academicJournals

Vol. 12(23), pp. 3590-3599, 5 June, 2013
DOI: 10.5897/AJB12.2586
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Review

Photodynamic therapy: A new light for the developing world

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Accepted 30 April, 2013

In one article, photodynamic therapy (PDT) was commended as the most suitable method for cancer therapy in the Developing World. PDT is cost effective and simple to use. Unlike chemotherapy, no special training is required for nurses, and no post treatment course in intensive care. No engineer, computerized dosimetry computations, or additional costs for isotope re-treatment are required, as in radiotherapy. There are no blood transfusions, or sophisticated operating theatres, as in surgery. Ironically, it is in the developing world that there appears to be very little awareness of and practice of PDT. Cancer sufferers are thus limited to chemotherapy, radiotherapy and surgery procedures that are relatively complex and costly, without distinctive advantage in cure or palliation. Is it possible that the low level of clinical practice in PDT in the developing world is related to the low level of articulation of what is admittedly a relatively new modality? However, this slow emergence of clinical practice in PDT when compared with advances in its developmental research was also observed in the developed world in the last two centuries or so. The purpose of this article was to advance the articulation of PDT, primarily among basic science researchers, clinicians and clinical scientists in the developing countries. It is also to advance the emerging new frontiers of the clinical applicability of the processes of photodynamic reactions in the fight against infectious disease epidemics, which are a more common occurrence in the developing world countries.

Key words: Photodynamic therapy, developing world, photosensitizer, bacterial infection.

INTRODUCTION

Photodynamic therapy (PDT) has been defined as the combined effect of a photosensitizing drug and light to produce biological damage of therapeutic value, under conditions where either the light or the drug operating alone have no effect (Bonnett and Berenbaum, 1989a). The procedure involves administration of the photo-sensitizing drug followed by irradiation at a wavelength corresponding to an absorbance band of the sensitizer. In the presence of oxygen, a series of events lead to direct tumor cell death, damage to the microvasculature, and induction of a local inflammatory reaction (Agostinis et al., 2011). Clinical studies revealed that PDT can be curative, particularly in early stage tumors. The approach has aroused considerable interest in scientific, medical and commercial circles in recent times, partly because it is conceptually very exciting, but also because there is no doubt that it works in destroying malignant or otherwise problematic tissue. The basis for PDT lies with two remarkable properties of the photosensitizing drugs that are used in the approach-their preferential accumula-

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tion in cancer tissue and the subsequent light triggered toxicity of the drugs (Henderson and Dougherty, 1992). In the last two decades, exponentially increasing basic research has appeared in the literature in the applications of photodynamic reactions against various diseases caused by bacterial colonization (Schastak et al., 2010; Dai et al., 2009), fungal infection (Lyon et al., 2011) and parasites (Barbosa, 2012), that are faced by many clinics in developing world countries, as well as those caused by entomological vermin (Amor and Jori, 2000, 2001). These have expanded the applicability of the photodynamic reaction beyond its initial focus on cancerous conditions based on destroying malignant or otherwise problematic tissue.The patent issued in 1992 for the photodynamic inactivation of viruses in blood cell-containing compositions (Horowitz et al., 1992), appears to have triggered more than seventy patents in this and related areas since then.

HOW DOES PDT WORK IN DESTROYING CANCER?

In the systemic approach, often used for deep-seated tumours, the patient is injected intravenously with a suitable preparation of the drug and confined to the dark. A period of time is allowed for the drug to accumulate preferentially in cancer tissue. The period varies from drug to drug, from as little as 3 h, to more than 72 h. After this period, most of the drug is in the cancer tissue. The differential in drug concentration between cancer tissue and the rest of the body varies from drug to drug, from as low as 6:1 to more than 20:1. A suitable dose of light is then administered. This presents little challenge for external cancers. For internal ones, optic fibre light delivery and guidance devices are often used. Drug rich tissue is destroyed immediately. Cancer tissue destruction persists without affecting drug deficient tissue. When the drug is completely cleared from the body the patient is allowed back into normal light environments (Okunaka et al., 1995; Serebrovskaya et al., 2009). For topical cancers such as skin cancers, topical ALA-PDT is more appropriate. Dead skin around the affected area is removed with an abbraisive tool. A cream formulation of ALA is applied, followed, after a suitable period of 6 to 24 h, by application of laser light. Necrosis of cancerous tissue starts immediately (Monfrecola et al., 2009).

Cancer tissue destruction is achieved because the drug absorbs the energy of light, generating highly reactive species, which cause biological damage (Neely et al., 1988; Hartley et al., 1988). The extremely short lifetime of singlet oxygen within the biological environment means that the treatment is highly localized, without systemic side effects. For this reason, PDT can be especially effective in disease sites close to vital organs, such as in cancers of the head, neck and the brain (Brown, 1999; Biel, 2007; Wilson and Patterson, 2008). Although necrosis has been recognized as the major photodynamic response, apoptosis has been described in PDT (Oleinick, 1998; Oleinick et al., 2002; Mroz et al., 2011).

THE MECHANISM OF PDT

Although tissue damage resulting from porphyrin photosensitised photodynamic action is generally regarded as arising from a number of different mechanisms, the major pathway is the singlet oxygen also referred to as the type II mechanism (McRobert et al., 1989; Mroz et al, 2010; Mroz et al, 2011) (Figure 1). The mechanism involves photo-excitation of the photosensitizer from its singlet ground state to its first singlet excited state, followed by intersystem crossing to the triplet excited state, whereupon it photosensitizes triplet ground state oxygen to give singlet excited state oxygen, a highly reactive species, which has been reported as the cause of chemical degradation for a variety of biological systems (Neely et al., 1988; Rezka, 1990; Mroz et al., 2011). This is illustrated in the Jablonski diagram of Figure 2, and in the mechanistic reaction equations 5 to 7. Some of the targets of singlet oxygen, for example, unsaturated lipids, cholesterol and certain amino acid side chains in proteins are important membrane components. It is thought that photochemical changes in these components are an important cause of tissue damage (Candide et al., 1998; Yanina et al., 2012). Some of the reported consequences of this cytotoxic action include, for example, inhibition of membrane bound enzymes, and interference with membrane transport resulting in impairment of the vital membrane permeability control barriers (Rezka et al., 1990; Goes et al., 1998; Hsieh et al., 2003; Weyergang et al., 2008).

