, with a high yield of singlet oxygen [38]. In addition, the energy transfer of excited molecules results not only in the generation of radical species, but also in emitted fluorescence. These properties make hypericin a potential, powerful diagnostic tool. The best results in this regard have been obtained in the photodiagnosis of small tumours of the urothelium [39-42]. However, only a limited number of studies have been reported in other forms of human cancer [43].

Purpurins contain the porphyrin macrocycle and have absorption bands of 630 – 715 nm. Rostaporfin also absorbs light at 700 nm, with an extinction coefficient of 40,000 $M^{-1}cm^{-1}$. This purpurin (Figure 11) has already found important application in the treatment of the macular degeneration, but it has also been proposed for cancer treatment (basal cell carcinomas, squamous cell carcinomas, breast adenocarcinomas, cutaneous Kaposi's sarcomas in AIDS patients) [44]. Another molecule, lutetium texaphyrin, an expanded metallo-porphyrin (Figure 12), is the subject of a clinical trial (NCT00005067) for interstitial PDT in patients with locally recurrent prostate cancer.

Palladium-bacteriopheophorbide (Figure 13) has been successfully used to treat human prostatic small cell carcinoma [45]. Within this group of photosensitisers, 2-[1-hexyl oxyethyl]-2-devinyl pyropheophorbide-a, also appears to be a very promising molecule (Figure 14). This drug, now in a Phase II clinical trial (NCT00281736) for the treatment of esophageal cancers, seems to cause only mild skin photosensitivity that

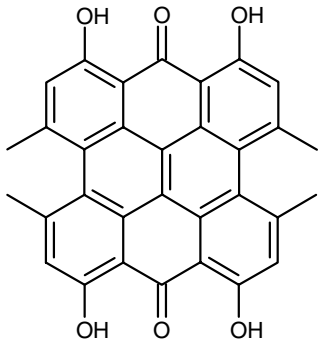

Figure 10. Hypericin.

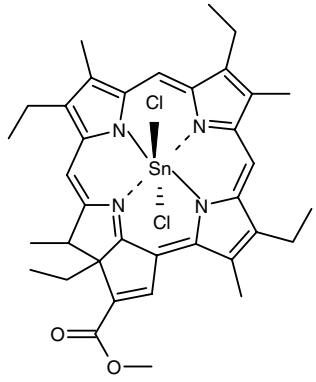

Figure 11. Purpurin.

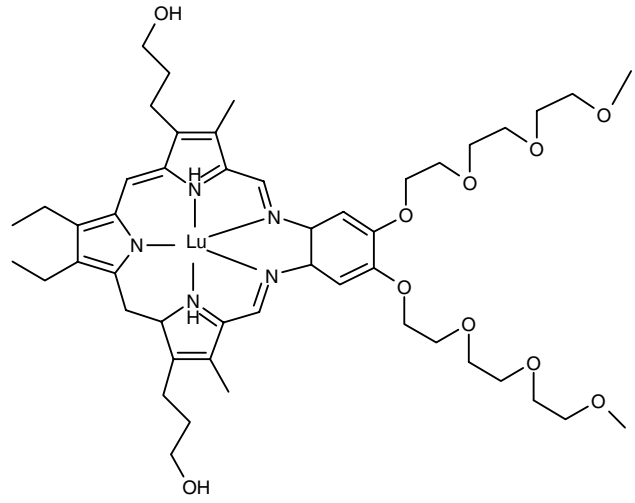

Figure 12. Lu(III) texaphyrin.

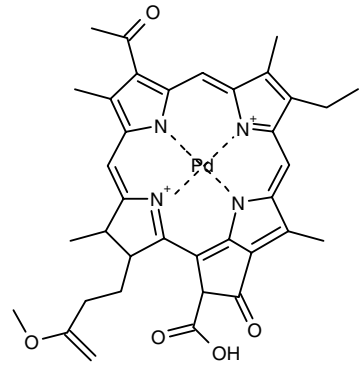

Figure 13. Palladium-bacteriopheophorbide.

declines in a few days [46]. This is an interesting report, as the prolonged photosensitivity observed in patients receiving porfimer sodium/m-THPC is a very incapacitating side effect.

