Second Generation Photodynamic Agents: A Review

By ETHAN D. STERNBERG, Ph.D. and DAVID DOLPHIN, Ph.D.

ABSTRACT

Over the last decade, laser treatment of neoplastic diseases has become routine. The ability of these light-induced therapies to effect positive results is increased with the utilization of photosensitizing dyes. The approval of Photofrin® in Canada as a first generation photodynamic therapeutic agent for the treatment of some forms of bladder cancer is being followed by the development of other agents with improved properties. At this time a number of second generation photosensitizing dyes are under study in phase III clinical trials. A review of the status of these trials along with mechanistic aspects is reviewed in this article. In addition, a review of the status of lasers to be utilized for photodynamic therapy gives some indication of which instruments could be considered for this therapy in the future.

INTRODUCTION

Photodynamic therapy (PDT) is a therapeutic modality that utilizes a photosensitizer and visible light to create a toxic environment to biological systems. PDT is utilized in fields ranging from cancer therapy to viral inactivation of solid tumors and the treatment of leukemia (Fig. 1). The historical perspective of this methodology in the field of cancer is not within the domain of this review but has been reviewed elsewhere.1 We will endeavor to enlighten the reader on the mechanistic aspects of the phototoxic effect, show how these relate to different therapeutic protocols, and review some aspects of recent clinical trials for both the first and second generation compounds being studied at a number of clinical sites throughout the world.

Photofrin® is the first generation compound for PDT and has become most familiar to the readers of this journal.2 It consists of an oligomeric mixture of hematoporphyrin, a nonmetallated derivative of the porphyrin found in hemoglobin (Fig. 2). This drug has undergone clinical trials throughout the world and has been found to be effective in treating a number of cancers.3 Trials for the therapy have been instituted in a number of hospitals throughout the world. Quadra Logic Technologies in partnership with Lederle Laboratories has recently filed a new drug application (NDA) in a number of countries including Japan, Belgium, and Denmark and will have filed in the rest of Europe and the United States by the time of publication of this review. A Notice of Compliance from the Canadian Health Protection Branch was received in April 1993 for the use of Photofrin® in the treatment of superficial bladder cancer. This was a very important landmark since it was the first approval for PDT anywhere in the world.

Photofrin® is activated at 630 nm using an argon pumped dye laser. This wavelength is chosen since the strong absorptions due to oxy- and deoxyhemoglobin in the visible region become weaker than the longest wavelength absorption band of Photofrin®.4 As the absorption of the natural chromophores (primarily the hemoglobins) decreases then the effective depth of penetration increases with increasing wavelength (Fig. 3). In addition to absorption, scattering of photons also limits the effective penetration depth. In practice, light at 690 nm travels twice as deep as that at 630 nm. On moving from 690 nm into the near infrared (800 nm) only an additional 10% effective depth of penetration is achieved. Clearly then, second generation photosensitizers will be most effective when they absorb and can be activated at wavelengths longer than that used to activate Photofrin® (630 nm). In addition, successful second generation photosensitizers must exhibit less prolonged skin photosensitivity than seen with Photofrin®, where a patient's skin may remain photosensitive to strong sunlight for up to 4–6 weeks.5 The side effects, small in comparison to those seen with chemotherapy or radiation therapy, could limit the use of Photofrin® in non-life threatening indications.
FIG. 1. Feline T cells were treated with 2 µg/ml of BPDMA and 20 J/cm² of light. (A) Normal cells. (B) Initial damage on close inspection shows small holes. (C) These holes increase in size after time. (D) Cell membranes are completely ruptured. Reprinted with permission from North et al. Blood Cells 18:129-140.

