Porphyrins and the Photodynamic Therapy of Cancer

As Porfirinas e a Terapia Fotodinâmica do Cancro

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The radiotherapy and the chemotherapy, normally used for the treatment of cancer, can induce dangerous side effects due to their indiscriminately destruction of both normal and tumor tissues. Photodynamic therapy (PDT) is a new technique for the treatment of various types of malignant tumors. PDT is based on the ability of some porthyrins and porphyrin-like chromorphores to be accumulated selectivity in tumor tissues; tumor necrosis can be obtained by irradiation of the neoplastic area with light of the appropriate wavelength. Until now a few thousand patients have been successfully teateo by PDT worldwide. This article reviews the synthesis, main characteristics and mechanism of action of the main porphyrin-like chromophores used in PDT.

A radioterapia e a quimioterapia, normalmente usadas para o tratamento do cancro, podem original efeitos secundários perigosos, devido à destruição indiscriminada que provocam nos tecidos normais e cancerígenos. A terapia potodinâmica (PDT) é uma técnica nova para o tratamento de vários tipos de tumores malignos. A PDT baseia-se na capacidade que algumas porfirinas e compostos afins têm para se acumularem selectivamente nos tecidos cancerígenos; a destruição dos tumores é obtida através da irradiação da área neoplástica com luz de comprimento de onda apropriado. Até hoje, alguns milhares de doentes têm sido tratados com sucesso pela PDT, por todo o mundo. Neste artigo é feita uma revisão sobre a sintese, principais características e modo de acção dos principais compostos do tipo porfirínico usados em PDT.

Introduction

Photodynamic therapy is a form of photochemotherapy (PCT) that combines visible light, molecular oxygen and a porphyrin-based photosensitizing drug to achieve an efficient therapeutic effect. In this method of cancer treatment neither the light nor the drug has any independent biological effect. In PDT, dangerous side effects in normal tissues which are common in chemotherapy and radiotherapy, can be avoided since the photosensitizer selectively accumulates in the tumor tissues. New developments in lasers and fiber optics have made possible the treatment of different kinds of tumors, both internal and external.

It has been known for over seventy years that some porphyrins have a natural tendency to selectively localize in malignant tumors compared with normal tissue. Upon activation with red light, these porphyrins become very toxic to the surrounding environment; it is believed that they sensitize the production of singlet oxygen and other types of radicals that are very toxic to the tumor cells. Therefore, porphyrins have been successfully used as photosensitizers for PDT. The phototoxicity of the porphyrins and derivatives is mainly determined by their photophysical and photochemical properties and by their degree of tumor selectivity. The basic PDT treatment consists of injecting a patient with the porphyrin sensitizer and then waiting a certain time for the photosensitizer to localize in the tumor tissues. The porphyrin containing tumor cells fluoresce in ultraviolet light and this can be used for detection of neoplastic tissues. The activation of the photosensitizer, by irradiation of the tumor with light of the appropriate wavelength, leads to the generation of cytotoxic species and destruction of the tissues. Photofrin® is currently the photosensitizer used worldwide in PDT and it has been successfully used in over ten thousand patients since its first preparation in 1981. However, due to its complicated composition and weak absorption in the red light region, new improved substances have been investigated as potential photosensiti

zers for PDT. Second generation drugs have been proposed and some are already in Phase I/II clinical trials. In this article an historical view of PDT is first given, followed by discussion of the mechanism of action and structural features of the second generation of photosensitizers with potential use in PDT (for recent reviews see [1-6]).

Historical

In 1900, Raab [7] for the first time reported the use of acridine (**1**) on paramecium for the detection and treatment of tumor cells. Three years later, Jesionek and Tappeiner [8] demonstrated the use of eosin (**2**) in the treatment of human tumors. In 1908, Hausman [9] first noted the photodynamic properties of hematoporphyrin-IX (HP) (**3**) and described the treatment of cancer cells in rats. In the following twenty years, a large number of compounds were used to induce cancer in laboratory animals. In 1942, Auler and Banzer [10] and Figge [11] showed that HP (**3**) had a great affinity for malignant tissue. Some other porphyrins, e.g. protoporphyrin-IX (PP) (**4**), mesoporphyrin-IX (MP) (**5**), deuteroporphyrin-IX (DP) (**6**) and coproporphyrin (**10**), were classified as naturally occurring cocarcinogenic compounds. Other fluorescent compounds such as eosin, fluorescein, rhodamine, dihydrocollidine, methylene blue, thioflavin, toluidine blue and riboflavin were also used, but none exhibited such a strong affinity for the neoplastic tissues as did the porphyrins. In the 1950s, many other authors [12] demonstrated that porphyrins have a tendency to accumulate in both animal and human tumor tissues and that they could be used for the detection and delineation of neoplastic tissue. However, the early studies in humans required large dosage of HP (**3**), [12c] which increased the danger of photosensitivity of the patients, sometimes causing severe skin necrosis upon exposure to strong light.

In the early 1960s, Lipson et al. [13] introduced an improved technique using a hematoporphyrin derivative (HPD) for the detection and treatment of malignant tumors in humans. They demonstrated that HPD had a greater tendency to accumulate in malignant tissue than did HP (**3**) and also that it had superior fluorescent properties. To prepare the HPD product, Lipson et al. [13c] dissolved HP (**3**) in a solution of 19:1 glacial acetic acid/concentrated sulfuric acid, filtered the resulting mixture and treated the porphyrin solution with 3% sodium acetate. The presumably acetylated products were collected by filtration and dissolved in saline solution containing sodium hydroxide. The final pH was adjusted to 7.4 with hydrochloric acid and the HPD product was stored at -30°C, in the dark. Since 1961 many investigators have used HPD in combination with light for selective *in vivo* tumor destruction.