Porphycenes (isomers of porphyrins) have unsuitable activation wavelengths (< 635 nm) for PDT, although 9-acetoxy-2,7,12,17-tetrakis-(methoxyethyl)-porphycene (Figure 15) has been assayed for potential topical application in skin lesions [47]. Because of the high fluorescence yields of porphycenes, they are considered potentially useful as diagnostic tools.

Anthracycline compounds exhibit reasonable tumour selectivity, and members of this group, such as doxorubicin, are widely used in chemotherapy, although adverse side effects are common. Some of these compounds have demonstrated additional phototoxicity, raising the potential of combination therapy in which comparable antitumour activity could be achieved with a lower drug dose, thereby reducing harmful side effects [48].

Indocyanine green (ICG), a tricarbocyanine dye, is a special case amongst photosensitisers. This compound (Figure 16) is already used in humans, where it is used as an angiographic agent, under the name of Cardiogreen. This molecule appears to have very interesting and rare photochemical property of an absorption band in the near-infrared region [49], and is non-toxic in humans. The photoactivation of ICG near 800 nm results in significant production of reactive oxygen species, and the infrared light (from a compact and inexpensive infrared laser source) guarantees the deepest penetration into tissues [50]. Despite the absence of controversial results or adverse reaction, this molecule is not particularly appealing to researchers, as the number of studies, even in model systems appears to be rather limited [51-56].

8. Photodynamic therapy in cancer preclinical studies

8.1 Molecular/cellular research

Successful clinical outcomes obtained with PDT over the years have prompted investigations, in order to develop an understanding of the cellular and molecular responses resulting from the photodynamic action on cells and tissues. It is now

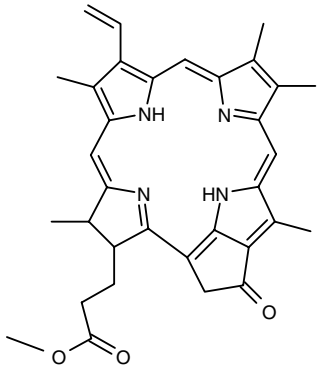

Figure 14. 3-devinyl-3-(hexyloxy-ethyl)pheophorbide.

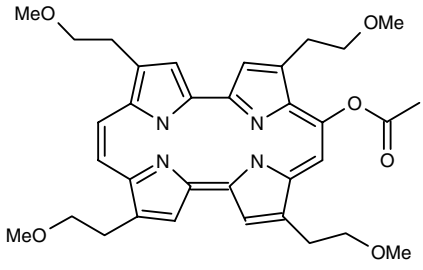

Figure 15. 9-acetoxy-2,7,12,17-tetrakis-(methoxyethyl)-porphycene.

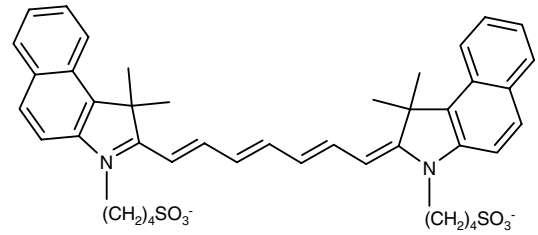

Figure 16. Indocyanine green.

generally acknowledged that only a full understanding of the effects caused by PDT at the molecular level will allow the adjustment to adjust, and ultimately set the optimal operational conditions required to improve the efficacy of the therapy. To date, few studies have been carried out in model systems and, rather surprisingly, most of this work is concerned with sensitisers that have not been approved for use in humans by the various health authorities worldwide. The therapeutic effects of PDT depend on many factors (the photosensitiser: type, distribution within normal and neoplastic tissues, specific subcellular distribution, time span between injection and treatment; the light: wavelength, continuous or intermittent irradiation, fluence rate, geometrical distribution of the light; and the target tissue: properties of the specific tissue, localisation), so it is surprising that approved drugs have not been the object of more systematic and multidisciplinary investigations. Targeted and coordinated cancer research-oriented programmes dealing with porfimer sodium, m-THPC and ALA (including its derivatives) are rare, despite a consensus that there is much room for improvement in therapeutic efficacy, even with the abovementioned drugs. In fact, the efforts spent in searching for new drugs and the number of experiments done to characterise their behaviour in living cells exceed those made to better unravel the cellular responses and molecular effects caused by the few drugs presently employed in human therapy.

