Contents

Preface

PHOTODYNAMIC THERAPY (PDT) of malignancies is another treatment modality for the management of cancer patients to be added to surgery, radiotherapy, chemotherapy, immunotherapy and targeted molecular therapies. In addition, PDT has the potential to be employed to treat nonmalignant diseases including bacterial and viral infections. This book presents the history, basic science, including the molecular and cellular mechanisms of PDT, methodology, and clinical outcomes for PDT treatment of diseases of the head and neck. The authors, all experts and pioneers in their field, discuss the indications for PDT treatment with their advantages and pitfalls. As PDT is an approved therapy for treatment of head and neck cancers in many countries in the world, this text provides the clinician and basic researcher with an understanding of PDT and how to successfully employ it for the successful treatment of head and neck cancers as well as its potential use for treatment of noncancerous conditions. This comprehensive book is unique in that no other scientific text has devoted itself to the presentation of PDT treatment of head and neck and upper aerodigestive tract disease, a treatment area that has its own unique treatment issues.

Merrill A. Biel

ONE

The Roswell Park History of PDT: 1972 to the Present

A PERSONAL PERSPECTIVE

Thomas J. Dougherty

CURRENT STATUS

Photodynamic therapy (PDT) development has had a long and convoluted history. Therefore, I begin with the current status and then explain (from my own experience) how we arrived here. As readers of this volume already know what PDT is, I will not describe it, nor discuss its mechanism of action, but note that there are some really interesting new developments in this area (eg, PDT-induced immunologic effects).[1,2]

Health Agency Approvals (as of 2006)

Photofrin®:

- Obstructive esophageal cancer (palliative intent)—this first PDT approval occurred in 1993 in Canada and 1995 in the United States.
- Early stage, microinvasive lung cancer (curative intent)
- Endobronchial lung cancer (palliative intent)
- High-grade dysplasia in Barrett's esophagus (curative intent), the most recently approved (2004).

The only other cancer indication of which I am aware at this time is for Foscan® (m-THPC) in Europe for head and neck cancers.

However, in addition to the above, there are numerous "off-label" studies (ie, non-approved) of Photofrin-PDT, for example, basal cell carcinoma, head and neck cancers, prostate cancer, and as an adjunct treatment with surgery for mesothelioma, brain cancers, and head and neck cancers (etc). These are being carried out in numerous centers as once a drug is approved by the FDA they have little control of how it is used. However, the company selling the drug cannot advertise it or even discuss its off-label uses. This will bring on a reprimand from the FDA to cease and desist.

ALA (protoporphyrin precursor) is approved for potentially cancerous actinic keratosis.

Companies that have/had the license to Photofrin® include Oncology Research and Development (1981–1985), Johnson & Johnson (1985–1987), Lederle/QLT (1987–1990), QLT (1990-2000), and Axcan (2000-current). Some details of the change of sponsors are discussed below.

T W O

Principles of Photodynamic Therapy-Induced Killing of Tumor Cells

Nancy L. Oleinick
David Kessel

PHOTODYNAMIC THERAPY (PDT): AN INTRODUCTION

PDT is a treatment for cancer and certain nonmalignant conditions that employs a photosensitive drug that is 'activated' by light in the visible range, producing a lethal oxidative stress and cell death in the targeted tissue.[1-4] This procedure differs from surgery and ionizing radiation as it can be directed with great specificity toward malignant tissue. But unlike chemotherapy, PDT requires that the precise location of a neoplastic lesion be known. It has been estimated that malignant cells can remain at surgical margins in as many as 50% of cases,[5] so the use of PDT as a surgical adjuvant might be a reasonable approach.

The FDA has approved PDT with the photosensitizer Photofrin® for treatment of esophageal and lung cancer.[6] In other countries, protocols have been approved for other indications, including the use of Foscan® for treatment of head-and-neck cancer in Europe, and there are ongoing clinical trials in bladder, brain, skin, head-and-neck, gastrointestinal, genitourinary, and other cancers.[6] PDT has also been approved for the treatment of certain noncancerous conditions including age-related macular degeneration (using the photosensitizer Verteporfin®), actinic keratosis (using Levulan®), and Barrett's esophagus (with Photofrin®).[6]

There are three components to PDT.[1-3] A photosensitizing agent is administered intravenously or topically, and after a suitable time to permit selective drug accumulation, selected sites are exposed to visible light at a wavelength of light corresponding to an absorbance band of the sensitizer. The third component of PDT is oxygen. In a series of steps abbreviated in Equations 1 through 4, the light energy absorbed by the photosensitizer is transferred to molecular oxygen to form the highly energetic singlet molecular oxygen, which is

the primary damaging species of PDT. The ground-state photosensitizer is regenerated and available to absorb more light. In order for this to occur, the tissues need to have a sufficient level of oxygenation.

Photosensitizer + Light → Singlet-state Photosensitizer (1)
Singlet-state Photosensitizer → Triplet-state Photosensitizer (2)
Triplet-state Photosensitizer + Oxygen → Singlet Oxygen + Photosensitizer (3)
Singlet Oxygen + Cellular Target → Oxidized Cellular Target (R-OOH) (4)

One of the advantages of PDT is the high degree of specificity offered. Most photosensitizers accumulate preferentially in malignant or other abnormal tissue in comparison to the surrounding normal tissues, for reasons that still remain largely obscure. Moreover, light can be precisely focused onto a selected region. Because of the strong oxidative stress produced, the PDT response is unhindered by the usual modes of resistance to conventional cancer treatments, and there is no evidence for limits on the doses that can be tolerated by patients, such as occurs with ionizing radiation. PDT also can be used in combination with conventional treatments. A final but very important factor is that both the photosensitizer and the wavelength of light used are inert and, therefore, harmless, eliminating systemic toxicity.[1-3]

Limitations of PDT also exist. In spite of attempts to elicit an immune or vaccine response with PDT,[7,8] at present it remains a local treatment. The photosensitizers can distribute in a tumor unevenly, allowing some regions to escape photodynamic damage. The photosensitizers can remain in the skin for up to several weeks, making the patient sensitive to sunlight; in practice, this is a problem only for Photofrin®. The penetration of photoactivating light through human tissue increases with wavelength and is most efficient at the longer wavelengths (red and infrared light). The energy of the photon decreases with increasing wavelength, so that at wavelengths above 800 to 850 nm, formation of the photosensitizer triplet state is inefficient. Although the longer wavelengths of light can penetrate deeply into tissues, local regions of high optical density can limit the exposure of certain regions to irradiation. Sensitizers in current use tend to absorb in the vicinity of 600 to 800 nm. Because Photofrin® and some other porphyrins absorb only weakly in this region (the extinction coefficient of Photofrin® is approximately 5000 at 630 nm), newer photosensitizers have been developed with much higher extinction coefficients at wavelengths greater than 650 nm that permit deep penetration of light into tissues.[9-11]

In tumor-bearing animals and in the clinic, PDT can yield a complete tumor response within a few days. There are three processes that contribute to successful treatment.[1-3, 9] (1) PDT can directly damage and kill the malignant cells of the tumor, generally resulting in a 2 to 3 log reduction in viable tumor cells. (2) PDT causes profound changes in the tumor vasculature, including blood flow stasis, vascular collapse, and/or vascular leakage, that can result in indirect killing of malignant cells. (3) PDT can promote release of cytokines and other inflammatory mediators from treated cells that induce an inflammatory response and recruit additional host cells to the tumor. The contribution of each mechanism to the overall tumor response depends on the photosensitizer, the tumor, and the treatment parameters (eg, the dose of photosensitizer and the amount (fluence) of light). One critical parameter is the fluence rate, that is, the rate at which photons impinge

on the tissue. It has long been known that the delivery of oxygen in vivo to the treatment site can be a limiting factor in PDT. Molecular oxygen is consumed in the photodynamic process in order to generate singlet oxygen (*Equation 3*), which is further fixed in oxidized substrates (*Equation 4*). High light fluence rates can deplete the targeted tissue of oxygen, limiting the impact of further photoirradiation. Decreasing the rate of light delivery, or using on-off cycles, can slow oxygen consumption, permitting reoxygenation of tissues. This results in a reduced rate of vascular blockage, allowing more oxygen to be delivered via the circulation, producing a greater overall response (more tumor cures) at a lower total light dose. Lower fluence rate irradiation can thereby produce a higher level of tumor cell death.[12]

CHEMICAL AND BIOCHEMICAL PROPERTIES OF THE PHOTOSENSITIZERS USED IN PDT

It was initially thought that only the complex mixture termed "HPD," initially described by Schwarz, could yield a selective in vivo PDT response, but it was quickly learned that this property is shared by many related agents.[1] These include porphyrins and related structures, for example, benzoporphyrins, chlorins, pheophorbides, purpurins, and phthalocyanines. All of these agents have relatively hydrophobic ring systems that can bring about drug localization in cellular membranes.[3,4,9,10] One critical element is the ability of the agent to be a photosensitizer, that is, it must have photophysical properties that permit formation of reactive oxygen species upon irradiation.[6] Some photosensitizers are not porphyrins; for example, hypericin, a photosensitizer derived from St. John's wort, which is under investigation in Europe and Singapore.[11] A large number of PDT protocols employ the heme precursor 5-aminolevulinic acid (ALA) which is metabolically converted to a photosensitizer: protoporphyrin IX (PpIX), an intermediate of the heme biosynthetic pathway.[13,14] Upon administration of ALA (systemically, orally, or topically), PpIX is generated over the first 2 to 4 hours, reaches a maximum level, and then is lost, either by metabolic conversion to heme through the introduction of iron catalyzed by the enzyme ferrochelatase or by diffusion out of the cell. While PpIX remains at the site of its formation in the mitochondria, it is an efficient photosensitizer and can also be used for fluorescence detection of tumors. The latter are often more efficient in the generation of PpIX than are normal tissues.[13,14] Esters of ALA have also been employed to enhance cellular uptake of ALA.[15]

