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Photodynamic Therapy:
You down with PDT? Yeah you know me!

by

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Abbreviations

ALA- Aminolevulenic acid
FDA- Food and Drug Administration
HPD- Hematoporphyrin derivative
LED- Light emitting diode
PDT- Photodynamic Therapy
UV/Vis- Ultraviolet/Visible light spectroscopy

Table of Contents Page

Abstract

Photodynamic therapy is used as a treatment for a variety of diseases, ranging from lung or esophageal cancer to psoriasis. Photosensitizing agents, applied by either injection or ointment, are activated by light of a specific wavelength and singlet oxygen is released, causing damage to nearby cells. The advantages and disadvantages of photodynamic therapy will be discussed.

Introduction

Around the turn of the last century, Charles Raab, a German medical student, made an interesting discovery in the culture of *Paramecium* and acridine he had prepared. Sunlight seemed to induce the death of paramecia. Specifically, an acridine solution of 1:20,000 would kill paramecia exposed to direct sunlight in 6 minutes, and diffused light took 60 minutes to kill the paramecia, while in the dark there was no lethal action whatsoever. His boss, Hermann Von Tappeiner, ran experiments that showed oxygen was also essential in killing the paramecia [1]. Then, 95 short years later, the US FDA approved Photofrin as the first drug used in photodynamic therapy.

Obviously, some steps were skipped over in the preceding paragraph, and this paper will serve to elucidate questions the reader may have concerning PDT. Photodynamic therapy is the treatment of disease by highly reactive oxygen intermediates, which are generated by the interaction of light and a photosensitizing drug [2]. Porphyrins are most commonly used as photosensitizers, but, by definition, any chemical which induces oxygen damage as a result of exposure to light is a photosensitizer. Light from a laser, LED, or lamp, in the range of 600-700 nm, is usually used to activate the photosensitizer, for reasons to be discussed later.

Several diseases can be treated with PDT, such as Bowen's disease, Kaposi's sarcoma, squamous cell carcinoma, and macular degeneration. All of these diseases occur externally, so it is easy to use a laser or lamp to activate the photosensitizer at the site of affliction. However, light can also be channeled to an internal tumor via fiber optics, so PDT is a viable treatment for several internal diseases as well.

Unfortunately, there are several drawbacks to the use of photosensitizers, the most obvious being the excessive sensitivity to sunlight on the remainder of the non-infected

areas. Other problems include interactions of singlet oxygen with DNA and other biological tissues in the area of treatment, and the lack of penetration due to the absorption of light [7].

Light Sources

Common light sources are tunable dye lasers, LEDs, and lamps. Tunable lasers are desirable because their light can be focused and is, by definition, monochromatic. Argon, excimer, and copper vapor lasers are all commonly used in the visible red region between 630 and 690 nm. Lasers are used when the target area is highly specific, such as regions of the eye afflicted by macular degeneration. Also, laser light can be directed to internal tumors by fiber optics. LEDs can also be used to deliver light to tumors, as they can be attached to the end of a catheter, and snaked throughout the body. Mesothelioma, a cancer of the lungs due to asbestos inhalation, can be treated this way [4]. If the patient requires treatment over a broad area, a lamp would be used. Sources range from xenon arc lamps to a simple slide projector with a red filter attached [5]. Diseases, which cover a large area of the body, such as psoriasis, may be treated in this manner.

Mechanism of Action

Just as chlorophyll in plants utilizes energy from sunlight to produce sugar, photosensitizers utilize energy from light to produce toxic oxygen species. Singlet oxygen, produced by PDT, induces apoptosis in cells which have weak or no defense mechanisms, such as viral cells. The first step is reduction of the mitochondrial potential, followed by a drop in ATP level and a decrease in cell respiration. Finally DNA fragmentation, appearance of apoptotic bodies and eventually loss of plasma membrane

integrity lead to the death of a cell [3]. When the radical scavengers trolox or alpha-tocopherol succinate were present during irradiation, apoptosis was prevented, as was phototoxicity. Addition of either scavenger 60 minutes after irradiation provided only partial protection from apoptosis and phototoxicity; this protection was abolished if the addition was delayed for 100 minutes. These results are consistent with a model whereby long-persisting photoproducts continue the initiation of apoptosis for approximately 100 minutes after irradiation has ceased [4].

