Photodynamic Therapy in Cutaneous Malignancy

A thesis submitted for the degree of Doctor of Medicine

by

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Declaration

I declare that I have personally performed the major part of the work relating to this thesis. The involvement of other individuals is detailed in the following pages, related to collaborative work. Sections of the thesis have been published and these references are listed separately. I confirm that the entire thesis has not been previously published or submitted for any other higher degree.

Colin A. Morton

Collaborative work

The inventors of the light unit used in the clinical trials of photodynamic therapy for non-melanoma skin cancer, Drs. C. Whitehurst and J.V. Moore, advised on the calibration and use of the light source. On-site monitoring of equipment was undertaken by the University Dept. of Medical Physics initially by Dr. H. Moseley, then by Dr. D. Keating. The design, performance and analysis of these experiments, however, were undertaken by myself under the supervision of Professor R. M. MacKie.

The in-vivo studies with mice were undertaken by myself under the supervision of Dr. A. Oseroff, Head of the Department of Dermatology, Roswell Park Cancer Institute in Buffalo, NY. The study of photodynamic therapy in cutaneous T-cell lymphoma was conducted by Dr. Oseroff with my role (during a 6 week visit) being to jointly undertake the final analysis of patients and then solely to collect and interpret the entire study data, both written and photographic.

Statistical advice for the Glasgow trials was provided by Dr. J. McColl, University Department of statistics, and for the American studies by Dr. J Whitaker, Dept. of Dermatology, Buffalo, NY..

Acknowledgements

I would like to take the opportunity to express my thanks to Professor R. M. MacKie, whose expert guidance was available throughout this work. Her great interest in the studies contained in this thesis and enthusiasm towards the development of photodynamic therapy in dermatology were a source of considerable encouragement to me.

I wish also to thank Dr. A. Oseroff, Buffalo, for welcoming me to his unit for a short, but intense, period of research and analysis at Roswell Park Cancer Institute, to a department which was the first to recognise the potential of photodynamic therapy in dermatology.

The studies in Glasgow received no external funding. The visit to Roswell Park Cancer Institute, Buffalo, was sponsored by awards from the Royal College of Physicians and Surgeons, Glasgow, and the British Association of Dermatologists, London.

Publications

Some of the work involved in this thesis has appeared in print in the publications listed below:

1. Morton CA, Whitehurst C, Moseley H, et al. Development of an alternative light source to lasers for photodynamic therapy: Clinical evaluation in the treatment of pre-malignant non-melanoma skin cancer. Lasers Med Sci. 1995; 10: 165-71.
2. Morton CA, Whitehurst C, Moseley H, et al. Comparison of photodynamic therapy with cryotherapy in the treatment of Bowen's disease. Br J Dermatol. 1996; 135: 766-71.
3. Morton CA, Whitehurst C, McColl JH, et al. Photodynamic Therapy for Basal Cell Carcinoma - Effect of Tumour Thickness and Duration of Photosensitizer Application on Response. Archives of Dermatology. 1998; 134: 248-9.

Abstracts

1. Morton CA, Whitehurst C, Moseley H, Moore JV, MacKie RM. Photodynamic therapy - clinical evaluation of a non-laser light source. Br. J Dermatol 1995; 133 (Suppl 45): 62.
2. Morton CA, Whitehurst C, Moore JV, MacKie RM. Comparison of photodynamic therapy with cryotherapy in Bowen's disease. Photodermatol Photoimmunol Photomed 1996: 12; 38.
3. Morton CA, Whitehurst C, Moseley H, Moore JV, MacKie RM. Photodynamic therapy for Bowen's disease and basal cell carcinoma - an effective therapy? Br. J Dermatol 1996; 135 (Suppl 47): 22-3.
4. Morton CA, Whitehurst C, Moseley H, Moore JV, MacKie RM. Photodynamic therapy for basal cell carcinoma - tumour thickness a predictor of response. Br. J Dermatol 1996; 135 (Suppl 47): 66.

Summary

Introduction: The aim of this thesis was to critically evaluate the potential of photodynamic therapy (PDT), using a novel non-laser light source and a topically active photosensitizing agent, 5-aminolaevulinic acid (5-ALA), for use in the management of skin cancer. PDT primarily acts via the activation by visible light, of a photosensitizer located within the neoplastic tissue, to produce activated oxygen species, especially singlet oxygen, in order to promote tumour destruction.

Lasers have been the usual source of irradiation in PDT although coherence of light is not required. Recent developments in light technology have enabled the production of a relatively inexpensive non-coherent light source of similar intensity and wavelength to laser. The development of a topically active agent, 5-aminolaevulinic acid (5-ALA), converted within cells to the active photosensitizer, protoporphyrin IX, can now avoid prolonged generalized photosensitivity reactions which limited the development of PDT as a practical clinical therapy.

Published clinical data on ALA-PDT available at the commencement of this thesis related predominantly to the use of lasers in open studies, with short term follow-up. The potential of non-laser light had received only limited interest and no randomized comparison trials of PDT had been undertaken.

Methods: Local hospital ethical committee approval was acquired for the clinical studies. A prototype light source, incorporating a 300W xenon short arc discharge source, (Cancer Research Campaign, U.K.), with a proven efficacy from *in-vitro* and *in-vivo* studies comparable to laser, was used in all U.K. studies. 5-ALA (20% w/w) was applied 4 or 6 hours (Bowen's disease and basal cell carcinoma) or 24 hours (cutaneous T-cell lymphoma) pre-

illumination. All studies using the prototype source were performed in the U.K. by myself. Two murine studies were undertaken by myself in Buffalo, NY where I also participated in a clinical study of ALA-PDT in cutaneous T-cell lymphoma (CTCL).

Chapter outline: Chapter 1 reviews the development of both the photosensitizers and light sources for PDT along with a detailed description of the photodynamic reaction and its acute and chronic effects. Chapter 2 reviews the literature on the use of PDT in cutaneous malignancy with comparison of individual study protocols.

Chapter 3 summarizes the clinical trials of topical ALA-PDT using the prototype xenon source, with output filtered to 630±15nm. The studies and principal findings are listed below:

1. Actinic keratoses and Bowen's disease - Pilot study: 24 lesions, 100% initial clearance, 2 recurrences over 12 months, pain absent/mild during therapy in 22 lesions, local anaesthesia required in only one ulcerated lesion. This confirmed the clinical efficacy of ALA-PDT using the prototype lamp.

2. ALA-PDT vs. cryotherapy in Bowen's disease - Randomized comparison study: 40 lesions, PDT at least as effective as cryotherapy with a higher probability of clearance after one treatment with PDT. Cryotherapy was associated with more frequent adverse effects with ulceration and wound infection, effects not observed following PDT.

3. Effect of tumour thickness and time of 5-ALA application on BCC response - Comparison study, 53 lesions, tumour thickness predicted therapeutic response ($p<0001$). PDT most effective for BCC up to 1mm in depth (CR = 100%) with a higher clearance rate for thicker lesions (1-2mm) when 5-ALA application was increased from 4 hours (CR = 43%) to 6 hours (CR = 100%).

