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COMMENTARY

PORPHYRIN LOCALIZATION: A NEW MODALITY FOR DETECTION AND THERAPY OF TUMORS

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This commentary describes recent progress in the use of porphyrins for tumor detection and therapy. In 1961, Lipson *et al.* [1] described porphyrin localization in human tumors following injection of a product termed "HPD" (hematoporphyrin derivative), synthetically derived from hematoporphyrin (Fig. 1). Although accumulation of porphyrins at neoplastic loci had been described in 1924 [2], there were few reports (summarized in Ref. 3) on the phenomenon prior to the work of Lipson. HPD was found to be a superior localizer but was, initially, something of a laboratory curiosity. Developments in instrumentation, described below, have now made feasible use of HPD in the clinic for both tumor localization and therapy.

Cells that have accumulated porphyrins are photosensitized, i.e. subsequent exposure to light has cytotoxic consequences. An excess production of endogenous porphyrins occurs in a pathologic state called porphyria [4], which is associated with substantial light-catalyzed damage to skin and other tissues. If porphyrin accumulation could be targeted to tumors, adjoining normal tissues would be spared from phototoxicity.

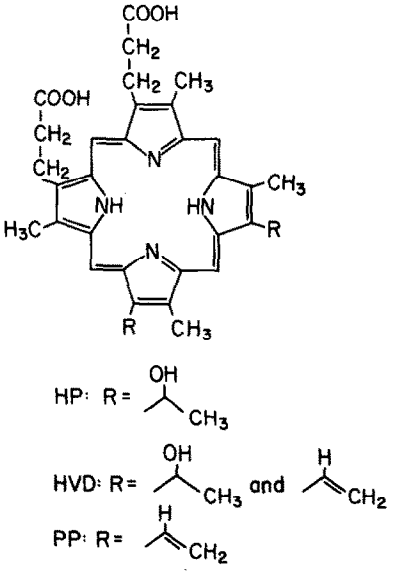

Fig. 1. Porphyrin structures. Abbreviations: HP, hematoporphyrin; HVD, hydroxyethyl-vinyldeuteroporphyrin; and PP, protoporphyrin.

The use of HPD in tumor therapy is based on the ability of one or more of its components to be *localized*, i.e. selectively retained at neoplastic loci. The light-catalyzed destruction of photosensitized tumors has been termed "photoradiation therapy". A 1975 report [5] described the use of photoradiation therapy for eradication of experimental tumors in the rodent. Mice bearing subcutaneous tumors were treated with 2.5 to 5 mg/kg of HPD, and tumor loci were irradiated 24–48 hr later with 15 mW/cm^2 of red light (620–640 nm) obtained from a filtered xenon lamp.

HPD photoradiation therapy also successfully eradicated a broader spectrum of animal tumors: those spontaneously arising in pet cats and dogs. With a laser-fiber optic combination, light at an appropriate wavelength was directed into tumors not readily accessible to external irradiation [6]. No resistance to phototoxicity was encountered; the major limitation of tumor destruction was the degree of light penetration into neoplastic tissues.

HPD has now been used clinically both for tumor localization (via fluorescence endoscopy) and for photodynamic tumor destruction. Before turning to a discussion of methodology and results, studies on porphyrin properties and porphyrin–cell interactions will be described.

HPD is prepared from hematoporphyrin (HP) by a two-step process. HP is first acetylated with a 9:1 acetic–sulfuric acid mixture [3, 7]. Since HP contains two *sec*-hydroxyl groups (Fig. 1), which can be either acetylated or dehydrated, this step yields several products. These include the mono- and diacetates of HP, the monoacetate of the HP dehydration product, hydroxyethyl-vinyldeuteroporphyrin (HVD), and a further dehydration product, protoporphyrin (PP) [8, 9]. Structures of these compounds are shown in Fig. 1. Positional and diastereo isomers add to the complexity of this mixture.

