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Tumour photosensitizers: approaches to enhance the selectivity and efficiency of photodynamic therapy

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Abstract

While Photofrin, the photosensitizer currently in clinical use for photodynamic therapy (PDT) of tumours, has been shown to be both efficacious and safe in the treatment of a variety of human cancers, its chemical heterogeneity and low absorbance in the phototherapeutically useful wavelength range (600–850 nm) make the development of new photosensitizers with improved characteristics desirable. A suitable manipulation of the molecular structure of porphyrins offers several interesting possibilities for controlling the optical and photophysical properties of the photosensitizer, as well as its biodistribution between tumour and peritumoural tissues or at the subtissular and subcellular level. The achievement of these goals may also be facilitated by the association of the photosensitizer with selected delivery systems, opening the way to a qualitative and quantitative improvement of PDT.

Keywords: Tumour photosensitizers; Photofrin

1. Introduction

The recent regulatory approval of Photofrin as a tumour-photosensitizing agent for the photodynamic therapy (PDT) of lung, oesophageal and bladder cancer opens new challenges to both clinicians and basic investigators since time now seems ripe for a qualitative improvement of the technique along different guidelines, such as: (a) definition of PDT protocols which yield optimal results with specific tumour types (e.g. tumours at different anatomical sites, or having different thickness, histological features, degree of vascularization, etc.) and assessment of the potential of PDT to compete with existing therapies for the same tumours; (b) enhancement of the efficacy of PDT (e.g. increased selectivity of tumour targeting possibly through conjugation of the photosensitizer to tumour-specific delivery systems; intraoperatorial application of PDT for the sterilization of the tumour bed after surgical resection); and (c) expansion of the scope of PDT to treat conditions other than malignancies, including atheromas, restenosis of arteries after angioplasty, psoriasis and sexually transmitted diseases, viral or microbial infections and blood banking [1].

Most pre-clinical and clinical studies have been performed so far with Photofrin II, a chemically prepared derivative of haematoporphyrin, and an impressive body of information has been collected on the in vitro/in vivo behaviour of this drug [2–4] in spite of its intrinsic limitations, such as the large degree of chemical heterogeneity and the low molar extinction coefficient in the red spectral region [5]. These data were used as a basis to develop and test a large number of second generation tumour-localizers and -photosensitizers, from which a handful of phototherapeutic agents has been selected and presently are in phase I/II clinical trials (see Table 1). Unlike Photofrin, all newly proposed PDT agents are characterized by a high degree of chemical purity and a high molar extinction coefficient at the absorption maximum in the red spectral region, which is larger by one or two others of magnitude than that typical of Photofrin at 630 nm. This article reviews the common features and specific properties of such photosensitizers in an attempt to draw some conclusions of general interest.

2. General properties of a photodynamic tumour sensitizer

Some properties which have been identified as typical of an efficient photodynamic tumour sensitizer are listed in Table 2. Such properties can be operationally subdivided into physico-chemical, photophysical, pharmacological and phototherapeutic. It is obvious from Table 2 that the success of a PDT treatment requires an optimal interplay among a number of several different parameters and none of the presently available tumour photosensitizers meets all requirements to a satisfactory extent. However, porphyrins and their analogs (chlorins, phthalocyanines, naphthalocyanines, porphycenes) are endowed with two favourable features:

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Table 1
Tumour photosensitizers presently used in clinical trials for PDT of tumours

Photosensitizer	Remarks	Absorption maximum in the red (nm)	Molar absorptivity [a] ($M^{-1}s\,cm^{-1}$)	Reference
Photofrin	contains mainly covalent Hp oligomers, plus Hp, Pp and HVD	630	3 200 [b]	[4]
Benzoporphyrin derivative	requires lipid-based delivery systems	690	43 000	[6]
Monoaspartyl-chlorin e_6	fast clearance from tumour/skin	675	47 000	[7]
m-Tetrahydroxyphenyl-chlorin	to be administered in alkaline solutions or water/DMSO mixtures		35 000	[8]
Sn(IV)-etiopurpurin	requires lipid-based delivery systems	660	28 000	[9]
Zn(II)-phthalocyanine	requires lipid-based delivery systems	675	243 000	[10]

Hp = haematoporphyrin; Pp = protoporphyrin; HVD = hydroxyethyl-vinyl-deuteroporphyrin
[a] Data for monomeric compounds, with the exception of Photofrin
[b] Approximate value due to inhomogeneity of the preparation and scarse reproducibility of monomer-oligomer equilibria

1. The possibility to modify the chemical structure at different loci and with various levels of complexity, including the size of the macrocycle and the extension of the aromatic electron cloud, the coordination of metal ions with the four central nitrogen atoms, and the nature of the peripheral substituents and/or axial ligands (see Fig. 1). This allows a remarkable flexibility in the design of the photosensitizing agent, so that different levels of hydro-/lipo-philicity, tendency to undergo aggregation, subtissular or subcellular distribution, spectroscopic and photophysical properties can be imparted to the photosensitizer molecule [11].