4. Light dose/response study of ALA-PDT for Bowen's disease - Randomized comparison study, 122 lesions, 25-125J/cm^2 of red light (630±15nm) was

more effective than lower doses in clearing lesions and minimizing recurrence ($p<0.001$).

5. ALA-PDT for large patch Bowen's disease - Open study, 40 lesions, 88% initial clearance after 1-3 treatment sessions, with inferior response for larger lesions. Four recurrences over 12 months of follow-up.

6. Red vs. green light for ALA-PDT in Bowen's disease - Randomized comparison study, 61 lesions, with complete response rate at 12 months using red light of 88%, compared with 48% using a theoretically equivalent dose of green light ($p=0.002$).

Chapter 4 examines the macroscopic and microscopic skin changes induced by ALA-PDT using the prototype lamp. Oedema and erythema developed during therapy and settled over 48 hours. Although occasional blister formation occurred, ALA-PDT only induced epidermal ulceration following therapy in one patient. Whilst there are no observed specific histological changes of ALA-PDT, both necrosis and apoptosis occur. Microscopic evidence of scar formation developed in a few basal cell carcinomas, but rarely occurred after PDT to other lesions.

In chapter 5, two murine studies were undertaken to determine whether a photoproduct of PPIX, activated by 670nm light, can be detected *in vivo* and alter PDT response. The initial study of cutaneous damage induced by 635 with or without 670nm light indicated phototoxicity attributable to a photoproduct at 670nm. Dual wavelength illumination appeared to be responsible for an improved tumour response to ALA-PDT in the second study. These pilot studies suggest a therapeutic benefit from inclusion of 670nm in the light spectrum in ALA-PDT.

Chapter 6 summarizes the potential for ALA-PDT in the treatment of CTCL. Previously restricted to 2 case reports, this is described in detail for patch, plaque and tumour stage disease in patients with multiple lesions. Complete clearance was reported for 50%, 56% and 33% of 14 patch, 18

plaque and 21 tumours following 2 treatment sessions with increased response following further treatments. Five lesions recurred during a 12 month follow-up period. This gives an overall response rate of 45% which requires comparison with existing modalities to determine the place of PDT in CTCL management.

Overview: This thesis progresses the development of ALA-PDT in cutaneous malignancy. Randomized comparison or large open studies have permitted the refinement of protocols, assessment of clinical efficacy and adverse reactions, and monitored long term follow-up. Several studies confirm the efficacy of a compact non-laser source in PDT which, combined with topical application of photosensitizer and protocols to permit day/out-patient therapy, endorses ALA-PDT as a practical clinical modality.

Topical ALA-PDT is confirmed as an effective therapy for certain non-melanoma skin cancers, in particular, Bowen's disease and superficial BCC. PDT offers the advantages of being non-invasive, well tolerated in slow healing sites, and tissue sparing, leaving the skin surrounding the tumour intact and functional. ALA-PDT is demonstrated to be particularly useful for large superficial tumours and for lesions in anatomical sites where disfigurement from conventional therapies may be a particular risk. An initial assessment of ALA-PDT in CTCL also suggests that this may be a useful additional modality.

The poor efficacy of 4 hour ALA-PDT for lesions greater than 2mm in depth represents a major limitation for its use in nodular BCC and other potentially metastatic lesions, such as squamous cell carcinoma. New photosensitizers and the further refinement of treatment protocols may increase the depth of action of ALA-PDT. Utilizing the immunological effects of PDT may also allow improvements in response at tissue depths beyond that predicted by light attenuation and photosensitizer absorption.

The rising incidence of cutaneous malignancy and the requirement to provide effective, yet well tolerated therapy with good cosmesis, suggest that ALA-PDT, using a relatively inexpensive non-laser source, can be an effective additional modality, although its current clinical use should be restricted to in-situ squamous cell carcinoma and superficial basal cell carcinoma.

Abbreviations

5-ALA	5-aminolaevulinic acid
AK	Actinic keratoses
$AlPcS_n$	Sulfonated aluminium phthalocyanines
ArPDL	Argon pumped dye laser
AuVL	Gold vapour laser
BCC	Basal cell carcinoma
BpD-MA	Benzoporphyrin derivative monoacid
CuVDL	Copper vapour-dye laser
CTCL	Cutaneous T-cell lymphoma
He/Ne	Helium-neon laser
HpD	Haematoporphyrin derivative,
Nd:YAGL	Neodymium:Yag-dye laser
NPe_6	N-aspartyl chlorin e_6
IL-6	Interleukin 6
IL-10	Interleukin 10
PDT	Photodynamic therapy
PpIX	Protoporphyrin IX
SCC	Squamous cell carcinoma
$SnET_2$	Tin etiopurpurin
mTHPC	Meso-tetra-(hydroxyphenyl)-chlorin
$TPPS_4$	Tetraphenylporphine sulphonate
Xe	Xenon
ZnPc	Zinc phthalocyanine

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Chapter 1 - Photodynamic therapy - development, mechanisms of action and applications

1.1 - Introduction

Photodynamic therapy (PDT) is the activation of a photosensitizing drug by visible light to produce activated oxygen species within the neoplastic/dysplastic tissue. The photosensitizer or precursor requires to first be introduced into the dysplastic tissue and should be retained there at higher concentration than in the normal surrounding tissue to permit a disease-localizing therapeutic effect. This tissue is then exposed to light of a wavelength appropriate for absorption by the photosensitizer. Through several photophysical pathways, reactive oxygen species harmful to cell function, including singlet oxygen, are produced and eventual tissue destruction results.

Despite the concept of photodynamic therapy being first described almost 100 years ago, the development of PDT as a potential clinical modality has occurred only during the past 20 years with topical therapy first reported as recently as 1990. Section 1.2 outlines the history of the development of PDT.

A comprehensive understanding of the mechanisms involved in PDT tumour destruction has yet to emerge. Variations in treatment protocol are likely to determine the relative importance of the various cellular and vascular events occurring during PDT to achieve a therapeutic effect. Sections 3-7 of this chapter summarize current knowledge of these mechanisms with emphasis on those known to be relevant to the *in-vivo* PDT response.

PDT has been used to successfully treat a variety of cutaneous and non-cutaneous neoplasms with a few non-neoplastic applications also under assessment. World-wide, PDT has been approved for clinical use in one or more countries for the treatment of tumours of the bladder, lung, oesophagus,

stomach and cervix. PDT is licensed for the treatment of early superficial neoplasia and the palliation of late disease of these organs. Section 1.8 summarizes the potential non-cutaneous applications of PDT and current interest in its use for inflammatory cutaneous disorders.

