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The History of Photodetection and Photodynamic Therapy¶

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ABSTRACT

Light has been employed in the treatment of disease since antiquity. Many ancient civilizations utilized phototherapy, but it was not until early last century that this form of therapy reappeared. Following the scientific discoveries by early pioneers such as Finsen, Raab and Von Tappeiner, the combination of light and drug administration led to the emergence of photochemotherapy as a therapeutic tool. The isolation of porphyrins and the subsequent discovery of their tumor-localizing properties and phototoxic effects on tumor tissue led to the development of modern photodetection (PD) and photodynamic therapy (PDT). This review traces the origins and development of PD and PDT from antiquity to the present day.

INTRODUCTION

Light has been employed in the treatment of disease since antiquity. However, it is only relatively recently that it has been used to any significant degree in medicine and surgery. In the latter part of the twentieth century it has been used in many different forms, including phototherapy for neonatal jaundice, the combination of psoralen molecules and ultraviolet A light (PUVA) in dermatology, photodynamic therapy (PDT) and photodetection (PD) which are the subject of this article.

PDT is a promising treatment for cancer and other nonmalignant conditions, which involves the administration of a photosensitizing agent (usually a porphyrin-based compound) followed by exposure of the tissue to visible nonthermal light (400–760 nm). When the photosensitizer is illuminated with light of the appropriate wavelength, the molecule is excited. This produces a series of molecular energy transfers leading to the liberation of singlet oxygen, a highly reactive and cytotoxic species, resulting in cell death (1). Photosensitizers are often taken up by malignant or dysplastic tissues with some selectivity, and light delivery can be targeted to the appropriate tissue (2). The combination of drug uptake in malignant tissues and selective light delivery has the potential to provide an effective tumor therapy with efficient cytotoxicity and limited damage to the surrounding normal tissue (3).

However, although the concept of PDT has been known for about 100 years, it is only since World War II that it has become familiar to the English-speaking world. Much of the pioneering work was performed in Europe and, therefore, the early literature was published in German, French and Danish texts. In view of this, the origins of PDT are perhaps not widely known, and with the exception of the occasional review (4) the literature regarding the history of PDT is rather sparse. In this review we aim to document the history and development of phototherapy, PD and PDT from antiquity to the present day, highlighting the major milestones and guiding the reader to further reviews detailing all aspects of the current status of PDT in depth.

PHOTOTHERAPY AND PHOTOCHEMOTHERAPY

The use of light as a therapeutic agent can be traced back over thousands of years. It was used in ancient Egypt, India and China to treat skin diseases, such as psoriasis, vitiligo and cancer, as well as rickets and even psychosis (5,6). The ancient Greeks employed whole-body sun exposure or heliotherapy in the treatment of disease, and lying nude in the sun was a popular pastime. The famous Greek physician, Herodotus, who was regarded as the father of heliotherapy, emphasized the importance of sun exposure for the restoration of health. However, it was not until recently that the therapeutic effects of sunlight were widely used in medicine.

In the eighteenth and nineteenth centuries in France, sunlight was used in the treatment of various conditions, including tuberculosis, rickets, scurvy, rheumatism, paralysis, edema and muscle weakness (7). Phototherapy was further developed by the Danish physician, Niels Finsen, who at the turn of the last century described the successful treatment of smallpox using red light, which prevented suppuration of the pustules (8). He then went on to use ultraviolet light to treat cutaneous tuberculosis and developed the use of carbon arc

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Abbreviations: AK, actinic keratose; ALA, 5-aminolevulinic acid; AlSPc, aluminum chlorophthalocyanine sulfonate; AMD, age-related macular degeneration; BCC, basal cell carcinomas; DHE, dihematoporphyrin ether; DMBA, dimethylbenz(a)anthracene; HpD, hematoporphyrin derivative; PD, photodetection; PDT, photodynamic therapy; PpIX, protoporphyrin IX; PUVA, psoralen ultraviolet A; SCC, squamous cell carcinoma; SnET2, tin ethyl etiopurpurin; mTHPC, tetra(*m*-hydroxyphenyl)chlorin; mTHPP, *meso*-tetra(hydroxyphenyl)porphyrin.

phototherapy in the treatment of this condition for which he was awarded a Nobel Prize in 1903.

Phototherapy describes the use of light in the treatment of disease, photochemotherapy, on the other hand, involves a combination of the administration of a photosensitizing agent followed by the action of light on tissues in which the agent is localised. This form of therapy also dates back over 3000 years when the Indians used psoralens in the treatment of vitiligo, and in the twelfth century the Egyptians employed different psoralens in the treatment of leucoderma (9). More recently, in 1834, Kalbrunner isolated the chemical bergapten or 5-methoxypsoralen from bergamot oil but did not use it in any therapeutic application. It was not until the 1970s that psoralens, activated by ultraviolet A light (PUVA therapy), were used clinically in the treatment of psoriasis and more recently for vitiligo and in immunotherapy (6).

PHOTODYNAMIC THERAPY

The concept of cell death being induced by the interaction of light and chemicals has been recognized for 100 years. This was first reported by Oscar Raab, a medical student working with Professor Herman von Tappeiner in Munich. During the course of his study on the effects of acridine on malaria-causing protozoa he discovered that the combination of acridine red and light had a lethal effect on Infusoria, a species of paramecium (10). This was a chance finding as the experiment had been performed during a thunderstorm when the ambient lighting conditions were unusual. He went on to demonstrate that this effect was greater than that of either acridine alone, light alone or acridine exposed to light and then added to the paramecium. Raab had, therefore, discovered the optical property of fluorescence and concluded that it was not the light but rather some product of the fluorescence that induced *in vitro* toxicity. He postulated that this effect was caused by the transfer of energy from light to the chemical, similar to that seen in plants after the absorption of light by chlorophyll. In a second publication, shortly afterwards, von Tappeiner concluded with a prediction of the potential future application of fluorescent substances in medicine (11).

The first report of parenteral administration of photosensitizer in humans was in 1900 by Prime, a French neurologist, who used eosin orally in the treatment of epilepsy. He discovered, however, that this induced dermatitis in sun-exposed areas of skin (12). This discovery then led to the first medical application of an interaction between a fluorescent compound and light in which von Tappeiner, together with a dermatologist named Jesionek, used a combination of topical eosin and white light to treat skin tumors (13). Together with Jodlbauer, von Tappeiner went on to demonstrate the requirement of oxygen in photosensitization reactions (14) and in 1907 they introduced the term ''photodynamic action'' to describe this phenomenon (15).

