

# Photodynamic Therapy (PDT)

## 3. New Approaches and Newer Applications

*Bhaskar G Maiya, G Hariprasad and L Giribabu*



**Bhaskar G Maiya** is in the School of Chemistry, Central University, Hyderabad.



**G Hariprasad** is a former graduate student of the Central University, and is currently pursuing his post-doctoral research work at the US Department of Veteran Affairs Medical Center, Columbia, MO (USA).



**L Giribabu** is presently a Senior Research Fellow in the School of Chemistry, Central University, Hyderabad.

Application of photophysical principles to problems at the interface of chemistry and biology is a unifying research theme of these authors.

In this concluding article, three emerging aspects related to PDT are presented: (i) new approaches to PDT of cancer, (ii) PDT diagnostics, and (iii) new clinical applications.

### Introduction

In Part 1 of this series on photodynamic therapy (PDT)<sup>1</sup>, we discussed salient features of this new modality against cancer with special emphasis on the physical and biochemical principles involved. The subsequent article was devoted to the design and clinical applications of porphyrinic and non-porphyrinic photosensitizers for PDT. In recent years, several new approaches to PDT, new developments in PDT diagnostics and new clinical applications (other than cancer management) of PDT have emerged. The present article provides an overview of these new aspects related to PDT.

### New Approaches to PDT

Initially, we defined PDT as an anticancer modality in which an intravenously injected photosensitizer, after accumulation in the tumor, is irradiated by light. However, recently it has been discovered that it is possible to generate the photosensitizer inside the tumor by the topical or even oral application of the so-called 'pre-photosensitizer'. In addition, hybrid molecules in which a photosensitizer is covalently linked to a chemotherapeutic drug are being developed for 'combination therapy' (i.e., chemo + photo) against cancer. These two developments have taken place very recently and are discussed here.

### PDT with ALA

A fascinating new approach to PDT is concerned with the

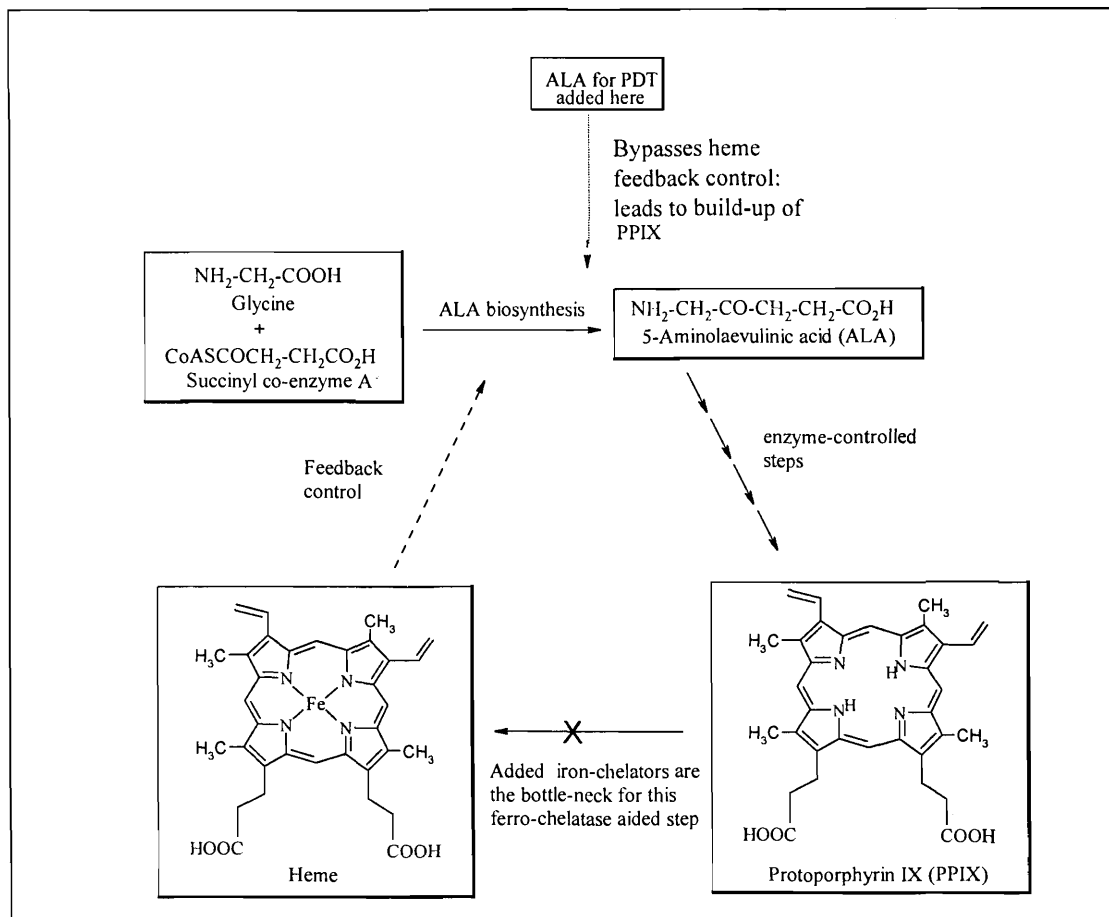
<sup>1</sup> Previous articles in this series are:

1. Basic Principles, *Resonance*, Vol. 5, No.4, p. 6, 2000.

2. Old and New Photosensitizers, *Resonance*, Vol. 5, No.6, p.15, 2000.

**Figure 1. Mechanism of protoporphyrin IX build-up after administering ALA.**

application of 5-aminolaevulinic acid or ALA. ALA itself is not a photosensitizer, but it is a key metabolic precursor in the biosynthesis of the body's own naturally occurring, iron containing porphyrin, heme (see *Figure 1*). In normal cells, ALA synthesis is under tight feedback control by the intracellular levels of heme produced, which inhibits ALA synthetase, the enzyme responsible for the biosynthesis of ALA. However, if extra ALA is provided, together with iron chelators to inhibit the action of another enzyme called ferrochelatase (which is capable of complexing with iron), this bypasses the heme feedback control mechanism. This results in the accumulation of intracellular protoporphyrin IX (PPIX). This non-metallated precursor of heme (and remember, not heme itself!) is a power-



ful photosensitizing agent that can be detected by its characteristic fluorescence. Interestingly, using either oral or intravenous (IV) administration of ALA, it has been found that PPIX fluorescence levels are higher in malignant cells than in control healthy cells. Thus, ALA, by producing intracellular PPIX, may also be used in the fluorescence detection of cancers that might otherwise be missed (or detected via more involved magnetic resonance imaging (MRI techniques). Interestingly, ALA can also be applied topically to treat a range of malignant dermatological conditions. Thus, the poor localization of PPIX can be overcome when it can be made *in situ* using ALA. Peak PPIX levels are reached several hours after ALA is administered, compared to up to 96 hrs for tumor localization of a sensitizer like HpD. Also, because PPIX clears from the body within 24 hrs (presumably, via the normal heme catabolic pathway), there is less risk of photosensitivity.

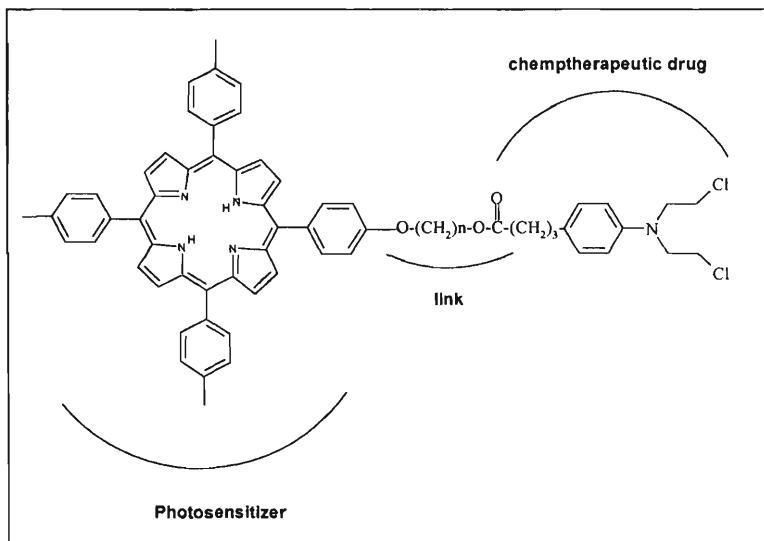
Porphyrin-chemotherapeutic drug conjugates permit the chemotherapeutic-drug-mediated conventional therapy to be carried on both in the presence ('light-on') and absence ('light-off') of light.