Singlet oxygen is not a radical, but is simply oxygen in an excited, more reactive state. It can undergo several oxidative reactions, such as the "ene-reaction", a Diels-Alder type addition, or addition to activated double bonds. Finally, in the presence of a reducing agent, an electron can be transferred to singlet oxygen, yielding superoxide [5]. All of these reactions contribute the toxicity of singlet oxygen to a non-mammalian cell, and its non-specific mechanism severely hinders the drug resistance.

Photosensitizers

The first photosensitizer was extracted from blood, using sulfuric acid and alcohol, by Meyer-Betz in 1913 [6]. He noticed severe phototoxicity upon self-injection of hematoporphyrin, which lasted over 2 months. Since then, hematoporphyrin derivatives have been created that decrease toxicity yet increase effectiveness. The first photosensitizing drug approved by the FDA, Photofrin, or porfimer sodium, is pictured below in Figure 1, along with other HPDs.

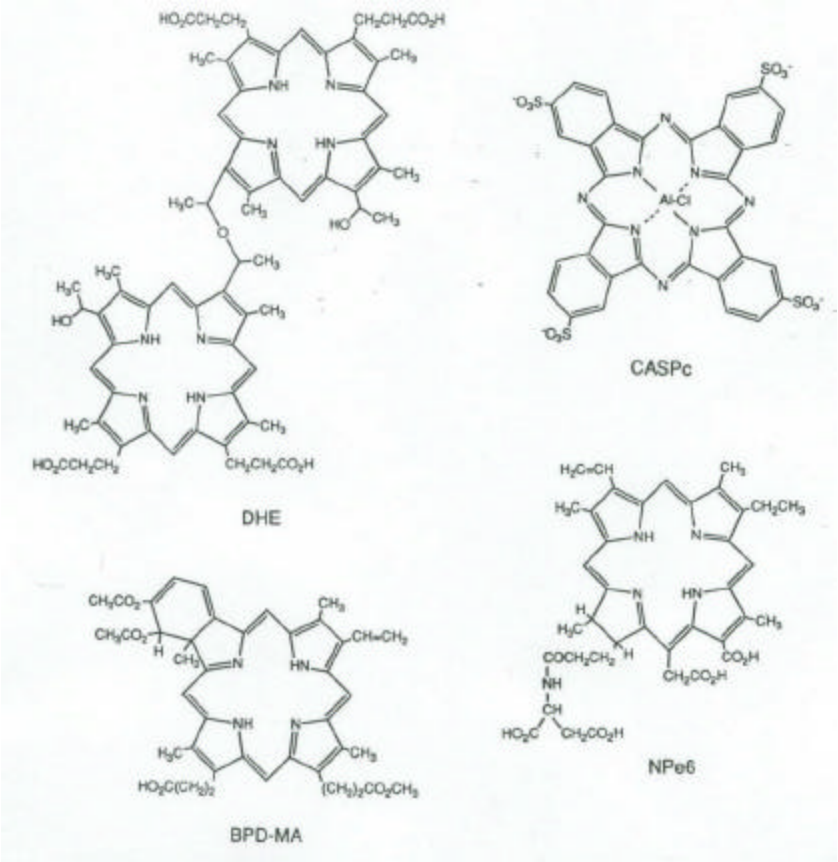


Figure 1- Structural formulas for tetrapyrrole photosensitizers. DHE is dihematoporphrin ether (a component of porfimer sodium), BPD-MA is benzopophyrin derivative monoacid ring A, CASPc is chloroaluminum sulfonated phthalocyanin, and NPe6 is mono-l-aspartyl chlorin e6. Adapted from [3].

The name porfimer is derived from the components of photofrin. The first part comes from the components, porphyrins. The "imer" ending denotes that it is a polymer, ranging from 2 to 8 subunits and linked by ether bonds.