THE PHOTODYNAMIC EFFECT

The processes by which light creates a phototoxic effect in the presence of a dye are still under debate. There are two categories of energy transfer that may occur. The first step for both of these is absorption of light by a dye. Typically the dye is an extended aromatic system such as merocyanine, methylene blue, or a variety of porphyrin derivatives (Fig. 4). An excited state of the dye is generated that can interact with a biomolecule via an electron transfer mechanism resulting in the destruction of the biomolecule and the bleaching of the dye (Fig. 5). This is known as a Type I photo process and the formation of radicals during such processes may result in additional damage due to subsequent radical chain or oxidation reactions. A more

FIG. 2. Photofrin® is a purified fraction of oligomers of hematoporphyrin.

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R = \text{H or } \text{CH}_2 = \text{CH}_2 \\
\text{and } \eta = 0-7
\]

FIG. 3. Relative effective light dose versus wavelength (nm). Light penetrates tissue better in regions near the infrared. Only a moderate increase is found in going from 700 to 800 nm.
interesting process occurs when the energetically excited chromophore, after intersystem crossing from the singlet excited state to the triplet state, interacts with ground state triplet oxygen. This interaction results in the formation of singlet oxygen ($^1O_2$) and the dye returns to its original singlet ground state ready to absorb another photon and to initiate another catalytic cycle and the formation of more singlet oxygen. Although singlet oxygen has a short lifetime (approximately 6 μsec in water and a little longer in cell membranes), it has the ability to readily react with a wide variety of biomolecules as shown in Fig. 6. These latter reactions can cause the destruction of various biological components and shut down numerous biological processes. Singlet oxygen is generated catalytically and the rate of singlet oxygen generation depends on some of the criteria described below. In the case of several phototoxins the quantum yield for singlet oxygen production is around 0.7. 3 Three criteria determine the efficacy of a sensitizing dye, in a non-biological system. The molecular extinction coefficient ($ε$) measures the ability of a sensitizer to absorb light falling on it at a specific wavelength effectively. The quantum yield of bleaching of the photosensitizer determines its lifetime when undergoing a Type I photo process, and the quantum yield for singlet oxygen production measures the ratio for the number of photons absorbed to the number of singlet oxygen molecules produced. These three criteria are relatively easy values to measure, however, relating these parameters to in vitro and especially in vivo cytotoxicity is difficult.

The environment of a phototoxin not only determines which biological systems are most disrupted by PDT but also determines the efficiency of the sensitizer, because of photobleaching and the photo properties of the tissue. This synergism complicates the biological studies in that it is possible that the site of the greatest concentration of the dye may not define the site of cell death. Thus the development of dyes for PDT differs from the classical development of pharmaceutical compounds where one hopes to find a drug that interacts with a specific binding site and then further modify the drug to interact more effectively with that binding site.  8

**PHOTOTOXINS IN BIOLOGY**

Many photodynamic agents have been investigated for their abilities to induce a phototoxic effect. Compounds that are near or currently in clinical trials range from a totally synthetic tetrahydroxytetraphenylchlorin (1) developed by Bonnert and Berenbawn 9 and zinc phthalocyanine (3) being used by CIBA-Geigy 10 to the modified natural product monoaasparylchlorin ε, (4) being investigated by Nippon Petrochemical, 11 tin etiopurpurin (5) synthesized by Morgan et al., 12 and benzoporphyrin derivative monoacid (BPDMA) (5), which we at QLT and

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**FIG. 4.** (top) Methylene blue; (middle) merocyanine; (bottom) tetraphenylporphyrin.

**FIG. 5.** Two types of reactions can occur from the photoexcited state of a sensitizer 5. They may react directly with a substrate via electron transfer and bleach (Type I), or undergo intersystem crossing, react with oxygen, and generate singlet oxygen.

**FIG. 6.** Biomolecules such as cholesterol (A), methionine (B), guanidine (C), and histidine (D) give a number of products ranging from hydroperoxides to sulfides.
Lederle Laboratories are developing\(^1\) (Fig. 7). All of these compounds have absorption maxima beyond 650 nm and extinction coefficient at their maximal long wavelength absorption at least 10-fold over that of Photofrin® at 630 nm. Figure 7 illustrates the near red absorption maxima of several of these newer photosensitizers.