Various investigators, using high performance liquid chromatography (HPLC) techniques, demonstrated that HPD is a complex mixture of compounds. The major components have been identified as HP (**3**), PP (**4**), hydroxyethylvinylporphyrin (HVD) (**7** and **8**), diacetoxyethyldeuteroporphyrin-IX (DAD) (**9**), and aggregates (dimers and higher oligomers) of porphyrins [14]. In 1981, Dougherty et al. [15] used gel exclusion chromatography to purify the HPD material. In this way they were able to remove most of the monomeric porphyrins and therefore obtain a purified high-molecular weight aggregate that accounted for essentially all of the photo-activated tumoricidal activity of HPD. In addition, the skin photosensitivity of animals receiving an equivalent tumoricidal dose (when activated by light) of this purified active component was greatly reduced compared to HPD. Thus, an improvement in therapeutic ratio was achieved using this purified material, known today as Photofrin®. This purified HPD material has been the subject of numerous studies in an attempt to unambiguously identify its active compounds. Dougherty et al. [16] first described the active compound of HPD as dihematoporphyrin ether (**11** and isomers). A few years later, Kessel [17] proposed dihematoporphyrin ester (**12** and isomers) as the active component of HPD. Some authors [18] have proposed a mixture of the two dimers (**11**) and (**12**) for the active component of HPD. Other studies have suggested that the active species in HPD is a mixture of dimers and higher oligomers linked by ether, ester and even carbon-carbon bonds [19]. More recently it was reported [20] that the ratio monomer:dimer:oligomer for HPD is 22:23:55 and for Photofrin® is 14:19:67.

The rights to Photofrin® belonged to Oncology Research and Development, Buffalo, from 1981 until 1985 whereupon an agreement for transfer of the patent was negotiated with Johnson & Johnson. In 1987 the rights to Photofrin® were sold to Quadra Logic Technologies (QLT), Vancouver. The QLT company together with American Cyanamid/Lederle Laboratories are currently obtaining world-wide marketing approval for Photofrin®. The first Phase III clinical trials using Photofrin® began only in 1988. In 1993 the Canadian Health Protection Branch announced the first approval for the use of Photofrin® in the treatment of superficial bladder cancer [2]. In 1994 approvals occurred in Holland and Japan, in 1995 in the US, and in 1996 in France.

The photodynamic effect

Porphyrin-induced photodynamic damage to cells results in modification of many cellular components and functions. For example, inhibition of transport across the cell membrane, inactivation of enzymes, impaired protein synthesis, vascular damage, effects on DNA, membrane swelling and complete membrane

lysis, and vessel constriction and degeneration have all been reported [21]. It has also been observed [22] that, while the cell plasma membrane may be altered due to HPD photodynamic damage, it may not be a primary event. Effects at the nuclear and mitochondrial membranes may be of primary importance to the ultimate demise of the cell. Interestingly, not all effective photosensitizers cause the same primary effects [23]. Ackerman et. al. [24] showed that singlet oxygen, a short-lived electronically excited state of molecular oxygen, is the effective cytotoxic agent in many photodynamic reactions. *In vivo* experiments have shown [25] that oxygen is required in order to have an effective photodynamic reaction. More evidence includes inhibition of cell killing when quenchers capable of trapping oxygen radicals are added [26], and enhanced killing in D_2O-containing systems [27].

Upon activation with red light, an electron of the porphyrin nucleus is excited from the ground-state to an excited singlet state (Figure 1). The electron then can either return to the singlet ground-state with the emission of light (heat, fluorescence), or it can change its spin via intersystem crossing (ISC) to give the triplet state, which has a slightly lower energy [28]. The decay of the triplet to the singlet ground-state is slow because according to the spectroscopic selection rules, the transition is forbidden. The metastable triplet porphyrin sensitizer therefore has time to react with its chemical environment, transferring energy to produce forms of oxygen known to be lethal to cells. There are two possible mechanisms for the transfer of the energy of the sensitizer [29]. In the designated Type II photooxygenation mechanism, the excitation energy is transferred to molecular oxygen in its ground-state (triplet) to produce highly reactive (toxic) singlet oxygen. In this process the photosensitizer acts catalytically since its function is to absorb light energy and to transfer it to molecular oxygen, regenerating ground state photosensitizer. Singlet oxygen is a powerful oxidant that reacts with a variety of biological molecules and assemblies [30].

In the so-called Type I mechanism the excited triplet state of the sensitizer may react, for example by abstracting a hydrogen atom from a substrate molecule, or undergo electron transfer. The latter may be followed by the formation of, for example, the superoxide ion and radical anion, and the hydroxyl and hydroperoxyl radicals. Some of the radicals produced by these reactions can subsequently react with oxygen to produce a wide variety of oxidized products such as peroxides. The first excited singlet state of the porphyrin can also participate in an electron transfer process with a biological substrate, resulting in the photobleaching of the photosensitizer and the destruction of the substrate [31]. Although some work has been done to identify the photoproducts of HPD photosensitizing reactions in model systems [32], *in vivo* products are not yet identified. Recently, the *in vivo* formation of a chlorin-type photoproduct from protoporphyrin-IX (**4**) was reported [33].