HEMATOPORPHYRIN AND PORPHYRIN LOCALIZATION IN TUMORS

Porphyrins were identified in the mid-nineteenth century, but it was not until the early twentieth century that they were used in medicine. Hematoporphyrin was first produced by Scherer in 1841 during experiments investigating the nature of blood. Dried blood was heated with concentrated sulfuric acid, the precipitate was washed free of iron and then treated with alcohol (16). However, the fluorescent properties of hematoporphyrin were not described until 1867 (17) and it was named hematoporphyrin in 1871 (18). Hausmann (19) in Vienna performed the first studies of the biological effects of hematoporphyrin. In 1911, he reported on the effect of hematoporphyrin and light on a paramecium and red blood cells and described skin reactions in mice exposed to light after hematoporphyrin administration (19). In particular, he described acute, subacute and chronic photosensitivity changes and some phototoxicity with intense light. The first report of human photosensitization by porphyrins was in 1913 by the German, Friedrich Meyer-Betz. In order to determine whether the same effects could be induced in humans as well as mice, he injected himself with 200 mg of hematoporphyrin and subsequently noticed prolonged pain and swelling in light-exposed areas (20).

The first report of fluorescent porphyrin localization in a malignant tumor appeared in 1924 when a Frenchman, Policard, from Lyon observed the characteristic red fluorescence of hematoporphyrin in an experimental rat sarcoma illuminated with ultraviolet light from a Woods lamp (21). Although the fluorescence was correctly attributed to porphyrin localization within the tumor, it was initially thought to be caused by secondary infection, as similar fluorescence had been observed in bacterial cultures.

There were no further publications until 1942 when Auler and Banzer (22) from Berlin described the localization and fluorescence of exogenously administered porphyrins in malignant tumors. This finding prompted Figge and Weiland (23) to further investigate the tumor-localizing properties of porphyrins in an attempt to develop this application in the diagnosis and treatment of tumors. In 1948 they administered a range of porphyrins, including hematoporphyrin, coproporphyrin, protoporphyrin and zinc hematoporphyrin, to 240 mice with experimentally-induced and transplanted tumors and 50 non–tumor-bearing mice. Porphyrin localization was found in each tumor type and with each porphyrin. The fluorescence appeared within 24–48 h of administration and persisted for 10–14 days. The fluorescence was not seen in normal tissues, other than lymph nodes, omentum, fetal and placental tissue and healing wounds. This affinity for lymphatic tissue led Figge to conclude that porphyrins coupled to radioactive compounds may have a potential use in the treatment of lymphatic leukemias (24).

TUMOR DETECTION USING HEMATOPORPHYRIN AND PORPHYRINS

After a paucity of medical research during the war years the first reports of attempts to localize human tumors with fluorescent porphyrins appeared in the early 1950s. In 1951, Manganiello and Figge (25) studied the effects of hematoporphyrin in three patients with head and neck malignancies but fluorescence was not detected. This failure was ascribed to the proportionately lower doses of photosensitizer given to humans (30–120 mg) as compared with those in previous animal experiments and the fact that the patients had been previously irradiated. In 1955, Rassmussan-Taxdal *et al.*

(26) studied the effects of intravenous infusions of hematoporphyrin hydrochloride administered to patients before the excision of a variety of benign and malignant lesions. Typical red fluorescence was observed in seven out of eight malignant tumors but in only one of the three benign lesions. Tumor fluorescence increased in proportion to hematoporphyrin dose, and with higher doses it was possible to detect a breast cancer through intact skin and a colonic adenocarcinoma through the bowel wall. The authors concluded that this finding had major implications for tumor diagnosis.

In previous studies it had been observed that not only were tumors and lymphatic tissue fluorescent following hematoporphyrin administration but also the gall bladder and biliary tree. In 1955, Peck *et al.* (27) investigated this phenomenon further in both animals and humans to assess the potential application in biliary surgery. In all the animals studied marked red fluorescence was observed in the gall bladder and biliary tree, sufficient to allow a photographic record. In three out of five human subjects no tumor or lymphatic localization was seen following the administration of relatively low doses (30–120 mg) of hematoporphyrin. However, with a higher dosage (1000 mg) one patient exhibited a bright fluorescence of a cervical carcinoma and associated inguinal lymph nodes. The final patient had an indwelling T-tube following biliary surgery. Hematoporphyrin metabolism was studied by administration of 40 mg of hematoporphyrin intravenously, and fractionated bile samples were collected and analyzed for hematoporphyrin content by spectrofluorimetry. Fluorescence was detected in the bile within the first hour and maximal levels were observed at 2–3 h. Peck concluded that biliary structures could be visualized following hematoporphyrin administration and that this application required a lower dose and shorter time interval after administration than was needed for localization in tumors and lymphatics. Analysis of the administered and excreted porphyrins was not performed, and it was not considered that the apparent rapid biliary excretion of the administered porphyrin may have been caused by the excretion of different porphyrins to those that were retained in certain tissues for prolonged periods (27).

HEMATOPORPHYRIN DERIVATIVE

The previously described studies had demonstrated the potential role of hematoporphyrin as a diagnostic tool for cancers. However, a major disadvantage was the large dose necessary to produce consistent photosensitizer uptake, which also led to unacceptable phototoxicity. In 1955, Schwartz *et al.* (28) demonstrated that the hematoporphyrin used in previous studies was a mixture of porphyrins, each with different properties. He showed that, after partial purification, the pure hematoporphyrin produced localized only very poorly in tumors, whereas the residue left behind had great affinity for tumor tissue. Schwartz continued his experiments in an attempt to further purify this non-hematoporphyrin fraction. Amongst other processes he treated crude hematoporphyrin with acetic and sulfuric acids, filtering and then neutralizing with sodium acetate, before redissolving the precipitate in saline to produce a substance which became known as hematoporphyrin derivative (HpD). This substance was found to be approximately twice as phototoxic as crude hematoporphyrin, having a lethal effect on mice subsequently exposed to light. The nature of the reaction was similar to that previously demonstrated by Hausmann (19) with skin irritation, edema and erythema, leading to skin necrosis and death. Animals kept in the dark suffered no ill effects. The severity of the reaction was dependent on three factors, the drug dose, the duration of light exposure and the time interval between drug administration and light exposure.

Schwartz persuaded Lipson, from the Mayo Clinic, to discontinue his work on hematoporphyrin and concentrate on HpD studies. Along with Baldes, Lipson further demonstrated the property of tumor localization (29), and in the early 1960s they became interested in the potential use of HpD in tumor detection. Using experimental animal tumor studies they went on to show that HpD was more effective in tumor localization and differentiation from normal tissues than crude hematoporphyrin and using much smaller doses (30).

HUMAN TUMOR DETECTION STUDIES USING HpD

Previous studies had suggested that HpD may be useful in the detection as well as the treatment of human tumors. Lipson *et al.* (31) went on to study the potential localization of HpD in tumors in patients undergoing bronchoscopy or esophagoscopy for suspected malignant disease. Light of the appropriate wavelength to activate HpD (400 nm) was produced by a filtered mercury arc lamp and transmitted *via* a fiber optic cable to the endoscope. Tumor fluorescence was observed through a filter, which excluded reflected light from the mercury arc lamp. A total of 15 patients were studied, nine undergoing bronchoscopy, five undergoing esophagoscopy and one both procedures. HpD was given intravenously at a dose of 2 mg/kg body weight, 3 h before endoscopy. Of these 15 patients, 14 were found to have histologically proven malignancy of which 10 were detected by HpD fluorescence. Failure to detect the other lesions was ascribed to tumor inaccessibility preventing adequate light exposure. A number of different malignant tumors fluoresced, but the only benign lesion, an empyema, failed to demonstrate any fluorescence. Side effects caused by cutaneous photosensitization were only seen in one patient. This study was the first to demonstrate that tumor localization and fluorescence had a potential clinical use in the detection of human tumors.