### Chemo- + Photo-therapy

A new breed of porphyrin compounds are being explored for 'combination therapy' against cancer. In this approach, clinically used anti-cancer drugs are crafted onto the porphyrin photosensitizers and the resulting 'combination drugs' are expected to be beneficial in the co-lateral application of chemo- and photo-therapy. Such 'porphyrin-chemotherapeutic drug' conjugates permit the chemotherapeutic-drug-mediated conventional therapy to be carried on both in the presence ('light-on') and absence ('light-off') of light. They also enable the photodynamic therapeutic condition to prevail upon irradiation into the porphyrin absorption bands with the visible light. Synergistic effects towards tumor eradication are thus expected to predominate during the irradiation period. This has been referred to as the 'double war-head approach'. In addition, these new hybrids can also refine the ability of the porphyrin to home in on the cancerous cell/tissue by virtue of its conjugation to the chemotherapeutic drug, which would usually possess an inherent ability to localize in the cancerous tissue. These concepts have been realized recently and a few examples of 'porphyrin-



**Figure 2.** The 'double war-head approach'. Combination drugs wherein a PDT agent (porphyrin photosensitizer) is covalently linked to a chemotherapeutic drug (chlorambucil).



**Box 1. Tissue Auto-Fluorescence in Tumor Diagnostics**

Discrimination between the normal and cancerous tissue can be achieved by using the tissue auto-fluorescence itself. This method relies on the differences in the fluorescence spectral profiles of normal and cancerous tissues originating probably, due to the differential enzymatic activity in the corresponding cell. Some enzymes contain fluorophores like flavins, tryptophan, etc. However, this method uses the undesirable UV-light and generally has limitations when it comes to the current clinical scenario.

chemotherapeutic drug' conjugates are shown in *Figure 2*. As seen in these new hybrids, a porphyrin is linked to a chemotherapeutic drug (eg. chlorambucil – a DNA cross-linking 'mustard', currently marketed as an anti-leukemic drug).

**PDT Diagnostics**

To be effective, PDT relies on the clinician having an accurate knowledge of light dose, tissue optics and sensitizer levels. Measuring fluorescence from the sensitizer is a good way to assess all these parameters. It is best achieved by exciting long wavelength (visible region) absorption bands. This significantly reduces tissue auto-fluorescence (*Box 1*).

Fluorescence can be used for macroscopic detection of sensitizers in tissues using fiber optic probes, and for the microscopic studies of photosensitizer biodistributions. Researchers have developed a quantitative fluorescence microscopy imaging system, which uses a highly sensitive digital camera. This is capable of observing fluorescence from a sensitizer, eg., a water-soluble sulfonated aluminum phthalocyanine, in tissue sections (useful for monitoring sensitizer biodegradation) and individual cells (for investigating sensitizer pharmacokinetics). Recently, it has proved possible to quantify the sensitizer levels in biopsies

taken from patients. Clinicians are therefore now able to study how a patient's sensitizer distribution evolves on a macroscopic level, which is vital for tracking the mechanism of PDT.