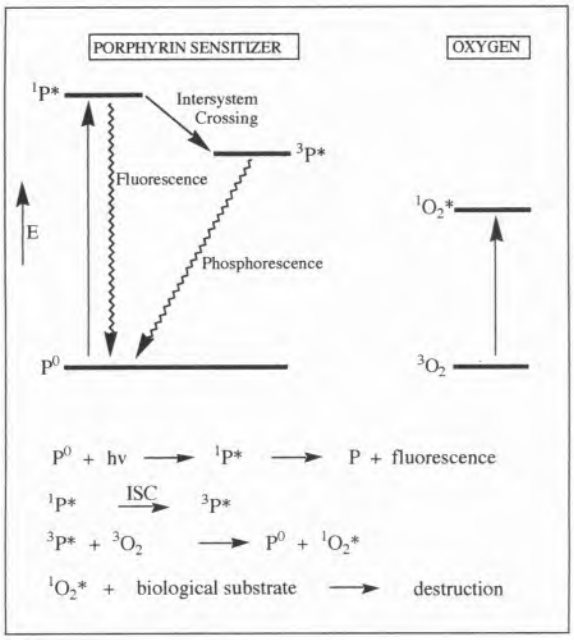


Figure 1 – Energy diagram (excluding vibrational levels) showing porphyrin and oxygen singlet and triplet states. P = porphyrin sensitizer, * = electronically excited state; 1 = singlet excited state, 3 = triplet excited state.

Most of the new photosensitizers for PDT have quantum yields for singlet oxygen production >0.5 which means that singlet oxygen can be produced in considerable amounts when the photosensitizer is activated by red light. The importance of the singlet oxygen mechanism gives rise to another problem for the photodynamic effects, which has to do with the concentration and consumption of molecular oxygen in the tumor cells; PDT induces vascular damage resulting in reduced microcirculation within a few minutes, which further reduces the oxygen supply to the tumor. Low vascular density tumors are more sensitive to oxygen consumption than highly vascularized tumors [34]. It has been reported that tumor oxygenation may be improved by breathing a perfluorochemical emulsion and carbogen (95% O_2, 5% CO_2) [30].

Most clinical studies use lasers as the source of red light, in order to take advantage of the high coupling efficiency to optical fibers which allows a beam of intense light to be delivered conveniently to many areas of the body. One of the more practical recent advances in PDT is the availability of diode lasers as light sources. The more compact diode lasers are much cheaper and have minimum power requirements. The reason for using red light rather than shorter wavelengths, which actually are absorbed more strongly by Photofrin®, is that the red light is considerably more penetrating through tissue [35] due in part to the presence in tissue

of strongly absorbing melanin and hemoglobin. Light scattering is also much greater at shorter wavelengths. The choice of 630 nm light, normally used in PDT, is therefore a compromise between optimal tissue transmission and Photofrin® absorption.

First generation photosensitizers

The original HPD material (also designated as Photofrin I) [13] and the purified HPD material known as Photofrin® (initially known as Photofrin II) [15] represent the first generation of photosensitizers. A number of problems in PDT are the consequence of the complex nature and instability of the first generation photosensitizers. They vary in composition from preparation to preparation and have been observed to degrade with time to yield inactive side products [36]. The commercial Protofrin® was originally shipped and stored at -78°C and only warmed to room temperature immediately prior to use, a freeze dried preparation is now available. Other disadvantages of the first generation photosensitizers are the weak absorption at 630 nm (presumably requiring larger doses of drug to obtain a satisfactory therapeutic effect), the poor selectivity for tumor tissues, and the long retention period (2-3 months) in cutaneous tissues, which causes skin photosensitivity. Research in the last 15 years has turned to the preparation of the so-called second generation photosensitizers for PDT. These are mostly pure compounds, with long wavelength absorptions and enhanced photoactivity compared with Photofrin®. Table 1 lists the long wavelength absorptions and extinction coefficients of Photofrin and some second generation photosensitizers. A new approach to increase selective localization of the photosensitizers in tumor tissues and reduce skin photosensitivity, consists of second generation photosensitizers bound to special carriers.

Table 1. **Long wavelength absorption maxima and extinction coefficients of some typical PDT photosensitizers**

Photosensitizer	λ_{max} (nm)	ε (L.mol^{-1}.cm^{-1})
Photofrin	630	3000
Chlorin p6	664	43000
Natural bacteriochlorin	785	150000
Benzoporphyrin	690	30000
Benzochlorin	660	35000
Azachlorin	670	50000
Zn-etioporphyrin	690	70000
Tetrabenzoporphyrin	685	120000
Zn-phthalocyanine	675	150000
Porphycene	630	52000

The basic requirements of a PDT photosensitizer are that it should be minimally toxic in the dark, have long wavelength absorptions ($\lambda > 630$ nm) with high extinction coefficients ($\varepsilon > 30000$ L.mol^{-1}.cm^{-1}), be preferentially localized in tumor tissue, have limited *in vivo* stability for rapid clearance after treatment, and have favorable photophysical properties. The most important photophysical properties of the photosensitizer are the energy ($E_T \geq 94$ kJ.mol^{-1}), lifetime ($\tau_T > 100$ ms), and quantum yield ($\phi_T > 0.4$) of the triplet state. For the singlet oxygen mechanism of tumor destruction, the quantum yield of singlet oxygen ($\phi_\Delta > 0.2$) is also an important parameter. It is also very important that the photosensitizer should have a short, high-yielding and inexpensive synthetic route leading to a single pure substance.