In a further study, in 1967, Lipson *et al.* (32) investigated the use of fluorescent bronchoscopy in 50 patients. Of 34 malignant tumors accessible to the bronchoscope, 32 exhibited fluorescence but none of the benign lesions did so. In the same year Gray *et al.* (33) studied cervical and vaginal lesions using HpD fluorescence techniques. Fluorescence was demonstrated in all but one of 34 malignant lesions, but disappointingly over half (13 of 23) of the benign lesions also fluoresced. Further histological review, however, revealed the presence of either carcinoma *in situ* or severe dysplasia in most of these lesions.

The following year the same group reported the use of HpD fluorescence in a large series of 226 patients, including 173 malignant tumors and 53 benign lesions (34). Fluorescence was detected in 84% of adenocarcinomas, 77% of squamous carcinomas and 62.5% of sarcomas. Increased

fluorescence was observed in the squamous lesions when compared with either the adenocarcinomas or the sarcomas which only displayed low levels of fluorescence. In contrast, only 22% of the benign lesions, which included leg ulcers, postirradiation ulcers and burns, exhibited low levels of fluorescence. Though a significant difference existed between the fluorescence in benign and malignant lesions, it was, however, concluded that the low sensitivity and specificity of this technique limited the use in tumor detection. However, it was proposed that fluorescence detection may have a role in the assessment of the extent of tumor invasion, especially during endoscopic procedures.

In 1971, two otolaryngologists, Leonard from Philadelphia and Beck from Iowa, reported a study of tumor detection using HpD in 40 patients with suspected head and neck tumors (35). The typical red fluorescence of HpD was observed in 29 patients with biopsy proven malignancy, furthermore, in 5 patients the HpD fluorescence was used to aid detection of the lesions and the choice of biopsy site. Of the remaining cases three benign tumors and four inflammatory lesions exhibited no fluorescence, but two out of four biopsies of ''normal tissue'' were fluorescent. This finding was ascribed to the presence of lymphoid tissue in the biopsy specimen. It was, therefore, concluded that HpD fluorescence was of no value in predicting tumor margins or in detecting malignancies covered by an intact mucosa. The authors considered that the affinity of HpD for lymphoid structures would lead to considerable confusion in the diagnosis of head and neck malignancies.

Despite this problem, Lipson and coworkers (36) continued to produce encouraging results using HpD fluorescence in the detection of early cancers and premalignant changes in the cervix. However, several cases of squamous metaplasia, chronic cervicitis and one case with no obvious histological abnormality also exhibited fluorescence.

Some years later coworkers at the Mayo Clinic assessed HpD in the localization of early-stage lung cancer (37,38). Successful detection of carcinoma *in situ* was described using a system in which violet light from a filtered mercury lamp was alternated at high frequency with white light, allowing both tumor fluorescence and near normal visualization during endoscopic examinations.

Similarly, in 1979, a krypton ion laser was developed, with a 405 nm wavelength to excite porphyrin during endoscopy (39). The potential application of HpD fluorescence using a krypton ion laser for localization of early lung cancer was demonstrated in an animal model by Hayata and Dougherty (40). In 1982, Hayata also used a similar system to study 36 patients with bronchial neoplasms and four with metaplasia (41). Following HpD injection, endoscopic examination revealed three of the early and 33 of the more advanced lesions. Three severely dysplastic lesions were identified and one mildly atypical lesion was not fluorescent. There were three false negatives that were attributed to blood or necrotic tissue obscuring the lesions.

Using HpD and filtered mercury arc lamp to study bladder tumors, in 1983 Benson *et al.* (42) demonstrated a positive correlation between the fluorescence of the resected specimens and the presence of tumor on histological examination, including some macroscopically normal areas containing carcinoma *in situ* or severe dysplasia. Normal urothelium showed no fluorescence and the optimal differential fluorescence between tumor and normal tissues was seen 2–3 h after HpD administration.

However, in order to have any significant clinical impact early macroscopically normal lesions need to be reliably identified. These lesions are, however, often extremely difficult to distinguish from normal tissues caused by autofluorescence. Despite the use of equipment to subtract background fluorescence this technique has not found a practical role in everyday clinical practice (43).

TUMOR TREATMENT

Following the early work by von Tappeiner and Jesionek (1903) little research on the clinical therapeutic applications of PDT was performed until nearly 70 years later. In a landmark paper the concept that the combination of tumor-localizing and phototoxic properties of porphyrins might be exploited to produce an effective treatment for cancer was first proposed in 1972 in The Lancet by Diamond *et al.* (44) from San Francisco. These authors had originally wondered whether porphyrins may potentiate the effects of X-irradiation, but having found that this was not the case, went on to test the hypothesis that hematoporphyrin may serve as a selective photosensitizing agent to destroy tumor cells exposed to light. The effect of light activation of hematoporphyrin was studied in an experimental rat glioma both *in vitro* and *in vivo.* Glioma cells in culture when exposed to white light for 50 min in the presence of hematoporphyrin underwent 100% cell death, this was determined by trypan blue uptake. When the same cell line was used to induce subcutaneous tumors *in vivo,* a similar effect was seen with a marked diminution in tumor volume following light exposure 24 h after hematoporphyrin administration. Tumor growth was suppressed for 10–20 days, but tumor enlargement then occurred from viable areas in deeper regions of the lesion. Histological examination showed coagulation necrosis in all but the deepest regions of the tumors. Neither hematoporphyrin nor light administration alone produced any effect. The authors concluded that PDT offered a new approach to the treatment of brain tumors and other neoplasms resistant to other existing forms of therapy.

One of the major milestones in PDT development occurred in 1975 when Dougherty and coworkers (2) at the Roswell Park Cancer Institute in Buffalo reported the first successful complete tumor cure following administration of HpD and activation with red light in the treatment of experimental animal tumors. Mice carrying spontaneous or implanted mammary tumors were given 2.5–5.0 mg/kg HpD and rats with implanted Walker 256 carcinosarcomas or bearing 7,12-dimethylbenz(a)anthracene (DMBA)–induced mammary tumors received 5–15 mg/kg HpD. Tumors were then exposed to red light from a xenon arc lamp for three 1 h periods over 5 days. Using a definition of cure as no palpable tumor at least 2 months after the last treatment, 48% of the transplanted mouse mammary tumors were cured. Similar results were observed with the rat DMBA–induced tumors using 15 mg/kg HpD with light exposure at 24 h. Lower doses of HpD or light failed to induce tumor regression, and neither drug nor light alone had any effect (2).