1.2 The development of photodynamic therapy

The term 'photodynamic therapy' was first used in 1907 by von Tappeiner following experiments using eosin and a combination of natural and artificial light in the treatment of skin cancer, lupus of the skin and condylomata of the female genitalia.[1] He realized the requirement for oxygen, describing the phenomenon as an oxygen-dependent photosensitization. Around this time, the first studies of the biological properties of haematoporphyrin were reported. Meyer-Betz[2] observed photosensitivity of light-exposed sites which persisted for more than two months following self-injection of the agent. The subsequent observation of selective localization of porphyrins to tumours[3] (1924) and the demonstration of a photodynamic action involving haematoporphyrin in tumours[4] (1942) raised interest in PDT. However, large doses of the crude photosensitizer were required and the consequent phototoxicity limited development. Partial purification produced haematoporphyrin derivative (HpD) which had greater tumour-localising properties. PDT using HpD in human cancer was reported in 1967 by Lipson *et al*[5]. The first human trial of PDT using HpD was performed by Dougherty *et al*[6] in 1978 in a variety of malignancies, with complete or partial response in 111 of 113 lesions. Tumours treated included carcinomas of the breast, colon, prostate, squamous cell, basal cell and endometrium, mycosis fungoides, chondrosarcoma and angiosarcoma. Many studies have subsequently reported the successful use of PDT with HpD or

its more purified form, porfimer sodium (Photofrin II), in the treatment of various cutaneous and non-cutaneous malignancies.[7-9]

Whilst Hpd and porfimer sodium require systemic administration, Kennedy et al[10] reported in 1990 a topically active agent, 5-aminolaevulinic acid (5-ALA), which is converted within cells to the active photosensitizer, protoporphyrin IX (PpIX). This avoids the problem of generalized photosensitivity which followed the use of HpD and could last 6-10 weeks.[11,12] PDT using topical 5-ALA now offers the potential of a practical, out-patient based, alternative treatment modality for dermatology.

1.3 Mechanisms of action - Photosensitizers and their delivery

Photosensitizers possess the ability to transform absorbed light energy (photoexcitation) into chemical energy. The initial stage of PDT involves delivering the photosensitizer to the target tissue. Photosensitizers are distributed to and retained by both normal as well as neoplastic tissues.[13] There is considerable variation between agents in the time interval between administration and peak sensitizer tissue levels, and in the duration of sensitizer retention in tissues, although much of this work relates to *in-vivo* experiments in mice.[14] Despite the wide distribution of systemically administered photosensitizer, rodent tumour models demonstrate a poorly understood, partially selective, tumour response. These responses appear to be even more pronounced in human malignant skin lesions. A gradient of differential sensitizer concentration between tumour and surrounding tissue has been demonstrated, but differs between sensitizer and tumour type. The photosensitizer chloraluminium phthalocyanine ($AlPcS_n$) is found following injection into mice in a ratio of 2:1 tumour:normal tissue concentration in UV-2237 fibrosarcoma, but 10:1 for the Colo 26 carcinoma.[14] Photofrin II tumour:skin ratios are usually less than 2:1 in transplantable rodent tumours

and certain normal tissues (liver, spleen) may retain more photosensitizer than tumours.[15-17]

For skin tumours in humans, the topical route of application of 5-ALA permits an alternative method of tumour selectivity. 5-ALA is a precursor, in the haem biosynthesis pathway, of protoporphyrin IX (PpIX), an endogenous photosensitizer not normally present within tissue in therapeutically useful concentrations.[18] Exogenous administration of 5-ALA, however, can increase the intracellular concentration of PpIX as 5-ALA is the first precursor of haem after the feedback control point and the conversion of PpIX to haem is relatively slow. Local application of 5-ALA is possible due to its increased passage, when in aqueous solution, through an abnormal epidermis thus restricting the photosensitization primarily to the tumour sites. This tissue selectivity in 5-ALA photodynamic therapy can be demonstrated by the detection of PpIX-induced fluorescence. Svanberg *et al*[19] report a 15:1 tumour:normal ratio in basal cell carcinomas (BCC) and in Bowen's disease 6 hours following the topical application of 5-ALA.

5-ALA induced PpIX accumulates, after topical or systemic administration, primarily in epithelia (epidermis, conjunctiva, oral, respiratory and vaginal mucosae, serosal surfaces, endometrium and urothelium) or in glands and ducts (sebaceous, mammary, salivary glands and seminal vesicles), but not in mesodermal tissues.[10] This may be due to different capacities of cells to synthesize haem or differences in feedback control. Proliferating, relatively iron deficient, tumour cells preferentially accumulate PpIX as iron is required for the final conversion of PpIX into haem. This is described as the biological selectivity of 5-ALA for tumour tissue.[20]

For exogenous photosensitizers, including Photofrin, where systemic administration is required, there remains only a limited understanding of the mechanism of increased tumour accumulation/retention. Porous tumour vasculature may favour accumulation and poor lymphatic drainage may

promote retention of the photosensitizer. Increased low-density lipoprotein mediated endocytosis activity of tumour cells may provide a more specific mechanism for uptake.[13] Selective retention may also be related to the decreased pH in tumours as sensitizers become more water soluble as pH is decreased and are thus less able to leave the tumour cell.[21]

The ideal photosensitizer should have a short half life, specific binding to the tumour, high quantum yield of singlet oxygen, and an activation spectrum between 700-800nm to optimise tissue penetration. Several new agents are under evaluation for photodynamic therapy; benzoporphyrin derivative monoacid (BpD-MA), meso-tetra-(hydroxyphenyl)-chlorin (mTHPC), N-aspartyl chlorin e_6 (NPe_6), tin etiopurpurin ($SnET_2$), tetraphenylporphine sulphonate ($TPPS_4$), zinc phthalocyanine (ZnPc) and sulfonated aluminium phthalocyanines ($AlPcS_n$).[22] Chlorins, which include BpD-MA, are reduced hydrophilic porphyrins and purpurins are chlorins with one reduced pyrrole group. Porphines and phalocyanines are synthetic porphyrins. These new photosensitizers are more potent than porfimer sodium and are more rapidly cleared following systemic administration, exemplified in BpD-MA which causes cutaneous sensitivity for only 3-5 days following systemic administration. Another group of photosensitizers are the cationic dyes, such as rhodamine 123, which are lipophilic, penetrating plasma membranes easily and accumulating less in skin than porphyrin-derived agents.[23] They thus have a potential to treat tumours and spare overlying epidermis. However, some of these agents exhibit toxicity in the absence of light, limiting their suitability.

The *in-vivo* pharmacokinetics and biodistribution of photosensitizers are affected by their water solubility, with hydrophilic agents requiring a suitable vehicle (e.g. oil in water emulsions) to facilitate their delivery, usually via serum components, to the tumour tissue. Lipophilic (hydrophobic) dyes , such as porfimer sodium, PpIX, HpD and the aluminium phthalocyanine -

$AlPcS_1$, localize to membrane structures (mitochondrial membranes, plasma and nuclear membranes and membranes of the endoplasmic reticulum), whereas more hydrophilic dyes, such as the aluminium phthalocyanines $AlPcS_{2a}$, and $AlPcS_4$, and NPe_6, localize to lysosomes. Both the phthalocyanines and porphyrins increase in hydrophilicity as the number of sulphonated groups increases. Amphiphilic photosensitizers have hydrophobic and hydrophilic regions and include the most potent PDT agents, mTHPC and BpD-MA, with respect to direct activity on neoplastic cells.[24,25]

The predominant mechanism of action of PDT is presumed to be direct tumour cell kill although in PDT with systemically administered photosensitizers, an important contribution to tumour destruction is achieved via damage to the vascular supply. Topical ALA-PDT, however, would appear to rely on direct tumour cell destruction to achieve a therapeutic effect, with a recent study demonstrating no direct vascular damage following this type of PDT.[26]

Most photosensitizers, given appropriate excitation, can be made to fluoresce, so that their distribution can be studied by fluorescence microscopy. Although naturally occurring PpIX contributes to the autofluorescence of normal skin, exogenous ALA-induced PpIX offers a means of enhancing contrast and its detection affords a valuable method of evaluating its method of action in PDT.[16] Fluorescence detection can also be used as a method of detecting neoplasia.