Thus, the insertion of two polar substituents (e.g. carboxylate, sulphonate or hydroxyl groups) on two adjacent rings of the macrocycle and the consequent presence of a hydrophobic matrix on the opposite side of the molecule (two unsubstituted rings) makes the photosensitizer an amphiphilic species; in this way, the porphyrin achieves a sufficient water-solubility, to allow its systemic injection in vivo, while it retains a high tendency to cross the lipid barrier of the cytoplasmic membrane of tumour cells and localize at endocellular sites [12]. In particular, a preferential targeting of lysosomes has been proposed to occur [13] for amphiphilic disulphonated phthalocyanines. Even in the case of deeply hydrophobic porphyrinoids, such as those having one or no polar substituents, systemic injection into the bloodstream is possible, provided the photosensitizers are pre-incorporated into suitable delivery systems (Table 3).

At the same time, the presence of electrically charged functional groups protruding from the pyrrole rings or bulky axial ligands perpendicular to the plane of the porphyrin molecule generates electrostatic repulsion and steric hindrance, thereby preventing the formation of aggregates [17] which would drastically inhibit the photosensitizing activity [18,19]. Actually, although the oligomeric components of Photofrin are known to give the main contribution to the tumour-localizing properties, it is now generally accepted that only monomeric porphyrinoids act as efficient photosensitizers of biological systems, especially in diffusion-controlled photoprocesses [20].

Typically, in neutral aqueous solutions at 10 μM concentrations, only about 50% of dicarboxylic haematoporphyrin and deuteroporphyrin exist in a monomeric state, compared with 100% monomerization for the octadicarboxylic uroporphyrin [17]. Similarly, Al(III)-phthalocyanines show a much smaller tendency to aggregate than the corresponding Zn(II)-derivatives owing to the presence of an additional orthogonal ligand (e.g. chlorine) for the Al ion [21]. An analogous inhibition of aggregation was obtained by fusion of out-of-plane cyclic hydrocarbon structures with the isoindole rings, of phthalocyanine, such as in Zn(II)-tetrabenzodibarreleno-octabutoxy-phthalocyanine [22].

2. The presence of absorption bands in the 600–850 nm wavelength region, corresponds with maximal light penetration into mammalian tissues [23]. For lightly pigmented tumours the transmission of incident light increases up to about 700 nm, while in the presence of an extensive pigmentation (such as in melanotic melanoma) only at wavelengths longer than 780 nm is some tissular transparency is observed [23]: as a consequence, pigmented melanoma is insensitive to PDT with Photofrin and undergoes an important photodamage only in the presence of naphthalocyanines which display an intense absorbance (ϵ about 500 000 $M^{-1}\,cm^{-1}$) at 780 nm, thereby efficiently competing with melanin for light absorption [24].

In general, the use of photosensitizers with a high extinction coefficient offers the possibility to inject smaller drug doses, which gives further advantages over Photofrin and other haematoporphyrin-related porphyrins which exhibit a weak absorbance above 600 nm (Table 1). An ingenious manipulation of the chemical structure of the photosensitizer often allows one to enhance the molar absorptivity, as well as to shift the absorption bands in order to obtain an optimal matching to the optical characteristics of any given tumour. Typical examples are summarized in Table 4. Clearly, the addition of one or two benzene moieties to each pyrrole or the insertion of additional double bonds into the 18 π-electron cloud of the porphyrin macrocycle results in a red shift of the absorption maximum and hyperchromicity. A "fine tuning"