Spectrofluorimetric assay of minced tissue samples was

also performed to determine the organ distribution of HpD. In normal tissues the greatest fluorescence was seen in the liver with a maximum tumor–normal ratio of 3.7:1 at 24 h following HpD administration. As a result of this finding tumors were treated at least 24 h after HpD administration. Toxicity studies in mice showed that exposure of the entire abdominal area to light 24 h after HpD administration produced a typical photosensitivity reaction with 50% mortality at 24 h. This mortality was greatly reduced when the drug to light interval was increased to 48 h, but at this time the tumor–liver ratio had decreased to 0.86 and the cure rate for the same tumor was reduced to 20%, less than half that seen at 24 h (2).

In the same year, Kelly, a urologist working at St. Mary's Hospital in London, UK, demonstrated that human bladder tumor cells transplanted into mice could be destroyed using PDT (45). Seven of 11 tumors implanted subcutaneously developed into tumor nodules and displayed typical red fluorescence following HpD administration. Subsequent light exposure produced ulceration and variable destruction of the tumor nodules with most (22 of 32) being totally or almost totally destroyed. There was also marked edema of the surrounding normal skin. However, when normal epithelium or smooth muscle was implanted little or no damage was seen following PDT.

EARLY HUMAN STUDIES

Another major event in the development of PDT occurred in 1976. Following the successful treatment of animal tumors using porphyrin-based PDT, Kelly and Snell proceeded to the first human study of the effects of PDT using HpD in five patients with bladder cancer. At cystoscopy, only limited tumor fluorescence was observed, but the resected specimens fluoresced brightly, indicating absorption of the short wavelength portion of the cystoscopic light source. Following total cystectomy, fluorescent microscopic studies of the specimens showed fluorescence restricted to malignant and premalignant lesions, including macroscopically normal areas, which were subsequently shown on histology to be dysplastic or malignant (46).

In one patient with extensive recurrent bladder carcinoma, following previous failed transurethral resections, radiotherapy and intravesical chemotherapy, a quartz rod connected to a mercury vapor lamp was used in an attempt to activate HpD and induce tumor destruction. Tumor necrosis was seen in the illuminated area and there was no effect in the unexposed areas following treatment (46).

In 1978, Dougherty reported the first large series of patients successfully treated with PDT. Twenty-five patients with 113 primary or secondary skin tumors, all of which were refractory to conventional treatment, were treated with HpD followed by exposure to red light from a xenon arc lamp 24–168 h later. Ninety-eight lesions completely regressed, 13 exhibited a partial response and only two were resistant to treatment. The primary tumors that showed a response included squamous cell carcinomas (SCC) and basal cell carcinomas (BCC) and malignant melanomas, and the metastatic lesions were from primary tumors of the breast, colon and endometrium. Side effects included sunburn, erythema, edema and in some cases skin necrosis, although these effects were reduced by increasing the time interval between drug administration and light exposure to at least 3 days. This study demonstrated that PDT could be used successfully in the treatment of various malignant tumors even where conventional therapies had failed (47).

Since then many more studies have been published confirming the clinical effectiveness of PDT in the treatment of a variety of tumors. In view of the superficial position and ease of light application skin tumors, both primary and secondary, are ideally suited to treatment with PDT. No special instrumentation other than a light source and delivery system is needed. Deeper and larger lesions may require multiple treatments, but smaller lesions are easily treated often leaving no scarring. Even in early series complete responses were seen in 70–80% of BCC, 50% of malignant melanomas, 20% of SCC and 80% of secondary tumors (48–51).

It was soon realized that PDT could be used bronchoscopically in the treatment of inoperable obstructing lung tumors refractory to radiotherapy, often with dramatic improvement in symptoms and pulmonary function (52–54). It was also found to be useful in the treatment of early lung cancers not suitable for resection (55,56). The first report by Hayata *et al.* in 1982 (55) described a significant bronchoscopic response in the majority of patients, but only one patient of 14 was cured.

Following Kelly's early report of PDT of a bladder tumor using HpD many clinical studies have assessed the use of PDT in transitional cell carcinomas of the bladder (46). Benson *et al.* (42) reported four cases of *in situ* carcinoma responding to PDT, and Ohi and Tsuchiya (57) published a series of 11 superficial tumors successfully treated using light delivery *via* a flexible cystoscope in 1983. Two years later Schumaker *et al.* (58) reported a series of 14 patients with carcinoma *in situ* treated with PDT. Eleven were disease free at 26 months and two of the three that subsequently relapsed were successfully retreated. In 1987, Prout *et al.* (59) treated 19 patients with bladder tumors of whom nine (47%) had a complete response with 37 of 50 individual tumors eradicated.

The potential of using PDT in the treatment of esophageal cancer was soon realized. In 1984, McCaughan *et al.* (60) reported the use of PDT in the treatment of seven patients with obstructing carcinoma in which all lesions responded, regardless of histological type, with good palliation in all cases. Two patients obtained relief of dysphagia for up to 11 months following treatment. The following year Hayata *et al.* (61) reported the effects of PDT in superficial esophageal lesions and early gastric cancer in patients who refused or were unfit for surgery. In the esophagus complete responses were seen in four cases, although three of these also received radiotherapy. Of 16 patients with early gastric cancer four were treated with PDT alone and all had a complete response. Twelve patients in this series later underwent resection, and a complete response was seen in five patients previously treated with PDT. More recently, in 1995, Sibille *et al.* (62) published a large series of 123 patients with esophageal cancer treated with PDT. HpD was administered, followed 72 h later by laser irradiation using a 630 nm dye laser (62). The complete response rate at 6 months was 87% with an overall 5 year survival rate of 25 $\pm$ 6% and a disease specific 5 year survival rate of 74 $\pm$ 5%. This represents a

significant survival benefit when compared with the outcome without treatment.

Over the last 20 years PDT has been successfully employed in the treatment of many other types of tumor. These include recurrent gynecological tumors (63), intra-ocular lesions (64,65), brain tumors (66,67), head and neck lesions (68,69) and rectal cancer (70).

MECHANISMS OF ACTION

Despite considerable investigation and debate (71,72) the mechanism of action of PDT is still not fully understood. PDT was initially shown to have effects at a cellular level (73) and subsequently on the tissue vasculature (74). The vascular response plays an integral role in tumor death with vessel shutdown and stasis starving the tumor of oxygen and nutrients and slowing of flow leading to thrombus formation (74,75). However, increasingly, the effects at a cellular level are being investigated and targeted.

The extent of photodamage and cytotoxicity is thought to be multifactorial, including the intracellular distribution and concentration of photosensitizer, light intensity and oxygen availability. Sites that have been localized include the plasma membrane, mitochondria, nuclei and lysosomes (76). It is easier to identify a specific target with newer sensitizers, as these tend to be a pure compound rather than a mixture. This is of importance as reactive singlet oxygen has a short lifetime and thus limited diffusion within the cell (77).