PDT-INDUCED CELL DEATH

Whereas high-dose PDT can cause cell necrosis (membrane destruction with release of cell contents into the environment), lower doses often initiate a cell-death process termed apoptosis. This is an elegant method for eliminating cells by taking advantage of an already existing process that is normally involved in programmed cell death. The literature on this topic has grown markedly since the first report of the induction of apoptosis by a photosensitizer and light.[16] PDT is an efficient inducer of apoptosis[3,16] in both cultured cells and in vivo. Triggering apoptosis, for example, by photodamage to anti-apoptotic proteins or by mitochondrial photodamage causing loss of cytochrome *c* into the cytosol, results in the initiation of this intrinsic cell death process. This is a very efficient proc-

THREE

PDT Laser Physics and Safety

Tom Mang

INTRODUCTION

Photodynamic therapy (PDT) is a minimally invasive therapy designed to treat conditions resulting from hyperproliferating tissues. It utilizes a drug, the photosensitizer, which is also a tumor localizer, and nonthermal, low-power, visible wavelength laser for the activation of the drug to produce the photodynamic effect. The only side effect of this therapy is that a patient who receives the drug will have some skin photosensitivity due to residual low levels of drug in the skin. This condition lasts approximately 1 to 4 weeks, dependent on the photosensitizer and the final dose used,[1] in which the patient is very sensitive to direct sunlight or extremely bright artificial lights (ie, flood lamps). PDT has been shown to destroy various types of cancerous tumors in clinical trials. Currently, investigators are using PDT to treat a variety of cancers including esophageal cancer, lung cancer, head and neck cancer, recurrent cutaneous breast cancer, recurrent brain tumors, HIV-associated Kaposi's sarcoma, squamous cell cancer, and basal cell carcinoma.

The treatment can be used at various stages of disease. Photodynamic therapy can and has been used in conjunction with other treatments including surgery, chemotherapy, and radiation therapy. It is a two-stage process in which the patient is given a systemic injection of the drug. The drug by itself does not have any effects, particularly those that are associated with chemotherapy. The drug is inactive until triggered by light. After a waiting period of approximately 40 to 50 hours (depending on the photosensitizer utilized) the laser aspect of the therapy is accomplished. This waiting period is necessary to allow the accumulation of the drug into the tumor and allow some clearance from normal tissues, to set up a favorable therapeutic ratio.

The drug, which is concentrated in the tissues through selective retention, is activated by the appropriate wavelength of light. The light, in all of the approved applications of PDT for oncologic use, is obtained by a laser. The activation of the drug results in selective destruction of the tumor with minimal damage to the surrounding tissue as a result of the tumor to normal tissue ratio and the selection of the correct light fluence and dose rate delivered from the laser.[2]

FOUR

Photodynamic Therapy (PDT) in Oral Cancer

Barry L. Wenig
David Goldenberg

ANATOMY AND PHYSIOLOGY

Most head and neck malignancies develop within the oral cavity and oropharynx. The complex anatomy of the region makes the diagnosis and treatment of these lesions particularly challenging.

Anatomy

The oral cavity is bounded anteriorly by the vermilion border of the lips and posteriorly by an imaginary perpendicular plane dropped between the soft and hard palate junction superiorly and the circumvallate papillae of the tongue inferiorly. The lateral boundaries are the buccal mucosa on each side consisting of the epithelial lining of the inner surface of the cheeks and lips. Structures of significance within these boundaries include the lips, the upper and lower alveolar ridges, the retromolar trigone, the floor of the mouth, the anterior two-thirds of the tongue, the hard palate, the gingivae, the teeth, and the buccal mucosa.

Neoplasia of this region can be both benign and malignant and generally originate from the mucosal lining although any of the underlying supporting tissues can result in tumor growth as well.

The major clinical concern of primary malignancies of the oral cavity rests with the propensity of these tumors to metastasize by lymphatic drainage. The patterns of drainage are of predictive value in the evaluation of a patient for metastases.

Physiology

The oral cavity is a complex organ comprising muscle, glands, teeth, and specialized sensory receptors. The orosensory and oromotor apparatus is critical for successful defense, reproduction, exploration and vocalization.[1] Somatosensory innervation of the oral cavity is provided by the maxillary (V2) and mandibular (V3) branches of the trigeminal nerve and by the glossopharyngeal nerve (IX). The mandibular nerve branches to innervate the oral mucosa of the cheek, anterior two-thirds of the tongue, mandibular dentition, gingiva, and anterior mandibular vestibule. Branches of the maxillary nerve innervate the hard and soft palate, the oral mucosa of the mandibular vestibule, the maxillary dentition, and the gingival. Somatosensory innervation of the posterior third of the tongue is provided by the glossopharyngeal nerve.

Oral motor functions include mastication, swallowing, respiration, and vocalization. Chewing, swallowing, and breathing are each produced by generators located within the brainstem and are influenced by descending inputs from a major regions of the neuraxis.

Motor coordination taking place on multiple levels is essential to enable the competing functions of chewing, swallowing, and respiration to coexist in a coordinated manner. Coordination must take place between motor groups and must also be found within the muscles themselves.

Gustatory or taste sensations are evoked by relatively low concentrations of chemical stimuli. Individual neural elements are usually sensitive to a variety of chemical stimuli. Receptor cells, afferent nerve fibers, and central neurons are responsive to diverse chemical stimuli that elicit different sensations in humans.

HISTORY OF PDT OF THE ORAL CAVITY

Photodynamic therapy (PDT) was initially described as a clinical treatment over a century ago.[2] In 1975 Dougherty et al[3] described the effect of hematoporphyrin derivative (HpD) in combination with red light destroying tumors in mice. Clinical trials ensued in both bladder cancer and skin malignancies,[4,5] resulting in the approval of Photofrin® for clinical use.

The efficacy of PDT for the treatment of malignancies is a function of the type of photosensitizer, the drug concentration and intracellular localization, the light dose, the dose rate, and the availability of oxygen. The singlet oxygen generated can directly kill tumor cells by inducing apoptosis and necrosis and by damaging the vasculature of the tumor and the surrounding healthy vessels resulting in indirect tumor kill by the induction of hypoxia and starvation. The outcome is dependent on all of these mechanisms and the relative contribution of each depends on the treatment regimen that is selected.[6] The ideal photosensitizers would be chemically pure having a preferential uptake in tumor, rapid clearance, and absorption at peak light wavelengths greater than 630 nm. PDT is considered to be a local rather than systemic therapy and is accordingly thought to be suitable only for localized disease. This indication makes it an excellent choice for treatment of malignancies of the oral cavity yet, traditionally, treatment has been limited to relatively small, accessible tumors. It can be used, however, in combination with debulking surgery for the palliative treatment of larger tumors.

Several advantages make it an excellent choice for use in oral cavity malignancies. Limited light penetration protects tissue immediately below as well as adjacent to tumor from phototoxic effects. This localized illumination with shielding of tissues results in tumor-specific treatment without resulting destruction of normal tissue. Resultant ulceration of the treated area resolves with minimal long-term sequelae such as fibrosis being seen. By sparing tissue architecture regeneration of normal tissue is expected as noncellular supporting elements (such as collagen and elastin) are preserved. Finally, retreatment is possible and repeatable without concern for excessive tissue damage.

Early stage oral cavity malignancies (T1-T2) can be treated either with surgery or radiation therapy whereas combination therapy encompassing some combination of surgery, radiation therapy, and chemotherapeutic agents is generally reserved for late stage disease (T3-T4). PDT appears to

be equally as effective as curative surgery or radiation therapy for small, superficial tumors with reportedly high cure rates[7,8] and it does have a role in the salvage and palliative treatment of large, previously treated tumors as well.[9-11]

Biel[12] reported that PDT is effective in the treatment of carcinoma in situ (*Cis*) and T1 carcinomas of the oral cavity including the palate, floor of mouth, and posterior pharyngeal walls. Less success was achieved with lesions that were deeply infiltrating probably as a result of the inability to deliver adequate laser light to the bed. Other authors[13-15] report similar observations and results.

PHOTOSENSITIZING AGENTS IN ORAL CANCER

Early detection of oral malignancies leads to improved outcomes and survival. Although oral leukoplakia is a clinically descriptive term it is nevertheless considered to be a precancerous lesion with a prevalence of between 1 and 4% in the general population. Malignant transformation rates are reported to be 1 to 7% for homogenous thick leukoplakia, 4 to 15% for granular or verrucous leukoplakia, and 18 to 47% for erythroleukplakia.[16] Photosensitizers selectively localize in the areas of disease and render the tissues fluorescent. These result in the following advantages: utilization of these agents as noninvasive diagnostic markers, employment of sensitizers as monitors following treatment, and initiation of treatment to selectively destroy targeted cells only.

Using Photofrin® in the form of a topical application Chang and Wilder-Smith[17] evaluated 20 patients with oral neoplasms. Differentiation was made between tumors and adjacent healthy mucosa with 25% displaying hyperkeratosis, 45% squamous hyperplasia, and 30% squamous cell carcinomas. The authors determined that the predictive value for their method of fluorescent study was 95.65% correct for the macroscopic diagnosis and 97.50% correct for the microscopic diagnosis.

Endogenous photosensitisation is a mechanism by which naturally occurring substances produced by the body that generate photosensitive molecules can be exploited to induce therapeutic levels of the photosensitiser. One such molecule is 5-aminolevulinic acid (ALA) (Fig 4-1), a naturally occurring intermediate in the heme biosynthetic pathway and precursor of the photosensitising agent protoporphyrin IX (PpIX) (Fig 4-2).[43] In order to obtain fluorescence, 5-ALA, is administered exogenously to the cells. In a normal cell, 5-ALA is taken up by the mitochondria and converted through various steps into protoporphyrin-IX (PpIX), a fluorophore. 5-ALA is a second generation photosensitizer. Its photoactive derivative, protoporphyrin IX, is metabolized within 1 to2 days, eliminating prolonged skin photosensitivity even when administered IV.