Type I mechanism

$$^1P \xrightarrow{h\upsilon} {}^1P^* \text{ (short lived)} \quad (1)$$

$$^1P^* \longrightarrow {}^3P^* \text{ (longer life times)} \quad (2)$$

$$^3P^* + LH \longrightarrow P^{\cdot-} + L^{\cdot} + H^+ \quad (3)$$

$$P^{\cdot-} + O_2 \longrightarrow O_2^{\cdot-} {}^1P \quad (4)$$

Type II mechanism

$$^1P \xrightarrow{h\upsilon} {}^1P^* \text{ (short lived)} \quad (5)$$

$$^1P^* \xrightarrow{ISC} {}^3P^* \text{ (longer life times)} \quad (6)$$

$$^3P^* + {}^3O_2 \longrightarrow {}^1P + {}^1O_2^* \quad (7)$$

Where, P = photosensitiser, LH = lipid molecule and * in

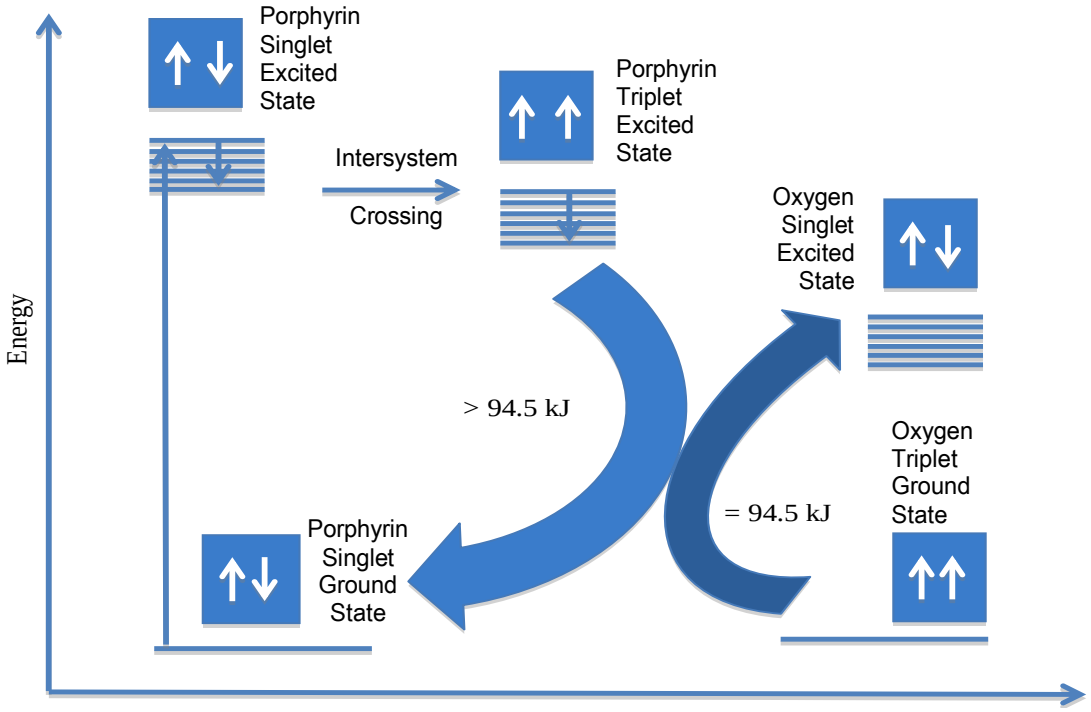

Figure 1. Jablonski diagram showing the formation of singlet oxygen via a porphyrin triplet state with energy greater that the oxygen singlet state of 94.5 kJ. White arrows represent electron pairs, parallel arrows represent triplet states while antiparallel ones represent singlet states. The two large antiparallel arrows represent the photosensitization of triplet state oxygen by the triplet state photosensitizer.

indicates excited states. Scheme 1 shows the two major mechanisms for photodynamic reactions, type I and II.

In the radical mediated mechanism also referred to as type I, the photosensitizer is excited to the triplet state as in the first step of the singlet oxygen mechanism, whereupon it interacts directly with biomolecules causing irreversible chemical changes in them. The triplet state photosensitizer, for example, may remove an electron from a lipid molecule in cell membranes giving a lipid cationic radical and a photosensitizer anionic radical (Girotti, 2001). The anionic photosensitizer radical may then interact with oxygen to produce a superoxide radical and a singlet state photosensitizer.

The cationic lipid radical may lose a proton to give a neutral radical. Such lipid molecule radicals may eventually react with a variety of protein molecules embedded in the membranes. The superoxide radical can generate such species as hydrogen peroxide. These two mechanisms are referred to as type I and II (Singh et al., 1992; Feix et al., 1991; Feix and Kalyanaraman, 1991; Mroz et al., 2010, 2011).

PDT DRUGS IN USE

The majority of PDT drugs belong to the unique family of macrocyclicporphyrin, and phthalocyanine type of compounds (Kessel, 1995; Oleinick, 2001). A number of drugs have been licensed for use in PDT (Dougherty, 2000; Oleinick, 2001). Photofrin currently enjoys the widest approval, in a number of countries, including Canada, Denmark, France, Finland, Germany, Iceland, Italy, Japan, Netherlands, United Kingdom and the USA, for a variety of cancers, including lung, oesophageal, bladder, gastric, cervical, skin and brain cancer (Brown, 1996, 1999). Photofrin belongs to the first generation of PDT drugs in that it is a mixture of a number of porphyrin compounds that are ether and ester linked oligomers of haematoporphyrin, its composition is not stable and may vary from production batch to production batch (Singh et al., 1992).

Second generation drugs now in use

Second generation drugs now in use, pre-clinical and clinical trials include Foscan from Scotia (Bonnett and Djelal, 1993), Visudyne from QLT (Levy et al., 1994), Lutex from Pharmacyclics (Woodburn et al., 1998), Pc4 from Case Western Reserve (Ahmad et al., 1998), Purlytin from Miravant (Kaplan et al., 1998), NPe6 from Nippon (Taber et al., 1998), HPPH from Roswell Park Cancer Institute (Dougherty, 1998) and amino laevulinic acid from DUSA (Marcus, 1996). A number of second generation drugs are under development including boronated porphyrins, which are designed for use in boron