Table 2
Main features of an efficient photodynamic agent for tumours

Property	Related structural and biological features
Physico-chemical	
High chemical purity	Purification may be especially laborious in the presence of two or more peripheral substituents and/or chiral centers
Large molar extinction coefficient in the red	Extensive conjugation of π electrons along the macrocycle
Low tendency to aggregation in an aqueous milieu	Presence of electrically charged peripheral substituents or bulky axial ligands to the central metal ion
Photophysical	
Long triplet lifetime	Extensive monomerization (favoured by distribution in apolar regions of membranous systems)
High yield of $^1O_2$ generation and/or electron transfer to substrate molecules	Easy accessibility by molecular oxygen or close proximity to substrates with suitable redox potential
Pharmacological	
Efficient and selective targeting of the tumour tissue	Hydrophobic or amphiphilic properties; association with suitable delivery systems
Fast clearance from serum and healthy tissues	High affinity for serum proteins responsible for transport of dyes from peripheral tissues to liver
Low systemic toxicity	Lethal dose (LD-50) higher than ca. 300 mg kg^{-1} body weight
Phototherapeutic	
Efficient and preferential killing of malignant cells	Large concentration difference between tumour/peritumoural tissues and/or fast healing of any photodamaged healthy tissue
Lack of side effects	Minimal accumulation in skin to avoid cutaneous photosensitivity
Lack of mutagenic potential	No photoeffect on DNA

of the absorption properties can be also achieved: thus, partial hydrogenation of one or two pyrrole rings converts porphines to chlorins and, respectively, bacteriochlorins with a simultaneous bathochromic shift of 50–150 nm [26]; however, the introduction of eight alkoxy substituents in the β position of the phenyl ring of the phthalocyanine isoindoles, as well as the replacement of the central Ge(IV) ion by Pd(II), allows one to shift the absorption band to selected wavelengths in the 700–800 nm interval [27].

In any case, no appreciable cytotoxicity is shown by the photosensitizer or light alone, at least at the phototherapeutically active drug doses and fluence rates: only the combination of the two agents causes tumour damage [2,25]. Actually, LD-50 values for most porphyrinoids range between 200–500 mg kg^{-1} body weight as compared with injected doses lower than 5, and sometimes 1 mg kg^{-1} which are recommended in current PDT protocols. Moreover, no functional or morphological alterations have been found in porphyrin-loaded tissues [28]; one possible exception is represented by meso-tetra(4-sulphonatophenyl)porphine (TPPS4), for which kidney and neural toxicity has been claimed [29]. At the same time, irradiation of tissues with light wavelengths longer than 600 nm causes no detectable effects, provided the fluence rate is kept below about 150 mW cm^{-2} in order to avoid heat deposition in the tissue and the consequent onset of thermal damage.

In the next paragraphs the pharmacokinetic and photobiological parameters which control the efficacy of the PDT treatment will be discussed.

3. Selectivity of PDT action on the tumour tissue

The essential goal of PDT is to induce an efficient photosensitized necrosis of the tumour mass while minimizing the damage of the peritumoural tissues.

A first criterion to assess the potential selectivity of PDT treatment is the ratio between the photosensitizer concentration in the tumour and the tissue from which the tumour originates or into which the tumour grows. This ratio is hardly predictable since the determinants of porphyrin uptake and clearance by tumours are poorly understood and it is likely that the overall process is the resultant of various properties typical of most neoplastic tissues, including the leaky vasculature, high cell proliferation rate, lower pH value, and inefficient lymphatic drainage [4]. For Photofrin, reported ratios vary from 1–1.5 (skin tumours) to above 10 (brain tumours, as one would expect owing to the inability of porphyrin derivatives to cross the intact blood-brain barrier) [5,14]. In general, the selectivity of tumour targeting is enhanced upon increasing the degree of hydrophobicity of the photosensitizer or by imparting amphiphilic properties to its molecule [12]. In order to induce a more specific localization of the injected photosensitizer in the tumour tissue, one can take advantage of the intrinsic features of the malignant cells (Table 5). While the potential of these approaches has not been fully exploited, encouraging results have been obtained in several cell and animal studies [16,30], so that further investigations along these directions are certainly warranted. In particular, there are indications [14–16] that the association of the porphyrinoid compounds with monoclonal

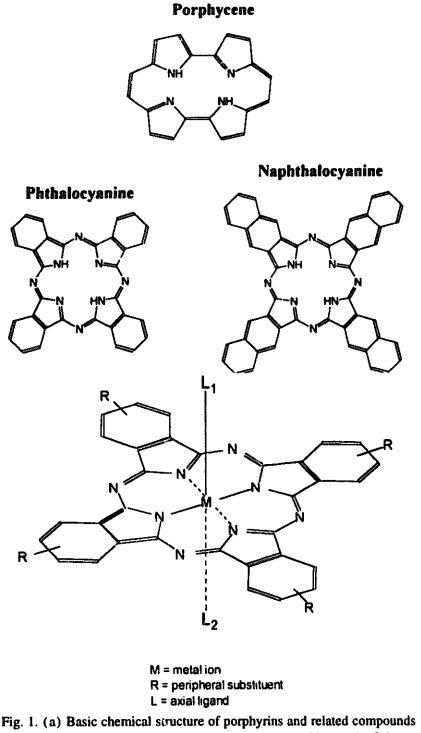

Fig. 1. (a) Basic chemical structure of porphyrins and related compounds with enhanced absorbance at wavelengths longer than 600 nm. (b) Scheme of the chemical structure of a metallo-phthalocyanine indicating possible peripheral substituents (R) and axial ligands (L) to the metal ion.