Two sites of destruction are generally acknowledged to be important to the in vitro damage to cells. The cell membrane is susceptible to a number of assaults by singlet oxygen.\(^4\) Proteins within the membrane, on PDT treatment, may be modified and have their ability to channel ions disrupted. Although lipid peroxidation can occur routinely on PDT the changes in membrane permeability in models such as red blood cells seem to be associated with damage to proteins.\(^5\) Membranes within the cell such as those associated with the lysosomes have also been suggested as sites of cell destruction. A further site of destruction is associated with mitochondria.\(^6\) Isolated mitochondria have shown that photosensitization by Photofrin® created a loss of oxidative phosphorylation that could be traced to oxidation of thiols, in carrier proteins, by singlet oxygen.\(^7\) The modification of other enzymatic processes has also been observed in vitro. For instance, aldehyde-3-phosphate dehydrogenase has been shown to lose the integrity of the SH sites in the active center after PDT.\(^8\) Changes in activity, conformation, and fluorescence along with an increase toward protease susceptibility are some examples of such destruction in this extensively studied enzyme. Magnetic resonance imaging (MRI) has shown a dramatic drop in ATP levels immediately after PDT,\(^9\) probably as a result of cell repair mechanisms being activated. Even nuclear enzymes are affected by PDT.\(^10\) In the case of DNA repair, photodynamic treatment of L929 cells generates nuclease activity that is normally controlled by ADP-ribosylation. The effect of direct single-strand damage to DNA has been noted with select photosensitizers in some systems. Recent studies suggest a dominant form of destruction associated with the DNA may correspond to interruption of microtubulin formation by a low-density lipoprotein delivered photosensitizer such as with Photofrin®.\(^1\)\(^1\)

The site at which tumor damage occurs is related not only to where the sensitizer causes damage in the cell but also to the morphology of the tumor. It has been observed depending on the hydrophilicity of the photosensitizer, that a higher concentration can be found around the tumor as compared to that of surrounding tissue.\(^2\) This increase in concentration may be accounted for by either specific uptake by a lipoprotein-associated mechanism as seen with other drugs or by simple physical morphology of leaky tumor vasculature and poor lymphatic drainage. Richter et al. have shown that low-density lipoprotein (LDL) bound BPDMA reduces the amount of drug in the skin while destruction of the tumor remains the same.\(^3\) It is known that LDL binding sites are higher on cancer cells and it is a topic of intense interest in the field of drug delivery.\(^4\) Formulation of a drug may be important in optimizing this increase in drug localization perhaps by an LDL delivery system. In the case of BPDMA, the formulation that is utilized for clinical trials are unilamellar vesicles (liposomes) to help solubilize the hydrophobic drug and direct it to its lipoprotein-dominated delivery mechanism.\(^5\) Liposomes are also being investigated for use in the delivery of zinc phthalocyanine,\(^6\) a compound that normally has extremely poor solubility even in organic solvents. The water-soluble NEP6 also accumulates in tumors although it is readily taken up in the blood by serum albumin.\(^7\) Perhaps even in this case, initial delivery of the drug is dominated by the lipoprotein fraction of blood even though a majority of it binds to the serum albumin.