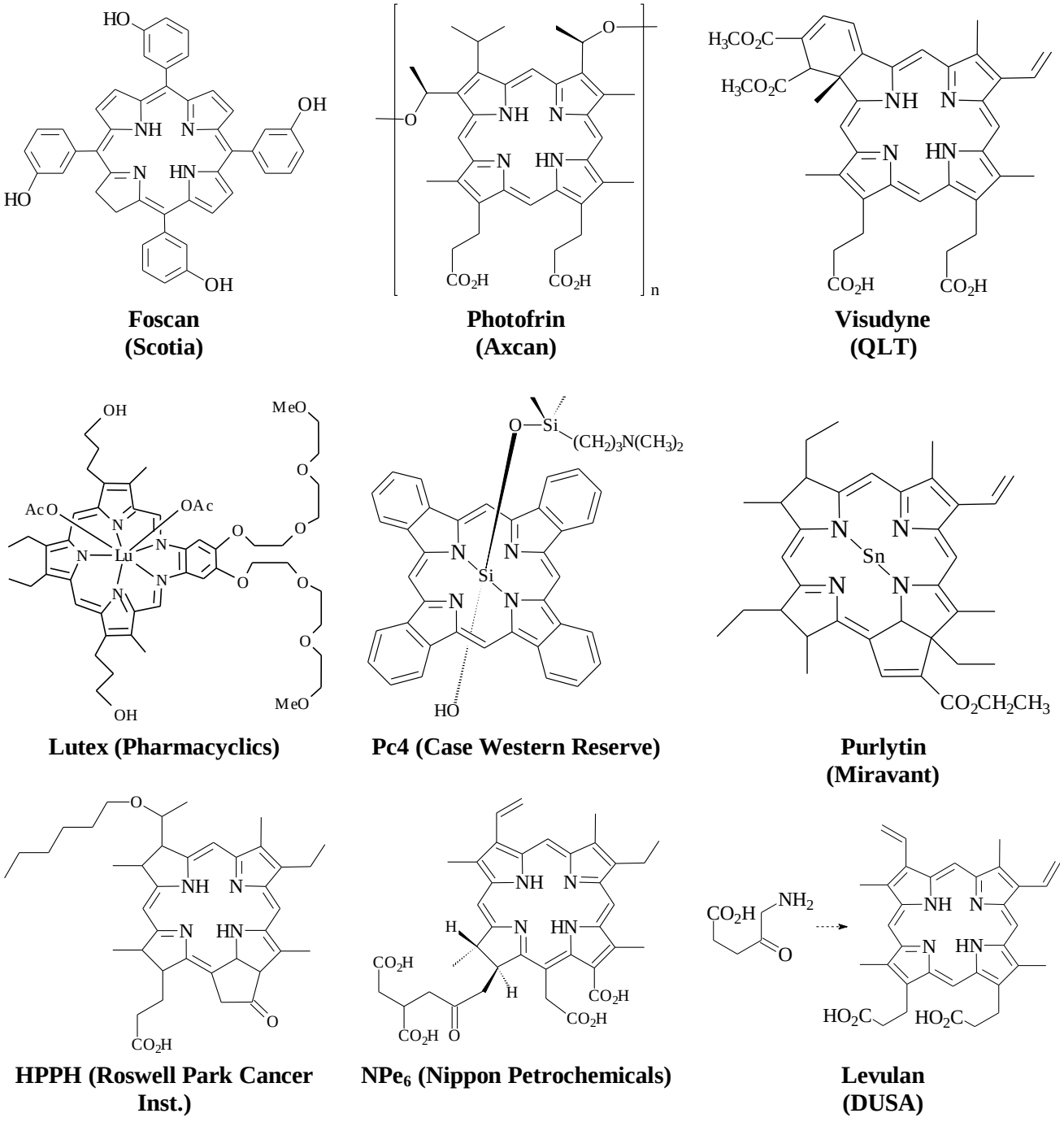

Figure 2. Structures of the leading second generation drugs now in use (the suppliers are indicated in brackets).

neutron capture therapy (Hill et al., 1995).

Some of the desirable features of second generation drugs

Some of the desirable features of second generation drugs include lack of toxicity in the dark, selective uptake by cancer tissue and short-term retention to avoid the persistence of photosensitivity. Other important features include triplet state energy greater than 94 kJ/mol, intermediate lipid water partition coefficient (Engelmann, 2007; Mizuno et al., 2011), pure single chemical compounds, and light absorption in the red or far red part of the visible spectrum. The last feature derives from the high depth of penetration of tissue by radiation in the red region of the light spectrum and lower energy, and it is being used as a design feature for third generation drugs

(Bonnett and Berenbaum, 1989b; Kinsella, 2001).

Third generation photosensitisers are in experimental development

Third generation photosensitisers are in experimental stages. These have two chromophores; one chromophore rapidly picks up two photons in the infrared region of the light spectrum and converts them into a single photon. This photon is then absorbed by the sensitizer's second porphyrinic chromophore. Such sensitisers are expected to be able to generate singlet oxygen from the triplet state of the porphyrinic chromophore of the photosensitizer, even though infrared excitation is being used. Experiments on this work started in the early nineties (Sessler et al., 1993; Wang, 2004; Josefsen and Boyle, 2008).

PDT VARIATIONS

A number of variations of PDT have been reported, including normal PDT, ALA-PDT, and radiation sensitization. Normal PDT has already been described. In ALA-PDT (Pollock et al., 2004; Wiegel and Wulf, 2006; Gaál et al., 2012) amino laevulinic acid (ALA) is administered either orally or topically. The high concentration levels of ALA disrupt the feedback controlled haeme biosynthesis and this leads to an abnormally high, and phototoxic level of protopophyrin IX, in excess of the enzymatic rate of chelation with Fe^{2+}. Irradiation in the presence of this high level of protopophyrin IX in cancer tissue with suitable light leads to the normal PDT response (Kennedy and Pottier, 1992; Marcus, 1996; Van den Berg et al., 1999). In radiation sensitization (Franken et al., 2012), the treatment of cancer with boron containing porphyrin or phthalocyanine drugs based on the reaction of the stable ^{10}B isotope with slow neutrons, which generates an ionizing fluence of high energyα-particles together with the recoil ^{7}Li particles, has been demonstrated. This type of radiation sensitization is known as boron neutron capture therapy (BNCT) (Barth et al., 1990). Recently BNCT was shown to be an effective alternative treatment modality for patients with glioma (Wang et al., 2010).

The scope of PDT has been extended beyond cancer therapy to include viral infections (Levy and Obochi, 1996), bacterial infections (Jori and Tonlorenzi, 1999) and other conditions (Hunt et al., 1998). The application of PDT to treat age related macular degeneration was approved in the USA and Canada (Husain et al., 1997) and the potential application of PDT to treat rheumatoid arthritis was demonstrated (Hunt and Chan, 2000). Applications of PDT for environmental antiseptic purposes (Bonnett et al., 1990; Brovko, 2009) and as pesticides (Wingo et al., 1997, 1998a, b; Amor and Jori, 2000, 2001) have also been demonstrated.

ALARMING CANCER STATISTICS

While incidence and mortality rates for most cancers are decreasing in developed countries, they are increasing in several less developed countries because of adoption of unhealthy western lifestyles such as smoking and physical inactivity and consumption of calorie-dense food (Jemal et al., 2010). The world health organization (WHO, 2008a; b) lists sixteen malignant neoplasms including mouth and oropharynx, esophagus, stomach, colon and rectum, liver, pancreas, trachea and bronchus and lung, melanoma and other skin, breast, cervix uteri, corpus uteri, ovarian, prostate, bladder, lymphomas and multiple myeloma, and leukemia. The top cancers in the world today are lung, stomach, breast, colon and rectum. The most common among men are prostrate, lung and bronchus, colon and rectum cancers. Among women, the top three are breast, lung and bronchus, and colon and rectum cancers (Amor and Jori, 2001).

