Chemistry behind the News

Photodynamic Therapy: The Sensitization of Cancer Cells to Light

by Jennifer Miller

All forms of cancer are characterized by the uncontrolled growth and spread of abnormal cells. If the spread of these abnormal cells is not prevented, cancer can result in death. Cancer is often detected by the formation of a malignant tumor whose potentially unlimited growth expands locally by invading tissue and spreads to the rest of the body by metastasis. The causes of cancer are diverse and include both external factors—radiation, chemicals, and viruses—and internal factors—hormones, immune conditions, and inherited mutations.

The most important step in preventing the spread of cancer is to kill the malignant cells. Photodynamic therapy, a promising, new approach for destroying malignant cells, takes advantage of light, oxygen, and a drug (photosensitizer) that preferentially localizes in rapidly growing cells (*1*–*3*). A photosensitizer is any molecule that uses radiant energy or light to elicit a specific response. The most well-researched photosensitizers of photodynamic therapy are hematoporphyrin derivative (Hpd) and its active component, Photofrin II (porfimer sodium). It has been known since the 1960s that Hpd, which is formed by acid catalyzed acetylation of hematoporphyrin (Figure 1) (*1*, *2*, *4*) and subsequent alkaline treatment, preferentially localizes in the tumors of mice and rats and can be detected by its fluorescence. At the same time, scientists found that Hpd has photodynamic activity—it can augment or induce a toxic reaction when exposed to light (*4*).

In the first step of treating cancer with photodynamic therapy (PDT), the drug is administered. After the drug has been injected, a period of incubation is required. During this incubation, the normal cells get rid of the drug and the malignant cells accumulate the drug through mechanisms not yet fully understood (*3*). This incubation optimizes the ratio of the concentration of the drug in malignant cells to that in normal cells, so that the malignant cells have a considerably higher concentration of the photosensitizer. Ranging from several minutes to a couple of days, the length of time required for this incubation depends on the particular photosensitizer.

Once the drug has reached the appropriate concentration, light is shone at the target tissue for 10–30 min (*3*). Initially, the light sources for clinical PDT were scientific argon-pumped dye lasers coupled to optical fibers, which could deliver about 3 watts of power at a wavelength of 630 nm. The development of light-emitting diode systems and solid-state diode lasers has greatly reduced the complexity and expense of the light systems (*1*).

In the absence of light, the drug is not toxic. In the presence of light, the drug is activated to produce singlet oxygen. Singlet oxygen reacts with biological molecules—amino acids, lipids, and steroids—disrupting the normal functions of the cell and causing the cell to die (*2*). To prevent the destruction of normal cells, the light source is shone directly at the malignant cells while avoiding the normal cells. Because these drugs preferentially accumulate in cancerous cells rather than in healthy cells, and light is shone directly at those target tissues, photodynamic therapy has selective toxicity and fewer side effects than do chemotherapy and radiotherapy.

Structure of Photosensitizers and Their Relationship to Biological Systems

When analyzed, Hpd is found to contain a mixture of oligomers (polymers of two to several structural units in length) and monomers of hematoporphyrin (Figure 1) (*1*). The oligomeric portion of hematoporphyrin, Photofrin II, is the active species for photodynamic therapy. The actual chain length of the oligomer, formed by both ether and ester linkages, is not known. Purification of Photofrin II by high-pressure liquid chromatography (HPLC) has proven to be difficult, and the complex mixture results in a broad profile. The most current mass spectroscopic data indicate that trimers of hematoporphyrin are the major constituents of Protofrin II (*1*).

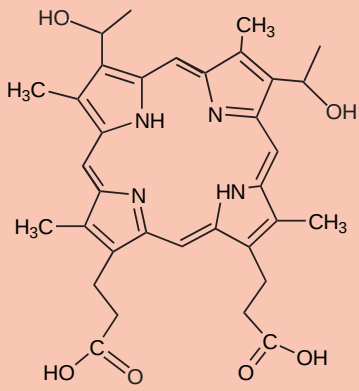

Figure 1. Hematoporphyrin

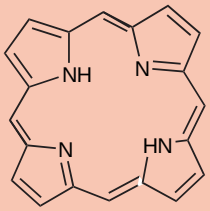

Figure 2. Porphyrin

Isolated from blood, hematoporphyrin is a member of a class of tetrapyrrole heterocycles referred to as poryphyrins. The several naturally occurring porphryins differ in the substituents of the macrocycle (Figure 2) and often contain a central metal atom. Found ubiquitously in

