Photodynamic Therapy Using Endogenous Photosensitization for Gastrointestinal Tumors

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Photodynamic therapy (PDT) is a novel approach in the treatment of carcinomas of the gastrointestinal tract. This review defines PDT, discusses means of photosensitization and considers the mechanisms by which PDT causes cell death of the target tissue while at the same time avoid damage to normal tissues. Additional considerations include the time of PDT application, activation of the photosensitizer, effectiveness and toxicity of PDT, potential need for additional modalities of treatment and concludes with application of PDT principals to the early detection of malignancy. Data regarding the long term effectiveness of PDT for digestive tract adenocarcinomas are lacking because this field is still in its infancy.

WHAT IS PHOTODYNAMIC THERAPY?

Photodynamic therapy (PDT)b, a relatively new and potentially important form of adjuvant treatment for cancers of the digestive tract, initially involves the preferential accumulation of a photosensitizing compound by malignant tissues. Suitable photodynamic sensitizers exhibit no spontaneous toxicity unless and until they are excited by light at a wavelength corresponding to an absorbance band of the sensitizer. Excitation leads to cellular destruction, primarily mediated by singlet oxygen [1-8]. This form of molecular oxygen is toxic and results in both direct tumor cell kill as well as lethal effects due to vascular occlusion. A number of reports indicate that PDT is effective in treating malignant tumors in experimental animals. There also are an increasing number of preliminary reports of PDT use for the treatment of human tumors, including carcinoma of the oral cavity, esophagus, stomach, rectum, biliary tract, tracheobronchial tree, lung, skin, breast, brain, bladder, female genital tract, Kaposi’s sarcoma, retroperitoneal sarcomas, mesothelioma and carcinomatosis of the peritoneal cavity [1, 9-24]. Previous treatment of tumors with radiation therapy and/or chemotherapy does not appear to alter responsiveness to PDT. Similarly, application of PDT does not preclude the subsequent use of radiation therapy and/or chemotherapy.

PHOTOSENSITIZATION

There are two approaches to the administration of photosensitizers. One is exogenous and involves administration of the photosensitizer intravenously and relies on selective uptake of the drug by the target tissue, or tumor. The second is to rely on endogenous photosensitization whereby the target tissue converts a “precursor drug” into a photoreactive compound.

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bAbbreviations: PDT, photodynamic therapy; ALA, 5-aminolevulinic acid; PpIX, protoporphyrin IX.
Exogenous photosensitization:

The most frequently used exogenous sensitizer in clinical studies of PDT for gastrointestinal tumors is Photofrin, a somewhat purified proprietary preparation of “hematoporphyrin derivative,” a complex mixture of various porphyrin monomers, dimers and polymers derived from mammalian blood. This photosensitizer, which is given intravenously, is efficacious. However, Photofrin can lead to anaphylactic reactions [23] and prolonged photosensitization of normal skin. (“Photofrin” is used in this review to refer both to hematoporphyrin derivative and Photofrin II).

Endogenous photosensitization:

An alternative approach to photosensitization of a cancer is the induction of a chemical porphyria, a novel means whereby PDT is effective as a result of endogenous production of the photosensitizer [25]. This involves the oral administration of 5-aminolevulinic acid (ALA), which is absorbed into the blood stream and converted by cellular enzymes to protoporphyrin IX (PpIX). ALA is a naturally occurring five carbon amino acid [26] and is the first committed intermediate in the heme biosynthetic pathway (which occurs in mitochondria) [27]. This reaction has been observed in cultured cells, whole animals and selected tumors of humans. ALA per se is not a photosensitizer but rather its end product, PpIX, is the photoreactive species.

By normal feedback control, heme inhibits the activity of ALA synthase, the first and rate-limiting enzyme of the biosynthetic pathway, thereby preventing the cell from drowning in an excess production of its own porphyrins. This negative feedback control can be bypassed in certain types of malignant cells exposed to an excess amount of exogenous ALA. The ALA is continuously metabolized by these cells, leading to an over-production of several porphyrins, predominantly PpIX. Excess accumulation of PpIX occurs because of the enzyme make up of certain malignant cells. The latter have a relatively low activity of ferrochelatase [28-32], which catalyzes the insertion of an iron atom into PpIX. This forms heme (which is not photoreactive), thereby inhibiting photodynamic activity. Another factor leading to augmented PpIX synthesis is the increased activity of the rate limiting enzyme porphobilinogen deaminase in various malignant tissues [1, 33-35]. Hepatic synthesis of PpIX from ALA is quite efficient and it is likely that there is subsequent transport of PpIX by the blood stream to peripheral sites. However, isolated cells can synthesize PpIX upon exposure to ALA and PpIX is limited to a subcutaneous injection site of ALA in mice and humans [27, 36].