In 1991, it was shown that PDT causes an apoptotic response in cells (78) and this provided an explanation for the widespread efficacy of PDT. Apoptosis is an energy dependant process in nucleated cells and equates to programmed cell death. It has been suggested that this is primarily caused by mitochondrial damage (79). The initial photooxidative injury triggers a number of responses, including direct cytotoxicity within the tumor microenvironment (80). This, in combination with endothelial cell damage, results in microvascular collapse and hypoxic death (75,81).

Although such immediate effects are important to the initial ablation of the tumor mass PDT has been reported to have an effect on the immune system. It can both activate and suppress the response. The inflammatory process is characterized by the release of a wide range of inflammatory mediators with recruitment of leukocytes after PDT and amplification of their activity. The induction of an acute inflammatory response and the generation of tumor-specific immunity also play a decisive role in achieving long-term control (82,83).

All of these principles are reviewed more extensively in several review articles (71,72,80,84).

ALTERNATIVE PHOTOSENSITIZING AGENTS USED IN PDT

The majority of photosensitive molecules have a heterocyclic ring structure similar to that of chlorophyll or hemoglobin. Light energy is captured in the form of photons and the energy is transferred to other molecules resulting in the liberation of short-lived energetic species that interact with biological systems and produce tissue damage (1). An ideal photosensitizer must be biologically stable, photochemically efficient, selectively retained in the target tissue relative to surrounding normal tissue and should have minimal toxicity other than to the treated area.

The majority of photosensitizers are derivatives of hematoporphyrin, a synthetic porphyrin synthesized from heme. In 1983, Dougherty (85) demonstrated that crude hematoporphyrin contains a range of different porphyrins and, when converted to HpD by acetylation further porphyrins are produced, such as protoporphyrin and hydroxyethylvinyldeuteroporphyrin. The following year he proposed that the active component of HpD was composed of two porphyrin units linked by an ether bond (86). The chemical formula bis-1-[3(1-hydroxy-ethyl)deuteroporphyrin-8-yl] ethyl ether (I) was proposed, and the compound was given the abbreviated name dihematoporphyrin ether (DHE). Further analytical studies by Kessel *et al.* (87) and Dougherty (51) suggested that the active component of HpD comprised a mixture of porphyrin rings, between 5 and 8, linked by a number of ether and ester bonds. Although the exact nature of this compound is still controversial, the active component of HpD has retained the name DHE. It is available commercially as ''Photofrin®'' (porfimer sodium, Axcan Pharma, Montreal, Canada), a heterogenous mixture of porphyrins, many of which are not active as tumor sensitizers.

Although Photofrin is the most commonly used photosensitizer it has significant side effects. Therefore, major effort has been invested in the development of new sensitizers. In particular, there was a need for new compounds that absorbed light at longer wavelengths to assist tissue penetration, greater PDT efficiency, selective tissue localization and self-limiting minor skin photosensitivity. To this end many other sensitizers have been described.

Phthalocyanines

These compounds have a structure similar to hematoporphyrin. Instead of four pyrrole units linked by methine carbon atoms, a ring of four isoindole units are linked by nitrogen atoms. In 1986, Bown and coworkers (88) at the National Medical Laser Center in London showed that aluminum chlorophthalocyanine sulfonate (AlSPc) produces more prolonged photosensitization than HpD, but less skin sensitivity in ambient light. A subsequent study by Chan *et al.* (89) demonstrated that AlSPc produces less toxicity on exposure to ambient light but greater toxicity on exposure to red light when compared with HpD. Further studies by the same group, using AlSPc-induced PDT in the treatment of rat bladder tumors, demonstrated that the drug is eliminated from the deeper muscle layers more quickly than the superficial layers of the bladder wall leading to an increased concentration in the mucosa and lamina propria at 24 h. It has since been proposed that this may potentially be exploited to produce a superficial necrosis without underlying muscle damage following light administration (90).

Meso-tetra(hydroxyphenyl)porphyrins

Tumor-localizing and sensitizing effects of the *meso*-tetra(hydroxyphenyl) porphyrins (mTHPP) were reported in 1986 by Berenbaum and coworkers (91) who demonstrated improved light absorption at the red end of the spectrum. The meta-isomer was found to be 25–30 times more potent than HpD in tumor destruction when activated by red light

(625 nm for HpD; 648 nm for mTHPP). In a study comparing the effects of PDT on skeletal muscle using different types of mTHPP, together with HpD and Photofrin II, the meta-isomer was demonstrated to be the most tumor-selective sensitizer (92). However, the major problem with these photosensitizers is the unacceptable degree of skin photosensitization and damage to underlying muscle layers when used in the treatment of epithelial lesions (92). Commercially available as tetra(*m*-hydroxyphenyl)chlorin, (mTHPC), this chlorin photosensitizer (93) is one of the most active photosensitizers requiring very low drug and light doses for efficacy. It had showed some promise in the treatment of head and neck cancers but failed to achieve regulatory approval in the United States and Europe, and the manufacturers Scotia Pharmaceuticals (Guildford, UK) withdrew the product in January 2001.

5-Aminolevulinic acid

A more recent development has been the concept of endogenous photosensitization. Many years ago it was observed that skin photosensitivity and other side effects seen after exogenous porphyrin administration were similar to those experienced by patients suffering from hepatic porphyria. These observations led to the discovery that the administration of certain drugs and chemicals can produce a porphyria-like syndrome in normal animals. This condition, known as chemical porphyria, has been the subject of several review articles (94,95). Although initially used as an experimental model in porphyria research, in 1990 Divaris *et al.* (96) in Kingston, Ontario, proposed chemical porphyria as a novel means of endogenous photosensitization for PDT.

Endogenous photosensitization involves the administration of 5-aminolevulinic acid (ALA), a naturally occurring intermediate in the heme biosynthetic pathway and precursor of the photosensitizing agent protoporphyrin IX (PpIX) (97). Although ALA has no intrinsic photosensitizing effect, in 1992 Pottier and coworkers (98) from the Kingston group demonstrated that ALA is metabolized by tissues to the potent photosensitizer PpIX. It has been shown by various authors that sufficient PpIX can be synthesized by exogenous ALA administration to produce a photodynamic effect on exposure to light, both *in vitro* and *in vivo* (96,99–102).

ALA has significant potential advantages over HpD and other photosensitizers, including more rapid photosensitizer clearance, leading to a shorter period of skin photosensitivity, usually no more than 24 h (98) and oral administration. It has been shown to produce selective photosensitizer accumulation in the mucosa of hollow organs (100,103) and may, therefore, be a superior photosensitizer for the treatment of dysplastic or noninvasive disease.

Texaphyrins

The texaphyrins are synthetic water-soluble porphyrin-related compounds that are activated by far red light (720–760 nm) (104). This sufficiently penetrates the tissues to activate the sensitizer, even in the presence of flowing blood. A phase-I trial of Antrin® (motexafin lutetium, Pharmacyclics Inc., Sunnydale, CA) performed following preclinical work demonstrated selective and efficacious atherosclerotic plaque resolution. Using different light and drug doses in 47 patients, 51 procedures were performed (105). Therapy was well tolerated and there were few side effects, including transient parasthesias and minor self-limited skin rashes. Although the study was performed to determine dose and light parameters, there were some observed clinical benefits. There was a small improvement in symptoms and angiographic measurements. Clinical trials are also being conducted for use in recurrent breast cancer and age-related macular degeneration (AMD) (106).