5-ALA has been successfully used in the diagnosis and treatment of neoplastic tissues [16] and a number of studies from Europe and Asia display its utility in the

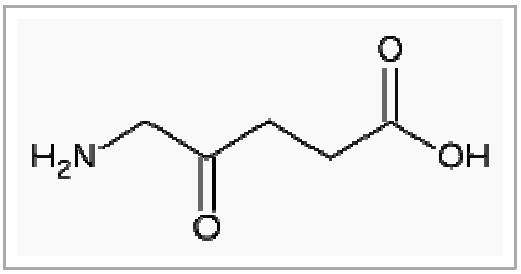

FIG 4-1. *Molecule is 5-aminolevulinic acid (ALA).*

FIVE

Photodynamic Therapy of Early Laryngeal Cancer

Merrill A. Biel

CARCINOMA OF THE LARYNX accounts for 25 to 30% of all carcinomas of the head and neck.[1] Early carcinomas of the larynx (Cis, T1, T2) and severe dysplasia are presently treated with either radiation therapy or surgery alone. Five-year cure rates achieved with this therapy is 75 to 90%.[2] Radiation therapy has the advantage of preserving the physical integrity of the larynx, thereby preserving the voice. Radiation therapy, however, has significant disadvantages even when small laryngeal fields of radiation are used. These disadvantages include discomfort and mucositis during and for potential prolonged periods after therapy, permanently altered voice quality, dysphagia, chondroradionecrosis of the larynx and trachea, and the extensive length of therapy (6-7 weeks).[3,4] Surgical therapy for early carcinomas, that is T1 and T2, of the larynx includes performing a partial cordectomy or hemilaryngectomy. Although cure rates are high, surgical removal of portions of the vocal cord or hemilarynx results in significant alteration of the quality of voice.[5]

Severe dysplasia and Cis may also be treated with either radiation or limited surgery with either microsurgical techniques or laser excision. Le reported on 82 patients with Cis of which 15 were treated with vocal cord stripping with a 56% local control rate; 13 treated with extensive laser resection/hemilaryngectomy with a 71% local control rate; and 54 treated with radiotherapy with a 79% local control rate. Anterior commissure involvement was a significant negative prognostic factor. Subjective voice quality was good to excellent in 73% of patients who underwent vocal cord stripping; 40% of those who underwent extensive resection and 68% who underwent radiation therapy.[6] Zeitels reported on 7 patients with Cis undergoing microsurgical resection. Two patients developed subsequent microinvasive cancer requiring more aggressive treatment.[7] Smith reported on 25 patients with Cis treated with surgical resection with an 88% cure rate.[8] Sittel reported on laser excision of vocal cord cancers and noted a significant effect on voice with anterior commissure resections even when done in a staged fashion.[9] Review of 10 reports of laser excision treatments of Cis demonstrated a 82.5% control rate in 177 patients. Many

required multiple laser excisions.[10] Damm reported on 29 patients with Cis treated with laser excision. 76% (22/29) required more than one laser excision for persistence of disease, 9 of which were in the anterior commissure. Two-year disease-free survival was 86%. Dysphonia was reported in all patients and none had improved voice over the pretreatment state.[11] The literature therefore demonstrates that surgical techniques to treat Cis are best limited to those patients where the Cis does not involve the anterior commissure or the bilateral vocal cords.

Garcia-Serra reported on 30 patients with Cis treated with radiotherapy with an 88% local control rate. Review of literature for radiotherapy of Cis demonstrated an 87.4% weighted local control rate at 5 years on 705 patients in 22 published reports.[10]

The optimal treatment for severe dysplasia and early carcinomas of the larynx would be one that is effective, safe, repeatable, minimally invasive, nonsurgical, and a less time-consuming therapy than radiotherapy. Photodynamic therapy is potentially such a treatment for severe dysplasia and early carcinomas of the larynx.

PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) is a minimally invasive treatment involving the use of a photosensitizing drug and laser light for the treatment of a variety of cancers.[12] When administered, these compounds are accumulated and retained to a greater degree in malignant tissues than normal tissues. The drugs remain inactive until exposed to a specific wavelength of light. The light, usually from a laser, is transmitted through specially modified fiber optics and activates the drug. The resulting photochemical reaction results in the production of oxygen radicals thereby destroying diseased cells with little effect on normal tissues. To date, PDT has been used to treat carcinomas in many organs and Photofrin-based PDT has been approved by the United States FDA to treat early and end stage endobronchial and esophageal squamous cell carcinomas and Barrett's dysplasia. In particular, the use of PDT to treat early carcinomas of the head and neck has been promising.[12-20]

The generally accepted mechanism of action of PDT is that there is an energy transfer process from the light activated or excited triplet state of the photosensitizer to oxygen producing singlet oxygen which, in turn, causes irreversible oxidation of some essential cellular component. It has also been shown that the vasculature changes within the tumor necrosis subsequent to PDT result in ischemia that is responsible for tumor necrosis. Either or both are sufficient to explain the remarkable necrosis of tumors within 2–5 days following PDT with HPD or Photofrin.

Photofrin^{R} (porfimer sodium), like HPD, concentrates in malignant tissue, is activated by penetrating light (630 nm + 3 nm), produces fluorescence, and is photochemically efficient. Like its predecessor HPD, Photofrin^{R} has produced only one major adverse reaction as a result of its use: light sensitivity. In animals, it requires about twice as much Photofrin^{R} as HPD to produce skin photosensitivity.

Photodynamic therapy has been demonstrated to be effective in the treatment of early carcinomas of the head and neck.[12-20] Furthermore, preliminary studies on the treatment of benign laryngeal papillomatosis with PDT have demonstrated this treatment to be safe and effective.

The advantage of PDT treatment for early carcinomas of the larynx is the abili-

ty to preserve normal endolaryngeal tissue while effectively treating the carcinomas. This results in improved laryngeal function and voice quality. Furthermore, PDT requires a short duration of therapy as compared to radiation therapy, is repeatable and carries less risk than surgical therapy, and is performed as an outpatient noninvasive treatment. Importantly, the use of PDT does not preclude the use of radiotherapy or surgery in the future for new primary or recurrent disease. If proven effective in a multi-institutional clinical trial, PDT may become the first-line therapy for treatment of early carcinomas of the larynx.

Multiple centers have reported Phase II study data on the use of Photofrin-based PDT to treat Cis-T2 carcinomas of the larynx[12-20] (Table 5-1). Freche[16] reported on 32 patients with T1 vocal cord carcinomas treated primarily with PDT. Twenty-five of 32 patients obtained a complete response for a complete response rate of 78%.[16] Feyh treated 12 patients with Cis-T2 laryngeal carcinomas. Eleven of 12 patients obtained a complete response for a complete response rate of 91%.[14] Gluckman treated 2 patients with T1 carcinomas of the larynx both of which obtained a complete response.[15] Schweitzer treated 10 patients with Cis-T2 carcinomas of the larynx of whom 8 obtained a complete response for an 80% complete response rate.[18]

The largest single study of the treatment of laryngeal carcinomas with long-term follow-up has been performed by Biel.[12] One hundred and ten patients with recurrent or primary CIS, T1N0, and T2N0 laryngeal tumors were treated with PDT for cure with Photofrin-based PDT at Abbott Northwestern Hospital (Minneapolis) from February 1990 to November 2005. Three patients had recurrent CIS, 92 patients had T1N0 carcinomas of the true vocal cord of which 25 were radiation failures, and 15 patients had T2N0 carcinomas of the true vocal cord of which 8 were radiation failures. All patients underwent a single microlens light treatment and most T2 tumors also underwent cylindrical diffuser implants into the paraglottic space. The age range was 39 to 88 years. All patients were treated according to specific protocols in accordance with FDA and IRB approvals. Pretreatment evaluation included a history and physical examination and endoscopic examination with tumor mapping and biopsy. CT or MRI scanning of the larynx was used for staging prior to treatment of tumors greater than T1 or radiation failures. Photofrin (Axcan Pharma-

Table 5-1. Summary of Published Results with Photofrin PDT of Early Squamous Cell Cancer of the Larynx

Study	Patients	Lesion and Site	Drug, Dose, mg/kg	Response, *n*		
				Complete	Partial	None
Feyh et al. (14)	12	T1 and T2, larynx	Photosan III	11	1	0
Freche et al (16)	32	T1, larynx	HPD, 3	25	7	0
			Photofrin, 2			
Schweitzer (18)	10	T1, larynx		8	2	0
Gluckman (15)	2	T1, larynx		2	0	2
Biel (12,19)	110	Cis, T1, and T2, larynx	Photofrin, 2	110	10	0

S I X

Interstitial PDT Cancer Treatment

Christian S. Betz
Colin Hopper

LIMITATIONS OF SURFACE ILLUMINATION PDT

The effectiveness of surface illumination PDT is limited by the depth of penetration of light within the tissue. Longer wavelengths have a greater depth of penetration although against this is the feature of lower quantum yields with increasing wavelength. Oxygen requires 94 kJ/mol to raise it from the triplet ground state to the excited singlet state. In photodynamic therapy this energy is acquired from the photosensitiser which has itself been raised to a high energy state. Only light-absorbing compounds which can emit energy greater than 94kJ/mol are therefore capable of activating ground state oxygen. This corresponds to an absorption wavelength of around 850 nm: photosensitisers are therefore only suitable for photodynamic therapy if their activation wavelength is below this figure. This equates to a depth limit of approximately 1.5 cm for surface illumination.[1] Most of these problems can be overcome if light can be delivered directly into tissue using interstitial techniques.