1.4 Mechanisms of action - Light delivery

Light of appropriate wavelength for activation of the photosensitizer is required in the target tissue. A 'therapeutic window' between 600-800nm exists with shorter wavelength light undergoing significant absorption by

endogenous chromophores in the skin, primarily haemoglobin, and longer wavelengths having insufficient energy to participate in photochemical reactions.[27]

Light fluence in tissue decreases exponentially with distance, affected by optical scattering and absorption by endogenous chromophores, both parameters differing between tissues. Whilst 630nm light may penetrate up to 6mm, compared to 1-2mm for light at 400-500nm,[28,29] the therapeutically effective maximum depth of PDT will depend on sufficient light dose being delivered to the tissue to achieve a photodynamic reaction. The therapeutically effective depth of PDT in the skin is therefore likely to be considerably less, at 1-3mm at 630nm, depending on the tissue.[13]

Most clinical applications of PDT have used red light of 630nm to achieve adequate penetration. 5-ALA-induced photosensitivity has a porphyrin-like spectrum with maximum excitation at 410nm and three smaller peaks at 510, 545 and 580nm.[30] Using shorter wavelength light could thus achieve more efficient activation of PpIX, but at the expense of depth of therapeutic effect. The new photosensitizers listed above have peak absorption wavelengths beyond 650nm and an extinction coefficient (measure of their ability to absorb light) at their maximal long wavelength absorption, at least 10-fold greater than porfimer sodium at 630nm.[22]

The biological effect in PDT is proportional to the amount of light energy reaching the tissue multiplied by the absorption coefficient of the photosensitizer.[31] As the concentration of photosensitizer in tissue and the light dose absorbed by the photosensitizer are often not known, dosage is usually estimated from the energy fluence. This assumes that a given dose of photosensitizer will be distributed in a tissue in the same way and that tissue oxygenation does not become a limiting factor in the photodynamic process.

A further complication in calculating the 'photodynamic dose' relates to the dosimetry of the light source used. To permit comparison of the light dose used from different laser and non-laser sources, the concept of total effective fluence has been developed. Total effective fluence rate (E_d) is defined at a specified tissue depth , *d* , by the equation:

$$E_d = \int I(\lambda) T_d(\lambda)\, A(\lambda)\, d(\lambda)$$

where: $I(\lambda)$ is incident spectral irradiance from light source at wavelength λ; $T_d(\lambda)$ is optical transmission through tissue to depth *d* at wavelength λ and $A(\lambda)$ is absorption by sensitizer at wavelength λ.[32] As the emission spectra of laser and non-laser sources are very different, measurements of total power or irradiance are insufficient to characterize each source as they do not quantify the dose of appropriate wavelength light at the required depth in tissue available for photodynamic therapy. This model has been used to compare the non-laser lamp used in this thesis with other sources including laser (Chapter 3).

Calculation of the requirements for light in PDT are further complicated by self-shielding and photo-bleaching. Self-shielding concerns the absorption of light by superficially placed photosensitizer thus limiting further tissue light penetration. Photo-bleaching is the process of photo-destruction of the sensitizer during light exposure, a helpful event in counteracting self-shielding. As there appears to be a threshold photodynamic dose to produce tissue necrosis,[33,34] photo-bleaching may also be useful if it occurs in normal tissue before this threshold is reached, but is undesirable in tumour tissue. Although the ratio of photsensitizer in tumour:normal tissue may not always exceed unity, selective tumour treatment can still be realized by selective light delivery.

Most studies of PDT have used laser sources to provide light of sufficient intensity and at the appropriate wavelength for drug activation, with the opportunity of delivery to internal body surfaces via fibre optics, which

can also allow for the illumination of thick tumours via implanted applicators. The most widely used lasers for PDT have been argon ion- and copper vapour-pumped dye lasers.[7-9] They can produce 1-3 watts of red light (at 630nm) for use in PDT. However, their expense, specialist support requirements, size, and limited availability has stimulated a search for cheaper, portable light sources that could be easily used in clinical practice. Laser diode arrays may provide a cheaper source of a fixed intense narrowband light. However, although they are able to produce 3-4 watts of light between 670-690nm, they cannot currently produce sufficient intensity of light in the 630nm region.

The recent development of topical ALA-based PDT,[10] has accelerated the need for an effective simple method of light delivery in PDT. Most noncoherent sources are based around incandescent or arc lamp sources. Modified slide projectors, by filtering out light below 600nm, have been used by several groups,[35,36] although the light filtered out may account for over 60% of the light energy available, leaving relatively inefficient sources of light, particularly if light of narrow-wavelength is desired. Sziemes *et al*[37] recently described a new source, a 1200W metal halogen lamp, although this also emits a relatively broad band of light between 600-800nm. The lamp which I evaluate in this thesis, incorporating a xenon short arc source, represents a novel approach to this requirement for a powerful, yet portable non-coherent source of light which is tuneable over a wide wavelength range and can provide therapeutically effective levels of power output within a narrow bandwidth.

1.5 - The photodynamic effect

The rationale of the therapeutic efficacy of PDT is based on the cytotoxic action of products generated by excited photosensitizers. The

excited photosensitizer can react directly with tissue constituents through the so-called type I process, yielding radicals or radical ions, or through the type II process in which energy is transferred to singlet oxygen. When a photosensitizer absorbs light of the appropriate wavelength it is converted from a stable electronic structure (ground state) to an excited singlet state. This short-lived singlet state may undergo conversion to a longer-lived excited triplet state (lifetime 10^{-3}-10s), which is the photo-active species responsible for the generation of cytotoxic products. This may either directly react with substrate by hydrogen atom or electron transfer to form radicals (type I reaction), or the triplet state can transfer its energy to oxygen directly to produce singlet oxygen (lifetime $0.6x10^{-6}$s in a cellular environment), which is highly reactive in biological systems (type II reaction), causing photo-oxidation and cell death.[38,39] Evidence for the involvement of the superoxide ion in some aspects of PDT damage suggest that both Type I and Type II reactions occur with ratio dependent on the photosensitizer, substrate and oxygen concentrations.[40]

Whilst the most important path leading to tumour necrosis in PDT appears to be that of singlet oxygen (type II), direct measurement in complex biological systems is difficult. The complete process, with excitation of photosensitizer, transfer of energy through intersystem crossings, to excitation of oxygen from its triplet state and the subsequent quenching of singlet oxygen through cytotoxic mechanisms, takes place in a time scale of microseconds.

1.6 - Acute effects of photodynamic therapy on tissues

The effect of photodynamic therapy on cells depends on the concentration and localization of the sensitizer and its efficiency in that environment, the light dose reaching the cell and the oxygen supply.