In 1990, the top five cancers among male South Africans were basal cell skin, prostate gland, oesophagus, lung and squamous cell skin cancers. Among females, they were cervix, breast, basal cell skin, squamous cell skin; and oesophagus cancers (Sitas, 1992, 1994; Du Plessiset al., 1999). However, as shown in Figure 3, WHO data for the period ending in 2008, shows a different trend altogether (WHO, 2008).

CLINICAL PRACTICE

Although the clinical practice of PDT was slow to catch up with advances in drug development, clinical trials and research, it has subsequently moved so rapidly in the developed world that a number of specialized and accredited training programs are now available (Sitas, 1992, 1994; Du Plessis et al., 1999). Traditional training in clinical PDT takes the medical student through classroom experiences, supervised hospital experiences, and through standardized examinations. In the class-room, students learn the principles of PDT, the drugs used, their mode of activity, metabolism and safety. They learn the physics and safety of light and of the laser systems used. They learn about the animal and cell models used in preliminary testing of new drugs. Clinical education on PDT includes ear, nose and throat (ENT), oral, dermal, urological, bronco-oesophageal and neuro-logical indications, and are led by the respective experts. PDT complications also form part of the curricula. The power of video-based demonstrations of PDT derives from the condensation of a treatment, which ordinarily takes a number of days to a single video session. Such a demonstration should prepare students for a series of live demonstrations by various experts. The examination takes the form of theory and practice to test both knowledge and ability. The certificates awarded enjoy recognition by a growing number of respected societies for standards of ethics, practice and safety (Dilkes, 1999).

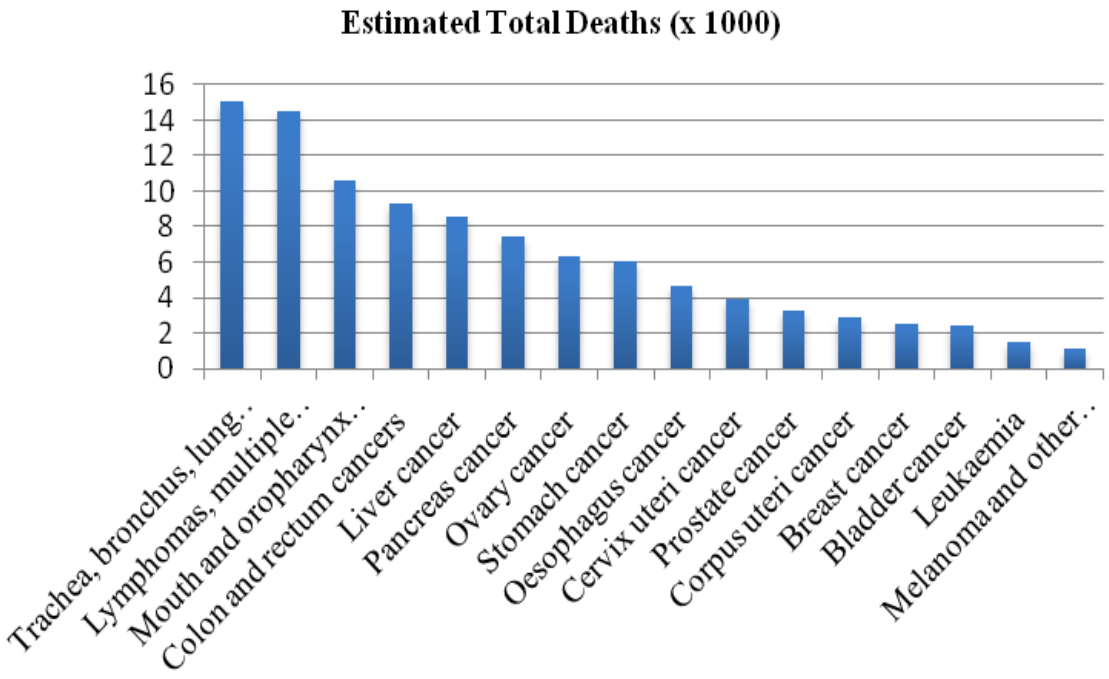

Figure 3. Rankings of malignant neoplasms in South Africa by the end of 2008. The data was obtained from the update of estimates of deaths using the methods of previous revisions carried out by WHO for 2002 and 2004 (Lopez et al., 2006; WHO, 2004). Mortality estimates are based on analysis of latest available national information on levels of mortality and cause distributions as at the end of 2010 together with latest available information from WHO programs, IARC and UNAIDS for specific causes of public health importance.

Towards clinical practice

A frequently asked question is "If PDT is so good, why then is it taking so long to reach the clinic?" There are no easy answers. It is a new modality and the various ethical and administrative agencies have proceeded cautiously in sanctioning its clinical use. There have also been a number of competing modalities developed during the same period. PDT has thus had to demonstrate superiority or at least comparability. Finally, the number of treatment parameters to be specified in PDT is significantly more than in conventional or competing new modalities for cancer. However, although photofrin has achieved widespread approval, a number of significantly superior new drugs are now being examined for approval for a wider variety of indications. This is expected to catapult PDT well into the clinic in this decade. It is generally agreed however that although PDT will prove extremely useful in this century, there is a great need for a great number of clinicians to move into the center stage of the modality (Kato et al., 1999). In the developing world, this simply means introducing PDT in the clinic through awareness and appropriately designed short learning programmes.

Awareness of PDT

The awareness and acceptance of PDT among clinicians has always been a major issue. A study suggested that in the UK, the percentage of clinicians that are not aware of PDT is less than 20%. This study also showed that the frequency of clinical publications in PDT worldwide has doubled over the past twelve years. However, the study also showed that the percentage of PDT related abstracts at the annual meeting of the British Association of dermatologists was less than 0.05%, and that the percentage of PDT related abstracts at the annual meeting of the American Society of Clinical oncology was less than 0.005% (Brown, 2000). How can the profile of clinical PDT be raised? Some of the key suggestions include the organization of more robust, multi-centre clinical trials, increased marketing by PDT companies, wider questionnaire research to ascertain levels of interest and understanding, publication of simple and informative booklets for clinicians, publication of simple handbooks on PDT, wider circulation of PDT magazines among clinicians, increase clinical content of PDT web-sites, and more PDT work to be published in specialist clinical journals (Brown, 2000).