Tin ethyl etiopurpurin

Tin ethyl etiopurpurin (SnET2, Purlytin, Miravant Medical Technologies, Santa Barbara, CA) is a chlorin photosensitizer that has been used for the treatment of cutaneous metastatic malignancies. A clinical study in patients with recurrent chest wall disease after treatment for breast carcinoma showed promising results (107). Eight patients with a total of 86 lesions were treated at a single treatment session using 1.2 mg/kg with 660 nm light administered 24 h later. There was a 92% complete response rate and an 8% partial response rate. This was 100% recorded for smaller lesions less than 0.5 cm in size. No photosensitivity reactions were reported. Kaplan *et al.* (108) reported a 100% response rate for 6 months in three patients and one patient was disease free 2 years after treatment. Another report of treatment for skin malignancies outlined trials being performed aimed for U.S. FDA approval that has not yet been granted (109). Similar response rates to previous studies were achieved, but there was an incidence of skin photosensitivity of 10–15% at one or more months after treatment.

A multicenter, placebo controlled, double-blind phase-III study using SnET2 for 'wet' AMD has recruited 934 patients in 59 centers in the United States and closed in December 1999. The results have not yet been published, and as yet, FDA approval has not been granted for this application.

Benzoporphyrin derivative monoacid ring A (verteporfin, Visudyne)

This was first synthesized in the mid-1980s at the University of British Columbia in Vancouver (110) with the intended use of treatment of cancers. However, it has been used primarily for the treatment of AMD. This is a form of retinal disease that causes severe and irreversible vision loss and is the major cause of registrable blindness in people over the age of 50 in the western world. It is estimated that there are 400 000 new cases in the U.S. and Europe each year. AMD affects the macula, the area of the retina that enables sharp, central vision, thus difficulties in reading, driving and recognizing faces result. The advanced form of the disease, 'wet' AMD, is caused by the new growth of abnormal blood vessels. The neovasculature is leaky and eventually damages the macula. Conventional treatment used thermal laser to cauterize the vessels but was associated with irreversible vision loss. Phase-III clinical studies with 609 patients treated in 22 centers showed that patients treated with verteporfin were more likely to experience stabilized vision than those given placebo at 12 month follow up (61 *versus* 46%) (111). The protocol involved intravenous administration followed by activation at 690 nm light 5 min later through an ophthalmoscope generally using a diode laser. Verteporfin is

cleared relatively quickly from the body, and patients are instructed to avoid direct sunlight and wear sunglasses with a low (4%) transmittance of visible light for 2–5 days after treatment (111). Visudyne has now been approved for use in 26 countries.

N-aspartyl chlorin e6

A phase-I study using *N*-aspartyl chlorin e6 showed it to be an effective and safe photosensitizer for PDT (112). Eleven patients with 14 superficial cancers were treated with an escalating dose of sensitizer (0.5–3.5 mg/kg) followed by activation with 664 nm light. At doses less than 1.65 mg/kg there was only short-lived tumor regression, but 66% of sites were tumor free at 12 weeks with doses greater than 2.5 mg/kg. However, this came at the expense of tissue selectivity. There were no significant side effects other than transient skin photosensitivity. This is despite the drug remaining in the plasma for up to 6 weeks (113). Phase-II trials are also being carried out in Japan for use in endobronchial tumors. Thirty-nine lesions in 35 patients have been treated and a 95% response was seen. There were complete responses in 84% of the trials (114).

RECENT CLINICAL TRIALS

Over the past 10 years the use of PDT in the treatment of benign and malignant lesions has increased dramatically. The first health agency approval for PDT was granted for Photofrin in Canada in 1993 for use in bladder cancer. Indeed, Photofrin is now licensed in many countries for the treatment of cancers of the lung, bladder, cervix and esophagus. In the following section recent clinical trials for PDT using Photofrin and other agents are now summarized.

Esophagus

In 1995, Lightdale *et al.* (115) published the results of a prospective, randomized, multicenter trial that compared Photofrin-PDT with Nd:YAG thermal ablation for the palliation of partially obstructing esophageal tumors. Two hundred and thirty-six patients were treated, with a similar relief of dysphagia, but there was a more prolonged response in the PDT arm (32% at 1 month *versus* 20%). Nine patients had complete responses with PDT compared to only two treated with Nd:YAG. Fewer treatments were required for PDT than Nd:YAG (mean 1.5 *versus* 2.4) and there was a smaller incidence of esophageal perforations (1 *versus* 7%). It was on the basis of these results that the FDA granted approval for Photofrin in the U.S. for the treatment of advanced esophageal cancer. This was a significant event as it paved the way for approval in other countries and for use in other indications.

The largest study of the use of Photofrin for Barrett's esophagus has been published by Overholt *et al.* (116). He treated 100 patients, 87 of whom had dysplastic Barrett's esophagus. Patients were followed up for a mean of 19 months (range 4–84 months). The results were encouraging with 43% having complete eradication of the entire Barrett's segment and the remainder demonstrating a 75–80% replacement with squamous epithelium. Dysplasia was eliminated in 78 patients, although two patients developed high grade dysplasia (HGD) underlying the neo-squamous epithelium. Thirty four percent of patients developed significant esophageal strictures that required formal dilatation. Over the period of follow up there was recurrence of dysplasia in almost 20% of cases that required further treatment with PDT.

ALA has been used in the treatment of Barrett's esophagus because of the relative mucosal selectivity. After an early report by Barr *et al.* (117) demonstrating potential efficacy, ALA-PDT has been shown to be effective in the treatment of this premalignant condition (102,118). Gossner *et al.* (118) have treated 32 patients with HGD and mucosal cancer ($uT_1N_0M_0$ on endoscopic ultrasound examination). Dysplasia was eradicated in all patients with HGD, and there was complete remission of the cancers in 17 of 22 patients (77%). The treatment failed to eradicate tumors that were greater than 2 mm in depth. Remissions were maintained during follow up of 1–30 months (mean 9.9 months). Ackroyd *et al.* (102) used green (514 nm) light for the treatment of Barrett's esophagus with low grade dysplasia (LGD) (102). This group also used a lower dose of ALA (30 mg/kg) than most other studies. Thirty-seven patients were treated with a single session of ALA-PDT, with a 30% median reduction in the area of Barrett's, and in all patients the dysplasia was eradicated in the treated area. This remains the only prospective randomized placebo-controlled trial of PDT published to date.