As the diffusion distance of singlet oxygen in cells is estimated to be only 0.1μm, cell damage is likely to be close to the site of its generation.[39] The intracellular distribution of the photosensitizer will therefore influence the target sites and depends on their relative lipophilicity/hydrophilicity and polarity.[24]

For lipophilic photosensitizers, including Photofrin, protoporphyrin IX and the phthalocyanines, inhibition of mitochondrial enzymes may be the key event in PDT cell death. However, the inactivation of membrane transport systems, depolarisation of the plasma membrane and inhibition of DNA repair enzymes may precede inactivation of the mitochondrial, cytosolic and lysosomal enzymes.[39,41-42] The release of lysosomal hydrolytic enzymes are presumed the main event in promoting cell death for hydrophilic agents such as the sulphonated phthalocyanines, tetraphenylporphine, and mono-l-aspartyl chlorin.[43] Certain cationic sensitizers accumulate preferentially in mitochondria due to electrical potential gradients across the membrane.[23,44]

Following *in vitro* PDT using photofrin II, several inflammatory mediators are released including several eicosanoids ($PGF_{2\alpha}$, 6-keto-$PGF1_{\alpha}$, arachidonic acid) and histamine.[45-47] These substances may contribute to the vascular effects of PDT *in-vivo*. The level of circulating photosensitizer is the main determinant for vascular photosensitivity with tumour cures reported in mice after exposure to light immediately after photosensitizer injection when blood drug levels were high and tumour cell localization absent.[48] No significant differential in sensitivity between normal and tumour vessels has been reported with vascular shut-down in rodent tumours only preceding that of the surrounding tissue at sub-therapeutic PDT doses.[49] Vascular photosensitivity has been observed *in-vivo* with several anionic agents, including porphyrins, purpurins, phthalocyanines and chlorins.[50-52] Activation of these photosensitizers results in the reduction of blood flow both via vasoconstriction, thrombus formation and embolization, with a low rate of

reperfusion observed.[50] The resulting tumour hypoxia may limit the cellular photodynamic process. The initiating events in PDT-induced vascular damage in humans remains unknown.

Lymphocytes, plasma cells and histiocytes infiltrate PDT-treated tissue suggesting an immune response. However it remains unclear whether this represents a specific PDT response, or is simply related to the degree of inflammation present.[53] At present, it remains unclear whether cytokines (Interleukin 1-beta, Interleukin 2 and tumour necrosis factor-alpha) noted for up to 50 days following bladder PDT treatment were merely a function of the associated inflammation or a specific PDT response.[54]

1.7 - Late effects of photodynamic therapy

Generalized photosensitivity up to 6-10 weeks following injection of Photofrin has been reported[11,12] although newer systemic photosensitizers under development are likely to have shorter intervals of skin sensitivity with murine studies reporting loss of skin sensitivity within 2 weeks for AlPcSn, 9 days for SnET2 and by 24 hours for NPe6.[55-57]

Preservation of the structural and function of collagen after Photofrin- and $AlPcS_n$-PDT is superior to that after hyperthermia[58] and probably contributes to the good cosmesis after PDT of skin lesions.

Nuclear damage has been observed in PDT with single strand breaks, alkali-labile lesions, DNA-DNA and DNA-protein crosslinking and sister chromatid changes. *In-vitro* studies with haematoporphyrin derivative and phthalocyanines, whilst demonstrating nuclear damage, have shown no mutagenic activity above background levels.[59] The mutagenic potential of PDT appears to be much lower than for x-ray or UVC treatment. While nuclear damage can be induced by PDT, as there are also many cytoplasmic and membrane targets for photooxidation, cell death is presumed to occur

rather than the induction of mutagenic or carcinogenic phenotypes. The importance of nuclear effects in cell killing in PDT thus appear to be minor, with membrane disturbance probably having a predominant role in porphyrin-mediated cell inactivation. To date, only one tumour, a melanoma, has been reported, arising on the scalp of an 82yr. old female with extensive actinic damage and several squamous cell carcinomas,[60] who had 1 year previously received 4 sessions of ALA-PDT to that site. Whether or not PDT was causative or coincidental to melanoma development is unknown, but it emphasizes the need for careful review of study patients following PDT to establish the frequency of late complications and in particular any neoplastic effects.

1.8 Applications of photodynamic therapy

A wide variety of potential applications for PDT in medicine are now emerging. Clinical studies have reported the therapeutic action of PDT in various cutaneous and non-cutaneous neoplasms.[7-9] PDT can be used for the curative treatment of early, superficial neoplasia, especially tumours lining body surfaces which can be accessed by light either directly or via an endoscope. It may also be applications as an adjunct to surgery or in the palliation of advanced malignancy.

Cutaneous applications

Non-melanoma skin cancer, Kaposi's sarcoma and cutaneous T-cell lymphoma can all respond to PDT (Chapter 2). Improvement in plaques of psoriasis following photodynamic therapy have been reported using systemic haematoporphyrin derivative and ultraviolet light,[61] 630nm laser light,[62] or broadband visible non-laser light.[63] Boehncke *et al*[64] have also reported treatment of chronic plaque psoriasis in three patients using ALA-PDT, with

an outcome comparable to that achieved using dithranol. However, a recent dose-response study[65] has failed to demonstrate a regime that provides for a predictable efficacy, possibly related to variation in photsensitizer uptake between plaques in the same patient and between patients despite standard quantities of agent being applied.[66]

Two patients with alopecia areata have also been reported as responding to haematoporphyrin plus UVA light after 8 -10 weeks of thrice weekly therapy with coarse terminal hair regrowing after 12-16 weeks.[67] Control sites treated with UVA alone showed no response.

Non-cutaneous applications

Phase I-II trials of PDT have shown complete eradication of tumour in around 90% of patients with early stage lung cancer with disease-free survival of up to 11 years.[68-70] PDT may also convert inoperable into resectable tumours, and reduce the extent of surgery required.[71]

PDT for squamous cell carcinomas of the oesophagus, confined to the mucosa/muscularis mucosa, achieves a 5-year survival of 90-100%.[72] It may also be superior to laser therapy for the palliation of advanced oesophageal carcinoma.[73] Complete remission of 34/207 inoperable or recurrent advanced tumours of the oesophagus and stomach using haematoporphyrin has been reported.[74] PDT has been observed in several centres to be effective in promoting the clearance of metaplasia and dysplasia in Barrett's oesophagus.[75]

Although PDT is effective for in-situ carcinoma of the bladder, early experiments, using haematoporphyrin and dihaematoporphyrin, were associated with high recurrence rates and the risk of reduction in bladder capacity which usually resolved by 3 months, but could be permanent.[76-80] As an *in-vivo* study with ALA suggests detrusor muscle damage and fibrosis is less likely[80], ALA-PDT may be preferable for bladder tumours.