**HOW DOES ACTIVATION OF THE PHOTOSENSITIZER KILL CELLS?**

Photosensitizers exhibit a characteristic absorption spectrum. Upon irradiation of cells containing the photosensitizer with a specific wave length of light corresponding to the absorbance maxima of the sensitizer, the electronic configuration of the sensitizer is raised to a higher energy level (excited state). This excess energy can be converted to heat, to fluorescence emission, or via an intersystem crossing to the “triplet” state from which energy can be transferred to oxygen in tissues. This results in the formation of “singlet” molecular oxygen, a highly reactive, short-lived (half life: 10^-6 sec) cytotoxic agent [37, 38]. The latter reacts with amino acids, unsaturated fatty acids and nucleic acids, resulting in cell damage [1]. Other reactive oxygen species can be formed (superoxide, hydrogen peroxide and the hydroxyl radical) [39-41], but PDT effects on tumor tissue appear
to be mediated largely by singlet oxygen [41, 42]. The yield of singlet oxygen depends on the oxygen concentration in the tissues [43]. While cells exposed to ionizing radiation in the absence of oxygen are 2-3 times less sensitive than cells exposed under aerated conditions, isolated cells are completely insensitive to PDT in the absence of oxygen [43]. The nature of cellular changes produced by PDT are also different than X-irradiation [6].

**Cellular effects:**

The oxidative injury mediated by PDT involves various subcellular targets. PDT increases the expression of stress proteins and heme-oxygenase (a rate limiting enzyme in heme metabolism) as well as the release or increased production of eicosanoids, tumor necrosis factor, interleukins, serotonin and histamine [44-47]. Depending on the photosensitizer used, PDT can affect mitochondria and lysosomes, although lysosomal hydrolases are inactivated by the photochemical treatment before escaping the lysosomal compartment [48]. Photodamage can also be detected at the plasma and endoplasmic reticulum membranes, and can affect DNA, resulting in the rapid initiation of apoptosis [43, 49-55, 56]. Photofrin appears to mediate oxidative stress through protein kinase-mediated signal transduction pathway(s) to activate early response genes [57]. ALA-induced porphyrin formation is more specifically localized to the mitochondria of certain types of normal and malignant cells in laboratory animals, and this includes mitochondria of endothelial cells of tumor tissue [28]. However, various photosensitizers act differently as a result of different patterns of intracellular localization of the sensitizers [43], since singlet oxygen reacts at its sites of origin. The subcellular distribution of sensitizers has been studied mainly in in vitro systems, although it has been proposed that patterns of biodistribution matter little with respect to in vivo effectiveness [44].

**Vascular effects:**

Eventual vessel occlusion seems to be a general phenomenon associated with PDT using Photofrin [44]. The time frame that a decrease in blood occurs is variable, ranging from within 10 sec to 10 min in experimental tumors [58, 59]. It is not clear if this range is due to characteristics of the specific tumor model and/or technical features related to the application of PDT. Nevertheless, phototoxicity from Photofrin appears to involve the destruction of the tumor vasculature, an effect which appears to be selective, even in regions of tumor where the photosensitizer concentration is similar to that in normal adjacent tissue [43]. Thus, the effect of Photofrin may be mainly an indirect one, derived from the destruction of the tumor vasculature [60]. Vascular destruction itself, without any contribution from direct tumor cell kill, can lead to cures of experimental tumors. Given the importance of oxygen availability on phototoxicity, the rapid formation of hypoxic cells resulting from vascular damage increases the likelihood that some tumor cells will escape direct photodestruction [61, 62]. If a photodynamic agent has primarily vascular effects, there is a theoretical disadvantage of hypoxic, but still viable, cells persisting at the interface of necrotic and surrounding well perfused regions [63].