Although some tumor localization occurs when this mixture of products is injected into tumor-bearing animals, prior hydrolysis in alkali yields a much more effective material [10]. The final step in preparation of HPD is, in fact, hydrolysis of the acetylated mixture in 0.1 M NaOH for 60 min [3, 5–7]. The acetate groups are lost, and additional dehydration and other reactions occur. The final product contains

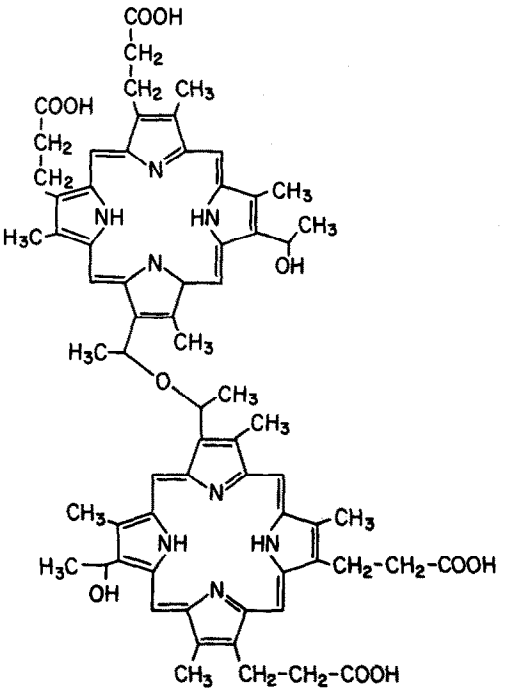

Fig. 2. Dihematoporphyrin ether.

three components that are *not* tumor localizers:HP, HVD, and PP [10–14]. These products are potent photosensitizers, but they are not selectively retained at neoplastic loci.

There are, however, additional components, constituting 20–30% of HPD, which are responsible for *in vivo* tumor localization. Dougherty *et al.* [15] have provided evidence recently that these localizing components are ethers formed from two HP molecules (Fig. 2). This structure is one of several possibilities suggested by Bonnett and co-workers [10]. A variety of isomeric ethers can be formed, and it is not yet known whether these products exhibit a spectrum of localizing activity. As is evident from this description, HPD is not a single compound, a concept which might be inferred from a casual reading of the literature.

It was first suggested by Dougherty *et al.* [16], and later confirmed by others [11, 12], that porphyrin aggregation plays a role in the tumor localization process. The extent of this aggregation can be assessed via absorbance [17, 18] and fluorescence emission spectra [18, 19]. The tumor-localizing component of HPD is aggregated more extensively in aqueous solution than any other porphyrin in the product, and the resulting aggregates are poorly bound to plasma proteins [20]. These aggregates present a hydrophilic surface to the environment and, unlike all other HPD components, cannot be extracted from an aqueous solution with octanol [20, 21]. The substantial fluorescence of porphyrin localized by tumors indicates that these aggregates dissociate after being accumulated, since the aggregates have very low fluorescence yields [19].

Photodynamic destruction of cells is mediated via excited products formed when porphyrins absorb photons of a wavelength at or near one of the porphyrin absorption bands. In decreasing order of intensity, these bands occur at approximately 400 >> 500 > 530 > 570 > 630 nm. Molecular oxygen reacts with excited porphyrin molecules to form the singlet excited state of oxygen, $^1O_2$ [22]. This potent oxidizing agent catalyzes porphyrin phototoxicity in well-oxygenated systems [23, 24]. Other activated oxygen species including H_2O_2, hydroxyl radicals and superoxide may also be formed [25]. In poorly oxygenated or hypoxic systems, excited porphyrin molecules can react directly with other substrates to form cytotoxic free radicals [22], although there have been no reports describing porphyrin cytotoxicity *in vivo* under hypoxic conditions.

Studies using micellar systems [26–28] and liposomes [29–31] have provided information on interactions involving activated porphyrin and oxygen species. In these model systems, phototoxic intermediates have a limited capacity to diffuse to nearby sites to catalyze photodamage [28, 29]. At low drug concentrations, porphyrin-mediated photodynamic destruction of liposomes involves the singlet oxygen pathway; free radicals catalyze lysis at higher drug concentrations [30, 31]. The porphyrin/lipid ratio is an important factor here. When this ratio is low, monomeric porphyrins predominate and catalyze photosensitization via formation of singlet oxygen. At higher porphyrin concentrations, aggregates predominate and the free radical pathway of photodamage becomes more important, even in well-oxygenated systems [26].

Cytotoxicity results from the interaction between these various activated species and biologically-important molecules. The intracellular site of porphyrin accumulation is one determinant of the locus of such damage. When cells are incubated with sufficiently hydrophobic porphyrins, the initial site of drug accumulation, and of light-catalyzed cellular damage, is the cell membrane [32–36]. The resulting damage to membrane transport systems is correlated with loss of cell viability [32].