It may not only be the actual destruction of cancer cells that results in tumor ablation during PDT but also that of the micro- (neo)vasculature.\(^8\) Some control over the biological behavior of the photosensitizer associated with its structure has been observed. A series of modified aluminum phthalocyanines (Fig. 8) in which the parent molecule has sulfonation ranging from 1 to 4 has shown that the more lipophilic monosulfonated compounds are marginally more effective than the tetradsulfonated compound in terms of absolute phototoxicity in vivo.\(^9\) However, in vitro experiments show that the very hydrophilic tetradsulfonated material has poor photosensitizing abilities. Some property of the in vivo delivery system to the vasculature has increased the more hydrophobic compound’s efficacy. Such possible vasculature destruction has been demonstrated by a number of researchers. Morgan et al. have shown that radioactive microspheres are held up to a greater extent in tumors after PDT.\(^10\) MRI has been used to show that the relaxation coefficients ($T_1,T_2$) of water around the tumor are dramatically changed soon after PDT.\(^11\) A new technique that utilizes laser doppler measurements can generate information much like that given by MRI about blood flow of a recently treated tumor.\(^12\)
Photodynamic therapy in mice in contrast has shown immunosuppression as indicated by sensitivity to dinitrofluorobenzene. Observation of patients after successful treatment for bladder cancer showed an increase in chronic inflammatory cells. Further observations have shown a relationship between the cytokines, interleukin-1β, interleukin-2, and tumor necrosis factor-α concentrations and the level of PDT treatment in patients. More direct evidence of cytokine release was found by Evans et al. who observed "tumor necrosis factor" release by macrophages after PDT. Bellnier has used combination therapy of PDT and tumor necrosis factor to increase tumor response. Thromboxane has also been found to be released shortly after PDT. It is also suspected that nitric oxide, better known as endothelium releasing factor (EDRF), which is related to guanylate cyclase, may be a consequence of macrophage activation. This is not surprising in that at least in the case of Photofrin®, a minor constituent of the drug is protoporphyrin IX, a cofactor in the cascade in nitric oxide formation.

LIGHT DELIVERY DEVICES

In the last several years QLT and Lederle have set up total laser systems that meet international clinical standards. Among the areas to review were the safety, equivalency of clinical lasers to a reference standard, compatibility of existing fiber optics and power meters, verification of manufacturers specifications, conformance with international regulatory requirements, and suitability for the clinical setting. This is an ongoing process.

For PDT many types of lasers have been evaluated. The most common type in use is the argon pumped dye laser (APDL). This has been used exclusively in the QLT/Lederle Photofrin® Phase III clinical trials in North America. Recently, APDLs such as the Lambda Plus from Coherent Medical have been engineered specifically for PDT with Photofrin® as the toxin. It produces upward of 1.9 W from the tip of the fiber at 630 nm, which is more than enough power for most clinical requirements. Other lasers that have been considered for use with Photofrin® are the copper vapor pumped dye lasers and the gold vapor lasers. These pulse systems suffer from several problems such as lack of technical compatibility for the clinical setting, developing light dosage standards to relate to the APDLs, and high peak pulse energy output that tends to degrade the optical fibers.

The utilization of a KTP YAG to pump a dye in the region of 630 nm has been achieved and an example of such a system has been made by Laserscope. At the primary output of 1066 nm this laser can be utilized for general YAG surgical applications. When the output in frequency doubled to 532 nm it can pump a dye laser to a number of standard frequencies. This laser provides a high frequency pulse of ~10 kHz with a relatively low power output of 1 kW, which should prove to maintain fiber integrity. The last several years have seen the development of a new type of laser that has the potential to be compact, require little or no cooling, and should work directly from a wall plug. These solid state diode lasers have shown that clinically significant output (~3 W) can be achieved at 630 nm and thus may be considered in the future for use with Photofrin®.

For second generation compounds the diode laser, KTP YAG pumped dye lasers, and the standard APD laser are being reviewed. Clinical trials at present are concentrating on utilizing research type APDL. However, as the second generation drugs move through clinical trials it becomes more likely that a diode laser designed for the drug's specific wavelength will be developed.

In the case of psoriasis or cutaneous cancers it may be practical to use nonlaser light sources such as arc lamps, fluorescent tubes, and light emitting diodes since the light does not have to be concentrated into a small area. These light sources will require all the clinical equivalency studies, which have been necessary for the lasers.