A proposed advantage of chemically-induced porphyria with ALA is that the phototoxic effect relies primarily on direct cell kill, whereas other photosensitizing agents appear to rely more on vascular effects [64, 65-67]. However, it is becoming apparent that ALA has definite vascular effects but the data are difficult to compare because of the number of confounding technical variables involved in these studies. For example, the vascular effects are related to the duration and intensity of the light source used to activate PpIX derived from ALA [68, 69]. Adding to the controversy are magnetic resonance spectroscopic studies suggesting that direct cellular damage from PDT per se occurs well before the changes observed with tumor hypoxia, which usually occur later.
and are mostly attributable to vascular damage [70]. If this is the case, it suggests that cellular destruction caused by PDT occurs by a dual synergistic mechanism.

On the one hand, distinction between a direct cell killing effect and a vascular effect of PDT may appear to be an academic argument, as long as destruction of the tumor occurs. Hypoxic regions exist in many experimental tumors due to variability in vascularization, yet PDT is still effective [71]. Thus, cell death may not occur immediately from photodynamic effects, but later as a result of a local circulatory effect. On the other hand, the distinction has importance to the technical aspects of PDT. The shift of cells into hypoxia does not necessarily imply that they are protected from further PDT damage. Hypoxia may be reversible, depending on light treatment dose and activating light fractionation schemes that permit re-establishment of blood flow during short intervals (but this may depend on the individual tumor, sensitizer dose, etc) [44]. Thus, reoxygenation permits additional PDT treatment which might be more efficient in destroying remaining viable tumor cells.

**HOW CAN PDT AVOID DESTRUCTION OF NORMAL TISSUE?**

Since ALA-induced PpIX is not completely specific for malignant tissue, there will always be some PpIX-induced photosensitization of normal tissues. This effect is mainly caused by the relatively slow conversion of PpIX to heme and might place normal tissues at risk for photodestruction. However, such damage can be avoided by relying on bleaching of the photosensitizer. ALA-induced PpIX is rapidly photobleached; that is, PpIX is destroyed by auto-oxidation. Thus, a low concentration of tissue PpIX can be photobleached before the photodynamic threshold for tissue damage occurs. This phenomenon makes it possible to "overdose" the treatment field to get maximum light penetration without causing serious damage to normal tissue. However, malignant cells will only be destroyed if sufficient PpIX accumulates so that there is a loss of viability before photobleaching can reduce the PpIX concentration to a non-toxic level [27, 64, 72, 73].

**HOW SOON AFTER ADMINISTRATION OF ALA IS PDT APPLIED?**

A very practical issue is knowing when the concentration of PpIX in the target tissue reaches not only a sufficient level for PDT to be effective but also a level substantially greater than the surrounding normal tissue. Our studies of actual tissue concentrations of PpIX after administration of ALA indicate that the time of peak PpIX levels occurring in both normal and malignant tissues can vary by several hours among patients [74]. The importance of this observation is that it may explain why some adenocarcinomas of the gastrointestinal tract appear to be unresponsive to PDT using ALA [8]. Serial measurements of actual PpIX tissue concentrations prior to PDT treatment is impractical because of the involved time for such determinations. However, advantage can be taken of the fact that photosensitizers fluoresce. PpIX fluoresces to a salmon pink color in response to blue light. In humans, it has been shown that more than 96 percent of fluorescing porphyrin after administration of ALA is PpIX [13, 75]. Gross visualization of porphyrin fluorescence does not always correlate with the actual tissue concentrations [76]. This is related to tissue pigmentation, fluorescence quenching and the lack of quantitative sensitivity of the human eye. However, changes in tissue concentrations of PpIX can be quantitated in humans by applying spectrophotometric methodology [77]. The advantage of the latter is that it offers a practical means for determining the most favorable time for starting PDT because relative changes in fluorescent signals correlate with changes in tissue concentrations of PpIX [77]. At the time of surgery, we use a sterile dual fiber optic cable (one fiber for delivery of blue activating light and one fiber for detection of fluorescence) connected to a spectrophotofluorometer.
Our studies with ALA in humans indicate that significantly greater concentrations of PpIX occur in adenocarcinomas of the gastrointestinal tract than in skin, skeletal muscle, intestinal muscle, mucosa and fat [74].