The light penetration through tissues increases (typically doubles from around 550 nm to 630 nm and then again from 630 nm to 750 nm) and the scattering of light decreases with the wavelength. It might be expected that photosensitizers with the longest wavelength absorptions would be more adequate for the treatment of bulky tumors. However, there is a limit for the value of the maximum wavelength of absorption of the photosensitizer due to the requirement for singlet oxygen generation. The "phototherapeutic window" has been reported to include light in the 600-1000 nm spectral region. The photosensitizer energy of the triplet state should be greater than the energy of the singlet oxygen, which is 94 kJ.mol^{-1} (1270 nm), for efficient singlet oxygen production. For some potential photosensitizers with absorptions near 1000 nm, the quantum yield of singlet oxygen is zero probably because of their low energy of the triplet state ($E_T < 94$ kJ.mol^{-1}). It has also been observed that some long-wavelength absorbing drugs containing extended π-systems are less kinetically stable and more subject to photobleaching [5]. Therefore, the photochemical limitation to the long wavelength of a photosensitizer is about 800 nm. Light of this wavelength penetrates, in lightly pigmented tissues, up to 2-3 cm.

Second generation photosensitizers

Since the the early 1980s new improved photosensitizers related to Photofrin® have been synthesized and reported [1-6, 37]. These are the so-called second generation photosensitizers and some of them are already in Phase I and II clinical trials. These photosensitizers are based on cyclic tetrapyrrole molecules with intensified long-wavelength absorptions. Only the metal-free or the diamagnetic metal complexes (e.g. Al, Zn, Sn, Si, Ge, Ga, Cd) of these macrocycles can be used in PDT, since a paramagnetic ion would quench the photochemistry necessary for sensitization.

The first second generation photosensitizers consisted of pure porphyrin dimers and trimers linked by ether, ester, and carbon-carbon double bonds, in a

mimic of the presumably active components of Photofrin® [38]. However, these compounds showed low photoactivity compared to Photofrin®. By judicious substitution of the porphyrin ring it is possible to move the long wavelength absorption band of the porphyrin spectrum further to the red region. However the effect is small and the extinction coefficient of the longest wavelength absorption is only modestly increased. Therefore research has turned to the synthesis of chlorin and bacteriochlorin type macrocycles. Reduction of the porphyrin macrocycle (for instance with diimide) leads to the formation of chlorins (λmax = 650-690 nm) (Figure 2) and bacteriochlorins (λmax = 720-760 nm) which have red-shifted, high extinction coefficient absorptions, and which allow in principle the use of lower doses of photosensitizer. It has also been reported that chlorins do not cause severe cutaneous photosensitization as porphyrins usually do [39]. Natural chlorins, such as chlorophyll-a (**13**) and bacteriochlorophyll-a (**14**) have been used as photosensitizers for PDT [40]. Some chlorophyll-a derivatives were prepared mainly by demetallation, cleavage of the phytyl group, and reactions on the isocyclic cyclopentanone ring of (**13**) [41]. For example chlorin e_6 (**15**) [42], N-aspartylchlorin e_6 (**16**) [43], chlorin p_6 (**17**) [44] and its lysyl derivative (**18**) [45] are all active in PDT. Chlorin e_6 (**15**) is obtained from chlorophyll-a (**13**) by demetallation, transesterification using methanol/5% sulfuric acid, cleavage of the cyclopentanone ring with sodium methoxide/methanol, and basic hydrolysis of the resulting methyl esters. Chlorin p_6 (**17**) is obtained from purpurin-18 methyl ester (**19**), a chlorophyll-a degradation product, by simple hydrolysis of the anhydride ring and the methyl esters. Lysyl chlorin p_6 (**18**) is prepared by treatment of purpurin-18 (**19**) with lysine in a mixture of dichloromethane, pyridine, and water. The bacteriochlorin (**20**) has a long-wavelength absorption at 815 nm, and has shown promising anti-tumor activity [46]. The bacteriochlorin (**20**) is also prepared from purpurin-18 methyl ester (**19**). The anhydride ring in (**19**) is first replaced with the imide ring in (**20**) by reacting (**19**) with lysine ethyl ester followed by cyclization of the open chain product with Montmorillonite K10 clay suspended in dichloromethane. The β-vinyl group is then converted into the β-formyl group by reaction with osmium tetroxide/sodium periodate. This conversion produces a 30 nm red shift in the optical spectrum whereas the imide ring produces a 40 nm red shift relative to the open chain amide product. The *vic*-dihydroxy-bacteriochlorin (**20**) is produced by the reaction of the β-formyl-chlorin analogue with osmium tetroxide/pyridine, and then with hydrogen sulfide gas, which cleaves the intermediate osmate complex. Many other potential photosensitizers have been prepared from compound (**19**) since it is possible to open the anhydride ring of (**19**) with a number of different kinds of nucleophiles, such as amino acids.