The desired result of PDT is the death of cancer cells. When the photodynamic action on living matter (cells or tissue) is severe, the effect is the rapid or even instantaneous death of the cell. In the situation where immediate effect is non-lethal, a number of molecular pathways are activated, involving the immediate or delayed activation of alarm signals. This process is particularly relevant to cells comprising tissue layers below the surface of the tumour, as the amount of light that penetrates into tissues decreases rapidly with the depth. The number and type of signals that can be elicited are different and depend on many factors, including the optical, pharmacodynamic and pharmacokinetic properties of the photosensitiser, the environment and the particular genotype of the cell. The cell genotype and the PDT dose, in particular, have been found to account for the pathway by which the cell death occurs (i.e., apoptosis or necrosis). Not unexpected, was the finding that the nature of death rapidly shifted from apoptosis to necrosis when the intensity of photodynamic action was increased [57-60]. For PDT-mediated cell death, it is surprising that only a limited number of major observations have been obtained with porfimer sodium [61-69], m-THPC [70-73], ALA and ALA derivatives [74-77] (i.e., widely used drugs to cure cancers) compared with the number of those obtained with the group of other photosensitisers that are not or, at least not yet, widely employed in clinical practice.

A good review of preclinical studies of the intracellular effects caused by the activation of the most common photosensitisers in cell lines was reported in 2004 by Almeida *et al.* [78]. With regards to apoptosis, it was suggested that under sublethal photodynamic conditions, both receptor-mediated and mithocondria-mediated apoptotic pathways could be activated. Another important conclusion was that the switch from one cell-death pathway was dependent essentially on the nature and subcellular distribution of the sensitiser. In addition, the balance between the cellular activity of pro- and antiapoptotic members of the Bcl-2 family was seen as being capable of ultimately setting the resistance limits of cells to PDT-induced apoptosis.

The relationship between PDT and antitumour immunity is another large area that has been extensively covered in the literature by others [79], and will not be discussed here.

8.2 Photodynamic therapy, MAPKs, regulation of major transcription factors and protein expression

8.2.1 MAPKs

The function of MAPKs in response to PDT has also been investigated in various systems (cell and photosensitisers). The MAPKs include the ERKs, the c-Jun *N* cellular signal regulated kinases, the c-Jun *N*-terminal kinases/stress activated protein kinases (JNK/SAPKs), and the p38 MAPK. Each of these kinases is involved in signalling cascades that ultimately regulate the gene expression in response to external stimuli, including PDT-mediated oxidative stress [80]. Although activation of ERKl/2 was found to protect cells from porfimer sodium PDT [81], and p38 MAPK and JNK are considered responsible for the induction of apoptotis, it appears that the role of individual MAPKs in cell death is dependent on cell type and physiological context [80].

The evidence that has been collected indicates that the effect of PDT on the activity of MAPKs may also depend on the particular cell line and/or photosensitiser used. This issue has been specifically addressed by Tong *et al.* [81,82] and more recently confirmed by results obtained using a benzoporphyrin derivative [83] or hypericin [84,85] as a sensitiser.

8.2.2 AP-1 transcription factors

AP-l is a homo- or heteroodimeric protein complex composed of various proteins belonging to the Jun, Fos, Maf and activating transcription factor (ATF) families. The AP-1 transcription factors are activated by stress and typically recognise either cAMP responsive elements and/or 12-*O*-tetradecanoylphorbol 13-acetate responsive elements. AP-l transcription factors have been related to both the induction and prevention of apoptosis, depending on the tissue and on its developmental stage.

Although PDT with various porphyrin-based sensitisers has been reported to enhance the levels of mRNA of *c-fos*, *c-jun*, *c-myc* and other genes, and the half-life of *c-fos* and *c-jun* transcripts in different types of cells [86,87], the identification of the exact signalling cascade responsible for AP-1 activation still awaits clarification.