A CASE FOR PDT AND VARIATIONS IN THE DEVELOPING WORLD

In the Developing World, there is a need to start the debate on PDT among clinicians, perhaps by conducting

extensive PDT awareness campaigns and research. This should provide the basis for rational programming and capital investment in key centres of excellence, in order to launch a focused effort in PDT. It would then remain for this effort to demonstrate the performance of PDT against traditional modalities, the ultimate acid test, as in the Developed World. In addition, these centres of excellence should provide platforms for the exploration of emerging applications of PDT in areas other than cancer and other accepted applications.

Breast cancer

Breast cancer is a significant oncological issue because it is diagnosed in over a million patients a year. Treatment paradigms have shifted to emphasize protocols that require expensive equipment and treatment rooms, aimed at preserving the breast. However, due to a lack of equipment and facilities this option is only rarely offered to poverty stricken patients like those in the Developing World. PDT may play a role in allowing for greater breast conservation, based in part on the emerging success of partial breast radiation (Allison et al., 2006).

PDT against antibiotic resistant bacteria

PDT has been studied to demonstrate its effectiveness in eradicating Gram- positive and negative antibiotic resistant bacteria including those in biofilm formations (Biel, 2010). The antibacterial effectiveness of PDT is an important issue for the developing world where bacterial infections pose a more serious threat to healthcare than in the developed world. PDT for localized infections was demonstrated by Dai et al. (2009), who observed that the advantages of PDT include equal killing effectiveness regardless of antibiotic resistance, and the absence of induction of resistance to PDT treatment and that the disadvantages include the cessation of the antimicrobial effect when the light is turned off, and less than ideal selectivity for microbial cells over host tissue. Unlike anticancer PDT, microbial selectivity therefore remains a research issue in antimicrobial photodynamic therapy.

Photodynamic antimicrobial chemotherapy

Given the ever increasing problem of antibiotic resistance in nosocomial pathogens, it is important to promote alternate technologies that may be more effective than current antibiotics. Photodynamic antimicrobial chemotherapy (PACT), a technology based on the use of a photosensitizer activated by visible light illumination and found to be effective against most types of microbial pathogens, including those resistant to antibiotics was reviewed recently (Nakonechny et al., 2011). PACT has been extensively studied and has demonstrated efficacy in the laboratory, in several animal models and in the treatment of periodontal disease and additional clinical trials should be initiated, particularly in the developing world.

PACT in wound management

The loading of the photosensitizers*meso*-tetra(N-methyl-4-pyridyl)porphyrin tetra tosylate (TMP), methylene blue (MB) and TMP with sodium dodecyl sulphate (SDS) into, and release from hydrogels composed of the polyelectrolyte poly(methyl-vinyl-ether-co-maleic acid) crosslinked in a 2:1 ratio with polyethylene glycol (PEG) were investigated as a potential rapid photodynamic antimicrobial chemotherapy (PACT) treatment for infected wounds using iontophoresis as a novel delivery method (Fallows et al., 2012). These results support the contention that the iontophoteric delivery of TMP and MB using anti-adherent, electrically-responsive, PEG-cross-linked PMVE/MA hydrogels is a potential option in the rapid PACT treatment of infected wounds. This methodology may be ideal for the treating of wounds in remotely located clinics in third world countries as it is rapid and effective, generally requiring a single application and no further dressing and retreatment.

PACT for fire burn wounds

Informal settlements are a common feature of developing world countries. Fire burns are very common in the clinics that serve patients from informal settlements. Burns are one of the most common injuries in both children and adults (Brigham and McLoughlin, 1996). The primary goals of acute burn wound management are prevention of infection and the promotion of optimal wound closure by re-epithelialization. With the open method of wound care for second degree burns, antimicrobial agents are routinely used to minimize bacterial proliferation and fungal colonization (Harry et al., 2005). *Pseudomonas aeruginosa* is considered one of the most notorious pathogens that represent life-threatening risk in nosocomial environments, mainly in patients with severe burns. *In vitro* tests showed that antimicrobial photodynamic therapy (aPDT) was effective against a clinical isolate of *P. aeruginosa* with resistance to multiple antibiotics (Hashimoto et al., 2012).

The absence of the development of bacterial resistance

The significant promise of PACT and aPDT for developing countries is not only its effectiveness against diseases of microbial origin, such as the treatment of

teeth with apical periodontitis (Silva et al., 2012) but also the absence of the development of bacterial resistance (Tavares et al., 2010).

Even as PDT quickly embraced nanotechnology for benefits such as drug administration, delivery and enhancement of efficacy, the effectiveness of nanoparticle based aPDT was demonstrated with the study of the *in vitro* effects of poly(lacticco-glycolic acid) (PLGA) nanoparticles loaded with thephotosensitizer methylene blue (MB) and light against *Enterococcus faecalis* (ATCC 29212) (Pagonis et al., 2010).

Other areas proposed for clinical application

Clearly therefore, PDT appears to represent an efficacious alternative modality for the treatment of localized microbial infections by using either *in situ* or topical application of the photosensitizer followed by irradiation of the photosensitizer-loaded infected area, using a wavelength of light that matches the red region absorption maxima of the photosensitizer. The areas proposed for clinical application of antimicrobial PDT include the treatment of chronic ulcers, infected burns, acne vulgaris and a variety of oral infections (Jori et al., 2006). Furthermore the application of photodynamic reactions can be exploited to address environmental problems of high significance, including the decontamination of waste waters, the disinfection of fish-farming tanks and the control of populations of noxious insects. Such diversified applications take advantage of the availability of a truly large number of porphyrin derivatives with chemical structures that can be tailored to comply with the physical and chemical properties, as well as the biological features of several environments. In addition, the unique property of porphyrins to absorb essentially all the wavelengths in the emission spectrum of the sun allows the promotion of processes largely based on natural light resources with significant energy saving and low impact on the ecosystems (Coppellotti et al., 2012).

CONCLUSION

PDT is accepted as a treatment for a number of diseases, including several forms of cancer and some infectious diseases, and it is approved in many countries. In addition there has been an upsurge in the number of articles published on PDT each year, both clinical and basic. Therefore after several decades since its discovery, PDT is still an emerging technology with increasing potential. Many experimental investigations have been performed all over the world, some of them are duplicated. In recent times, the potential for clinical PDT in the developing world has been widely expressed in a number of reviews. Three of the major reasons given for this view include the cost-effectiveness of most of the PDT technologies, low infrastructure and equipment requirements and the rapidly emerging effectiveness against Gram-negative and positive bacteria including those that are resistant to antibiotics. Probably due to the very low knowledge and awareness of PDT and associated technologies in the Developing World, there is very little or no clinical practice. Increasing the awareness and knowledge of these technologies will increase clinical practice in the Developing World. In the Developing World, PDT may be advanced among clinicians by conducting extensive PDT awareness campaigns and research, to provide the basis for rational programming and capital investment in key centres of excellence, in order to launch a focused effort in PDT. An important contribution of these centres of excellence would be in emerging applications of PDT in areas other than cancer and other accepted applications.