Some synthetic chlorins and bacteriochlorins have been found to be good photosensitizers for PDT. For example, *meso*-tetrakis(*m*-hydroxyphenyl)chlorin (**21**) has been used in clinical trials since 1991 [5, 47]. Chlorin (**21**) was prepared by reduction, with diimide, of the *meso*-tetrakis(*m*-hydroxyphenyl)porphyrin, which was synthesized from the corresponding *meso*-tetrakis(*m*-methoxyphenyl)porphyrin by cleavage of the methyl ethers with BBr_3 at low temperatures. The *meso*-tetrakis(*m*-methoxyphenyl)porphyrin is prepared in moderate yields, in a one step reaction, by condensation in acidic media of pyrrole and *m*-methoxybenzaldehyde. Although simple to prepare, chlorin (**21**) causes skin photosensitivity of the patients, presumably in part due to its different structure (it has a *meso* rather than a β substituted macrocycle) from the natural occurring chlorins. A disadvantage of using chlorins and bacteriochlorins as photosensitizers for PDT is their re-oxidation back to porphyrins, with loss of the intense long-wavelength absorption necessary for deeper tissue penetration and greater photosensitizing activity. Furthermore, *in vivo* oxidation of the photosensitizers may result in the formation of a new chromophore absorbing at a different wavelength, thus reducing the photodynamic efficiency. In order to prevent dehydrogenation, chemically stable chlorin macrocycles having an isocyclic ring or containing a keto or geminal alkyl groups [48] were synthesized. Examples are the oxochlorin (**22**) [49], the benzoporphyrin derivative monocarboxylic acid (BPDMA) (**23**) [50] and the etiopurpu-

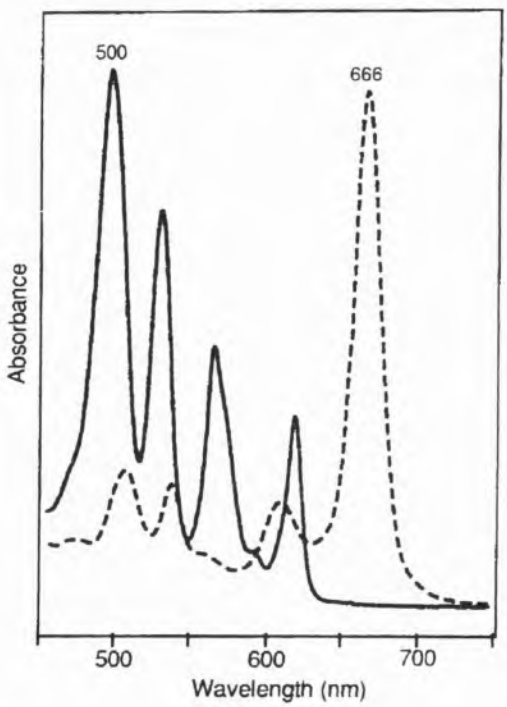
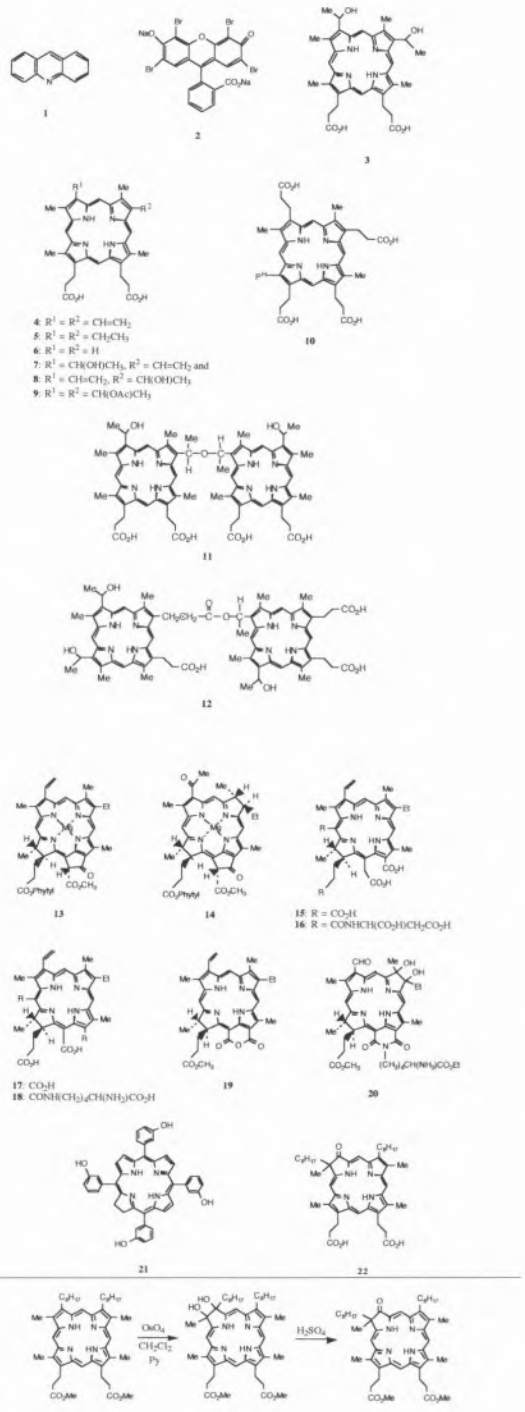


Figure 2 – Typical optical spectra (450-750 nm), of a porphyrin (—) and a chlorin (– – –).