Skin

After the early studies using HpD (48–51), the majority of clinical studies have involved the topical application (or occasionally intra-dermal injection) of ALA to skin lesions. ALA is ideally suited for use in the skin because of its superficial localization. Most of the lesions treated have been superficial BCC, SCC and actinic keratoses (AK). In an early study, ALA-induced PpIX-mediated PDT was used in the treatment of six cases of *in situ* or early invasive SCC (119). All six showed a complete response. However, two SCC that were elevated approximately 10 mm above the skin surface showed only a partial response (greater than 50% loss of tumor volume) even after repeated weekly treatments. AK also responded well to treatment with 9 out of 10 lesions showing a complete response after a single treatment. These authors then went on to treat over 300 BCC, with a complete response rate of 79% at 3 months following a single treatment (120).

Approximately 40% of SCC, the second leading cause of skin cancer deaths in the United States, begin as actinic (or solar) keratoses. A landmark event occurred in September 2000 when the FDA approved the Levulan Kerastick (ALA–HCl for topical solution, 20%, DUSA Pharmaceuticals, Valhalla, NY) system for the treatment of AK. This topical system is licensed for use with the Blu-U® blue light PDT illuminator, a special radiation source designed to provide a uniform distribution of blue light to the affected areas. In two phase three controlled multicenter clinical trials (121) treatment with Levulan Kerastick plus the Blu-U blue light resulted, at week 8, in complete responses in all treated lesions in 63–69% of the 243 enrolled patients. The corresponding response rate in patients treated with vehicle plus Blu-U blue light was 13–14%. Among patients whose per-

sistent lesions at week 8 required a second treatment, all the treated lesions responded completely in 43% by week 12 *versus* in 4% of patients who were retreated with vehicle and Blu-U blue light. With one or two treatments, 88% of patients had 75% or more of their AK lesions cleared. The long-term effect of ALA-PDT in the treatment of solar keratoses has been shown to be beneficial with a projected disease-free rate of 71% (122).

Cairnduff *et al.* (123) carried out a phase-I trial using topical ALA for SCC *in situ* (Bowen's disease), SCC, BCC and metastatic breast adenocarcinoma (123). The response in Bowen's disease was good with a complete response seen in 89% with 18 months follow up. The response in BCC was less successful with only 50% regression. Metastatic nodules responded poorly. The authors concluded that PDT was good for large or anatomically difficult areas of Bowen's disease. In addition, the cosmetic result after treatment is good (124).

Large cutaneous malignancies can present a therapeutic challenge. Morton *et al.* (125) treated 40 large patches of Bowen's disease (maximum diameter over 20 mm) and 40 large BCC with topical ALA. There was an initial clearance rate of 88% for both groups, but there were four recurrences of patches of Bowen's within 34 months. Three patients with multiple BCC had 52 of 58 lesions cleared with only two recurrences. The authors proposed that PDT should be considered as a first line therapy.

To improve the penetration of ALA, debulking nodular carcinomas and application of dimethylsulfoxide topically prior to PDT has been tried (126). At a mean of 17 months (range 12–26 months) 113 of the 119 lesions were still in complete response. In an attempt to improve the penetration of ALA into the skin, lipophilic esters of ALA have been produced (127). It is thought that these will produce higher levels of ALA in the tissue as ALA has poor bioavailability. However, this has not yet been borne out clinically (128).

ALA-PDT has also been used in the treatment of hand and foot warts (129). The results were significantly better than with cryotherapy, the standard treatment. In the only randomized double-blind trial of ALA-PDT and PDT-placebo for recalcitrant warts (130), the relative reduction in area was 100% for the ALA group *versus* 71% in the placebo group. ALA has also been used in the treatment of psoriasis and acne (131,132). The results are disappointing in psoriasis because of unpredictable clinical response and patient discomfort. In selected patients with acne, PDT causes phototoxicity to sebaceous follicles, prolonged suppression of sebaceous gland production and a decrease in follicular bacteria.

Bladder

The first license for Photofrin was granted in Canada in 1993 for use following transurethral resection for papillary tumors. PDT is an option in patients who have refractory tumors rather than cystectomy. Initial responses to treatment tend to be good, but 70–80% of patients relapse within a year (133,134). There are also significant side effects of bladder contractures. Bladder cancer tends to be a superficial condition, and for this reason ALA may be a preferable sensitizer. Kreigmair *et al.* (135) used intravesical ALA in 10 patients with refractory superficial transitional cell carcinoma. After 10–12 weeks, four patients had a complete remission and two a partial remission. Fifty percent of patients had progressive disease that required cystectomy after a mean follow up of 15 months (range 6–27 months). It has been shown that with repeated PDT treatments, it is possible to limit or inhibit progression of disease and cure a proportion of patients (136). Twenty of 24 patients had halted progression of refractory tumors, and seven were rendered disease free. The advantage of intravesical treatment, when compared to systemic, is that there is no cutaneous photosensitization.

Lung

PDT is an attractive treatment option for lung cancer as it is tissue sparing, especially in patients with limited respiratory function. In advanced cancers with airway obstruction it is equally effective when compared to Nd:YAG laser for palliation of symptoms, but the PDT effect appears to last longer before disease relapse (137). A multicenter trial has been reported, but not published, comparing the two modalities, there was a more durable response with PDT (61 *versus* 36% at 1 month), and the conclusion was that PDT was superior to Nd:YAG for the relief of dyspnea, cough and hemoptysis.

In early or radiographically occult tumors PDT offers a curative option. There is a low risk of lymph node involvement in these tumors and the treatment is tissue sparing and does not preclude surgery at a later date. Several studies have managed to achieve cure rates between 69 and 100% (reviewed by Sutedja and Postmus [138]). The optimum response appeared to be in patients with small tumors less than 1 cm in length (97.8 *versus* 42.9% for tumors larger than 1 cm) (139).

Head and neck cancer

Biel (140) has reported the largest series of PDT for head and neck cancer. One hundred and seven patients were treated with Photofrin PDT. Cure for early cancer of the vocal cords (T1 and *in situ* disease) was possible in 25 patients after a single treatment. There was only one recurrence in 79 months of follow up. The cure rate for patients with early oral cavity tumors was 80% after 70 months, but all patients responded initially. For patients with advanced disease the use of PDT as an adjunct with radiotherapy improved cure rates. Interestingly, PDT did not appear to impair wound healing. Biel also reviewed the literature for early SCC of the head and neck, demonstrating an 89.5% complete response rate.

For superficial tumors of the larynx and oropharynx similar results were obtained with a single treatment (141). Of 19 patients treated 17 patients had a histological complete response over a follow up period of 13–71 months.

Brain

Intracranial tumors have traditionally been difficult to treat because of access and the morbidity associated with radical excision. Glioblastomas and astrocytomas also tend to have foci of tumor that spread beyond the main tumor field, thus

recurrence rates are high. Radiotherapy has some effect but cannot be repeated. Thus the prognosis for these patients is generally very poor.

Muller and Wilson (142,143) at the University of Toronto, Canada, have shown prolongation of survival in patients with malignant gliomas. With surgery alone the median survival was only 20 weeks, but addition of PDT to the regimen raised survival to 30, 44 and greater than 61 weeks for patients with recurrent glioblastoma, malignant astrocytoma and astrocytoma–oligodendroglioma, respectively. In patients with newly diagnosed tumors, PDT after subtotal resection appears to be safe and can prolong survival. The best results were obtained when the light dose was greater than 1260 J (142,143).