SAFETY CONSIDERATIONS—TISSUE TOLERANCE

In order to effectively treat interstitially, it is necessary to be confident about PDT effects on normal tissue. This is of particular relevance in the head and neck with its abundance of vital structures. A number of safety studies have been carried out on different photosensitisers[2-4] and have demonstrated the ability of normal tissue to recover from the PDT insult. Bone treated with PDT does show some slightly impaired healing. It is not entirely clear what mechanism is involved, but microvascular damage with reduced blood supply to the healing site is possible. Fortunately, no long-term problems were seen with bone healing and this is obviously of great importance for any treatment in the oral cavity. Of particular relevance was the study by Kübler, who used m-THPC on rabbit carotid and femoral vessels as well as the vagus and femoral nerves. In a dose escalation study he found that at high drug doses (0.3 mg/kg) and a short drug light interval (24 hours) with a light dose of 20 J/cm^2 there

was edema, some thrombosis, and disruption of the endothelial layer. He also reported up to 75% demyelination, but importantly, neither caused any clinical distress and no vessel rupture was seen.[5] It is clear from these studies that PDT is safe in close proximity with blood vessels in the normal setting. It would be quite reasonable to speculate that in a clinical setting, where tumor was eroding the vessel wall, PDT might precipitate acute hemorrhage. However, as long as tumor is close but not eroding the arterial wall, treatment can be carried out safely. These studies have been performed on normal tissue models, so caution should be used when extrapolating from these animal studies to head and neck cancer where patients may have already been heavily pretreated with surgery radiotherapy and chemotherapy.

Interstitial PDT (Fig 6-1) has been successfully used in the treatment of internal organs such as the pancreas, prostate, and brain, so there is a reasonable amount of data to suggest iPDT is safe in the head and neck.

DRUGS IN iPDT USE

Any PDT treatment can be carried out interstitially just as long as the activating wavelength can be delivered through an optical cable. Most clinical studies in the head and neck have used either Photofrin, phthalocyanines, or Foscan, although several other drugs have been investigated in other pathologies—for example, the bacteriochlorin m-THPBC has been used in the treatment of liver metastases from colon cancer[6] and Pd-bacteriopheophorbide (Tookad) and metallotexaphyrin (Lu-Tex) are being investigated for the treatment of prostate cancer.[7-9]

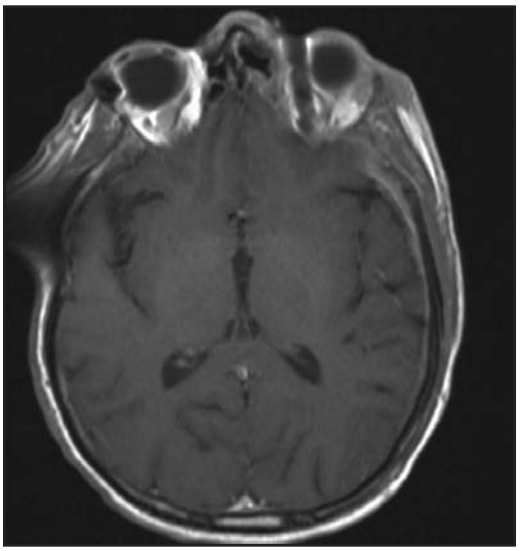

FIG 6-1. *Foscan PDT treatment in the posterior orbit using interstitial light delivery through titanium needles. Eyesight was preserved in this patient.*

DOSIMETRY

There are two main methods of delivering light—point sources and diffuser fibers (Plate 10). The tissue effects of these when used interstitially can be simulated using mathematical modelling; this can be quite useful in the calculation of light distribution and extent of treatment effects (Plate 11). There are limitations with these techniques which are related to the exact nature of tissue and the extent to which light can pass through the tissue. There is really no substitute for real-time monitoring of light distribution which can be done in a number of ways. Isotropic detector fibers can be used to monitor light within tissues during treatment.[10] Recently a more innovative approach has been described by Gross which uses BOLD MR as a marker of photodynamic activity.[11] None of these methods, however, is so far able to accurately predict the actual effect on the tissue but histologic confirmation, although such evidence is hard to come by in hu-

man tissue and would certainly not be available in all cases.

Planning

The aims of planning are to identify the total volume of disease and then ensure that light is delivered at sufficient energy to trigger the PDT effect to this tumor volume. The accurate evaluation of tumor extent is usually carried out using MRI, CT, ultrasound, and PET CT and a similar approach is taken to radiotherapy planning where the target volume includes a rim of normal tissue. The advantage of PDT over radiotherapy is, of course, the absence of cumulative toxicity with repeat treatment.

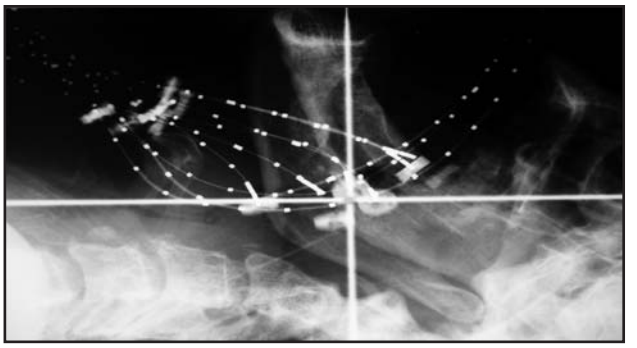

FIG 6-2. *Use of transparent conduits to facilitate diffuser fiber placement in the treatment of a recurrent tongue tumor.*

Fiber Positioning

Positioning of the fibers can be performed freehand, or in more sensitive areas under image guidance with MR, CT and ultrasound. One of the techniques for doing this has been described by Jäger et al where titanium needles are placed in the target tissue under MR guidance in accordance with the London rules for iPDT.[12] These are loosely based on the Paris system for radiotherapy and state that light delivery systems should be parallel, equidistant and deliver consistent light along their length.

An alternative approach is with an afterloading system that is very much adapted from brachytherapy techniques. Diffuser fibers can be fed through a series of plastic conduits and complex volumes of tumor can be treated (Fig 6-2).

ANAESTHETIC CONSIDERATIONS

There are a few important points regarding patients undergoing iPDT under general anaesthesia. Overall, the patient population may have already had extensive surgery and radiotherapy. This often makes airway management problematic and an experienced airway anaesthetist is essential for these cases. There are the added complications of slightly subdued lighting and if the treatment takes place in an MR or CT scanner, the surroundings may not be that familiar. In addition, the use of protective glasses makes the reading of color-coded drugs and monitors difficult. Care must also be taken with the use of a pulse oxymeter as this uses a red HeNe laser that can cause blistering of the finger if left in place too long.

LIMITATIONS ON EFFECTS

Proximity to major vessels where vessels may be invaded by tumor is a great cause for concern and treatment in this situation is likely to result in catastrophic bleeding. Accurate preoperative assessment of vascular invasion is essential for safe treatment. When vascular invasion is present in a major vessel, we would advocate the prophylactic placement of a covered endoluminal stent (Fig 6-3).

The optical properties of tissue are also of obvious importance. Light transmission

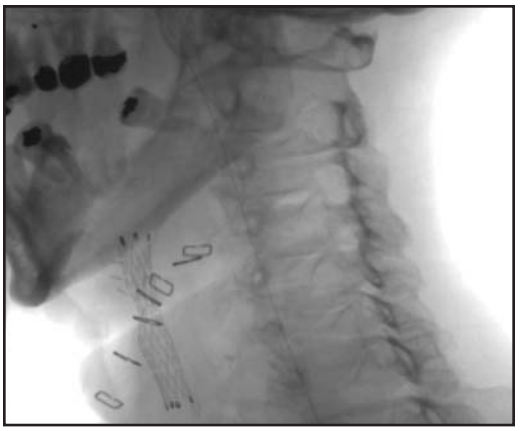

FIG 6-3. *Placement of a covered endoluminal stent to protect carotid artery from a blowout during PDT.*

through a vascular tumor would be very different to scarred or fibrotic tissue. This makes real-time dosimetry even more desirable.

CLINICAL STUDIES

Based on the theoretical and preclinical advantages addressed above, several groups have endeavored to bring interstitial Photodynamic Therapy into clinical reality (Table 6-1). As early as 1988, there had been a single, sporadic report on the use of interstitial PDT with hematoporphyrin derivative as a photosensitizer for the treatment of primary oral squamous cell carcinomas as well as their regional lymph node metastatases.[13] The authors state that all lesions treated except for those with bony involvements showed a good response to treatment, whereas the covering skin, connective tissue, and nearby organs seemed spared from major injury.