rin (**24**) [51], which have already been used in successful clinical trials. The main step in the preparation of oxochlorin (**22**) is the oxidation of the starting porphyrin with osmium tetroxide followed by an acid-catalyzed pinacolic rearrangement (Scheme 1). The treatment of porphyrins with osmium tetroxide and the migratory aptitudes in the pinacolic rearrangement have been reported [52]. It has been shown that the osmium tetroxide reaction can be directed to give bacteriochlorins if the substrates in the reaction are chlorins (e.g. in the synthesis of bacteriochlorin (**20**)). The BPDMA (**23**) was prepared from protoporphyrin-IX (**4**) by the Diels-Alder [4+2] cyclization reaction using dimethyl acetylenedicarboxylate as the dienophile, followed by reaction with base. Triethylamine and 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) are used to promote rearrangement of the Diels-Alder adduct intermediate to give the cis-cyclohexadiene product. Recently, some derivatives of BPDMA with good photodynamic activity have been reported [53]. The etiopurpurin (**24**) was prepared by the intramolecular cyclization of a *meso*-acrylic acid chain onto the adjacent β-position, under mild conditions. The *meso*-acrylic acid chain is introduced by Vilsmeier reaction (N,N-dimethylformamide/$POCl_3$) followed by the Wittig reagent (carbethoxymethylene)triphenyl phosphorane. More recently, other promising chlorin and bacteriochlorin macrocycles have been synthesized. Examples are the bacterichlorin (**25**) [54], the benzochlorin (**26**) [55], and the naphthochlorin (**27**) [56]. The bacteriochlorin (**25**) was prepared by the double cyclization of an acrolein and an acrylic acid chains on two adjacent *meso* positions of octaethylporphyrin (OEP), using trifluoroacetic acid. Compound (**25**) shows an intense absorption at 890 nm. Benzochlorins are in pre-clinical trials and have demonstrated good tumoricidal activity [57]. Benzochlorin (**26**) was prepared from OEP as seen in Scheme 2. The acrolein side chain was introduced by the Vilsmeier reaction, using 3-(dimethylamino)acrolein and phosphorus oxychloride. The cyclization of the *meso*-acrolein chain, in concentrated sulfuric acid, onto the adjacent β-position of the macrocycle afforded (**26**) in 40% overall yield. The naphthochlorin (**27**) was prepared from the readily available *meso*-tetrakis(*p*-methoxyphenyl)porphyrin (TMPP), as can be seen in Scheme 3. The intramolecular cyclization of the β-vinyl group into the *ortho* position of the adjacent phenyl ring afforded (**27**) in an overall yield of 50%. Research is now in progress to demetallate naphthochlorin (**27**) and to cleave the methoxyl groups in the presence of BBr_3, to generate the free-base naphthochlorin (**28**). The starting porphyrins OEP and TMPP are easily prepared in one step due to their high symmetry [58].

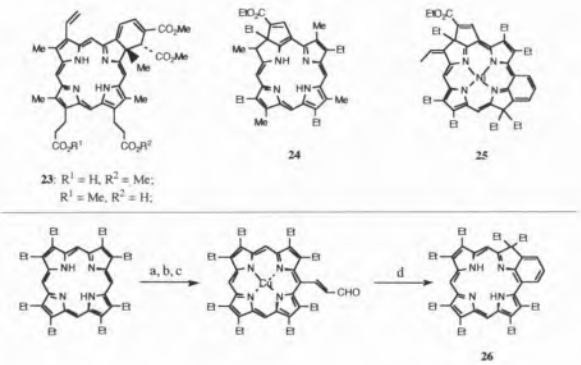
Second generation photosensitizers derived from phthalocyanines (**29**) [59] and naphthalocyanines (**31**) [60], such as (**30**) and (**32**), are currently in clinical trials and show good photobiological activity. Phthalocyanines (λmax = 680-720 nm) and naphthalocyanines (λmax = 760-800 nm) absorb light of longer wavelength with higher extinction coefficients compared to porphyrins, due to the extended conjugated π-system. Furthermore, phthalocyanines and naphthalocyanines absorb minimally in the wavelength region 400-



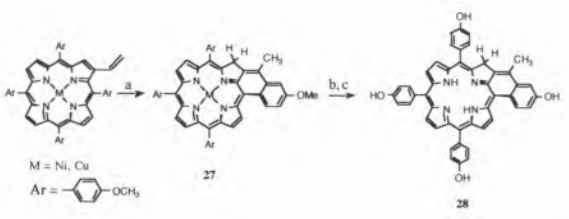
Scheme 1 – Vicinal hydroxylation to give a dihydroxy-chlorin and acid catalyzed pinacolic rearrangement to give the oxochlorin.

600 nm, so that induced skin sensitivity to sunlight is smaller with these compounds than with porphyrins. Both macrocycles (**29**) and (**31**) are readily prepared by reductive tetramerization of, respectively, phthalonitriles and naphthalene-2,3-dinitriles, or equivalent monomers. The tetramerization reaction occurs in the presence of a metal or metal salt which acts both as a template and an electron source. The disadvantage of phthalocyanines is their high *in vivo* photostability, which causes extended skin sensitivity in the patients.