The spectroscopy of HPDs is very interesting. HPDs have a molar absorptivity in the range of 2.2-5.0 $\times 10^4$ $M^{-1}cm^{-1}$ [10]. Near infrared phosphorescence has been used to determine the quantum yield of these molecules. The quantum yield is defined as the ratio of molecules that undergo the desired transition to the total number of molecules. Initially, HPDs had a quantum yield of 15-20%, but current treatments are as high as 36% [10]. Studies are currently running to find HPDs with the highest possible quantum yield, and researchers have succeeded in breaking the 40% barrier. However, these drugs have not yet been approved for treatment by the FDA [11].

A UV/Vis spectra of several HPDs is shown below in Figure 2. HPDs have a molar absorptivity in the range of $2.2\text{-}5.0 \times 10^4$ $M^{-1}cm^{-1}$ [10].

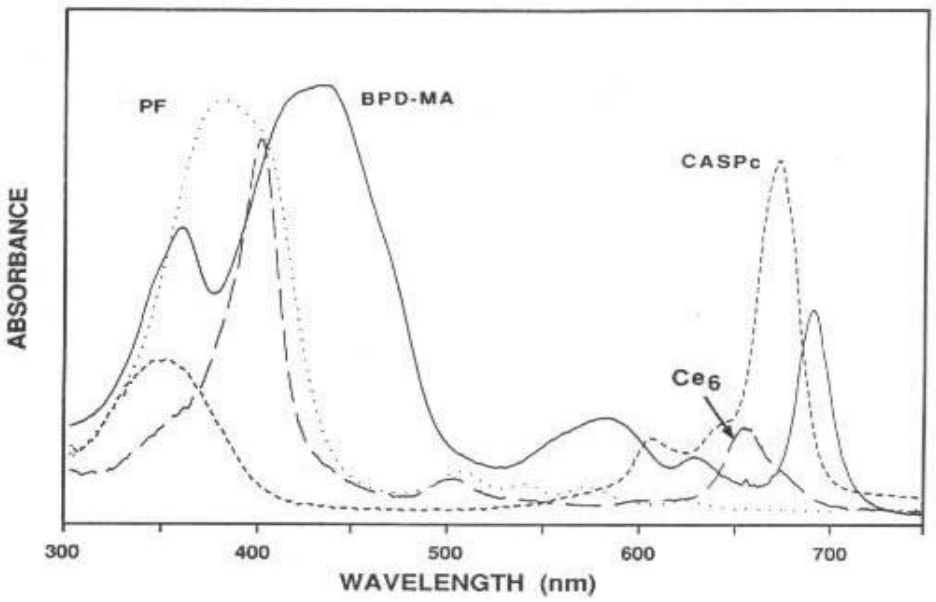
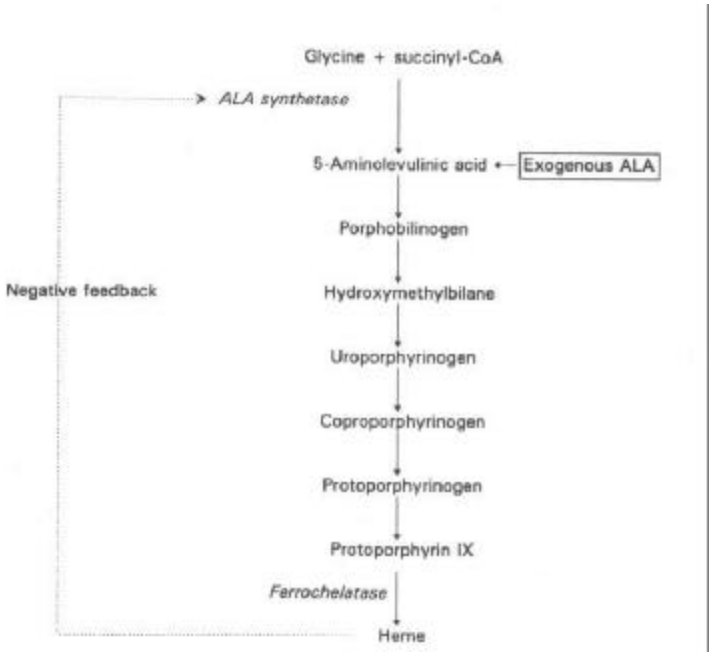


Figure 2- UV/Vis spectra of several HPDs. PF is Photofrin, or porfimer sodium. The absorption near 400 nm is due to the heme-like nature of these molecules. They are activated by light near 620 nm, so the quantum yield of PF is extremely low compared to other photosensitizers. Adapted from [3].