Fluorescence is also being used to monitor sensitizer photodegradation, or photobleaching. There are several mechanisms by which photobleaching occurs. First, the singlet oxygen ( $^1\text{O}_2$ ) produced oxidises the sensitizer to a non-fluorescent photoproduct (this could be an advantage too, see *Box 2* for details). Secondly, one of the sensitizer excited states, in a type I mechanism can attack various tissue components to form nonfluorescent ionic or radical products. Both routes lead to the loss of sensitizer fluorescence, which is easily detected. Because the amount of photodegradation should correlate with the amount of light absorbed by the sensitizer, measuring photodegradation by using fluorescence may provide a way to monitor treatment in real time.

Using fluorescence techniques to exploit sensitizer retention by tumors, however, had mixed results. Nevertheless, *in vivo* fluorimetry is proving much more promising. This is where ALA is used to induce PPIX production. Malignant cells are observed to have higher rates of porphyrin synthesis, so that feeding them with the porphyrin precursor ALA produces more fluorescence *in situ* than the surrounding normal cells. This has recently been used successfully to tumor diagnostics, giving superior results compared to conventional visual inspection.

### New Applications of PDT

The cancerous tumors in which photosensitizers tend to concentrate are characterized by a lot of cell multiplication (hyperproliferation) and growth of new blood vessels (neovasculature). Thus photodynamic therapy could be useful for other diseases with the same indications. In fact, there exist several diseases that are characterized by hyperproliferation and neovasculature. Among them, the most important one is atherosclerosis. Here, plaque builds up along the interior walls of arteries, decreases

#### Box 2. Turning a Handicap into an Advantage!

Photobleaching results in the loss of absorbance due to the photosensitizer. Since with irradiation there will be a progressive loss of sensitizer, this process can modify the reciprocity between photosensitizer level and light. Photobleaching therefore places a limit on the total amount of phototoxicity that can be produced by a given level of photosensitizer for a given light dose. While this is a problem, it can also be an advantage. Photosensitizer near the surface of the tissue will absorb light and decrease the illumination at a depth. As photosensitizer near the surface bleaches, more light will penetrate deeper into the tissue. Not an unwanted situation after all, as far as the real therapy is concerned, if not the photo-diagnostics!

Pharmacyclics' letetium texaphyrin is known to strongly bind to atherosclerotic plaque and is currently used for photo-angioplasty – that is, photochemically induced removal of plasty fatty deposits called plaque that accumulate on arterial walls.

the flow of blood and causes hardness of arteries. As observed with malignant cells, photosensitizers preferentially accumulate in atherosclerotic plaque rather than in the surrounding healthy cells. The reason for this is not yet clear but, recall that the way photosensitizers accumulate in cancerous tissue is due to their affinity towards low density lipoproteins (LDLs). Indeed, any rapidly dividing cell, healthy or unhealthy, has more receptors for LDLs than do quiescent cells. Thus, the photosensitizer can piggyback onto the LDLs, and the LDL and its passenger are picked up by a receptor. This process accounts for the preferential accumulation of photosensitizers in rapidly dividing cells, such as endothelial cells in new blood vessels and the LDL-loving macrophages present in atherosclerotic plaque. Pharmacyclics' letetium texaphyrin is known to strongly bind to atherosclerotic plaque and is currently used for photo-angioplasty – that is, photochemically induced removal of plasty fatty deposits called plaque that accumulate on arterial walls. Preclinical studies show that atherosclerotic rabbits retain lutetium texaphyrin 16-34 times more in plaque than in arterial walls. After treatment with light, up to 80% of the plaque is removed without damage to surrounding normal arterial walls.