Other reported photosensitizers for PDT that show strong absorption bands at long wavelengths include tetrabenzoporphyrin (**33**) [61], the azachlorin (**34**) [62], the texaphyrin (**35**) [63], and the porphycene (**36**) [64]. The tetrabenzoporphyrin (**33**) and other benzoporphyrin derivatives have been successfully used in *in vivo* PDT studies. However the literature methods for the synthesis of these type of compounds give low yields of impure products and new synthetic routes are currently being developed [65]. Azachlorins have a nitrogen atom in the place of one methine bridge of the chlorin macrocycle, which confers a higher extinction coefficient of the long wavelength absorption [66]. Although azachlorin (**34**) has not yet been tested in *in vivo* PDT studies, it has good photophysical properties



Scheme 2 – Synthesis of a benzochlorin. a) $Cu(OAc)_2/CHCl_3$; b) 3-DMA/$POCl_3$, CH_2Cl_2; c) $NaHCO_3$, sat. aq.; d) conc. H_2SO_4,2h, RT.



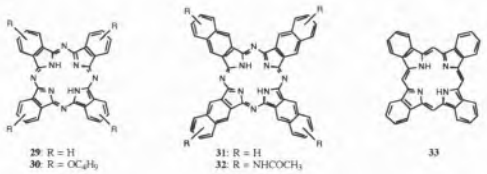
Scheme 3 – Synthesis of a naphthochlorin. a) dil. H_2SO_4; b) conc. H_2SO_4; c) BBr_3

for sensitization. Azachlorins, like azaporphyrins, are prepared from the naturally occurring bile pigment bilirubin. The bilirubin is first converted into biliverdin which reacts with zinc acetate and then with acetic anhydride to produce the corresponding zinc oxoniaprotoporphyrin. This compound is transformed into the azaprotoporphyrin by cleavage of the oxonia bridge with ammonia, followed by activation of the lactam oxygen with trimethylsilyl polyphosphate (PPSE) and recyclization in pyridine (Scheme 4). The resulting azaprotoporphyrin is converted into azachlorin (**34**) by reaction with singlet oxygen, reduction of the aldehyde functions, cleavage of the porphyrin glycol formed, reduction with sodium borohydride, and amide acetal Claisen rearrangement of the intermediate allylic alcohol. Texaphyrins are expanded porphyrins that absorb strongly in the 720-780 nm spectral region. The lanthanum complex of texaphyrin (**35**) has been shown to be an efficient singlet oxygen producer and photoactive in *in vivo* experiments. These type of compounds are prepared by the Schiff base condensation of diformyltripyrranes with *ortho*-diaminobenzene derivatives, followed by aromatization using a metal salt, a base and air. Porphycene (**36**) has a strong absorption in the red region of the absorption spectrum and was reported to be a good *in vivo* tumor localizer. However these types of compounds are produced only in fairly low yields from the coupling reaction of the corresponding diformyl-bipyrroles, in the presence of low-valent titanium complexes.

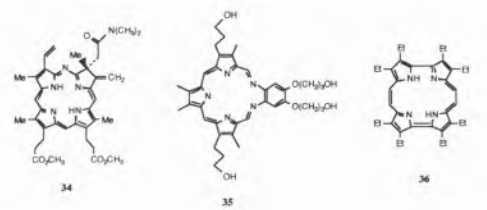
The porphyrin, phthalocyanine and porphycene macrocycles are all hydrophobic, so many of the reported new effective photosensitizers (e.g. (**24**), (**25**), (**26**), (**30**), (**33**), and (**36**)) are insoluble in water. In order to obtain water solubility for injection into the bloodstream, polar hydrophilic substituents were introduced, such as sulfonic acid, carboxylic acid, hydroxyl, and quaternary ammonium salt functionalities. Derivatives of chlorophyll-a (**13**) such as (**15**), (**16**), (**17**), and (**18**) contain hydrophilic carboxylic acid groups and exhibit good tumor localization. Amongst the *meso*-tetraarylporphyrins the 3-hydroxy- and the 3,4-dihydroxyphenyl derivatives have been reported to be 25-30 times as potent as Photofrin® in sensitizing tumors [67]. Positively charged porphyrins and phthalocyanines were shown to exhibit significant tumor photonecrosis [68]. The photodynamic properties of sulfonated aluminium (III) phthalocyanines (**29**, R = SO_3H) have been studied and reported to be significantly influenced by the degree of sulfonation (the mixture of the disulfonic acid derivatives is the most active photosensitizer) [69]. The photosensitizer properties for effective *in vivo* tumor localization are not yet completely understood but seem to be connected with the lipophilicity and aggregation behavior of the sensitizers. Highly lipophilic photosensitizers are poorly soluble and strongly aggregated in aqueous solutions, whereas hydrophilic ones generally show lower cell membrane penetrating properties. It has

been reported that macrocycles containing a suitable combination of hydrophilic (for solubilization in the aqueous media) and hydrophobic (for interactions with the lipidic part of cell membranes) substituents orient preferably in the cell membrane allowing a better tumor accumulation [6, 67b, 70]. The effect of varying the hydrophobicity of the macrocycle by changing the length of the alkyl side chains has been shown to have a significant response on *in vivo* tumor damage [71]. The attachment of hydrophilic carbohydrate structural units to the chlorin macrocycle [50] and the synthesis of neutral glycosylated porphyrins derived from *meso*-tetraphenylporphyrin [72] have been reported.