At present PDT requires the utilization of a laser coupled to a fiber optic system incorporated into an endoscope. Fiber optic systems have been developed with specific treatments in mind. In the instance of cutaneous cancer a GRIN lens is utilized to spread the light to a cone. This allows light dosages to be easily calculated by taking into account the transmitted power from the tip, the time, and the distance from the tip to the treatment field. Bladder cancer requires the incorporation of a sphere diffuser into the above system. Newer systems have utilized a balloon catheter to center the fiber as the balloon expands the bladder to be roughly spherical. This, in combination with an intrabladder detector, should ensure constant and even light dosimetry. Cylindrical geometries that are found in the esophagus or the bronchi can be treated by fibers developed with cylindrical output. A fiber with a cylindrical output tip of up to 2.5 cm in length is passed into the treatment area and placed parallel to the tumor. Improvements to this system include the development of a balloon catheter with detector probes on the wall. In combination with this a face of the balloon has been darkened generating a lantern effect. In the case of a very large tumor the fiber can be placed directly into it.

CLINICAL TRIALS

The details and complexity of preclinical studies and clinical trials are even more complicated for PDT than for a "traditional" drug since both drug and light are involved. Toxicity must examine the traditional "dark" effects as well as those generated on illumination. For the most part none of the drugs described above has been reported to have any inherent dark toxicity in doses ranging much higher (x10–x100) than the therapeutic dose. After stability and toxicity of the appropriate drug formulation are addressed, it is necessary in a Phase I clinical trial to investigate drug dosage, time of irradiation after injection, pharmacokinetics, human drug toxicity, and, most importantly, the extent of skin photosensitivity during and after
treatment. The development of most standard therapeutic agents generally does not have to correlate drug dosage to skin toxicity and light activation of drug. For these reasons initial clinical trials in PDT have been directed toward investigating the use of the drug for treatment of skin cancers. In these studies a combined review of toxicity toward the skin while deriving information about the light doses necessary to damage the tumor can be determined. Of course such information gives an indication of the efficacy toward the tumor, which is not generally the goal of initial clinical trials.

A profile of drugs in Phase I investigations to date has shown that after initial injection the drug takes some hours to accumulate in the tumor and the skin. One hopes to irradiate at some time after the injection so that therapy can proceed on the same day. Time of accumulation into the tumor as compared to the skin or other tissue may not be ideal for all the new drugs. In the case of Photofrin® a dosage of approximately 2.5 mg/kg is standard. Irradiation occurs some 48 to 72 hr after injection at a time when the drug has a higher concentration in tumor compared to that in surrounding tissue. A typical light dosage from the APDL is 150–250 J/cm² for treatment of a lung tumor. In the case of bladder cancer the light is diffused over the entire surface of the organ to give a light dosage of 150 J/cm². With the above dosage of drug, skin photosensitivity may last about 4–6 weeks. Most photosensitizers are also good fluorophores. This property may allow the clinician to eventually determine the level of drug in both the skin and tumor before and after irradiation, which could be useful both for determining the site of treatment and for calculating the appropriate light dosage during therapy.

Four second generation drugs are currently in Phase I clinical trials. These are BPDMA, with which the authors are most familiar, monoaopartylchlorin e₆, meso-tetra(m-hydroxyphenyl)chlorin (mTHPC), and tin etiopurpurin. Since much of the preclinical and clinical research is of a proprietary nature, information on the progress of these drugs toward a submission of a new drug application (NDA) is rather limited. None exists, as of yet, for the tin etiopurpurin.