Only since the beginning of the 21st century, scientific publications concerning the use of interstitial PDT for the treatment of head and neck tumors have come up more frequently. In 2001, two groups have reported on the successful use of interstitial PDT with hematoporphyrin derivatives and phthalocyanines in a limited number of patients with head and neck tumors of various stages.[14,15]

At the Department of Oral and Maxillofacial Surgery at the University College London Hospital, interstitial PDT using m-THPC as a photosensitizer was established in 1997 and has since then primarily been applied for palliative treatment of advanced head and neck tumors. In an original publication from 2004 by Lou et al,[16] the group presented the retrospective results of a 5-year experience in this field. In total, 45 patients with persistent or recurrent head and neck cancer unsuitable for further treatment with surgery, radiotherapy or chemotherapy were recruited and subsequently

Table 6-1. Medline-Listed Publications on Clinical Studies Using iPDT for the Treatment of Head and Neck Disease

Author	Year	Tumor Site	Photosensitizer	*N*	Main Results	Ref
Wang et al	1988	Oral cavity	Hematoporphyrin derivative	10	"Good response" of all tumors except for those with bony involvement	13
Stranadko et al	2001	Oropharynx/ Larynx	Hematoporphyrin derivative/ Al-Phthalocyanine	Fraction of 61	CR 57.4%; PR 37.7%	14
Tanaka et al	2001	Tongue	Porfimer Sodium	3	CR 66.7%; PR 33.3%	15
Lou et al	2004	Oral cavity/ Oropharynx	m-THPC	45	CR 20.0%; PR 53.3% (only recurrent SCCs treated)	16

SEVEN

Intraoperative Adjuvant PDT of Head and Neck Cancer

Merrill A. Biel

HEAD AND NECK squamous cell carcinomas with extensive soft tissue invasion are known to have high rates of local and regional recurrence. Recurrence rates of cervical nodes with extracapsular spread range from 21 to 58%.[1-4] Primary squamous cell carcinomas of the tongue base and hypopharynx also have local and regional recurrence rates of 43 to 71% despite aggressive combined surgical and chemoradiotherapy.[5-7]

When patients with squamous cell carcinomas develop large invasive soft tissue recurrences of their carcinomas following previous surgery, radiation, and chemotherapy, the likelihood of further recurrence following extensive surgical resection of the recurrent carcinoma is extremely high. In addition, these recurrences tend to occur within the first 6 to 12 months of surgical resection.[6-7]

Photodynamic therapy (PDT) has been successfully used to treat patients with early squamous cell carcinmomas of the head and neck.[8-9] This is due to the ability of the activating laser light to penetrate up to one centimeter into tissue resulting in destruction of microscopic tumor with relative sparing of normal tissue when the correct drug and light dose combinations are used. Employing this principle, PDT may be used as an adjuvant intraoperative therapy following resection of tumor in patients with recurrent and large infiltrating carcinomas of the head and neck, to potentially increase the rate of cure in this dismal disease by destroying microscopic residual disease and to provide for a greater likelihood of tumor-free margins of resection. This method of treatment may be clinically applied in two different ways: (1) PDT for curative intent following gross tumor debulking. The goal of this treatment is to achieve complete tumor eradication with preservation of normal vital structures such as the larynx and tongue; and (2) PDT of the surgical resection bed following *complete* resection of T3 and T4 tumors. The goal of this treatment is to increase local/regional disease control by increasing tumor-free resection margins and destroy microscopic skip lesion disease while preserving uninvolved normal structures.

INTRAOPERATIVE PDT PRECLINICAL STUDIES

Intraoperative PDT in the head and neck results in the exposure of vital blood vessels and nerves to the PDT treatment. In particular, the carotid artery, internal jugular vein and cranial nerves X to XII would be commonly exposed during PDT treatment. A partial or complete necrosis of these structures due to PDT treatment could result in catastrophic life-threatenting complications including arterial rupture or permanent cranial nerve damage.

Several preclinical studies have been performed to evaluate the effect of PDT on blood vessels and nerves. Grant et al used a rabbit carotid artery model to investigate the effect of disulphonated phthalocycanine and 5-aminolevulinic acid mediated PDT on these vessels. Three days following PDT all treated vessels demonstrated a complete loss of endothelium with death of the media smooth muscle cells. There was no vascular occlusion, hemorrhage, or thrombosis present. Re-endothelialization occurred in all vessels by 2 weeks. Furthermore, intraluminal hydrostatic distension tests performed on the vessels demonstrated no reduction in the pressure required to burst the vessels treated with PDT versus the nontreated control carotid arteries. They concluded that despite the full thickness vessel wall cell death, PDT treated arteries are not at risk for thrombotic occlusion, rupture or hemorrhage.[10]

Ris et al performed intraoperative PDT to the blood vessels and nerves in the thoracic cavity of mini-pigs using mTHPC as photosensitizer (0.1 mg/kg, 20 J/cm^2, drug-light interval of 12 hours to 6 days). Tissue damage was strongest at a drug-light interval of 12 hours and gradually became less at longer drug-light intervals and was absent by 3 days after drug injection. At a drug-light interval of 12 hours, there was severe damage of the aorta with thrombosis and necrosis of the tunica media and desquamation of the endothelium; however, there was no damage to nerves. At 24 hours, only minor changes were observed in the aorta and the vena cava responded with swelling of the endothelium and thrombosis of the vasa vasorum but did not shown any necrosis of the vessel wall or thrombosis.[11]

Kübler et al studied the effect of m-THPC PDT on large blood vessels and nerves in a rabbit model. The most severe reactions occurred at a drug dosage of 0.3 mg/kg, light dose of 20 J/cm^2 and 24 hour light interval. Blood vessels demonstrated severe edema, media hyperplasia with loosening of the endothelium and various degrees of local thrombosis. There was no breakdown of the vessel wall or any vessel ruptures. Nerves were altered by a 75% demyalinization but with no clinical symptoms.[12]

Biel studied the effect of Photofrin PDT on the carotid artery, internal jugular vein, and vagus nerve in dogs. This study demonstrated that at a drug dose of 2 mg/kg and a light dose of 50J/cm^2 at 150 mw/cm^2, there was no histologic effect on either the blood vessels or the vagus nerve. At higher light doses, greater than 75 J/cm^2, there was loss of the arterial media and endothelial sloughing.[13]

Kübler et al demonstrated in a rat skin flap model that the addition of Photofrin PDT does not affect wound healing.[14] However, Belmont et al in a rat fasciocutaneous flap model demonstrated that Photofrin PDT reduced the critical primary ischemic time of the rat fasciocutaneous flap, whereas white light illumination in the presence of Photofrin had no effect on the critical primary ischemic time.[15]

Importantly, preclinical studies have

demonstrated that intraoperative adjuvant PDT at the time of surgery reduces the incidence of local recurrence. Dilkes in a squamous cell carcinoma model and Davis in a mouse neuroblastoma model demonstrated that intraoperative PDT to the tumor bed prior to closing the wound resulted in a 50% reduction in the local recurrence rate.[16-17] Based on these preclinical studies demonstrating the potential for adjuvant intraoperative PDT to improve cure rates, several centers have performed human clinical studies to demonstrate the efficacy of adjuvant intraoperative PDT delivered to the tumor resection bed at the time of surgical resection.

INTRAOPERATIVE PDT HUMAN CLINICAL STUDIES

Biel performed intraoperative adjuvant PDT using Photofrin in 35 patients.[8] These patients were divided into two treatment groups: (1) PDT for curative intent following gross tumor debulking. The goal of this treatment was to achieve complete tumor eradication with preservation of normal vital structures such as the larynx and tongue; and (2) PDT of the surgical resection bed following *complete* resection of T3 and T4 tumors. The goal of this treatment was to increase local regional disease control by increasing tumor-free resection margins and destroy microscopic skip lesion disease while preserving uninvolved normal structures.

In the first treatment group, PDT for curative intent following gross tumor debulking, 17 patients were treated, 11 laryngeal and 6 oral cavity. Of the 11 laryngeal, 8 were supraglottic and 3 were glottic. The oral cavity lesions were tongue and floor of mouth. The treatment consisted of the patient receiving Photofrin 2 mg/kg preoperatively and 2 days after the injection the patient underwent general anesthesia and gross but incomplete resection of the tumor mass. Residual microscopic disease was confirmed with frozen section biopsies intraoperatively. PDT was then performed to the resection site using a microlens fiber at 75 to 80 J/cm^2 at 150 mW/cm^2. Cylindrical diffuser implantation 0.5 cm in length was placed wherever the location was safe to do so and illumination performed at 100 J/cm fiber length at 400 mW/cm fiber length. Most of the treatments were performed on an outpatient basis. For the laryngeal tumors treated, with follow-up to 69 months, there have been no recurrences (Plates 13 and 14). For the 6 oral cavity tumors, with follow-up to 58 months there was one recurrence that went on to conventional surgical resection and remains free of disease.

In the second treatment group, intraoperative adjuvant PDT of the surgical resection bed following *complete* resection of T3 and T4 tumors, 18 patients with recurrent infiltrating squamous cell carcinoma of the head and neck were treated. Each patient had undergone previous treatment of the primary lesion of the head and neck with surgical resection, radiotherapy, and chemotherapy. The initial primary carcinomas were in the larynx; tongue and floor of mouth; branchial cleft cyst; medial canthal skin and ethmoid sinus; and tonsil. The sites of recurrence included the pharyngoesophagus and anterior neck skin; mandible and neck; medial orbit, ethmoid and anterior skull base; neck skin, parotid, and lateral skull base; tongue and floor of mouth; and neck. In all cases, extensive skin involvement with tumor was present with deep infiltration into the soft tissues as determined by CT, MRI, and angiographic scanning. All lesions were determined to be surgically resectable.

EIGHT

Photodynamic Therapy (PDT) in Nasopharyngeal Cancer

I. B. Tan
H. J. Nyst
H. J. C. M. Sterenborg
R. L. P. van Veen
P. C. Levendag
D. J. Robinson
F. A. Stewart

DEMOGRAPHICS

Nasopharyngeal carcinoma (NPC) occurs sporadically in the western world, but is endemic in certain parts of Asia, such as southern China. The worldwide incidence of NPC is 45,976, with 19,616 new cases each year in China and 4,848 new cases in Indonesia.[1] In western countries the incidence is much lower with 49 and 538 new cases each year in the Netherlands and Germany, respectively, and an intermediate position for the Mediterranean basin.

Nasopharyngeal carcinomas are epithelial neoplasm's, classified in three different histopathologic types (WHO classification, 1993). Type I tumors are squamous cell carcinoma (SCC) with varying degree of differentiation. Type II are nonkeratinizing carcinomas and type III are undifferentiated carcinomas, collectively considered as undifferentiated nasopharyngeal carcinomas (UCNT).