The hydrophobic second generation photosensitizers, e.g. (**24**), (**25**), (**26**), (**29**), (**31**), (**33**), and (**36**), have been administered *in vivo* in an emulsifying agent, such as a polymer or a liposomal preparation [73]. Some of the transport agents used include Cremophor EL (CRM, polyoxyethyleneglycol triricinoleate), Tween 80 (TW80, polyoxyethylene sorbitan monoleate), and DPPC (dipalmitoyl phosphatidylcholine). It has been reported that the delivery system can greatly affect the uptake of the hydrophobic photosensitizers by the tumors [74], and there are problems concerning regulatory approval of these drug/delivery vehicle combinations. Carrier polymers, such as methoxy(polyethylene) glycol and polyvinylalcohol, have the disadvantage of exhibiting longer retention times in the serum [6]. Photosensitizers containing both hydrophilic and hydrophobic groups have also been coupled to delivery systems.



Scheme 4 – Synthesis of a azaporphyrin from a zinc oxoniaporphyrin, which is prepared from biliverdin. a) $Zn(OAc)_2$/THF; b) Ac_2O/THF; c) NH_3/CH_3CN; d) PPSE, Py



It is generally accepted that the patterns of biodistribution of the photosensitizers are correlated with the association to low-density lipoprotein (LDL) and high-density lipoprotein (HDL) receptors in *in vivo* normal and tumor tissues [75]. The plasma protein LDL is a natural carrier of porphyrins in blood and can incorporate more than 50 porphyrin molecules. The role of LDL in the accumulation of porphyrins in tumor tissues has been demonstrated in cellular models [76]. Cancer cells exhibit high levels of LDL receptors, which can account for the accumulation of porphyrins in tumor tissues. It has been reported that LDL can also be used as an efficient delivery system for the photosensitizers [77]. Certain drugs, such as compactin and lovastatin, known to increase the expression of the LDL receptor in cells, have been reported to significantly increase PDT efficiency [78]. *In vivo* studies have indicated the importance of both the photodynamic properties of the sensitizer and the choice of the delivery vehicle as determinants for PDT efficacy.

An interesting alternative to the injection of the photosensitizer is its generation within the tissue. It has been shown that 5-aminolevulinic acid (ALA), a biosynthetic precursor of heme, can be efficiently used in PDT [79]. The external administration of ALA leads to the formation of protoporphyrin-IX (**4**), the immediate precursor of heme, in photosensitizing concentrations. However, only certain type of cells, including cancer cells, can synthesize protoporphyrin-IX (**4**) after ALA administration, which accounts for the selectivity of ALA-induced phototoxicity in PDT. Most of the clinical studies performed using this technique have involved the topical application of ALA as a cream to skin lesions [80]. In a few cases ALA has been given orally or injected, which generalizes this type of treatment to internal tumors [81]. Since the protoporphyrin-IX (**4**) is synthesized within the mitochondria of living cells, it accumulates inside those cells and little or no protoporphyrin-IX is present in the general circulation. Therefore no skin photosensitivity is usually detected in this treatment. The rapid photobleaching of ALA-induced protoporphyrin-IX in normal skin, with the production of photoproducts, has been reported [82].

The New Approach

A major goal of PDT is to preferential destroy tumor cells while sparing normal tissues. The mechanism for tumor localization of the photosensitizers is poorly understood, and it seems to depend on many variables such as the photosensitizer, the delivery system and the type of tumor. A new approach for the selective delivery of the photosensitizers to tumor tissues has been developed, and it is designated as antibody-targeted photolysis (ATPL). This new approach uses photosensitizers coupled to monoclonal antibodies which bind specifically to the malignant cell surface

antigens [6]. The malignant tumor cells have different cell surface antigens from those of normal cells. Therefore such conjugates can eliminate skin photosensitivity and diminish toxic effects on the nontarget normal tissues. Since the monoclonal antibodies are responsible for the selective localization of the antibody-photosensitizer conjugate in the tumor tissues, the photosensitizers used are selected for their binding capacities and photophysical properties. The antibody-bound porphyrins and chlorins totally retain their photophysical properties [83]. A suitable functional group on the photosensitizer is required for the direct binding to the monoclonal antibodies. Carboxylic acid groups have been used to form amide bonds with the amino groups on lysine residues of the antibody. Several sulfonated macrocycles were coupled using N-hydroxysuccinimidyl-3-(2-pyridyldithio)propionate as the linking agent [84]. However, when the photosensitizers bind near the antigen binding sites, inactivation of the antibodies can occur. To retain the antigen-binding activities the photosensitizers have been coupled to carriers, such as polyvinylalcohol and low molecular weight dextrans [85]. The resulting conjugates have been reported to be efficient generators of singlet oxygen and very selective for tumor tissues. In the last ten years many different photosensitizers conjugated to antibodies have been used in the diagnosis and treatment of cancer [6, 86].

Conclusions

Although many new promising photosensitizers for PDT have been synthesized and reported in the last 15 years, biological activity tests are only known for a small part. Many major pharmaceutical companies have not demonstrated much interest in this area, probably because PDT effectiveness depends on a lot of factors. The effect of a particular photosensitizer on a specific type of tumor is difficult to predict and the same PDT conditions can have different effects on different types of tumors and patients. In the future new improved sensitizers will undoubtedly be reported. Work will be done to elucidate the photochemical mechanisms to generate the cytotoxic species and the photobleaching of the photosensitizers. A better control of the *in vivo* behavior of the sensitizers will allow a more widespread application of the PDT treatment. Finally, the application of PDT to inactivate viruses in blood, such as the herpes simplex virus (HSV) and the human immunodeficiency virus (HIV), is currently the subject of intense interest and research.

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