antibodies or LDL markedly reduces the skin levels of photosensitizer, thus preventing the development of generalized cutaneous photosensitivity, which often represents an undesired side effect of PDT [3].

However, even for relatively low-tumour/healthy tissue ratios of photosensitizer concentration, the selectivity of PDT action can still be achieved provided the peritumoural compartments are less responsive to red light-irradiation or heal more rapidly from photodamage as compared with tumour tissues [31]. Again, no general prediction is possible at the present stage of our research in this field, since the photochemical behaviour of a tissue is strictly dependent on its biochemical composition and physiological properties [32]; for example, since photodynamic action occurs via photooxidative steps, the local concentration of antioxidants plays a major role [3,14]. It must be also emphasized that the main cellular constituents, such as proteins, saturated or unsaturated lipids, nucleic acids and carbohydrates display a very different susceptibility to photosensitized oxidation [32]. A very interesting start in this direction was performed by Bown and coworkers who undertook a systematic study on the responsiveness of normal tissues, such as liver, pancreas or colon, to PDT [31,33].

4. Efficiency of the phototherapeutic treatment

Several authors independently reported [35,36] that the extent of PDT-induced tumour necrosis is related to the concentration of the photosensitizer in the neoplastic tissue. Only for relatively high intratumoural accumulation of the photosensitizer, may a decrease in the efficiency of tumour photoresponse occur due to the light-filtering action exerted by the dye molecules present in the superficial layers of the tissue. In this connection, it has been observed that hydrophobic porphyrin derivatives are accumulated in larger amounts and retained for longer periods of time by a variety of experimental tumours [4,25]. An important contribution to this enhanced uptake could be given by the association of such photosensitizers with lipid-type delivery systems

Table 3
Delivery systems used for the in vivo administration of tumour photosensitizers (see ref. 14–16 for a more detailed discussion)

Delivery system	Photosensitizer	Observed behaviour of the photosensitizer
Liposomes made by		
DPPC	Hp, ZnPc, $SnET_2$	Highly preferential delivery to lipoproteins in the serum
DMPC	ZnPc, $SnET_2$	Delivery to both lipoproteins and albumin
POPC, OOPC	ZnPc	Selective release to lipoproteins
Cremophor EL emulsion	$SnET_2$, ZnPc	Preferential delivery to serum LDL as compared to liposome-delivered dyes
LDL	Hp, ZnPc, BPD	Fast redistribution among all members of the lipoprotein family; targeting of malignant cells in the tumour tissue
Albumin	Photofrin	Exchange with lipoproteins in serum; large amounts recovered in the vascular stroma
Epidermal growth factor (EGF)	Hp	Efficient binding to EGF cell receptor
Antibodies	Hp, chlorin e_6, BPD	Highly selective targeting of tumour cells (most encouraging results in cell cultures)

Abbreviations: DPPC, dipalmitoyl-phosphatidylcholine; DMPC, dimiristoyl-phosphatidylcholine; POPC, monopalmitoyl-monooleyl-phosphatidylcholine; OOPS, dioleyl-phosphatidylserine; LDL, low-density lipoproteins; Hp, haematoporphyrin; ZnPc, Zn(II)-phthalocyanine; SnET2, Sn(IV)-etiopurpurin; BPD, benzoporphyrin derivative.

Table 4
Modulation of the absorption properties of porphyrinoid compounds through a manipulation of their chemical structure (data for monomeric dyes). See Ref. [25] for further details.