TREATMENT OPTIONS

Nasopharyngeal carcinomas are responsive to radiotherapy and chemotherapy and the first-line treatment for primary NPC is radiation.[2] As the nasopharynx is located in the midline and is surrounded by critical structures, efforts should be made not to overdose these important areas. The volume to be irradiated should include the nasopharynx, adjacent parapharyngeal, tissues and the cervical lymph nodes (level II-V). After about 40 to 60 Gy, the spinal cord should be shielded as a conservative estimate of the tolerance of the spinal cord

is about 50 Gy in 2-Gy daily fractions. An additional dose of 22 to 30 Gy is delivered to the nasopharynx proper. In general, 66 to 70 Gy for T1 and T2 lesions and 70 to 75 Gy for T3 and T4 tumors is required. Because of the likelihood of developing cervical metastases, all of the cervical lymphatics should be irradiated with 46 to 50 Gy to both sides of the neck in N0 patients or, a cumulative dose of 70 Gy applied to the nodes in case of positive lymph nodes at presentation (80% of the cases).[3]

If a "booster dose" is required for the nasopharynx after external irradiation, this can be delivered by stereotactic radiosurgery, intracavitary therapy, or three dimensional (3D) conformal or intensity-modulated radiation therapy (IMRT) techniques.[4-8] Brachytherapy has also been used to deliver a higher dose to a limited volume of the nasopharynx.[9-12]

The best treatment for early T-stage nasopharyngeal carcinoma is external beam radiotherapy in combination with brachytherapy or stereotactic radiotherapy. For advanced disease the combination of radiotherapy and neoadjuvant or concomitant chemotherapy is standard.[13,14] The combination of chemotherapy and radiotherapy is an attractive therapeutic option because of a possible synergy and the potential reduction of distant metastasis.[15-16]

RESULTS OF TREATMENT

Despite the radio-responsiveness of nasopharyngeal tumors, good long-term survival is only achieved for patients who have early primary tumors with minimal neck disease 67 to 71% 10 years' disease-free survival for T1, T2, and N0-1. Survival is poor for patients who have extended tumors and/or extended neck nodes 29 to 54% 10 years' disease-free survival for T3, T4, and N2, N3.[17-21]

Poor survival in the T4,N0-1 category is chiefly the result of the high local recurrence rate (63.8%), whereas for the T1-2,N2-3 category, it is the result of the high distant metastases rate (approximately 50%).[22]

RETREATMENT OF RECURRENT NASOPHARYNGEAL CARCINOMA

Usual treatment options for early stage recurrent or persistent NPC are surgery in combination with external radiotherapy, brachytherapy alone,[23-25] or in combination with external radiation,[26-29] or stereotactic radiosurgery.[30] The standard treatment for advanced stages of recurrent or persistent disease is chemotherapy followed by reirradiation, or concomitant chemo-reirradiation.[31] Pryzant et al[32] reported on 53 patients with locally persistent or recurrent nasopharyngeal carcinoma treated with reirradiation. Local recurrence was confined to the nasopharynx in 27 patients, and persistent tumor in 26 patients. The 5-year actuarial local tumor control rate was 35%, 5-year disease-free survival was 18%, and overall survival was 21%. Eight patients developed severe complications from retreatment, two involving the brain, one the spinal cord, and two the cranial nerves, all of which were fatal. The 5-year actuarial incidence of severe complications was 17%. The incidence of severe complications was related to the total cumulative dose of external irradiation.

Lee et al[33] described the incidence of late complications after reirradiation in 891 patients with local recurrence after definitive radiation therapy for nasopharyngeal carcinoma. After external reirradiation, brachytherapy, or a combination of both, a wide

NINE

Photodynamic Therapy for Esophageal Diseases

Wytske M. Westra
Kenneth K. Wang

RATIONALE FOR PHOTODYNAMIC THERAPY

Photodynamic therapy is ideally suited for the esophagus as it is so readily applied using the endoscope. Photodynamic therapy has always been used in areas where photoradiation can be easily delivered such as the skin where it was first applied by the ancient Egyptians and Chinese to treat skin diseases, such as psoriasis and vitiligo. This application also took advantage of the fact that phototherapy can be used to treat relatively focused areas of skin in a single application. The esophagus is easy for gastroenterologists to access via endoscopy and these endoscopes have the appropriate working channels that can pass the optical fibers needed for photoradiation. It also has the advantage of treating large areas of circumferential mucosa with a single application of light and to this day represents the technically simplest therapy available, for treatment of large areas of esophageal mucosa.

METHODS OF PHOTODYNAMIC THERAPY IN THE ESOPHAGUS

Photodynamic therapy (PDT) involves the local or systemic administration of a chemical (photosensitizer) that has a known propensity to photoexcitation when exposed to light of the appropriate wavelength. The absorption of light causes the photosensitizer to transfer from its ground state into an excited singlet state which can then either decay directly back to the ground state with fluorescence emission, or undergo further transformation into an excited triplet state. The latter can react with surrounding oxygen to form singlet oxygen, a highly cytotoxic molecule. In the esophagus, PDT has a few specialized requirements. Since the photosensitizer needs to react with surrounding oxygen to form singlet oxygen, an adequate level of molecular oxygen is needed to achieve maximal tissue damage. There is usually a requirement for the delivery of supplemental oxygen as the airway can be slightly compromised by the placement of an endoscope into the esophagus.

The Use of Photodynamic Therapy in the Management of Lung Cancer

Eric S. Edell

INTRODUCTION

Lung cancer continues to be the most common cause of cancer death in the United States accounting for more deaths than prostate cancer, breast cancer, and colorectal cancer combined. Of those patients diagnosed with lung cancer, fewer than 15% of them will survive this devastating disease. The opportunity to achieve long-term survival depends on resecting lung cancer at its earliest stage which, unfortunately, is a rarity in this disease.

The NIH sponsored studies at the Johns Hopkins University, Mayo Clinic, and Memorial Sloan-Kettering evaluating the effectiveness of screening chest x-ray and sputum cytology on lung cancer mortality. Unfortunately, there was no mortality improvement with the screening strategy; however, sputum cytology did detect more cancers in the experimental group than in the control group. It was also noted during the studies that, on occasion, patients with abnormal sputum cytology had no obvious cancer identified on routine bronchoscopic inspection. This dilemma led to the development of fluorescent detection devices that relied on photochemicals to facilitate the localization of these bronchoscopic occult cancers. Photochemicals were also known to cause cell death and thus are effective in treating cancers of the airway. The purpose of this chapter is to review the use of both photodynamic diagnosis and photodynamic therapy in the management of lung cancer.

PHOTODYNAMIC DIAGNOSIS

Photodynamic diagnosis is a term that refers to the use of devices which discriminate normal from abnormal mucosa by differences in the wavelength of fluorescence for each. Compounds such as dihematoporphyrin ether (Photofrin) and hematoporphyrin-derivative (HPD) are photochemicals that were originally used for this purpose.[1–4] These compounds accumulate in abnormal tissue at higher concentrations than normal tissue enabling localization of the abnormal tissue by detecting the fluorescence of the compounds when exposed to the appropriate wavelength of light. The amount of photochemical that accumulates in small superficial cancers of the airway is

Photodynamic Therapy in Skin Cancer of the Head and Neck

Alexander Kübler
Nicolas Hunzelmann

INTRODUCTION

More than one-third of all cancers in the United States are nonmelanomatous skin cancers.[1] Exposure to sunlight is the principal cause for this kind of tumor. For Caucasians, their incidence is strongly associated with age and cumulative ultraviolet B radiation.[2,3] However, exposure to chemical carcinogens, ionizing radiation, chronic ulceration, immunsuppression, or genetic defects also account for these tumors. Due to their genesis, most nonmelanomatous skin tumors are located in sun-exposed skin parts. Therefore, 80% of the squamous cell cancers and basal cell carcinomas of the skin are found on the hands, arms or the head and neck.[2] Even after a curative therapy the risk of subsequent skin tumors is high.[4] About 50% of the patients with a history of nonmelanomatous skin cancer will develop a new skin cancer at another site within the first 5 years independent of the primary therapy.[5] In patients with a genetic defect, immunsuppression (eg, after organ transplantation) or chemical-induced tumors, the risk of subsequent tumors can be even higher. These patients may suffer simultaneously from more than one and up to several dozen skin tumors or premalignant skin lesions at various sites, not only limited to the arms, head, or neck. On account of the link between skin tumors and ultraviolet B light exposure and the changing leisure activities in western societies, nonmelanomatous skin tumors are an emerging problem in dermatology and the most common form of cancer worldwide. An increase would also be expected with the aging of the population but there are also data indicating that the incidence is increasing in the younger population as well.[6,7]

Squamous cell carcinoma rates have increased 3% to over 10%.[7] In New Hampshire (US) the incidence of squamous cell carcinoma increased by 235% in men and 350% in women and incidence rates of basal cell carcinoma increased by more then 80% in men and women between 1979 to 1980 and 1993 to 1994.[8] More then 1 million cases of basal cell or squamous cell carcinomas occur annually in the United States.[9] Basal cell carcinoma incidence rates have increased 3% to 6% annually according to most studies throughout the industrialized world. There are marked

geographic differences in the incidence of basal cell carcinoma. These incidences are probably underestimated as nonmelanoma skin cancers tend to be underreported in cancer registries. The overall age and sex standardized annual incidence in Minnesota, USA was 146 per 100,000 and in Australia was 726 per 100,000.[10] In Wales (UK), the incidence of nonmelanoma skin cancer rose from 173 to 265 per 100,000 population between 1988 and 1998.[11] Since 2001, nonmelanoma skin cancer occupies second place in Russia; in 2004, 54.284 new cases were diagnosed, and the incidence of skin cancer was 38 per 100 000 population.[12]

Despite good accessibility of this superficial malignancy to diagnosis and treatment, there are many still unsolved problems. Skin tumors can be treated by surgical and nonsurgical methods. Dependent on tumor location, size, type (primary or recurrence), histology, patient morbidity, and preference different treatment methods like surgical excision, Mohs´ micrographic surgery, cryosurgery, curettage, laser ablation, or radiation therapy can be applied.[6] Up to now surgery has been the mainstay of therapy for nonmelanomatous skin tumors. In patients with large or multifocal tumors, a good cosmetic outcome after surgery can be difficult to obtain. This is especially true for lesions on the face or in patients with multilocalized lesions, when primary wound closure after surgery can be difficult to achieve, requiring reconstruction by plastic surgery, for example, local skin flaps, skin graft, or healing by second intention.