8.2.3 NF-κB

NF-κB is the generic name of a family of transcription factors that act as dimers and regulate the expression of genes involved in the inflammatory/immune responses, as well as in some aspects of cell growth, survival and differentiation. In most cells, the major proportion of NF-κB proteins is found within the cytoplasm in a silent state, bound to inhibitory proteins, called IκB's. The activation of NF-κB can be divided into two phases. The first phase involves cytoplasmic events leading to the activation of a kinase complex. This complex phosphorylates serine residues in the *N*-terminal region of the IκBs, resulting in their polyubiquitylation and proteasome-mediated degradation. The main consequence of IκB degradation is the translocation of NF-κB dimers to the nucleus, where they take part in transcriptional activation.

As far as NF-κB factor in PDT is concerned, only a few observations have been reported with porfimer sodium during the last decade [88,89]. Conversely, the effects of photoactivation of other sensitisers, such as hypericin, pyropheophorbide esters and BPD, on NF-κB have been recently reported [90-92]. All data indicate that PDT induces NF-κB to activate the transcription of various target genes, but information concerning the role of NF-κB in cell death is still limited and largely incomplete.

8.2.4 E2F and Rb transcription factors

Ever since its discovery, the *Rb* gene and the corresponding protein, pRB, have been considered extremely important in cancer research. The E2F transcription factors provided the key to our present understanding of *Rb* function in the regulation of the cell cycle and in tumour suppression. The complexes of cyclins D, A and E and their associated kinases are all involved in the phosphorylation of Rb during the cell cycle.

PDT has been reported to reduce the level of phosphorylation of Rb [93], thus inhibiting the transcription functions of the E2F factors. In addition, it has been reported that phthalocyanine PDT induces the expression of WAF1/CIP1/p21 protein in some model systems [94]. Both hypophosphorylation of Rb and the upregulation of p21 are considered negative regulators of the transition from G_1 to S phase, leading to G_0/G_1 arrest.

8.2.5 p53

p53 protein has emerged as a key tumour suppressor protein at the crossroads of cellular stress response pathways. Through these pathways, which can lead to cell-cycle arrest, DNA repair, cellular senescence, differentiation and apoptosis, p53 facilitates the repair and survival of damaged cells, or eliminates severely damaged cells from the replicative pool to protect the organism. Multiple functions can be performed by p53, many of which can be traced to its activity as a transcription factor. Because of these dynamic and multiple functions of p53, which are largely lost following mutations in the gene encoding p53, this molecule continues to be intensively studied in biomedical research, including in the fields of toxicology and pharmacology.

In PDT, the role of p53 has often been studied by comparing the response to photoactivation of $p53^{+/+}$ cells with that of related cell lines lacking the protein. Many reports indicate that photoactivation by various sensitisers (porfimer sodium, hyperycin and ICG) induces p53 expression [53,95,96].

8.2.6 Heat-shock proteins

Heat-shock proteins can protect cells from stress-induced damage. This heat shock response is known to be transcriptionally regulated in eukaryotic cells exposed to certain forms of environmental stress. Many proteins of this family have been reported to be induced by various photodynamic treatments [97,98]. It is interesting that following sublethal photodynamic treatment of MCF-7cells, the

expression pf heat shock proteins 60/70 is strongly induced. The stimulation of high levels of these proteins may contribute to subsequent resistance of these cells to various stresses (including further PDT) that would normally induce apoptosis [99].

Even though PDT has been the object of numerous molecular studies, clinical PDT has received negligible help from them. The scattered and heterogeneous body of work in this area has not been able to draw any useful conclusions.

9. Photodynamic therapy perspectives

So far, PDT appears to have many open perspectives. The most appealing one appears to be the discovery and introduction of new photosensitisers whose properties would progressively improve the efficacy and specificity of a particular cancer therapy. However, the invention of new drugs is not the only direction of development of PDT. One important field of study is photosensitiser delivery technology. Another perspective concerns the use of PDT, not only as a stand-alone modality, but also in combination with surgery, radiotherapy, immuno- and chemotherapy.