Small clinical studies indicate a primary, adjuvant or palliative role for PDT in gynaecological neoplasia, head and neck tumours, and even as adjunctive therapy following surgical debulking of cerebral gliomas and pleural mesotheliomas.[8]

The use of lethal photosensitization to kill microorganisms, although not a new concept, is receiving renewed attention with the development of PDT for clinical applications. The prerequisite for photosensitization of a microbial cell is the binding of porphyrin to the cytoplasmic membrane. Gram positive bacteria appear particularly susceptible, including methicillin resistant *Staphlococcus aureus* and *Propionobacterium acnes.*[81] The gram-negative bacteria *Helicobacter pylori*, has also been shown to be successfully killed in-vitro by PDT.[82] The susceptibility of the bacteria and yeasts responsible for periodontal disease[83] and the inactivation of parasites and viruses , including human immunodeficiency virus, by PDT in the sterilisation of blood components[84,85] extend the possible applications of this modality.

The development of PDT for the indications outlined above will depend not only on efficacy in both open and randomized clinical trials, but also on assessment of ease of administration, adverse effects and outcome of PDT in comparison with existing therapies.

Chapter 2 - The use of photodynamic therapy in cutaneous malignancy

2.1 - Introduction

The accessibility of the skin to light-mediated therapy and the high prevalence of cutaneous neoplasia led Dougherty *et al*[6] to treat non-melanoma skin cancer in the first human trial of PDT in 1978. Several further trials have show the efficacy of PDT in a variety of primary cutaneous neoplasms and cutaneous metastases. A detailed review of these open studies is given in this chapter. Haematoporphyrin derivative, Photofrin and more recently, 5-aminolaevulinic acid, have been the main photosensitizing agents used. The description in 1990 of PDT using topically administered 5-ALA,[10] thus avoiding the prolonged generalized photosensitivity that followed the systemic administration of HpD and Photofrin, has stimulated interest in the clinical potential of PDT for cutaneous neoplasia. Lasers have been the predominant light source in these studies, although there are a few reports of the use of incandescent sources, including adapted slide-projectors.

2.2 - Actinic Keratoses and Bowen's disease

Actinic keratoses and Bowen's disease can be treated with cryotherapy, curettage and electrodessication, topical 5-flourouracil or surgical excision although no therapy is completely curative and each has potential complications. Radiotherapy can be used for Bowen's disease although full tumour doses are required. These therapies may be impractical for patients with numerous or large lesions, or lesions in anatomically difficult areas. As photodynamic therapy is tissue-sparing, it is potentially very useful in these circumstances.

Bowen's disease appears to respond well to PDT using HpD or Photofrin. Three of the four trials included in Table 2.1 reported a 96-100%

initial complete clinical response. However, the number of lesions treated was small (2-8) in three of these studies. Robinson et al[87] reported two patients treated using the active component of HpD, di-haematoporphyrin ether (DHE). At a drug concentration of 2mg/kg and 25J/cm^2, 160/166 lesions were observed to clear, but only 45/90 further lesions cleared with 1mg/kg DHE and 50J/cm.^{2} A pre-clinical hypothesis of a direct reciprocal relationship between photosensitizer dose and light dose was thus not confirmed. There was also an absence of any reduction in generalized skin photosensitivity in this latter group.

The 10 studies performed since 1990 using 5-ALA as the source of photosensitization for the treatment of actinic keratoses and Bowen's disease (Table 2.1) emphasize the interest that PDT using a topically active agent has generated in treating pre-malignant cutaneous lesions. Kennedy et al[10] was first to use 5-aminolaevulinic acid, applying it to the tumour surface in an oil-in-water emulsion to promote absorption. He used a Kodak slide projector (containing a 500W tungsten lamp) modified only by the inclusion of a long-wave pass colour filter to eliminate wavelengths less than 600nm. A high irradiance, between 150-300mW/cm,^{2} however suggests that heating of superficial tissue may have occurred with an unknown effect upon the PDT treatment. Hyperthermia has been reported as being an effective monotherapy for Bowen's disease[96] and thus may have contributed to the therapeutic effect as 150mW/cm^2 is considered to be the threshold intensity above which hyperthermic injury may occur.[97]

Wolf et al[35] also used 5-ALA, a slide projector (a Leitz Pradovit incorporating a 250W tungsten lamp) and filtered wavelengths less than 570nm. Individual lesions were irradiated at 50-100 mW/cm,^{2} with all treated lesions still clearing. Infra-red emissions from these projector sources, despite the incorporation of heat rejection filters in the beam path, may contribute to tissue heating independent of the irradiance intensity. Stables

Table 2.1 Summary of treatment conditions and results of studies using PDT for actinic keratoses and Bowen's disease

Reference	Disease	No. of lesions	Drug and Dose (mg/kg)	Interval (hours)	Light dose (J/cm^2)	Light source + wavelength (nm)	CCR (%)	Recurr. no. (%)	Follow-up (mths)
Waldow *et al,*[86] 1987	Bowen's	3	Photofrin 2.0	72	40-60	ArPDL (630)	100	0	9
Robinson *et al,*[87] 1988	Bowen's	166	DHE 1.0-2.0	72	25	AuVL (628)	96	0	6
Kennedy *et al,*[10] 1990	AK	10	5-ALA,20%	3-6	54-540	Tungsten (>600)	90	0	6
	Bowen's	6	5-ALA,20%	3-6	54-540	Tungsten (>600)	100	0	3
Jones *et al,*[88] 1992	Bowen's	8	Photofrin 1.0	48	185-200	ArPDL (630)	100	0	12
Petrelli *et al,*[89] 1992	Bowen's	2	Photofrin 1.0	24-48	150-200	ArPDL (630)	100	0	12
Wolf *et al,*[35] 1993	AK	9	5-ALA,20%	4-8	30-100	Tungsten (>570 or unfiltered)	100	0	7
Cairnduff *et al,*[90] 1994	Bowen's	36	5-ALA,20%	3-6	125-150	CuVDL (630)	97	3 (9%)	18
Calzavara-Pinton,[91] 1994	AK	50	5-ALA,20%	6-8	60-80	ArPDL (630)	100	5 (10%)	24-36
	Bowen's	6	5-ALA,20%	6-8	60-80	ArPDL (630)	100	0	24-36
Svanberg *et al,*[19] 1994	Bowen's	10	5-ALA,20%	4-6	60	Nd:YAGL (630)	90	0	6-14
Figan *et al,*[92] 1995	AK	43	5-ALA,20%	20	300	Tungsten (>550)	81	0	6
	Bowen's	10	5-ALA,20%	20	300	Tungsten (>550)	50	2 (20%)	6
Lui *et al,*[93] 1995	Bowen's	3	5-ALA,20%	3	100	Tungsten (>570)	67	-	-
Meijnders *et al,*[94] 1996	AK	5	5-ALA,20%	3-6	75	ArPDL (633)	60	1 (20%)	17
Oseroff, (p. comm.)1996	Bowen's	12	5-ALA, 10-20%	4-5	75-200	ArPDL (630)	92	0	6
Szeimes *et al.,*[95] 1996	AK	36	5-ALA,10%	6	150	Tungsten (>580)	33	0	3

et al[98] recently compared the visible and infra-red power output of a Leitz Pradovit projector, and reported over 60% of the total power output was infra-red, in the spectral range 900-1800nm. In addition, the remaining visible output (400-660nm) is unlikely to include wavelengths all suitable for PDT with those bandwidths around the absorption peaks of protoporphyrin IX of greatest importance. Incandescent tungsten-halogen lamps are thus relatively inefficient sources of light for PDT.