REFERENCES

Ahmad N, Feyes DK, Agarwal R, Mukhtar H (1998). Photodynamic therapy results in induction of WAF1/CIP1/P21 leading to cell cycle arrest and apoptosis. Proceedings of the National Academy of Sciences of the United States of America, 95(12):6977-6982

Allison RR, Sibata C, Downie GH, Cuenca RE (2006). Photodynamic therapy of the intact breast. Photodiagnosis Photodyn. Ther. 3(3):139-146

Amor TB, Jori G (2000). Sunlight-activated insecticides: historical background andmechanisms of phototoxic activity. Insect Biochem. Mol. Biol. 30:915-925.

Amor TB, Jori G (2001). Photodynamic Insecticides: An Environmentally Friendly Approach to Control Pest Populations. Photodyn. News 4(1):6-8

Barbosa AFS, Sangiorgi BB, Galdino SL, Pitta IR, Barral-Netto M, Correia NA, Pinheiroc ALB (2012). Evaluation of photodynamic antimicrobial therapy (PACT) against Promastigotes Form of the Leishmania (Viannia) braziliensis: In: *In vitro* study, mechanisms for low-light therapy VII, Eds: Hamblin MR, Anders J, Carroll JD, Proc. SPIE, 8211, 82110N, doi: 10.1117/12.909409

Barth RF, Solloway AH, Fairchild RG (1990). Boron Neutron Capture Therapy of Cancer. Cancer Research, 50: 1061-1070

Biel MA (2007). Photodynamic therapy treatment of early oral and laryngeal cancers. Photochem. Photobiol. 83(5): 1063-1068.

Biel MA (2010). Photodynamic therapy, photodynamic therapy of bacterial and fungal biofilm infections. Meth. Mol Biol. 635:175-194

Bonnett R, Berenbaum M (1989). in 'Ciba Foundation Symposium 146: Photosensitising Compounds: Their Chemistry and Biological Use'. John Wiley & Sons, Chichester UK. pp. 40-59.

Bonnett R, Berenbaum M (1989). Tumour Photochemother. Spectr. 218:8-10

Bonnett R, Djelal B, (1993). m-THPC. Photodynamics 1(6):2-4.

Bonnett R, Buckley DG, Burrow T, Galia ABB, Savile B, Songca SP (1990). Photobactericidal materials based on porphyrins and phthalocyanines. J. Materials Chem. 3(3):323-324.

Brigham PA, McLoughlin E, (1996). Burn incidence and medical care use in the United States: estimate, trends, and data sources. J. Burn Care Rehabilit. 17:95-107

Brown S (1996). Scotia Plans to FastrackTemoporpfrin. International Photodynamics 1(5):5-6.

Brown JE (2000). Awarenes of PDT among clinicians. Photodyn. News 3(2):2-5.

Brown S (1999). Photodynamic therapy: A bright future. Photodyn. News, Special issue for clinicians: Towards the Routine Use of PDT, 1-2.

Brovko LY, Meyer A, Tiwana AS, Chen W, Liu H, Filipe CDM, Griffiths MW (2009). Photodynamic treatment: A novel method for sanitation of food handling and food processing surfaces. J. Food Protect.