9.1 Drug delivery

Routes of administration, biodistribution and elimination of available photoactive agents can be modified by drug delivery systems to optimise therapy. The extent of drug absorption into the general circulation is affected by the bioavailability of the drug (i.e., the fraction of a dose reaching the target tissue). The hydrophobic nature of photosensitisers limits their solubility in water and significantly hampers bioavailability. This is especially true for those photosensitisers, such as porfimer sodium and m-THPC, which are administered to patients by systemic injection. In addition, a selective increase in uptake of photosensitiser molecules by tumour tissue would be of great interest in cancer PDT, as the existing drugs are only moderately specific to cancer cells.

9.1.1 *Nanobiothecnologies and photodynamic therapy*

At present, the most promising application of nano-technology in medicine and pharmacotherapy is in drug delivery. In this regard, nanobiotechnologies have been applied to improve drug biodistribution and to overcome the many biological, biophysical and biomedical barriers to drug delivery. This technology can prove to be very useful in cancer therapy, allowing for effective and targeted drug delivery.

Nanoparticles may be defined as submicronic colloidal systems that are usually composed of polymers. Suspensions of nanoparticles are achievable because the interaction of the particle surface with the solvent is strong enough to prevail over differences in density, which frequently result in material either sinking or floating in a liquid. Depending on the preparation procedures and the specific need, nanospheres (matrix systems) or nanocapsules (reservoir systems) can be obtained. The drugs can either be directly incorporated during polymerisation or adsorbed onto preformed nanoparticles. Polylactic acid or polylactic-co-glycolic acid, nanoparticles have been satisfactorily used in PDT in preclinical studies [100-103], as well as gold [104] and silica [105], among others [106].

9.1.2 *Targeted molecular delivery systems and photodynamic therapy*

The significant and selective accumulation of a photo-sensitiser in tumour tissues would greatly enhance the success of PDT. This increased selective localisation, while increasing the local concentration of the photosensitiser, would also reduce the need for precise light dosimetry, and the concerns of toxicity. If this is possible, in principle, other highly photoactive substances that would not concentrate into tumour tissues on their own, could be targeted efficiently and used in therapy.

One strategy to achieve this result has been the exploitation of the properties of monoclonal antibodies (mAbs). Indeed photo-immunotargeting was proposed several years ago [107]. However, this requires conjugates with favourable photosensitiser-to-mAb ratios, (maximal photo-sensitiser incorporation, but with optical photosensitiser properties and antibody activities remaining unchanged). Although this approach has been effectively used in pre-clinical models [108,109], it is not without its problems, including difficult chemical synthesis, stability, barriers to delivery [110] and potential toxicity. For these reasons, the application of photo-immunotherapy has remained largely confined to the laboratory.

Another promising targeted strategy, based on the folate receptor abundance in some tumours, has been recently proposed. In this regard, some research groups have designed, synthesised and assayed various folic acid targeted porphyrin derivatives for selective therapy of folate receptor expressing cell lines and experimental tumours [111,112].

9.1.3 *Further concepts in drug delivery*

There are at least three additional innovative methods that have been proposed to improve photosensitiser delivery. However, none of these approaches has reached the stage where they can be used safely in humans.

9.1.3.1 *The magnetically guided drug delivery*

Recent observations report enhanced drug delivery by means of an external magnetic field. This field directs custom-designed, drug-filled nanocarriers to the targeted area in the cell culture [106]. This approach may lead to PDT treatments having as an additional advantage, the potential to increase the uptake of the photosensitising drug by malignant cells, while reducing its accumulation in normal tissues.

9.1.3.2 *Iontophoresis*

The delivery of photosensitisers could also make use of iontophoresis [113]. This modality may prove particularly useful in treating skin malignant lesions, in order to improve

the penetration of ALA. As this prodrug possesses a zwitterionic nature, the mechanism of its transport is essentially electro-osmosis, with little contribution from electromigration. However, targeted delivery of short-chain alkyl cationic esters of ALA, which could be administered in place of ALA, have been targeted into skin (pigs' ears), with excellent efficiency [114]. This *in vitro* delivery method seems to be particularly useful for skin, but conclusions about the efficiency and the kinetics of delivery should be taken with great care. *In vivo*, the barrier function of the skin may be unpredictable, and the local microcirculation may rapidly clear the drug from the viable tissue, thereby resulting in much lower drug concentrations than expected from *in vitro* data.