Cairnduff et al[90] and Calzavara-Pinton[91] treated Bowen's disease and actinic keratoses by PDT, using 630nm laser light. Dosimetry differed although irradiance was less than 100mW/cm^2 in each study. A high clearance rate on clinical examination was observed and longer follow-up periods (18 and 29 months respectively) indicated a recurrence rate of up to 10%. Calzavara-Pinton excised 20 of the treatment sites (17 from treated actinic keratoses and 3 from sites of Bowen's disease) with histological examination showing tumour remnants in 3 AK sites, but in no Bowen's disease site. All the other studies listed in Table 2.1, except Figan et al[92] (see below) defined clearance as the absence of clinically evident tumour and histological confirmation was not obtained.

Figan et al[92] used 5-ALA in combination with the iron chelator desferrioxamine in an attempt to improve efficacy via the induction of endogenous porphyrin synthesis. 20% 5-ALA was applied for 20 hours prior to illumination with an incandescent source (filtered output 540-720nm) at a light intensity of 150-250mW/cm.^{2} Histological conformation of clearance was obtained for all lesions of Bowen's disease, but not for actinic keratoses. Repeated treatments cleared only 5/10 sites of Bowen's disease with 2 subsequent recurrences after 1 and 4 months. As no comparison was made using this protocol without 3% desferrioxamine (dissolved with 5-ALA in the oil in water emulsion), the influence of the chelator cannot be assessed.

Szeimes et al[95] compared the efficacy of PDT for actinic keratosis at different body sites. Only 10% 5-ALA was used, applied 6 hours pre-illumination with filtered light (580-740nm) from a 1200W incandescent source (Waldmann 1200) with a light intensity of 160mW/cm.^{2} 12/17 actinic keratoses situated on the head healed following a single treatment in comparison with none of the 19 lesions on the arms and hands. This difference was probably due to an observed greater initial thickness of lesions on the arms and hands reducing 5-ALA and/or light penetration.

Stender and Wulf[99] treated three patients with actinic cheilitis using topical 5-ALA (20% in a basic cream, applied 3 hours pre-illumination) and a slide projector (individual lesions received 55J/cm^2). No evidence of recurrence was evident over the following 12 months.

2.3 - Squamous cell carcinoma

Squamous cell carcinomas (SCC) can be treated by locally destructive therapy (curettage and cautery, cryosurgery), radiotherapy, or surgery. Unlike the treatment of potential pre-malignant lesions such as actinic keratoses and Bowen's disease where recurrent disease can be easily re-treated, the ability of SCC to metastasize implies that inadequate initial treatment may affect prognosis. The highest achievable clearance rate is thus required. In experienced hands, all techniques are reported to give 5 year cure rates of approximately 90%[100]. A new treatment modality requires to achieve at least this result. Currently, no 5-year follow-up studies of PDT for any clinical application have been published.

Experience of treating SCC with PDT has been limited to date with published studies listed in Table 2.2. Five studies used haematoporphyrin derivative or Photofrin, with light from argon lasers. Overall, 36/44 (82%) of

Table 2.2 Summary of treatment conditions and results of studies using PDT for squamous cell carcinoma

Reference	No. of lesions	Drug and Dose (mg/kg)	Interval* (hours)	Light dose (J/cm^2)	Light source + wavelength (nm)	CCR (%)	Recurr. - no. (%)	Follow-up (months)
Gregory and Goldman,[101] 1986	1	HpD 2.5	72	75	ArPDL (630)	100	0	3
Pennington *et al,*[102] 1988	32	HpD 5.0	72	30	ArPDL (630)	81	>50%	6
Keller *et al,*[103] 1989	2	HpD 2.0-3.0 /Photofrin 1.0	48-72	150-200	ArPDL (630)	100	0	-
McCaughan *et al,*[104] 1989	5	HpD 3.0 /Photofrin 2.0	48-144	20-30	ArPDL (630)	60	2 (40%)	12
Kennedy *et al,*[10] 1990	2	5-ALA,20%	3-6	54-540	Tungsten (>600)	0	-	-
Petrelli *et al,*[89] 1992	4	Photofrin 1.0	24-48	150-200	ArPDL (630)	100	0	12
Wolf *et al,*[35] 1993	6	5-ALA,20%	4-8	90-180	Tungsten (>570 or unfiltered)	83	0	7
Heinritz *et al,*[105] 1995	5	5-ALA,20%	6-8	150	ArPDL (630)	100	0	4-12
Lui *et al,*[93] 1995	2	5-ALA,20%	3	100	Tungsten (>570)	0	-	-

squamous cell carcinomas treated by systemic photosensitizer cleared although a high recurrence rate was observed in the 2 largest studies.

Four studies using 5-ALA as a topically applied photosensitizer have treated a total of 15 SCC. Two of the three studies using an incandescent light source failed to clear any tumours. In the third study, by Wolf et al,[35] 5/6 early invasive tumours were successfully treated. All 5 squamous cell carcinomas treated by Heinritz et al[105] by laser cleared, with histological confirmation and no recurrences during a 4-12 month review period.

Wolf et al[106] has also used topical ALA-PDT for the treatment of one SCC on the hand of a patient with xeroderma pigmentosum (XP). Although a single treatment was curative, erythema of the exposed area persisted for more than 2 weeks (normal 2-4 days). Persistent erythema is well known after ultraviolet irradiation (UVR) in patients with XP, but UVR exposure was unlikely in this patient who was in hospital throughout the 2 weeks with an incandescent light source used for the PDT. The persistant erythema remains unexplained and may limit the usefulness of PDT in XP despite its theoretical advantage of targetting membranous structures rather than via DNA damage.

Thus, although current studies of PDT for SCC thus suggest that this modality can be effective, results overall indicate efficacy short of 90% even during the short follow-up periods reported. Larger studies with extended follow-up are required, with comparison of different protocols before PDT can be considered as an alternative therapy for SCC.

2.4 - Basal cell carcinoma

A variety of therapies already exist for basal cell carcinomas (BCC) including surgical excision, curettage and electrodesiccation, cryotherapy, radiotherapy and Moh's micrographic surgery. Although it is extremely rare

for BCC to metastasize, inadequate therapy can allow recurrence and deep invasion. Recurrent disease can also be less responsive to repeat therapy with one study observing a 50% recurrence rate after retreatment of tumours treated inadequately at first presentation.[107] A review of studies published from 1947-87 on the outcome of the treatment of primary (previously untreated) BCC reported a cumulative 5 year recurrence rate between 1.0-10.1% (Mohs': 1.0%, cryotherapy: 7.5%, curettage and electrodessication: 7.7%, radiotherapy: 8.7%, and surgery: 10.1%).[108] As cost and man-power limits Mohs' surgery to only to a few indications, an average non-Mohs' 5 year recurrence rate of 8.7% indicates the limitation of currently available routine therapies. Photodynamic therapy will require to demonstrate not only efficacy, but also a comparable and preferably lower recurrence rate.