There is a published account on the treatment of 4 patients using mTHPC and it is known that additional patients have been enrolled in the study. mTHPC is somewhat hydrophilic and rapidly accumulates in malignant mesothelioma. The drug is delivered in a mixture of polyethylene glycol, ethanol, and water and is formulated just prior to injection. The ratio of drug in tumor as compared to surrounding muscle was determined to be 7:1 and 14:1 relative to skin. These ratios are higher than the 2–4:1 found for Photofrin® in humans. The circulating concentration of the drug 48 hr after injection was found to be 0.15 mg/ml, while the half-life of the drug in circulation was approximately 12 hr. Dosages ranged from 0.075 to 0.3 mg/kg with a light irradiation of 10 J/cm² at 650 nm. At the lower dosage 50% necrosis of the tumor was shown while the next higher dosage showed 80% necrosis and the final dosage 100% necrosis to a depth of 1 cm. Detailed histological profiles of the tumor and surrounding tissues described the extent and area of tissue damage. Description of skin photosensitivity studies was sparse and the studies appeared not to be carried out under controlled conditions with a solar simulator. However, some anecdotal data were noted. Skin photosensitivity appeared to begin some time after injection (3–10 days); it does appear, however, that significant clinical photosensitivity has been observed in some patients up to 10 days after treatment, which may place limitations on the use of this drug in PDT.

Phase I clinical trials for the water-soluble monoaopartylchlorin e₆ began at the same time as for BPDMA and were examined against the same skin tumors described below. Drug doses were assigned in the range 0.5 to 2 mg/kg with concurrent increases of light dosage from 12.5 to 200 J/cm². As in the case of BPDMA drug dosages were limited to the lower concentrations because of the initial positive responses found. Skin sensitivity in the lower dosages used (0.5 mg/kg) lasted up to 7 days. This drug has strong binding to serum albumin and it appears to circulate for a much longer time than other PDT drugs; in fact the drug is readily detectable 4 weeks after treatment while 80% of the initial concentration is detected in the blood 10 days after injection.

The second generation photosensitizer BPDMA is a semisynthetic porphyrin derivative that has been found to be effective in treating a number of cancers in vivo along with purging of viruses from blood. In the ongoing clinical trial information about the pharmacokinetics, skin photosensitivity along with tumor response has been reported. The patients had a range of cancers that included basal cell carcinoma, metastatic breast carcinoma, metastatic gastrointestinal carcinoma, and metastatic amelanotic melanoma. Drug dosages ranged from 0.25 to 0.5 mg/kg in the patients who were infused 3 hr prior to irradiation while light dosages administered by a laser tuned to 690 nm ranged from 50 to 150 J/cm² for the low drug dosages to 50 J/cm² for the high drug dosage. Sixty-four cutaneous tumors were treated and followed for several months after treatment. Overall tumor response was 63%, while 100% responses were noted for the higher drug and light dosages.

Since the major purpose of the first generation trial was to judge skin photosensitivity the most extensive studies for UV/Vis light were carried out with the utilization of a filtered xenon arc lamp source onto normal skin soon after light treatment of the tumors while UVB light was generated from fluorescent sun lamps. Testing of UVB sensitivity showed no response for the early patients, so these assays were discontinued. Marked skin photosensitivity to UV/Vis light was noted early after treatment. On the day of treatment the challenged light dose was reduced for the higher drug dosages so that minimal erythema dose (MED) could be noted. Close examination of light exposure on the patients ranging up to 9 days after treatment for high drug dosages shows a logarithmic loss of photosensitivity. An overall pharmacokinetic profile of drug serum concentration is reviewed in Fig. 9. It is noted that the drop of all drug dosage levels down from the maximum concentration corresponded to ~1 hr. The relationship between these results and the corresponding clearance of drug from skin has yet to be investigated.

All the drugs investigated to date have exhibited distinct clinical responses. The maximum effective drug dose corresponding to minimum skin sensitivity has yet to be determined for any of these materials. However, general approaches toward optimizing such a differential can be applied to all of them. Wilson and others have suggested that a minimum drug dose that would then be treated with a large light dose would illicit the best response over all clinical responses, especially if moderate bleaching of the drug occurs. Several other approaches may be considered when optimizing light dosage for clinical
responses, which could include fractionation of the light dosage.

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48. Private communication, R.P. Allen, University of California Davis, Medical School.
Second Generation Photodynamic Agents


Address reprint requests to:
Ethan D. Sternberg, Ph.D.
Department of Chemistry
University of British Columbia
2036 Main Mall
Vancouver, B.C., Canada V6T 1Z1