Not all photosensitizers are injected intact. 5-aminolevulenic acid can be given topically, and the body will convert it to protoporphyrin IX, a potent photosensitizer. The mechanism is shown below, in Scheme 1.



Scheme 1- Conversion of ALA to protoporphyrin IX, a photosensitizer. Adapted from [8].

As seen in Scheme 1, heme causes negative feedback on ALA synthetase. This system is circumvented by adding ALA after the inhibition step, so heme builds up but production is not stopped, as it normally would be. High levels of heme generate toxic radicals and other activated species which cause a general breakdown in the membranes of non-mammalian cells.

Protoporphyrin IX absorbs at 2 bands in the visible red range, 630 and 675 nm, so a broadband source such as a lamp is utilized. Unlike other photosensitizers, ALA doesn't cause long-term systemic problems. Another advantage is that treatment can be administered in one day, since the body absorbs and converts ALA quickly [2]. Other photosensitizers require multiple trips for treatment because of the long time necessary for systemic distribution.

Macular degeneration is a disease of the eye that occurs as people age. It was untreatable until Visudyne™ was approved by the FDA. Visudyne™ is the trade name for a benzoporphyrin derivative, verteporphyrin. It can be administered by injection or by eye drops. A cool laser, such as an excimer laser, which scans the eye and selectively targets the affected regions, activates it. Macular degeneration can be effectively treated by this method, a feat thought impossible just 5 years ago.

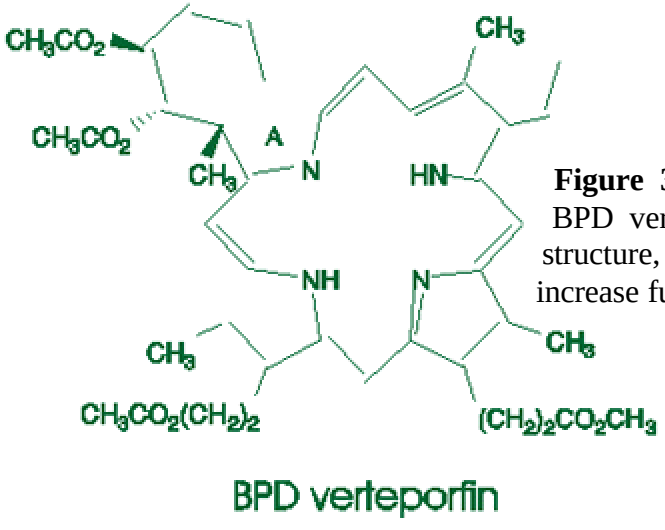


Figure 3- The structure of Visudyne™, or BPD verteporphyrin. It has a typical heme structure, with added sidegroups, which increase functionality. Adapted from [9].

There are downsides to this treatment though. Visudyne™ has the shortest lifetime of all photosensitizers, and patients who receive it still must wait 3-4 days before being exposed to sunlight. This is a significant improvement from the 2 months of sensitivity experienced by Meyer-Betz in 1913, but even shorter recovery times are desirable. Photobleaching is another shortcoming of photosensitizers. Photobleaching occurs when regular light stimulates the photosensitizer, and it expends its oxygen activating capabilities. The resulting singlet oxygen causes little damage to a mammalian cell, which is well prepared for oxidizing reactions, but photobleaching reduces the amount treatment available to the invading cell.

Conclusion

Photodynamic therapy is a relatively new, fast growing treatment for a variety of diseases. The singlet oxygen molecules generated are an effective treatment against many types of invading cells because they breakdown several types of membranes and essential tissues. Mammalian cells have defense mechanisms set up to combat this type of oxidative stress, so only the unwanted cells are treated. Various types of light sources can reach to all parts of the body for treatment, and assorted HPDs are effective for treating different diseases.

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