It is not yet known if the ratios between tumor and normal tissue concentrations can be improved in humans with intravenously administered ALA, although experimental studies show that the temporal kinetics of either oral or intravenous administration are similar [75]. Presently, oral intake of ALA by patients is preferable to intravenous administration, because the latter requires buffering to avoid adverse side effects. ALA is also poorly soluble in water and is chemically unstable at pH 7.4. However, there are several experimental ways to increase PpIX synthesis in response to ALA [22, 78], which may ultimately become clinically applicable. Intravenous administration of pure PpIX is also presently impractical because PpIX is only slightly soluble in water at physiological pH [27].

**ACTIVATION OF THE PHOTOSENSITIZER**

Porphyrrins absorb light at several wavelengths, but the most effective excitation wavelength is in the 405 nm (visible blue light) range. However, 630 nm (visible red light) is most often used for PDT because of its greater penetration in tissues [10, 79, 80]. The light source most often used to activate PpIX is a laser, not only because of its sharply defined wavelength, but also because the light bundle of a laser shows little divergence, making it possible to focus sharply. Specific laser photoactivation of sensitized cells is not one of either photocoagulation or photothermal ablation (vaporization) [64, 81], but rather like a switch to activate PpIX.

The optimal dose of light used to activate a photosensitizer in human cancers is not known and consideration of this issue is complex. Light dose is expressed as the delivered quantity (exposure dose) in J/cm², but the absorbed dose depends on the spectrum of the light source, irradiation geometry, depth in the tissue, light scattering in the tissue, concentration of the photosensitizer in the tissue, hemorrhage within the tumor as well as other factors [82, 83], making the absorbed dose difficult to calculate.

The PDT response is dependent on both the drug concentration and the light dose (conc*J/cm²) [72, 78, 84-86]. A “threshold” PDT dose must be exceeded for necrosis to occur. Since porphyrins are degraded (bleached) by light, a weaker response occurs at low drug concentrations. In order to obtain a PDT response at lower cellular drug concentrations, the light exposure must be increased. If a proper photosensitizer dose is used, then differential uptake by tumor should allow destruction of tumor and protection of normal tissue even at very high light doses because the level of photosensitizer in normal tissue would be below the photodynamic threshold for necrosis [73]. Thus there are at least 3 variables: degradation of the sensitizer (bleaching), differential tissue uptake, and threshold effects.

There is also controversy concerning total light dose and cell kill; some investigators have proposed that cell kill is proportional to the light dose which is independent of the power of the laser [87, 88]. Others suggest that the effect of PDT is inversely related to the strength of the light source [37, 89, 90], but this may be a characteristic of specific tumor types.

**IS PDT EFFECTIVE IN DESTROYING DIGESTIVE TRACT TUMORS?**

There is no question that proper application of PDT results in destruction of adenocarcinomas of the digestive tract [7, 8, 24, 91]. However, it is only recently that PDT has been systematically applied to these tumors and thus, necessary long term data relating to effectiveness are not yet available. However, there are limits to PDT and the major limiting factor is the depth of tumor kill.
The depth of penetration of 630 nm light in tissue ranges from 0.2 to 2 cm [15, 22, 43, 92, 93]. The mean depth of destruction of rectal and sigmoid adenocarcinomas in patients receiving Photofrin amounts to 0.6 cm with a range of 0.3 to 1.5 cm following intraluminal insertion of an optical fiber 1 mm into the tumor [94]. Among factors that limit light penetration are the presence of blood clot and necrosis within the tumor and absorption of light by the photosensitizer itself (a phenomenon called "self-shielding"). These factors may also limit the effectiveness of an interstitial light delivery system in which the laser light fibers are directly inserted into the tumor.

It appears that the main benefit of PDT at present for gastrointestinal tumors may be fourfold: 1) local control of microscopic deposits remaining after what seems to be a curative resection; 2) removal of relatively small deposits remaining after debulking surgery; 3) primary treatment for small mucosal lesions; or 4) palliative treatment. While PDT is not the stick of dynamite that everyone wishes to have available, it still may have an important, albeit somewhat, limited role. More promising are newer experimental photoreactive agents that are sensitive to longer wavelengths of light (> 660 nm), which will result in deeper tissue penetration. An important aspect of using PDT for palliation is that PDT can be repeated in multiple successive sessions.