Photodynamic therapy (PDT) has become an important alternative therapeutic option. As most tumors of the head and neck are easy accessible for direct laser light illumination, PDT has been used in the head and neck since the beginning of the clinical application of PDT. Cutaneous lesions, premalignant lesions of the oral mucosa, and solid tumors have been treated so far. PDT is also successfully employed for the treatment of a number of nonmalignant diseases. PDT is effective and has advantages over traditional treatment modalities.[5] Tumor destruction with preservation of surrounding normal tissues provides excellent cosmetic effects which is especially important in skin tumors.[13-15] PDT is convenient and well tolerated by patients. The most frequent adverse effect is photosensitivity which can be controlled by restriction of light exposure for the period of time that it is present. For medical personnel PDT is also relatively simple procedure.[16]

For the treatment of skin tumors, intravenous applied, systemic photosensitizers like Photofrin or Foscan can be used as well as the topical applicable photosensitizer aminolevulinic acid (ALA). Due to the limited penetration depth of a topical applied photosensitizer and due to its hydrophilicity, ALA is mainly used for very superficial skin tumors not thicker than 2 mm. Compared to that, systemic photosensitizers are mainly used for tumors thicker than 2 mm.

SYSTEMIC PHOTOSENSITIZERS

In using intravenously applied photosensitizers, drug accumulation is not limited to the superficial areas of the tumor as it is for ALA.[13-17] Instead the therapy depth depends only on the light penetration capability into human tissue, which is dominated by the activation wavelength and optical properties of the illuminated tissue.[18] For several studies on intravenous photosensitizers for PDT of skin tumors, hematoporphyrin derivatives (HPD, DHE, Pho-

tofrin), photosensitizers of the first generation, were applied. Hematoporphyrin derivatives are activated at 630 nm which causes a maximum light penetration depth into the skin respectively a therapy depth of maximum 5 to 7 mm. Various drug and light dosage combinations have been used ranging from 0.5 to 5 mg/kg and 25 to 288 J/cm^2. The clinical results of these studies are difficult to compare due to the various histologic diagnosis as the distinguish treatment parameters.[19-36] There are only very few reports about the PDT of squamous cell cancer (SCC). Most of them have shown good clinical results with a complete response rate of up to 100%. Only the results of Pennington et al, who have used extreme low doses of light, were disappointing.[26] Therefore a wide range of tumor response rates between 0 and 100% is notified which varies by tumor histology and tumor location. But there is a clear tendency that by reducing the drug dosage and increasing the light energy a better selective response and better cure rate can be obtained.[32] So most investigators reported about satisfying response rates and good cosmetical results for superficial and nodular basal cell cancer (BCC) by using hematoporphyrin derivatives. But Photofrin-mediated PDT seems not to be suitable for the treatment of morphoeic BCC.[37]

The reason for the very reserved clinical application of intravenous administered photosensitzers in skin PDT might be the long-lasting systemic photosensitivity of the patients. Due to the intravenous application of the drug a systemic photosensitivity of the patient occurs which forces the patient to stay indoors, out of bright daylight for up to several weeks, depending on the photosensitizer and the drug dosage. For Photofrin this period can last up to 4 weeks, why this photosensitizer is not a real treatment option for skin tumors so far. New second-generation photosensitizers have been introduced for clinical application. These drugs are activated at a longer wavelength which results in a deeper penetration in biological tissues. They also have much faster systemic elimination which shortens the photosensitivity period.

Benzoporphyrin derivate (BPD) a photosensitizer of the second generation, has also be used for the treatment of skin tumors so far.[38] By treating 27 patients with 107 primary nonmelanomatous skin cancers and skin metastasis at various drug and light doses, a complete response rate of 57% and a partial response rate of 22% with a good cosmetic outcome could be obtained.

Compared to Photofrin the period of photosensitivity when using Focan can be shorter than 2 weeks. This is the reason why Foscan has been used in several clinical trials for the treatment of skin tumors and other tumors so far.

Foscan is an approved second-generation photosensitizer for photodynamic therapy of recurrent head and neck cancer.[39,40] The active pharmaceutical ingredient of Foscan, Temoporfin, (m-THPC) is a lipophilic chlorin derivative with absorption peaks in green and red spectral bands (514, 532, and 654 nm). This compound is characterized by a high quantum yield of singlet oxygen formation and high photodynamic activity. The unique photochemical properties of Foscan allow administration of a relatively low drug and light dose to achieve good therapeutic results.[41] There is a favorable tumor-to-normal tissue retention ratio. The longer excitation wavelength allows adequate destruction of deep tumors of up to 1 cm when using superficial illumination technique. Clinical trials of Foscan-mediated PDT demonstrated very good results in

Nursing Care of the Photodynamic Therapy (PDT) Head and Neck Patient

Carla Kane

INTRODUCTION

Photodynamic therapy (PDT) is a nonsurgical, tissue sparing, minimally invasive technique used to treat certain types of cancer. PDT has been FDA approved in the United States for esophageal and tracheobronchial cancers. PDT has been used in investigative study treatments including: cancer of the breast, colon, bladder, brain, cervix, and skin. PDT has been recognized in the medical field for treatment in the following conditions: age-related macular degeneration, dermatology, and Barrett's esophagus. PDT has been used in the treatment of early oropharyngeal primary and recurrent cancers, and palliative treatment of refractory head and neck cancers. PDT is also effective in the early recurrent carcinomas (Cis, T1, or T2) of the oral cavity, larynx, pharynx, and nasopharynx.

The primary advantage of PDT is that it causes minimal damage to healthy tissue surrounding the cancerous tumor. The side effects of PDT are minimal, easily managed, and are not permanent. PDT does not affect the white blood count, leaving the patient's immune system intact, allowing the patient a better chance to fight disease.

On the other hand, chemotherapy and radiation therapy can cause significant side effects including nausea and vomiting, fatigue, headache, internal bleeding, diarrhea, hair loss, and blood abnormalities. Some patients suffer from xerostomia (severe dry mouth) after radiation therapy, resulting in significant difficulty eating and even speaking for the rest of their life.

PDT can be used repeatedly to achieve the desired results. PDT does not exclude concurrent treatment such as surgery, chemotherapy, or radiation therapy. Between 2 to 4 weeks of time should be allotted before commencing radiation therapy. This waiting period will allow for the inflammatory response from the PDT to subside.

PDT is often conducted on an outpatient basis, allowing the patient to go home the same day as their procedure, reducing the overall health services consumption.

The downside of PDT is that for about 4 to 8 weeks, the patient could develop an extreme photosensitivity reaction if the proper light precautions are not adhered to. PDT is contraindicated in patients with porphyria or a known allergy to porphy-

Photodynamic Therapy of Recurrent Respiratory Papillomatosis

Mark J. Shikowitz
Bettie M. Steinberg
Virginia Mullooly

INTRODUCTION

The beneficial health properties of sunlight have long been established. Romans often had a special room or "solarium" for sunbathing. This represented an early form of heliotherapy. Hippocrates in the 4th century BC advocated the use of sunbaths for building up wasted muscles. He even incorporated a protective head cover, something we do during modern therapies.

There is evidence that a form of phytochemotherapy was practiced in some ancient civilizations. This involves the addition of a drug to the light therapy. In India, extracts of *Psoralea corylifolia*, now known to contain furocoumarins, was administered orally, followed by exposure to sunlight to treat vitiligo.[1]

The role of phototherapy as a useful medical treatment seemed to founder for a period of time. Niels Ryberg Finsen (1860–1904) was born in the Faroe Islands, but studied and worked in Copenhagen. He wrote a book, which was translated into English.[2] In it, he reported that sunlight or light from a carbon arc with a heat filter could be used to treat lupus vulgaris, a tuberculin skin condition. A Medical Light Institute in Copenhagen was named after him. Finsen received the Nobel Prize for his work in 1903.

Queen Alexandra brought the idea of light therapy to London. She was the president of the London Hospital in White Chapel and introduced the technique in early 1900. The light source was a carbon arc and filtered through water to dissipate the heat. The light department was still in operation into the 1920s.

Again, despite success in specific diseases, phototherapy did not advance as hoped. There were isolated packets of disease treatments, but there was no cohesive treatment theory. This included the beneficial role of light in treating rickets[3] and psoriasis[4] is well documented. The form of light treatment known to most of us is the therapy of jaundice in the newborn. Cremer et al (1958) made observations of the effect of sunlight and then artificial light on infants in the Rochford General Hospital in Essex. Up to this time, exchange transfusion was the only method for re-

ducing the effects of unconjugated bilirubin, which acts as a neural toxin.[5]

The use of photodynamic therapy (PDT), which requires three components; visible light, photosensitizer, and oxygen to kill tumor cells is not new. This technique of PDT was first used by vonTappeiner and Jesionek in 1903 with their treatment of tumors treated with eosin topically, and then exposed to visible light.[6,7] In 1905, Jesionek and vonTappeiner reported the extension of this work to include two light sources (sunlight and arc lamp) with various photosensitizers. As before, the application of the photosensitizer was local at or near the surface of the lesion. The results which were mainly on basal cell carcinomas appeared promising.[8] Despite this apparent success, no further reports of PDT immediately followed.