The most detailed studies of photochemical membrane–porphyrin interactions have been carried out with erythrocyte ghosts. At high light fluxes, substantial cross-linking of membrane proteins can be detected [37–41]. The procedure for estimating the extent of membrane protein cross-linking is relatively insensitive: a densitometric evaluation of stained polyacrylamide gels. Until more sensitive procedures are employed, it is difficult to determine whether membrane protein cross-linking is an early or a late event in phototoxicity. Photo-oxidation of other biologically important molecules, e.g. lipids [39, 42], could also have lethal consequences.

Porphyrins can also photosensitize other cellular components, e.g. lysosomes [43], mitochondria [43, 44] and nuclei [45]. Incubation of human NHIK carcinoma cells with HP results in DNA photosensitization [46], but an equilethal dose of X-ray irradiation produces substantially more single-strand DNA breaks than does the photodynamic procedure. Sister-chromatid exchanges are only slightly increased under conditions wherein substantial numbers of cells are killed by phototherapy [45, 47].

Damage to DNA may play a greater role in porphyrin-mediated phototoxicity as the cell–porphyrin contact time is increased [48].

Porphyrin phototoxicity to cells in culture is independent of the phase of the cell cycle [49]. Clinical reports have also described substantial porphyrin-induced photodamage to non-dividing cell populations, suggesting a lack of phototoxic cell-cycle specificity *in vivo*.

In comparing results of studies designed to probe modes of phototoxicity, it should be noted that there are both obvious and subtle differences between *in vitro* photosensitization and *in vivo* tumor localization and phototherapy. In the latter case, there is a 48–96 hr interval between HPD administration and irradiation, and only the "localizing" components of HPD remain associated with neoplastic cells, at loci representing a steady-state. In contrast, many *in vitro* studies have been carried out over short intervals, often with either non-localizing porphyrins or with HPD which, as noted above, contains many non-localizing components.

Cellular porphyrin uptake is clearly a requisite for any cytotoxic photosensitization process, since extracellular porphyrins are poor photosensitizers [12, 32, 50]. The more hydrophobic porphyrins are preferentially accumulated [26, 32] and partition into correspondingly hydrophobic loci [26, 51]. But such porphyrins are readily washed from cells by medium containing serum [52]. Tumor localization phenomena thus cannot be explained on the basis of initial porphyrin uptake rates.

When evaluating data involving either cellular or liposomal systems, it is often helpful to determine *which* components of a porphyrin preparation have been accumulated. This is especially important with complex mixtures such as HPD. Even when "single" components are employed, unexpected effects can occur. For example, preferential uptake of and photosensitization by contaminating hydrophobic porphyrins were detected when a commercial grade of HP was used for photosensitization *in vitro* [50].

Incubation of murine tumor cells with HPD *in vitro* results in the gradual accumulation of the localizing component [14], a result which may mimic tumor localization *in vivo*. Moan has reported that a 1-hr incubation with HPD mainly photosensitizes membrane loci of a human carcinoma cell line *in vitro*. But after an 18-hr incubation, macromolecule synthesis is the primary photosensitization target [40]. As the incubation time is prolonged, the localizing component of HPD is a more potent photosensitizer, while the non-localizing components migrate to intracellular loci where irradiation is progressively less toxic [53]. The latter result was also obtained with mesoporphyrin [52], a readily accumulated [32] but non-localizing compound.

Since HPD contains multiple photosensitizing components, pharmacokinetic studies might help elucidate the mechanism of tumor localization. The first detailed description of HPD distribution was reported from Dougherty's group, who examined the uptake of radioactive HPD in the tumor-bearing mouse [7]. Tumor retained more total porphyrin than did muscle; skin also accumulated porphyrins. This agrees with clinical reports, outlined below, which indicate substantial skin photosensitization by HPD. Mouse liver, kidney and spleen all retain substantially *more* HPD than neoplastic tissues. This result appears to rule out therapeutic plans involving covalent binding of porphyrins to directly cytotoxic agents, e.g. radioactive metals. The pharmacokinetics of the various HPD components in plasma has not, as yet, been described. It seems likely that aggregates of the di-HP ether will persist in the circulation after other plasma protein-bound porphyrins are cleared.

A few comments on studies with porphyrins *not* contained in HPD seem appropriate. Tetraphenylporphine sulfonate (TPPS) localizes in mouse tumors [54, 55]. But TPPS neurotoxicity [56] precludes use of this agent in man. Tumor localization by the octacarboxyl-substituted uroporphyrin has also been described [57]. It is surprising that this very hydrophilic compound can be localized by tumors, and the mechanism of this process remains to be ascertained. In this context, a study by Spears *et al.* [58] described HPD uptake by atheromatous plaques in rabbit aorta; this could have important implications for selective destruction of such plaques via inter-arterial laser surgery. Our studies on sections of human aorta indicated that only the HP component of HPD was accumulated by such plaques, with uroporphyrin accumulated even more effectively.* These results suggest that certain biologic structures might demonstrate an affinity for relatively hydrophilic porphyrins perhaps including some neoplastic tissues.