nature, these porphyrins are pigments, such as chlorophyll, heme, and bacteriochlorophylls, that function as coenzymes to catalyze chemical transformations (a coenzyme is any functional group that has been incorporated into an enzyme to participate in catalysis). With the help of coenzymes, difficult chemical reactions, which cannot be accomplished by the side chains of the amino acids in an enzyme, are achieved in biological systems. The presence of porphyrins enables enzymes to transport electrons, store oxygen, reduce oxygen, and destroy peroxides. Because of their important roles in living organisms and interesting chemical properties, porphyrins and polypyrrolic macrocycles are the most widely studied of all macrocycles (*5*).

Physical Chemistry of Photodynamic Therapy

Photodynamic therapy is based on the idea that the photosensitizer will absorb a photon of energy at a specific wavelength of light and transfer that energy to oxygen. In the case of Photofrin II, the longest wavelength of absorption is 630 nm (*1, 2*). Having absorbed a photon of energy, the drug is activated, and an electron of the drug becomes excited. The energy of this electronically excited singlet state (S_1) of the photosensitizer may be lost in a variety of ways as shown in the modified Jablonski diagram (Figure 3) (*1, 2, 6*). The excited molecule can discard its excess energy by fluorescing or by transferring its excess energy to surrounding molecules. If the singlet state participates in an electron transfer process with a biological substrate, the substrate will be altered into an unusable form, and the photosensitizer will be bleached in a process known as Type I photoprocess.

The excited photosensitizer can also undergo intersystem crossing from the S_1 state to become an excited triplet state (T_1). At this point, the triplet state can proceed one of two ways. It can either phosphoresce and slowly emit its energy, or it can react with ground state $^3O_2$ (triplet oxygen) to regenerate the photosensitizer in its ground state and produce excited singlet oxygen. Singlet oxygen contains two electrons of opposite spins that occupy an orbital of higher energy than its ground state. As a result, singlet oxygen is unstable, due to its excess energy. Several reports indicate that the principal toxic species formed during photodynamic therapy is short-lived (~6 μs in aqueous environments), highly reactive singlet oxygen (*1–4*). Evidence for the production of singlet oxygen is supported by the oxygen-dependency of photodynamic therapy: in the absence of oxygen, there is no tumor damage, and in the presence of an oxygen-singlet quencher (a compound that eliminates the energy of singlet oxygen), cells that have incorporated Hpd are not destroyed (*4*).

Mechanism of Importing the Photosensitizer

The most advantageous property of Photofrin II (and similar photosensitizers) is its preferential localization in tumor cells. In the search for new photosensitizers, the effectiveness of a drug is determined by the ability of that drug to preferentially accumulate in malignant cells. Unfortunately, the mechanism of uptake by cells is not well understood: the drugs are not recognized by a specific enzyme or receptor, and they do not bind to a specific "site" on tumor cells. However, photosensitizers have an affinity for lipoproteins, especially low-density lipoproteins (LDLs). If the drug binds strongly to LDL, the complex of LDL and photosensitizer can be imported into the cell by receptor-mediated endocytosis (*1, 3*). Endocytosis is the process by which cells ingest fluids, solutes, and other necessary nutrients (*7*).

In order to accumulate cholesterol for the synthesis of membranes, proliferating cells have high levels of LDL receptor activity. Uptake and retention of these drugs may be mediated by LDLs and their receptors, whose concentration on the membranes of cancer cells, and any other rapidly growing cell, is considerably higher than that of normal cells. As a result, photosensitizers bound to LDL will accumulate in rapidly growing cells at a much greater concentration than in normal cells. Although non-receptor endocytosis appears to play some role, LDL receptor-mediated endocytosis is the current working model for the mechanism of uptake of the photosensitizer.

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Second Generation Drugs and Additional Applications of PDT

Photofrin II is the only photosensitizer that has gained regulatory approval in Canada, Japan, France, Germany, the Netherlands, and the United States for the clinical use of photodynamic therapy (*3*). It is currently being used for treatment of esophageal, lung, cervical, gastric, and bladder cancers. Research is also in progress to find more effective photosensitizers. One objective is to find PDT drugs that can be activated at longer wavelengths which, in turn, will increase the penetration of the tumor by photodynamic therapy (Figure 4) (*1–4*). At longer wavelengths of light, absorption by heme proteins and other reactive compounds in the blood will not interfere during therapy. A number of these photosensitizers, which can be activated at longer wavelengths than Photofrin II, have already been synthesized. Unlike Photofrin II and Hpd, these new PDT drugs have been purified, and their structures are known. Using pure compounds whose structures are known will enable scientists to better understand how photodynamic therapy works.