Besides atherosclerosis, another exciting potential noncancerous application of PDT is in the treatment of eye diseases, particularly macular degradation. People with this condition (usually aged) lose their ability to see directly ahead! As the disease progresses, it causes blurred vision and eventually blindness. This is caused by abnormal growth of blood vessels in the area of macula, which is the central portion of the retina directly opposite the lens. The new blood vessels are very fragile and very leaky. Underneath the retina, they start to bulge, leak blood and fluid, and lift the retina off its membrane (it is the 'wet' variety). The current therapy (a crude one!) involves use of a 'hot' laser to seal the new blood vessels. Of late, photodynamic therapy has come to the rescue and a couple of new drugs are about to enter the market (eg. Verteporfin, SnET2, lutetium texaphyrin). A typical PDT treatment requires IV administra-



tion of the photosensitizer. A few minutes later, the drug is activated with light, producing cytotoxic oxygen species including  $^1\text{O}_2$ . These reactive species attack proliferating endothelial and red blood cells, triggering thrombosis, or formation of a blood clot. By producing this blood clot locally, the leaky membrane is dried thus enabling the retina to sit again on the membrane. The vision is back within minutes! Indeed, clinical trials with SnET2 have shown that some patients with a visual acuity of 20/200 improved to about 20/100 within a week after the treatment (Normal vision is 20/20 and people with visual acuity of 20/200 are officially blind!).

In addition to its use as a photosensitizer in red-light induced anti-cancer treatment, there is an interesting blue-light induced dermatological application of ALA. Recall that when administered topically, ALA localizes in epidermis (less than 2 mm depth) in the skin and generates PPIX. Because ALA is localized in epidermis, and not buried deep in some internal organ, the depth of penetration of light is not an issue. This has allowed a Canadian pharmaceutical company to develop an inexpensive illuminating device based on blue light for treatment against sun induced precancerous skin lesions called actinic keratoses with ALA. Two other dermatological applications are also in the offing: treatment of acne and hair removal. Blue light is used in these cases because it is 20 times more efficient than the red light in producing  $^1\text{O}_2$  with PPIX and is also less expensive to produce.

Thus, in a short period, PDT has progressed from oncology to cardiology, ophthalmology and dermatology. Applications in urology (enlarged prostate and prostate cancer) and gynecology (dysfunctional uterine bleeding) are also being examined. Researchers are now beginning to work on making the PDT agents select other targets such as bacteria. It is interesting that in this context, PDT can have an impact on infectious diseases if the photosensitizers can work preferentially against bacteria rather than mammalian cells. With the knowledge that certain bacterial cells have a high degree of negative charge, several

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## Suggested Reading

- [1] W M Sharman, C M Allen, J E van Lier, *Drug Discovery Today*, 4, 507, 1999.
- [2] A M Rouhi, *Chem. Eng. News*, 22 November 1998.
- [3] L Milgrom, S MacRobert, *Chem. Britain*, 45, May 1998.
- [4] F Thomas, *Scrip Magazine*, 42, May 1997.
- [5] G Mehta, S Muthusamy, B G Maiya, S Arounaguiri, *J. Chem. Soc. Perkin Trans. 1*, 2177, 1999.
- [6] B G Maiya, *Curr. Sci.*, 72, 808, (and references therein) 1997.

laboratories are using photosensitizer mixed with polycations to help penetrate the cell wall barrier. Recent results in this direction have shown that photosensitizers covalently linked to polylysine (positively charged amino acid chain) are taken up in large amounts within minutes by bacteria compared with hours by mammalian cells. Thus, in principle it will be possible to expose light and kill only bacteria and not the host cells. Therefore, PDT appears attractive for wound sterilization, dental care, etc., especially since it is a localized therapy.

Another upcoming use where PDT can be effective is in blood bank applications. It is based on the knowledge that several photosensitizers are able to preferentially bind to certain receptor sites. These can include those present in lymphocytes the excessive accumulation of which leads to leukemia and to enveloped viruses such as HIV that are responsible for the acquired immuno deficiency syndrome (AIDS). Unfortunately, most of the information on this important application is patented because of its obvious 'market value'. The principles of PDT in such blood banking applications are illustrated in *Box 3*.