Other sites

PDT has also been used in the abdomen and thorax as an adjuvant treatment, mainly for disseminated disease, after surgical debulking of the tumor site. A phase-II study for abdominal sarcomatosis in 11 patients achieved a response in 45%, albeit the follow up was relatively short (range 1.7–17 months) (144). Four patients with disease progression were still alive at the time of reporting.

Intrathoracic PDT for pleural cancers, especially malignant mesothelioma, has been used as an adjunct to surgery. In a phase-II study 40 patients given Photofrin-PDT 48 h prior to surgery, it showed increased survival (145). Patients underwent excision of the bulk tumor followed by intracavitary PDT. The overall estimated median survival of all stages was 15 months, but for patients with stage-I and -II disease it was 36 months. Advanced disease was associated with a 10 month median survival.

RECENT ADVANCES IN PHOTODIAGNOSIS USING ALA

ALA also appears to have potential for clinical use as a photodiagnostic or PD agent in conditions that require histological surveillance but are not readily visible. This facilitates targeted biopsy or treatment, especially in the case of premalignant conditions, such as Barrett's esophagus and carcinoma *in situ* of the bladder.

The majority of work in the bladder using ALA has been for photodiagnosis, as ALA-induced fluorescence is greater in tumor tissue compared with normal urothelium (146) and the recurrence rate of superficial bladder cancer after resection remains high, up to 75%. This is thought to be because of dysplastic foci remaining that are not visible on white light endoscopy (147). The technique has spread widely among urologists with the advent of commercially available systems. White light cystoscopy has been compared with PpIX fluorescence using an incoherent light source that provided blue light in addition to white light (148). Three hundred and twenty-eight cases undergoing transurethral resection or surveillance for primary or recurrent bladder carcinoma were examined. Using fluorescence cystoscopy 82 additional neoplasms were detected that were not visualized with white light. Thirty-one percent of these lesions were found to be poorly differentiated on histological examination. There were no serious adverse effects and no phototoxic reactions. Similar results have been obtained by other groups (149,150). Another advantage of ALA-induced fluorescence is that phototoxic reactions are not seen after intravesical instillation (151).

Topical application of ALA has also been used in the respiratory tract (152) and Barrett's esophagus (153) and orally in the gastrointestinal tract (153) and breast (154). Carcinoma *in situ* and dysplastic areas show a clear positive fluorescence, and sensitivity when correlated with histological biopsies is high. The relatively high level of false positive fluorescence tempers enthusiasm for this technique, and randomized trials are needed to compare this with conventional white light endoscopy and biopsy.

LIGHT SOURCES

The first light sources used in PDT were conventional lamps where the output was defined by the use of filters (155). A drawback with this was that there was often a significant thermal component, and also calculating the delivered light dose was difficult. These noncoherent light sources (they produce a spectra of wavelengths) are attractive in that they are easy to use and relatively cheap. However, the most common light sources used are lasers as these are the most convenient source of light for PDT. They are useful in that they produce monochromatic light of a known wavelength, light dosimetry is easy to calculate and the light can be passed down an optical fiber for localized treatment. The laser system used can be chosen according to the photosensitizer used and the desired depth of tissue necrosis required. In addition, the type of fiber used can allow adaptation of the irradiation to the target lesion, for example a microlens or diffuser fiber.

Several types of lasers have been used in PDT and include argon dye, potassium-titanyl-phosphate (KTP) dye, metal vapor and diode lasers. The most commonly used lasers are pumped dye lasers (either continuous wave or pulsed) and these can be powerful systems capable of producing up to 7 W. The argon dye (continuous wave) laser is a single unit and has been widely used because it is also possible to alter the wavelength of these systems to match the optimum absorption wavelength of the photosensitizer by adjusting the filters inside the laser, thus giving the option to use different photosensitizers. For example, the argon dye laser can easily produce wavelengths of 630 nm for HpD, 635 nm for the activation of ALA-induced PpIX and 652 nm for mTHPC. The other unit that is frequently used because of its power is the KTP dye laser. This is a modular unit (Laserscope, San Jose, CA) that utilizes a KTP laser (pulsed wave) to optically pump a specially designed laser unit, which in turn produces the commonly used wavelengths of red light (630 nm for Photofrin and 635 nm for ALA). The advantage of this system is that the KTP unit is a common surgical system, and it is relatively economical to purchase the PDT dye unit as an accessory. However, these systems are bulky and require external water cooling and separate power sources as they generate a lot of current. They are also very expensive.

Advances in semiconductor diode technology have meant that simple, compact and relatively cheaper systems can be used for PDT. These operate on standard electrical supplies and require no external cooling and so are relatively portable. A drawback of these is that the power output is limited. Diomed (Boston, MA) produce a 630 nm laser with a maximum output of 2.8 W at source for activation of Photofrin, but other diode systems emitting at 635, 652 and 730 nm are also available. These systems are licensed for use in Europe and the U.S. Diode lasers also only emit one wavelength of light, and so are very specific for the photosensitizer used.

Other nonlaser light sources available include the Blu-U light system that uses visible blue light for ALA-PDT of AK. A commercially available incoherent light source (D-light, Storz, Tuttlingen, Germany) and a special camera (Endovision Telecam SL, Storz) has also been developed for detection of PpIX fluorescence in urinary bladders. The advantages of these systems are that they are relatively inexpensive and simple to use.

CURRENT STATUS AND FUTURE OF PDT

Exactly a century after Raab's original observations, the clinical potential of PDT is finally being realized. PDT has been successfully employed in the treatment of many tumors, including skin cancer, oral cavity cancer, bronchial cancer, esophageal cancer, bladder cancer, head and neck tumors in addition to nonmalignant diseases. The mechanism of action continues to be defined along with many theoretical advantages over other cancer therapies.

A number of clinical trials have now been reported and PDT has been shown to have a potential role in both the curative treatment of early tumors and the palliative control of advanced cancer. However, PDT is not without problems and before it becomes widely adopted as a treatment for cancer these have to be addressed.

Despite there being a number of photosensitizers under investigation, at the present time only a handful are approved for clinical use. These are Photofrin, Levulan (ALA) and Visudyne (verteporfin). The first license was granted in Canada for Photofrin in 1993, and it remains the sensitizer with the most indications at the present time. Further long-term studies with other sensitizers will undoubtedly lead to licenses for clinical use, and this will facilitate the role that PDT will have in the treatment of both malignant and benign disease.

Future research will undoubtedly be directed toward the development of improved photosensitizers with increased tumor: normal selectivity and fewer side effects, in particular the systemic toxicity and duration of photosensitivity. Research is also focusing on more efficient light delivery and increased understanding of the optical properties of tissues, in addition to the effects of drug and light fractionation. Only when all these issues have been addressed will PDT fully realize its potential role as a major form of cancer treatment.

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