**TOXICITY OF PDT AND PHOTOSENSITIZERS**

The toxicity of PDT is site-specific, being dependent upon the organ being irradiated and the selectivity of the photosensitizer for target tissue over normal tissue. A universal and clinically important adverse effect of PDT is skin photosensitization that leads to sun burns [11]. Most photosensitizing agents are not concentrated _per se_ in the skin, but low concentrations can be found in the skin for several weeks. For example, Photofrin cannot be bleached sufficiently to achieve photoprotection of the skin [95]. Although the mean duration of photosensitization with Photofrin injection is 3 months, true sunburns have been observed as late as 9 months [9, 11]. In contrast, ALA-induced PpIX is almost completely cleared from human plasma by 72 hr of oral administration. An occasional patient has been reported to develop mild cutaneous phototoxicity as late as 48 hr after receiving ALA [91]. However, by keeping patients in subdued light for 48 hr, preventing exposure to photodiode monitors (for example, a pulse oximeter) and filtering operating room lights to prevent nonspecific photoactivation of PpIX, we have not observed any phototoxic reactions after ALA administration.

An initial concern about administration of ALA is that it might mimic a genetic disorder of heme metabolism known as acute intermittent porphyria. This condition is characterized by increased levels of ALA in plasma and cerebrospinal fluid. Clinical manifestations include vomiting, tachycardia, abdominal pain, peripheral neuropathy and, to a lesser extent, central neuropathy [97]. Yet, there is minimal systemic dark toxicity (that is, in the absence of light) of ALA given orally. Except for mild nausea and vomiting, which occurs in almost a quarter of patients after oral intake of ALA, these other manifestations of porphyria have not been reported following ingestion of ALA. Experimental studies show that high ALA concentrations may lead to changes in behavior, cell membrane function as well as neuromuscular and spinal cord transmission. These effects have not been observed in humans. No clinically significant renal, cardiac, hepatic, pulmonary or metabolic adverse drug reactions have been reported in humans to date [11]. However, transient and variable abnormalities of liver function tests occur in about a third of patients given ALA.

If there is poor differential localization of the photosensitizer between malignant and normal tissue, there exists the potential for unwanted tissue damage [91, 77]. This may be more likely with the use of Photofrin than ALA, but there are also more clinical studies
involving use of Photofrin. For example, esophageal strictures occurred in 35 percent of patients who were given hematoporphyrin derivative as the photosensitizer for PDT involving esophageal cancers [91]. We have not to date observed any clinically significant unwanted tissue damage in patients who receive ALA and focal applications of PDT within the peritoneal cavity.

**WILL PDT AVOID THE NEED FOR ADDITIONAL CANCER TREATMENT?**

Nuclear damage and/or repair is not believed to be a dominant factor in PDT induced cytotoxicity in cells sensitized with Photofrin [43]. However, PDT may cause indirect damage to DNA, which might explain in part why different sensitizers and cell lines behave so differently with respect to induction of mutations [43]. There has been some suggestions that mutagenesis may occur as a result of PDT [44] and incomplete tumor destruction by PDT may lead to clonal emergence of more malignant cells [96]. Spontaneous mutation of tumors may also make them resistant to PDT. A case report of multiple cutaneous metastases from a breast cancer indicated that all but one site were sensitive to PDT using hematoporphyrin derivative [98].

**APPLICATION OF PRINCIPALS OF PDT TO TUMOR DETECTION**

Another use for chemically-induced porphyria with ALA relates to the detection of malignant tissues by fluorescence. Excitation of a photosensitizer by an incident photon produces re-emission of a fluorescent photon, which can be used to localize the reaction [11]. This might enable detection of metastases not ordinarily evident [10]. There appears to be a correlation between the presence of local tumor and local fluorescence. Success has been reported in examining potential treatment fields exposed to Photofrin using UV light [10, 17, 27]. Gross detection of fluorescence using UV light works with Photofrin and ALA-induced PpIX, but this requires subjective assessment using the eye and the UV spectrum does not include the peak excitation wavelength (410 nm) of PpIX [99]. More sensitive detection of PpIX can be accomplished in patients using spectrophotofluorometric technology using specific excitation wave lengths [56]. Application of this principle may ultimately lead to a relatively simple method for detecting tumor spread and directing site specific, rather than random, biopsies in order to more accurately determine the stage of the tumor.

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