A commentary on porphyrin photosensitization would be incomplete without reference to light dosimetry, an important parameter of phototherapy. Porphyrin absorption spectra [59–61] and the optical properties of different tissues [62, 63] are important determinants of phototoxicity. Since tissue transmission of light increases with increasing wavelength, successful photoradiation therapy requires irradiation at the longest porphyrin absorption band, approximately 630 nm. More efficient photosensitization may occur upon irradiation at 400 nm, but poor tissue transmission of light at this wavelength effectively limits photodestruction to a one-cell depth [60, 61]. Porphyrins that can be excited at longer wavelengths, where tissue transmission is increased, would mediate tumor photodamage at greater tissue depths than can currently be obtained and would facilitate therapy of pigmented tumors. The role of hyperthermia in porphyrin photoradiation therapy has been explored [64]. At high light fluxes, local heating may contribute to cell kill, but photodynamic toxicity can be demonstrated in the absence of hyperthermia.

Porphyrin therapy holds promise for detection of early neoplasia in lung, bladder and perhaps elsewhere, for selective eradication of localized primary or recurrent tumors, for exploring the extent of tumor invasion prior to surgery (e.g. in the lung), and for tumor palliation. The lack of cross-resistance between photoradiation therapy and ionizing radiation or chemotherapy, and the uniform sensitivity of cells through the cell cycle, are encouraging. But

* D. Kessel, unpublished results.

phototherapy with HPD is not generally useful in advanced cancer, or in circumstances where tumor destruction will lead to acute medical problems, e.g. when tumor has replaced a portion of the chest wall.

Since tissues containing porphyrins fluoresce, use of fluorescence endoscopy for tumor localization at remote sites is feasible. For tumor localization studies, irradiation at or near 400 nm is satisfactory since this will elicit optimal fluorescence. Applications of this procedure in lung [65] and bladder [66] have been described. The instrumentation is based on a laser : fiber-optic combination which directs light of the appropriate wavelength through an endoscope. The resulting fluorescence at tumor loci can be detected via image-enhancing techniques.

Therapeutic applications of HPD-mediated photoradiation therapy have now been evaluated by several groups [67–75]. The publications cited below describe the advantages and limitations of the procedure. Successes were described for therapy of recurrent breast carcinoma [67, 68, 72, 73], early lung tumors and bronchial tumors where surgery was inadvisable or refused [69, 70], bladder tumors [71], primary skin tumors [72] and tumors of the head and neck [74]. A report on the beneficial results of surgery followed by photoradiation therapy for eradication of gliomas has been presented [75].

A variety of light sources have been employed in photoradiation therapy, but two predominate: filtered high-energy xenon lamps, and lasers emitting at or near 630 nm. Multiple quartz optical fibers can be coupled to the laser source, so that irradiation at subcutaneous sites can readily be carried out. Kennedy [72] has described the use of alternative light sources for photoradiation therapy, including the Kodak Carousel projector.

Transient skin photosensitization results from HPD therapy, and patients must therefore be protected from direct sunlight or other strong light sources for 30–60 days after drug injection [65–75]. Preliminary information suggests that use of the tumor-localizing component of HPD, dihematoporphyrin ether (Fig. 2), may reduce the total drug dose required for successful tumor photosensitization, with skin photosensitization correspondingly decreased [15].

Clinical successes with photoradiation therapy have provided the impetus for many exciting new developments in the field. An example of such progress is the report that hematoporphyrin, covalently bound to a monoclonal antibody, retains its capacity for photosensitization and the antibody remains capable of tumor recognition [76]. This procedure may be useful for targeting photosensitizers that would otherwise not localize in neoplastic tissues.

Other groups are currently attempting to elucidate the nature of the tumor-localization process and the sequence of events whereby photoradiation therapy causes tumor regression. Photodamage to tumor cells, blood supply, and host biologic systems contributes to the net therapeutic result, and the roles of these factors need to be explored. Damage to the tumor vascular system by HPD photoradiation therapy may be the major tumoricidal event [77].

Further progress in drug development is needed to provide porphyrins or other photosensitizers that, exhibit less skin photosensitization or are activated at longer wavelengths of light to which tissues are more transparent, thereby increasing the cell kill produced by a given light flux.

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