72(5):1020-1025
Candide C, Reyftmann JP, Santus R, Maziere JC, Goldstein S (1998). Modification of E-Amino Group of Lysines, Cholesterol Oxidation and Oxidized Lipid-Apoprotein Cross-Link Formation by Porphyrin-Photosensitized Oxidation of Human Low Density Lipoproteins, Photochem. Photobiol. 48:137-146.
Coppellotti O, Fabris C, Soncin M, Magaraggia M, Camerin M, Jori G, Guidolin L (2012). PorphyrinPhotosensitised Processes in the Prevention and Treatment of Water and Vector-Borne Diseases, Current Medicinal Chemistry, 19(6):808-819.
Dai T, Huang YY, Hamblin MR (2009). Photodynamic therapy for localized infections- State of the art, Photodiagn. Photodyn.Ther. 6(3):170-188.
Dilkes MG (1999). Training in PDT for Clinicians. Photodynamics News, Special Issue for Clinicians: Towards the Routine Use of PDT, 4-5
Dougherty TJ (2000). PDT in the 21st Century. Int. Photodyn. 3(1):1-3
Dougherty TJ (1998). New PDT studies at the Roswell Park Cancer Institute. Photodyn.. News 1(2):5-7
Du Plessis L, Dietzsch E, Van Gele M (1999). Mapping of novel regions of DNA gain and loss by comparative genomic hybridization in esophageal carcinoma in the Black and Colored populations of South Africa. Cancer Research, 59(8):1877-1883
Fallows SJ, Garland MJ, Cassidy CM, Tunney MM, Singh TR, Donnelly RF (2012).Electrically-responsive anti-adherent hydrogels for photodynamic antimicrobial chemotherapy. B, [Epub ahead of print]. J. Photochem. Photobiol.
Feix JB, Kalyanaraman B (1991). Production of singlet oxygen-derived hydroxyl radical adducts during merocyanine-540-mediated photosensitization: analysis by ESR-spin trapping and HPLC with electrochemical detection, Archives of biochemistry and biophysics, 291:43-51.
Feix JB, Bachowski GJ, Girotti AW (1991). Photodynamic action of merocyanine 540 on erythrocyte membranes: structural perturbation of lipid and protein constituents. Biochim. Biophys. Acta.1075: 28-35
Franken NAP, Hovingh S, Oei A, Cobussen P, Bergs JWJ, van Bree C, Rodermond H, Stalpers L, Kok P, Barendsen GW, Crezee J (2012). Radiosensitization with Hyperthermia and Chemotherapeutic Agents: Effects on Linear-Quadratic Parameters of Radiation Cell Survival Curves, Current Topics in Ionizing Radiation Research, Chapter 22: 469-494. Nenoi M (Ed.), ISBN: 978-953-51-0196-3, InTech, Available from: http://www.intechopen.com/books/current-topics-inionizingradiation-research/radiosensitization-with-hyperthermia-and-chemotherapeutic-agents-effects-on-linearquadratic-paramet
Gaál M, Otrosinka S, Baltás E, Ócsai H, Oláh J, Kemény L, Gyulai R (2012). Photodynamic Therapy of Non-melanoma Skin Cancer with Methyl Aminolaevulinate is Associated with Less Pain than with Aminolaevulinic Acid, ActaDermato-Venereoligica, 92:173-175.
Girotti AW (2001). Photosensitized oxidation of membrane lipids: reaction pathways, cytotoxic effects, and cytoprotective mechanisms. J. Photochem. Photobiol. B: Biol. 63(1):103-113.
Goes M, Lauteslager XY, Verhoeven JW, Hofstraat JW (1998). A Blue Excitable Charge-Transfer Fluorescent Probe and Its Fluorogenic Derivative, Eur. J. Org. Chem. 1998(11):2373-2377.
Hartley JA, Rezka K, Lown JW (1988). Photochem. Photobiol. 48:19-25.
Harry S, Soroff M, Adam J, Singer MD (2005). Initial management of minor burns. Israeli J. Emerg. Med. 5(4):7-16
Hashimoto MC, Prates RA, Kato IT, Núñez SC, Courrol LC, Ribeiro MS, (2012). Antimicrobial photodynamic therapy on drug-resistant *Pseudomonas aeruginosa*-induced infection. An *in vivo* study, Photochem. Photobiol. 88(3):590-595.
Hsieh YJ, Wu CC, Chang CJ, Yu JS (2003). Subcellular localization of Photofrin determines the death phenotype of human epidermoid carcinoma A431 cells triggered by photodynamic therapy: when plasma membranes are the main targets. J. Cell Physiol. 194:363-375.
Henderson BW, Dougherty TJ (1992). How does photodynamic therapy work? Photochem. Photobiol. 55:145-157.
Hill JS, Kahl SB, Stylli SS, Nakamura Y, Koo MS, Kaye AH, (1995).Selective tumor kill of cerebral glioma by photodynamic therapy using a boronatedphotosensitiser.Proceedings of the National Academy of Sciences of the United States of America, 92: 12126-12130.
Horowitz B, Valinsky JE, Geacintov NE, Williams B, Rywkin SB (1992). Photodynamic inactivation of viruses in blood cell-containing compositions, US Patent number: 5120649.
Hunt DWC, Chan AH (2000). PDT for Rheumatoid Arthritis. Photodynamics News 3(1):8-10.
Hunt DWC, Chan AH, Levy JG (1998). Immunological Aspects of Photodynamic Therapy.Photodynamics News, 1(3): 2-4.
Husain D, Miller JW, Kenney AG, Michaud N, Flotte TJ, Gragoudas EN (1997). Photodynamic therapy and digital angiography of Experimental Iris neovascularization using Liposomal benzoporphyrin derivative, Opthalmology, 104: 1242-1250.
Jemal A, Center MM, DeSantis C, Ward EM (2010). Global Patterns of Cancer Incidence and Mortality Rates and Trends, Cancer Epidemiology Biomarkers and Prevention, 19:1893-1907.
Jori G, Tonlorenzi D (1999). Photodynamic Therapy for the treatment of microbial infections.Photodynamics News, 2(1): 2-3
Jori G, Fabris C, Soncin M, Ferro M, Coppellotti O, Dei D, Fantetti L, Chiti G, Roncucci G (2006). Photodynamic therapy in the treatment of microbial infections: Basic principles and perspective applications, Lasers in Surgery and Medicine, 38: 468-481
Josefsen LB, Boyle RW (2008). Photodynamic therapy: novel third-generation photosensitizers one step closer? Brit. J. Pharmacol. 154:1-3
Kaplan MJ, Somers RG, Greenberg RH, Ackler J (1998). Photodynamic therapy in the management of metastatic cutaneous adenocarcinomas: case reports from phase 1/2 studies using tin ethyl etiopurpurin (SnET2). J. Surg. Oncol. 67(2):121-125.
Kato H, Patrice T, Dougherty T, Jocham D (1999). Questions and Answers, A Photodynamics News Worldwide Guide.Photodynamics News, Special Issue for Clinicians: Towards the routine use of PDT: 6-7
Kennedy JC, Pottier RH (1992). Photochem. Photobiol B, 6: 275-292. Marcus SL (1996). Int. Photodyn. 1(5): 2-5.
Kessel D (1995). PDT: Expanding the database. Int. Photodyn. 1(2):2-3.
Kinsella TJ, Colussi VC, Oleinick NL, Sibata NL, (2001). Photodynamic therapy in oncology, Expert Opinion on Pharmacotherapy. 2(6): 917-927.doi:10.1517/14656566.2.6.917
Levy JG, Jones CA, Pilson LA (1994). Preclinical and clinical development and potential application of benzoporphyrin derivative. Int. Photodyn. 1(1):3-5
Lopez AD, Mathers CD, Ezzati M, Murray CJL, Jamison DT, (2006). Global burden of disease and risk factors. New York, Oxford University Press, Available at http://www.dcp2.org/pubs/GBD.
Lyon JP, de Maria Pedroso ESAC, Moreira LM, de Lima CJ, de Resende MA (2011). Photodynamic antifungal therapy againstchromoblastomycosis. Mycopathologia.172:293-297
Marcus SL (1996). 5-aminolaevulinic acid. International Photodynamics, 1(5): 2-4
McRobert AJ, Bown SG, Phillips D (1989). In Ciba Foundation Symposium 146: Photosensitising Compounds: Their Chemistry and Biological Use, John Wiley and Sons, Chichester, UK, 4-16
Monfrecola G, Fabbrocini G, Pinton PC (2009). Photodynamic Therapy For Non-Melanoma Skin Cancers. Curr Can. Ther. Rev. 5: 271-280
Mroz P, Huang YY, Szokalska A, Zhiyentayev T, Janjua S, Nifli AP, Sherwood ME, Ruzie´ C, Borbas KE, Fan D, Krayer M, Balasubramanian T, Yang E, Kee HL, Kirmaier C, Diers JR, Bocian DF, Holten D, Lindsey JS, Hamblin MR (2010). Stable synthetic bacteriochlorins overcome the resistance of melanoma to photodynamic therapy. J. Fed. Am. Soc. Exp. Biol. 24:3160-3170.
Mroz P, Yaroslavsky A, Kharkwal GB, Hamblin MR (2011). Cell death pathways in photodynamic therapy of cancer. Cancers 3:2516-2539. doi:10.3390/cancers3022516
Mizuno K, Zhiyentayev T, Huang L, Khalil S, Nasim F, Tegos GP, Gali H, Jahnke A, Wharton T, Hamblin MR (2011). Antimicrobial Photodynamic Therapy with Functionalized Fullerenes: Quantitative Structure-activity Relationships. J. Nanomed. Nanotechnol. 2(2):1-9.doi:10.4172/2157-7439.1000109
Nakonechny F, Nisnevitch M, Nitzan Y, Firer MA (2011). New techniques in antimicrobial photodynamic therapy: scope of application and overcoming drug resistance in nosocomial infections, in: Méndez-Vilas A (Ed), Science against microbial pathogens: communicating current research and technological advances. 684-691

Neely WC, Martin JM, Barker SA (1988). Oducts and relative reaction rates of the oxidation of tocopherols with singlet molecular oxygen, Photochem. Photobiol. 48:23-428

Neely WC, Martin JM, Barker SA (1988). Photochemistry and Photobiology 48:423-428.