Photosensitizer class	Absorption maximum in the red (nm)	Extinction coefficient (M^{-1} cm^{-1})
Hematoporhyrin (18 π)	630	2 800
26 π — porphyrin	780	0 000
34 π — porphyrin	760	370 000
Al(III)-phthalocyanine	675	228 000
Si(IV)-naphthalocyanine	773	557 000
THP-porphine	646	4 000
THP-chlorin	650	22 000
THP-bacteriochlorin	735	91 000
Ge(IV)-phthalocyanine	678	205 000
Ge(IV)-octabutoxyphthalocyanine	761	233 000
Pd(II)-octabutoxyphthalocyanine	732	51 000
	828	279 000

THP = meso-tetra-hydroxy-phenyl

(Tables 1 and 3) and/or their preferential transport in the bloodstream by lipoproteins (Table 5). However, this rule has some important exception, since a few photosensitizers, such as mono-aspartyl-chlorin e_6 [7] and $TPPS_4$ [29], which are endowed with a good water-solubility, are excellent tumour localizers.

The efficiency of tumour treatment by PDT is also heavily influenced by the subtissular and subcellular distribution of the photosensitizer. This parameter is again dependent on the chemical structure of the dye [37]. Photofrin, probably because of its heterogeneous composition, is partitioned among several different compartments of the tumour. As a consequence, upon photoexcitation, this porphyrin induces the modification of various sites, including the blood vessels, malignant cells and non-vascular stroma [3]. In many cases, vascular damage appears to predominate [13].

An extension of these mechanistic studies to porphyrinoids with different physico-chemical properties strongly suggests [38] that albumin-carried dyes are mainly deposited in the extracellular matrix, hence they cause an early impairment of blood circulation. More hydrophobic dyes, which are largely transported by lipoproteins, are released inside tumour cells (Table 5), which strongly favours a direct early photodamage of such cells [3,25]. Recently, the role of macrophages in the overall photoprocess has been re-evaluated [39]; macrophages can efficiently accumulate highly aggregated material, as it is present in Photofrin, as well as liposome- or Cremophor-delivered porphyrinoids.

In any case, it appears that most porphyrin-type photosensitizers localize in the cell membranes [14,25]; therefore, photoinduced cell death is usually a consequence of membrane damage: mitochondria, rough endoplasmic reticulum, lysosomes and plasma membrane have been invoked as primary targets of the photoprocess [2,3,39], their relative importance probably depending on the distribution of the specific photosensitizer. It is also possible that the loss of cell survival reflects a co-operative effect arising from the simultaneous impairment of multiple sites, rather than being determined by the irreversible modification of one critical target. To obtain more precise information on this topic it may be important to use photosensitizers which are orientated toward a specific cell site. This approach is exemplified in recent articles showing the possibility to obtain specific targeting of lysosomes by association of the porphyrin with a lysosomotropic agent [40] or to selectively label the outer or inner mitochondrial membrane by an appropriate choice of the liposome carrier [41].

5. Conclusions

In spite of persisting uncertainties about detailed mechanisms leading to porphyrin-photosensitized cell and tissue

Table 5
Approaches to enhance the selectivity of tumour targeting by porphyrin-type photosensitizer

Approach	*Rationale*	*Specific example*
Incorporation of photosensitizer into serum LDL	Several types of neoplastic cells express a large number of LDL receptors	Hp, ZnPc [14]
Covalent binding of photosensitizers to monoclonal antibodies	Antibodies are directed against antigens specifically present at the surface of tumour cells	Hp, chlorin e6 [15,16]
Use of cationic photosensitizers	Mitochondria of tumour cells have an unusually high affinity for cationic compounds	Triphenylmethane derivatives [34]

Hp = haematoporphyrin; ZnPc = Zn(II)-phthalocyanine

necrosis, it is well ascertained that nuclear damage is a late event and has a minor influence on the photoprocess. This would rule out any risk of a mutagenic effect of PDT, which is especially important if the phototherapeutic treatment is to be repeated at relatively short time intervals or is used in combination with other therapeutic modalities. At the same time, the steadily accumulating information on the factors which modulate the pharmacokinetic behaviour and the cell/tissue distribution of photosensitizers, as well as the efficiency of tumour photosensitization makes it feasible to explore two potentially innovative developments of the technique:

i) The use of a combination of photosensitizers with different intracellular and intratissular localization patterns which act in a synergistic manner to improve the efficacy of PDT. In particular, it is important to assess the possibility to stimulate apoptosis of at least some types of neoplastic cells by PDT treatment, as it is suggested by recent findings [42].

ii) The definition of a variety of integrated PDT systems (photosensitizer, delivery system, irradiation modalities) which are tailored to the treatment of neoplastic cells with specific properties, such as the mitotic index, metastatic potential, invasiveness of the extracellular matrix, degree of pigmentation, etc. As mentioned earlier, there are now several possibilities for introducing predetermined physico-chemical, biological, photobiological and optical properties into the photosensitizer molecule by chemical synthesis.

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