In addition to increasing the efficacy of these photosensitizers, new applications for photodynamic therapy have also been found. In the case of atherosclerosis, plaque builds up along the interior walls of arteries, decreases the flow of blood, and causes the hardening of arteries. As observed with malignant cells, photosensitizers preferentially accumulate in atherosclerotic plaque rather than the surrounding healthy cells. When photodynamic therapy was used to treat atherosclerotic rabbits in preclinical studies, 80% of the plaques were removed without damaging the surrounding healthy tissue (*3*). These results suggest that photodynamic therapy can be used to treat patients with heart disease.

In summary, photodynamic therapy is a potential medical treatment for any disease associated with rapidly growing cells. Quadra Logic Technologies Inc. (QLT), which bought the rights to Photofrin II in 1988, is currently investigating the application of photodynamic therapy to a variety of diseases, including rheumatoid arthritis, age-related macular degeneration, and autoimmune and ophthalmology diseases. Because of its selectivity, minimal side effects, and multitude of applications, photodynamic therapy may become the mainstream remedy for life-threatening diseases.

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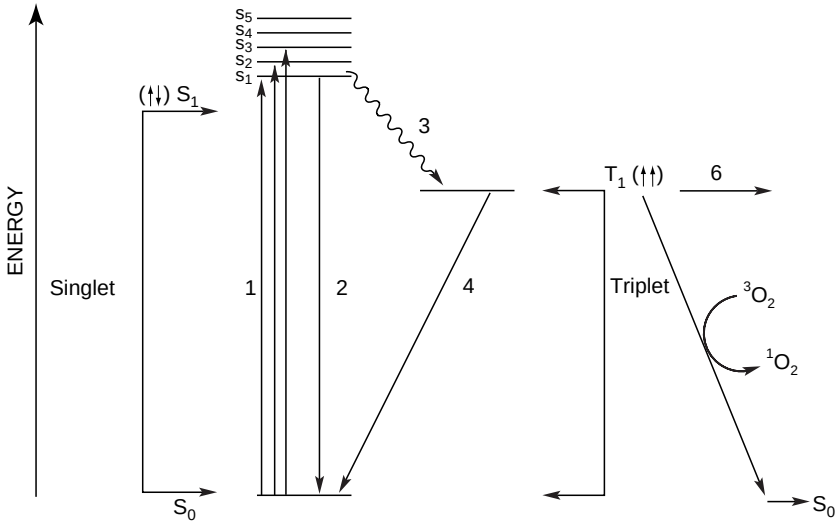

Figure 3. Modified Jablonski diagram for a typical photosensitizer showing: 1= Absorption of Light; 2= Fluorescence; 3= Intersystem Crossing; 4= Phosphorescence; 5= Production of Singlet Oxygen (Type II Photoprocess); 6= Hydrogen or Electron Transfer (Type I Photoprocess). A Jablonski diagram is a simplified representation of the electronic energy levels and radiative transitions of a molecule.

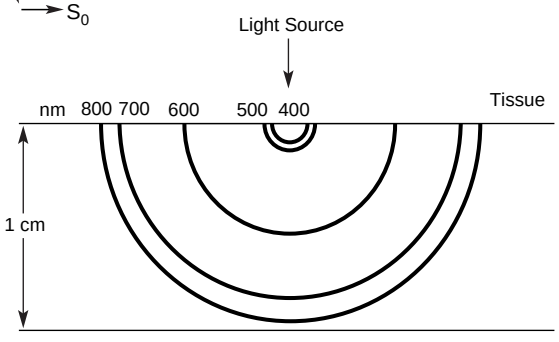

Figure 4. The relationship of the depth of penetration of light into a tumor and the wavelength of the light. Scattering and absorption are the predominant factors in limiting the penetration of light into the tumor. The depth of penetration doubles when the wavelength of light is increased from 550 to 630 nm (where Photofrin II is activated) and from 630 to 700 nm. Beyond 700 nm, the depth of penetration by light only increases another 10% by shifting into the infrared region (*1, 2*).