9.1.3.3 Ultrasound

Some studies have shown that non-thermal ultrasound energy can be applied for targeting or controlling drug release [115]. This relatively new concept of therapeutic ultrasound combined with drugs could lead, in the future, to revolutionary drug delivery systems relevant to PDT, but its application in PDT has not been studied even at preclinical levels.

10. Combined therapy

An attractive evolution of PDT is its combination with other types of therapy. Many possibilities have been explored, but nothing has been fully exploited, as most of the experimental research in this area is confined to the laboratory. Studies have been mainly concerned with the combination of PDT with antineoplastic drugs, with immunotherapy and with radiotherapy, although the combination with other substances, such as pharmacological inhibitors [116], and biological simulating factors [117], has been recently evaluated. Combined therapy with antiblastic drugs seems promising, as indicated by the expanding literature in this area (to cite the most recent papers, see ref. [53,118-121]. However, the findings reported in this field are predominantly descriptive, with a few exceptions [95], and require further studies.

In some situations, a combined effect is obtained by linking the photosensitiser directly to an anticancer drug [122] or to a specific antibody to target highly tumour-expressed receptors [123]. At the moment, this approach remains largely unexplored, but is apparently hampered by many practical and technical difficulties.

11. Expert opinion

The studies reviewed in this paper would suggest that the role of PDT in cancer treatment, albeit not prominent, should be a relevant one. This is not the case. With the notable exception of skin cancer, which recognises PDT as a mainstay for treatment, there is no firmly established advantage of PDT over conventional approaches. Obviously, there is more work to be done for PDT, in order for it to become a reliable therapeutic tool for cancer, as opposed to a simple 'hit or miss' approach.

PDT efficacy is dependent on many factors, including the chemical, physical and biological properties of the photosensitisers, the type and power of the light source, the precise dosimetry, the geometry of the irradiation, the time between the drug administration and exposure to light and the target cells. Indeed the study of these PDT-related variables requires multidisciplinary knowledge, and an assay consisting of a number of experimental conditions is so large that it is impossible without a coordinated effort. The ongoing search for new sensitisers and their subsequent biological characterisation has continued, but not all the conditions for the optimal use of the few approved drugs have been firmly established. Undoubtedly, the development of new drugs is very important; nonetheless, developing the field by optimising the therapies already available seems to be of even greater relevance.

In my opinion, it would be advisable to:

- Conduct well-coordinated, multidisciplinary studies dealing principally with photosensitisers already used in therapy, with the purpose of positively establishing the conditions for the optimal performance of each sensitiser in curing specific forms of cancers.
- Devise, develop, assay and validate new drug delivery methods. In this regard, both nanoparticle-based and tumour-targeted delivery technologies seem appealing approaches to be pursued.
- Explore and exploit the possibilities offered by combining PDT with other types of therapy. Most of the findings on combination therapy are essentially anecdotal, largely descriptive and confined to laboratories. Of particular interest is the case of chemotherapy where, under specific conditions, even synergy between the two therapies has been observed. This aspect deserves further study.
- It is known that PDT, while destroying tumour tissue by cellular necrosis, produces local, acute inflammation, attracting leukocytes. However, it is possible that the optimal PDT regimen for curing local tumours is different from the best possible PDT treatment for generating local inflammation and stimulating immune response. Additional investigations are necessary to establish whether PDT-induced antitumour immunity is clinically useful and can be used to benefit patients.
- Develop new PDT-related devices. More compact, inexpensive and PDT-oriented devices are needed including:
  - dedicated lasers, light sources and light dispensers;
  - precise and easy dosimetric apparatus;
  - instruments for alternative PDT administration as in 'interstitial therapy', in which light is guided, by CT scan, directly to tumours through suitable needles [124].

Acknowledgements

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