Okunaka T, Kato H, Konaka CA (1995). Clinical trial of photodynamic therapy for early stage lung cancer, international photodynamics, 1(2):4-6.

Oleinick NL, (1998). Apoptosis responses to photodynamic therapy. Photodyn. News 1(2):6-9.

Oleinick NL, Morris RL, Belichenko I (2002). The role of apoptosis in response to photodynamic therapy: what, where, why, and how, Photochem. Photobiol. Sci. 1: 1-21. DOI: 10.1039/b108586g.

Pagonis TC, Chen J, Fontana CR, Devalapally H, Ruggiero K, Song X, Foschi F, Dunham J, Skobe Z, Yamazaki H, Kent R, Tanner ACR, BDS, Amiji MM, Soukos NS (2010). Nanoparticle-based endodontic Antimicrob. Photodyn. Ther. J. Endodontics. 36(2):322-328

Pollock B, Turner D, Stringer MR, Bojar RA, Goulden V, Stables GI, Cunliffe WJ (2004). Topical aminolaevulinic acid-photodynamic therapy for the treatment of acne vulgaris: a study of clinical efficacy and mechanism of action. Braz. J. Dermatol. 151:616-622.doi: 10.1111/j.1365-2133.2004.06110.x.

Rezka K, Hartley JA, Lown JW (1990). Photosensitization by selected anticancer agents. Biophy. Chem. 35:313-323

Schastak S, Ziganshyna S, Gitter B, Wiedemann P, Claudepierre T (2010). Efficient Photodynamic Therapy against Gram-Positive and Gram-Negative Bacteria Using THPTS, a Cationic Photosensitizer Excited by Infrared Wavelength.PLoS ONE 5(7): e11674. doi:10.1371/journal.pone.0011674.

Serebrovskaya EO, Edelweiss EF, Stremovskiy OA, Lukyanov KA, Chudakov DM, Deyev SM (2009). Targeting cancer cells by using an antireceptor antibody-photosensitizer fusion protein, Proceedings of the National Academy of Sciences of the United States of America, 106(23):9221-9225.

Sessler JL, Mody TD, Hemmi GW, Lynch V (1993). Synthesis and Structural Characterization of Lanthanide (III) Texaphyrins. Inorg. Chem. 32: 3175-3187.

Silva LAB, Novaes AB, de Oliveira RR, Nelson-Filho P, Santamaria M, Silva RAB (2012). Antimicrobial photodynamic therapy for the treatment of teeth with apical periodontitis: A histopathological evaluation. J. Endodontics 38(3):360-366

Singh RJ, Feix JB, Kalyanaraman B (1992). Photobleaching of Merocyanine 540: Involvement of Singlet Molecular Oxygen. Photochem. Photobiol. 55:483-489

Sitas F (1994). Histologically diagnosed cancers in South Africa. South Afr. Med. J. 84(6):344-348.

Sitas F, Isaacson M (1992). Histologically diagnosed cancer in South Africa. South Afr. Med. J. 81(11):565-568.

Taber SW, Fingar VH, Coots CT, Wieman TJ (1998). Photodynamic Therapy Using Mono-L-aspartylchlrorin e6 (NPe6) for the Treatment of Cutaneous Desease: a Phase I Clinical Study. Clin. Cancer Res. 4(11):2741-2746.

Tavares A, Carvalho CMB, Faustino MA, Neves MGPMS, Tomé JPC, Tomé AC, Cavaleiro JAS, Cunha A, Gomes NCM, Alves E, Almeida A (2010). Antimicrobial photodynamic therapy: Study of bacterial recovery viability and potential development of resistance after treatment, marine drugs. 8:91-105.

Van den Berg H, Lange N, Jichlinski P (1999). A second generation precursor for protoporphyrin IX in photodynamic therapy and photodetection of early bladder cancer. Photodyn. News 2(1):4-8.

Wang P, Zhen H, Jiang X, Zhang W, Cheng X, Guo G, Mao X, Zhang X (2010). Boron neutron capture therapy induces apoptosis of glioma cells through Bcl-2/Bax, BMC Cancer, 10:661-671, http://www.biomedcentral.com/1471-2407/10/661.

Wang S, Gao R, Zhou F, Selke M (2004). Nanomaterials and singlet oxygen photosensitizers: potential applications in photodynamic therapy. J. Mat. Chem. 14:487-493. DOI: 10.1039/B311429E

Weyergang A, Kaalhus O, Berg K (2008). Photodynamic therapy with an endocytically located photosensitizer cause a rapid activation of the mitogen-activated protein kinases extracellular signal-regulated kinase, p38, and c-Jun NH2 terminal kinase with opposing effects on cell survival. Mol. Can. Therap. 7(6):1740-1750.

World Health Organization (2008a). The global burden of disease: 2004 update. Geneva, World Health Organization, Available at http://www.who.int/evidence/bod.

Wiegel SR, Wulf HC (2006). Photodynamic therapy of acne vulgaris using 5-aminolevulinic acid versus methyl aminolevulinate. J. Am. Acad. Dermatol. 54(4):647-651.

Wilson BC, Patterson MS (2008). The physics, biophysics and technology of photodynamic therapy, Physical and Medical Biology, 53(9), R61-R109.

Wingo PA, Landis S, Parker S (1998a).Using Cancer Registry and Vital Statistics Data to Estimate the Number of New Cancer Cases and Deaths in the United States for the Upcoming Year. J. Regist. Manag. 25:43-51.

Wingo PA, Landis S, Ries LA (1997). An Adjustment to the 1997 Estimate for New Prostate Cancer Cases. CA A Cancer J. Clin. 47:239-242.

Wingo PA, Ries LA, Rosenberg HM (1998b). Cancer Incidence and Mortality 1973-1995: A Report Card for the US. Cancer 82:1197-1207

Woodburn KW, Fan Q, Kessel D, Luo Y, Young S (1998). Photodynamic Therapy of B16F10 Melanoma with Lutetium Texaphyrin. J. Invest. Dermatol. 110:746-751.

World Health Organization (2008b).The global burden of disease: 2008 update. Geneva, World Health Organization, Available at http://www.bergfiles.com/i/bf4a0e04b9h32i0.

Yanina IY, Navolokin NA, Nikitina VV, Bucharskaya AB, Maslyakova GN, Tuchin VV (2012). Studies of lipid peroxidation of rat blood after in vivo photodynamic treatment. Proc. SPIE 8337, Saratov Fall Meeting 2011: Optical technologies in biophysics and medicine XIII, 83370G. doi:10.1117/12.923779.