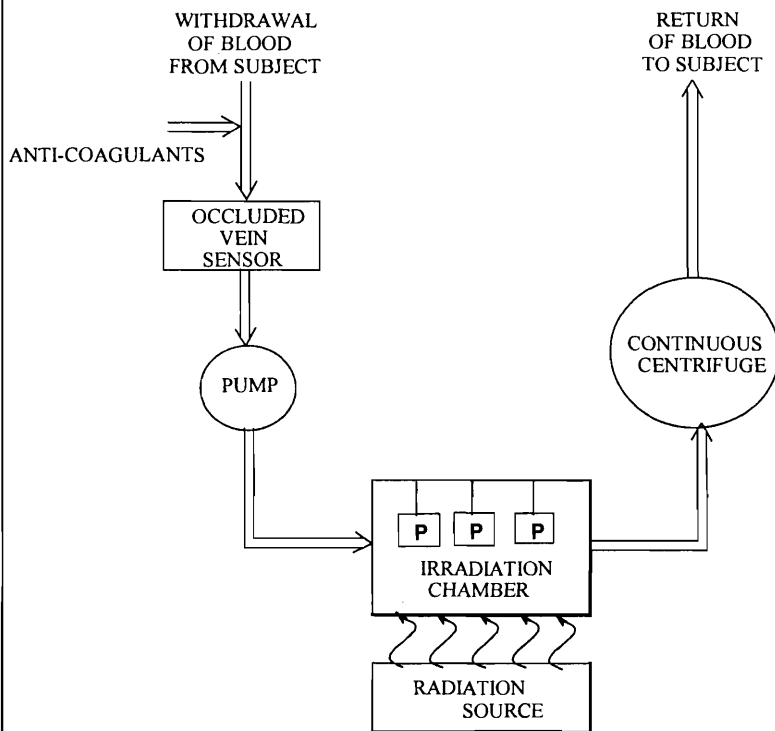
## Conclusions

In the new millennium, the need for new protocols for the treatment of cancer and other diseases is expected to become acute. With established therapies operating close to optimal levels, new therapies that can effectively combat cancer and other diseases are certainly necessary. PDT, as explained in this series of articles, is essentially a very simple concept that still offers possibility of being an effective and specific method of destroying malignant, premalignant and other benign tissues while sparing the surrounding healthy cells. Initial studies have shown that PDT is effective against cancer and other diseases. With the acceptance of the first generation photosensitizer Photofrin II<sup>®</sup> into the clinical world, a great variety of second generation photosensitizers are being tested against various pathogenic states. PDT could therefore be an important treatment of the future.





Box 3. Blood Banking Applications of PDT



*This new blood purification protocol involves 'on-line' irradiation of the contaminated (with lymphocytes, HIV, etc.) blood with visible light in the presence of a photosensitizer, P. The photosensitizer can either be impregnated on a solid support as shown or be injected directly into the blood stream flowing inside the irradiation chamber. The former method is preferred as it alleviates the problems associated with removal of the photosensitizer from the purified blood before its re-entry into the patient/storage bottle. The method is being tried out in applications such as bone-marrow purging, AIDS management, etc. Note that the*

*technology's application is not limited to blood alone. An imaginative use of it can as well be extended to purify other liquids infected by bacteria/virus; for example – water! Thus, an optimistic reader may visualize the neighborhood corner shops to soon display, inside a cool 'light-chamber', stacks of mineral water bottles that sport a tiny pink solid piece of polymer-bound porphyrin!!*

Notwithstanding the above optimism, a word of caution is justified. Although, PDT appears to be on the verge of becoming a mainstream treatment for various diseases and is reasonably risk free so far, its long term effects have not been looked at yet. Indeed, PDT has not really been around that long and hence its adverse effects after years of treatment are unknown. In addition, most current protocols of PDT are single treatments. What if it becomes more popular and clinicians start treating patients multiple times? Will resistance to the treatment develop? There are many such questions that are not answered so far. To sum up, it's not a fairy tale!

*Address for Correspondence*  
 Bhaskar G Maiya  
 School of Chemistry  
 University of Hyderabad  
 Hyderabad 500 046, India.  
 Email: bgmsc@uohyd.ernet.in