Recurrent respiratory papillomatosis (RRP) is a potentially life-threatening disease with a clinical course marked by multiple recurrences. Human papillomavirus (HPV) types 6 and 11, which cause RRP, exist as latent infections in morphologically normal tissue of the airway and are believed to be the source of recurrent disease.[9-12] To achieve cure, therapy must either eliminate latent infection or prevent its activation. The HPV is a commensal organism in humans, with widespread latent infection usually suppressed by the host immune system. Results of recent studies suggest that the immune response to HPV proteins is altered in patients with RRP.[13,14]

Numerous treatment modalities have been tried for RRP, with limited success in preventing recurrent disease.[15,16] These have included cryosurgery, hormones, steroids, antibiotics, autogenous vaccines, chemotherapeutic agents, interferon, and CO_2 laser excisions.[17,24] Each of these therapies has met with limited success. Initial report with interferon showed promise, but studies have shown continued therapy was required for even partial control.[19-21] Direct microlaryngoscopy with laryngeal forceps, CO_2 laser surgery and now the use of mechanical microdebriders are the most common methods of treatment today.[22,23] Our studies with PDT show it to be effective, but not ideal due to side effects.[18]

Current experimental treatments include adjuvant indole-3-carbinol, intralesional mumps vaccine[25,26] and intralesional cidofovir injections.[27] These therapies seem to be effective in a subset of patients but not all patients respond and the mechanism for the intralesional therapies are not known. In this chapter, we describe a possible mechanism for therapy with PDT or intralesional injections.

PHOTODYNAMIC ACTION

The ability of visible light damage or destruction of a living tissue with the addition of a photosensitizer was first observed by Oscar Raab in 1900. At that time, he was a medical student and spent time in the pharmacological laboratory of Professor Herman vonTappeiner working on the behavior of paramecia in the presence of small concentrations of acridine.[1,28] His initial findings were highly variable until he noticed that daylight seemed to be affecting the results. He then set up controlled experiments and showed that the acridine killed the paramecia in the presence of light but not in the dark. He also showed that light alone did not kill the paramecia in the absence of acridine.

vonTappeiner as well as others went on to develop this work. It was eventually recognized that the presence of oxygen was essential for the desired effects. vonTappeiner and Jodlbauer (1904) introduced

FOURTEEN

PDT of Bacterial and Fungal Biofilms

Merrill A. Biel

A BIOFILM is a complex aggregation of microorganisms marked by the excretion of a protective and adhesive matrix. Biofilms are often characterized by surface attachment, structural heterogeneity, genetic diversity, complex community interactions, and an extracellular matrix of polymeric substances.

Single-celled organisms generally exhibit two distinct modes of behavior. The first is the free floating, or planktonic, form in which single cells float or swim independently in some liquid medium. The second is an attached state in which cells are closely packed and firmly attached to each other and usually a solid surface. The change in behavior is triggered by many factors, including quorum sensing, as well as other mechanisms that vary between species. When a cell switches modes, it undergoes a phenotypic shift in behavior in which large suites of genes are up- and down-regulated.

Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. These first colonists adhere to the surface initially through weak, reversible van der Waals forces. If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion molecules such as pili.[1]

The first colonists facilitate the arrival of other cells by providing diverse adhesion sites and they begin to build the matrix that holds the biofilm together. Some species are not able to attach to a surface on their own but are often able to anchor themselves to the matrix or directly to earlier colonists. Once colonization has begun, the biofilm grows through a combination of cell division and recruitment.

Biofilms are usually found on solid substrates submerged in or exposed to some aqueous solution, although they can form as floating mats on liquid surfaces. Given sufficient resources for growth, a biofilm will quickly grow to be macroscopic. Biofilms can contain many different types of microorganism, for example, bacteria, archaea, protozoa, and algae; each group performing specialized metabolic functions. However, some organisms will form monospecies films under certain conditions.

The biofilm is held together and protected by a matrix of excreted polymeric

compounds called extracellular polymeric substance or exopolysaccharide (EPS). This matrix protects the cells within it and facilitates communication among them through biochemical signals. Some biofilms have been found to contain water channels that help distribute nutrients and signaling molecules.

Bacteria living in a biofilm usually have significantly different properties from free-floating bacteria of the same species, as the dense and protected environment of the film allows them to cooperate and interact in various ways. One benefit of this environment is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community. In some cases antibiotic resistance can be increased 1000 fold.[2] Kim Lewis of Northeastern University has discovered that a small fraction of cells within *E. coli* biofilms are dormant within the biofilm and almost immune to the effects of antibotics because of their very low level of metabolic activity. Once antibiotic levels drop, these dormant or "persister cells" become active and repopulate the biofilm. Persisters are not mutants, but phenotypic variants of the wild type.[3] The biofilm bacteria excrete toxins that reversibly block important processes such as translation, protecting the cell from bactericidal antibiotics that are ineffective against inactive targets. These toxins promote the creation of the persister cells.[1]

Biofilms have been found to be involved in a wide variety of microbial infections in the body, by one estimate 80% of all infections.[3] Infectious processes in which biofilms have been implicated include common problems such as urinary tract infections, catheter infections, middle-ear infections, sinusitis, formation of dental plaque, gingivitis, coating contact lenses, endocarditis, infections in cystic fibrosis, and infections of permanent indwelling devices such as joint prostheses and heart valves.[4]

In the head and neck area biofilms are a major etiologic factor in periodontitis, wound infections, oral candidiasis, and sinus and ear infections. Biofilms have been demonstrated to be present on the removed tissue of patients undergoing surgery for chronic sinusitis.[5-8] Patients with sinus biofilms were shown to have sinus mucosa that was denuded of cilia and goblet cells whereas normal controls without biofilms had normal cila and goblet cell morphology.[5] Importantly, the species of bacteria from interoperative cultures did not correspond to the bacteria species in the biofilm on the respective patient's tissue.[6] Thus, the biofilm, though a major cause of chronic sinusitis, was not present on routine culture and therefore was not treated. Due to the prevalence of biofilms as a cause of disease in the head and neck region and the significant bacterial resistance to conventional antibiotic therapies, new modalities of treatment are necessary to address this severe medical problem.

PERIODONTITIS

Periodontitis is a common infectious disease found in the majority of the adult population.[9] Periodontitis is an inflammatory condition of the periodontal tissues caused by bacterial infection that results in the progressive destruction of the periodontal connective tissue and resorption of alveolar bone. Conventional mechanical plaque removal (ie, tooth root scaling and planing) is clinically effective in most cases; however, antimicrobial treatment may also be required to achieve desired results.[10] Effectiveness of plaque, biofilm, and bacteria removal by mechanical means

can be improved on by the adjunctive use of antimicrobial agents. Numerous systemic and local antimicrobial agents have been evaluated for the treatment of periodontitis with various degrees of success.[11-15] Although the combination of mechanical and antimicrobial treatment has been shown to be more effective than either therapy alone, refractory periodontitis in many cases still remains. Periodontal biofilm is reportedly difficult to treat by either mechanical means or antimicrobial agents and is known to be contributory to chronic periodontitis.[16-20] Diminished clinical effectiveness of antibiotic therapy is reportedly due to the development of drug-resistant bacterial strains.[21-26] To address the clinical needs presented by ineffective mechanical treatment and the emergence of bacterial resistance to antibiotics, a number of investigators have examined the use of photodynamic therapy (PDT) as an alternative to antimicrobial treatments.

For the past several decades, photodynamic treatment has been reported in the literature to be effective in eradicating various microorganisms using different photosensitizers, different wavelengths of light, and different light sources.[27-38] PDT has been further studied to demonstrate its effectiveness for the eradication of both gram-negative and gram-positive antibiotic resistant bacteria.[38] Recent studies have demonstrated the effectiveness of PDT to eradicate bacteria associated with periodontitis including the anaerobic, gram-negative species *Porphyromonas gingivalis, Prevotella denticola, Fusobacterium nucleatum,* and *Capnocytophaga gingivalis;* the microaerophillic, gram-negative species *Actinobacillus actinomycetemcomitans* and the gram-negative, facultative anaerobic species *Eikenella corrodens*.[39-41] In addition, PDT treatment has also been demonstrated to be effective in controlling key virulence factors associated with periodontal bacteria such as endotoxins (eg, lipopolysaccharides) and enzymes (eg, proteases) which are primary contributors to the progressive tissue and bone destruction associated with periodontitis.[42] Most importantly, the development of bacterial resistance to PDT would appear to be unlikely as its bactericidal activity is due to conversion of cellular oxygen into singlet oxygen and other reactive species, such as hydroxyl radicals, which disrupt a number of normal cellular functions that result in cell death.[43-45]

Biel, Teichert, Sievert et al performed an in vitro experiment to determine the efficacy of PDT using a methylene blue (MB) based photosensitizer solution at various white light doses to destroy periodontal bacteria biofilms consisting of *Actinobacillus actinomycetemcomitans*.[46] This study demonstrated that white light doses of 10 and 20 J/cm^2 with MB concentrations of 200 and 300 µg/mL resulted in complete eradication of the *Actinobacillus actinomycetemcomitans* biofilm ($p < 0.05$) (Figs 14-1 and 14-2).

Metcalf et al performed an in vitro experiment to evaluate erythrosine mediated PDT of *Streptococcus mutans* biofilms using white light.[47-48] They demonstrated that one light treatment resulted in a 2 log reduction of the biofilm but that a fractionated light dosing scheme resulted in a 3.7 log reduction on the *Streptococcus mutans* biofilm.

Zanin, Lobo, et al performed an in vitro study of the effect of toluidine blue O-mediated PDT on *Streptococcus mutans, S. sobrinus*, and *S. sanguinis* biofilms. They demonstrated a 95% reduction of *S. mutans* and *S. sobrinus* and a 99% reduction of *S. sanguinis* biofilms using toluidine blue O at 0.1mg/ml at a light dose of 85.7 J/cm^2.[49]