A. PDT using Haematoporphyrin derivative and Photofrin

Table 2.3 summarizes the clinical experience of PDT for BCC using systemic photosensitizers. No study has published recurrence rates up to 5 years, although Keller et al[103] reported no recurrent tumours 4 years after PDT to 6 BCC.

The seven published studies of the treatment of BCC by PDT using haematoporphyrin derivative or Photofrin report widely differing initial clearance rates of 52-100%. Dougherty et al,[109] Waldow et al,[86] and Keller et al[103] cleared all lesions (17 in total), many treated BCC already failures of conventional surgery, with only one recurrence. Pennington et al[102] cleared only 11/21 BCC with all tumours recurring during the subsequent 6 months. Although failure in this study may have been due to the low dose of light employed, a similarly poor outcome was reported by Buchanan et al[111] despite a higher light dose at a previously effective concentration and duration of photosensitizer. The subtype of BCC treated in these early studies was not stated. A laser light source was used in all these studies

Table 2.3 Summary of treatment conditions and results of studies using PDT and systemic photosensitizer for basal cell carcinoma

Reference	No. of lesions	Drug and Dose (mg/kg)	Interval* (hours)	Light dose (J/cm^2)	Light source + wavelength (nm)	CCR (%)	Recurr. - no. (%)	Follow-up (months)
Dougherty *et al,*[109] 1981	5	HpD 2.5-5.0	>96	35-140	Xe (600-700), ArPDL (635)	100	0	7-12
Tse *et al,*[110] 1984	40	HpD 3.0	72-96	38-180	Xe (600-700), ArPDL (630)	82	2 (5%)	12-14
Waldow *et al,*[86] 1987	6	Photofrin 1.5-2.0	24-72	40-60	ArPDL (630)	100	1 (17%)	4-24
Pennington *et al,*[102]1988	21	HpD 5.0	72-120	30	ArPDL (630)	52	52 (100%)	6 (mean)
Buchanan *et al,*[111]1989	5	Photofrin 2.0	72	100	AuVL (628)	60	24 (40%)	12
Keller *et al,*[103]1989	6	HpD 2.0-3.0 /Photofrin 1.0	48-72	150-200	ArPDL (630)	100	0	48
Wilson *et al,*[112]1989	151	Photofrin 1.0	48-72	72-288	ArPDL (630)	88	13 (9%)	29 (mean)

although Dougherty et al[109] and Tse et al[110] treated a few lesions with a broad-band xenon arc lamp with comparable success rates.

Wilson et al[112] at Roswell Park Cancer Institute in Buffalo appreciated the possible impact on efficacy of the histological type of BCC treated. They treated 75 superficial, 52 morphoeic and 24 nodular BCC, with clearance of 133/151 tumours. During a follow-up period of 29 months however, 80% of recurrences arose from morphoeic tumours, with only 4% of the superficial or nodular types recurring.

The Roswell Park Group have also treated 588 superficial and nodular BCC in 9 patients with basal cell naevus syndrome (BCNS).[114] Photodynamic therapy was undertaken using Photofrin 1mg/kg 48-96 hours prior to illumination with an argon laser. Complete clinical response rates in the adults treated ranged from 82-98% (mean 95%) after a mean follow-up of 28 months. The opportunity to treat multiple lesions at a single visit (4 lesions/20 minutes and 40 per session) along with excellent cosmetic results encouraged the group to suggest that PDT might have a role in the management of such patients despite the generalized photosensitivity induced by Photofrin.

B. PDT using a topical photosensitizer

Santoro et al[115] in 1990 evaluated the effectiveness of topical meso-tetraphenylporphinesulphonate in the treatment, by PDT, of 292 lesions with superficial BCC. They reported complete clearance of 94% although with a 20% recurrence rate during the following 24 months. No further studies using this photosensitizer in cutaneous malignancy have been reported, possibly due to its major disadvantage of poor tumour selectivity when topically applied, necessitating its application strictly to the tumour only.

Since 1990, over 1000 basal cell carcinomas have been treated by PDT using topically applied 5-ALA (Table 2.4). Initial clearance rates for

Table 2.4 Summary of treatment conditions and results of studies using ALA-PDT for basal cell carcinoma

Reference	No. of lesions	Dose (mg/kg)	Interval (hours)	Light dose (J/cm^2)	Light + λ (nm)	Subtype	CCR (%)	Recurr. no. (%)	Follow-up (months)
Kennedy *et al,*[10] 1990	80	20%	3-6	54-540	Tungsten (>600)	superficial	90	0	2-3
Wolf *et al,*[35] 1993	37	20%	4-8	30-60	Tungsten (>570	superficial	97	1 (2%)	3-12
	10	20%	4-8	90-180	or unfiltered)	nodular	10	0	3-12
Cairnduff *et al,*[90] 1994	16	20%	3-6	125-250	CuVDL (630)	superficial	88	6 (43%)	17 (mean)
Calzavara-Pinton,[91] 1994	23	20%	6-8	60-80	ArPDL (630)	superficial	100	2 (9%)	24-36
	30	20%	6-8	60-80	"	nodular	80	2 (8%)	24-36
Svanberg *et al,*[19] 1994	55	20%	4-6	60	Nd:YAGL (630)	superficial	100	0	6-14
	25	20%	4-6	60	"	nodular	64	0	6-14
Warloe *et al,*[113] 1995	393	20%	3	40-125	CuVDL (630)	superficial	97	9 (2%)	36
	326	20%	3	40-80	"	nodular	75	12 (5%)	36
Figan *et al,*[92] 1995	34	20%	20	300	Tungsten	superficial	97	0	6 (mean)
	22	20%	20	300	(550-700)	nodular	59	0	6 (mean)
Lui *et al,*[93] 1995	8	20%	3	100	Tungsten (>570)	superficial	88	3 (43%)	2-3
Oseroff (pers. comm.) 1996	108	10-40%	4-5	75-200	ArPDL (630)	superficial	92	0	6-40
	14	10-20%	4-5	75-200	"	nodular	78	0	12-34

tumours classed as superficial was 88-100% and for nodular BCC a range of response between 10-80% was observed. In each study the subtype of BCC has been decided predominantly by clinical assessment with histological confirmation usually obtained only in one representative lesion in each patient. Clearance was determined only by clinical assessment of treated sites in the study of Kennedy et al[10] whilst Wolf et al,[35] Cairnduff et al,[90] Warloe et al[113] and Figan et al[92] only biopsied sites following treatment if they had clinical doubt over clearance. Calzavara-Pinton[91] biopsied 11/23 superficial and 18/23 nodular BCC four weeks after treatment with this representative sample of 29 lesions considered to be complete clinical responses at the time of biopsy. Histological examination revealed tumour remnants in 1/11 superficial and 7/18 nodular BCC sites suggesting a potential reporting error for clinical clearance of 9% and 39% for superficial and nodular lesions respectively. Lui et al[93] excised all 8 superficial BCC 7-12 weeks following treatment and discovered tumour in 3/7 (43%) lesions initially considered clear (clinical grading at time of excision not stated) which they interpreted as early recurrence. The absence of histological evidence of clearance for many of the lesions treated in these studies therefore limit assessment of the efficacy of PDT and complicate interpretation of recurrence rates which probably include several tumours which were only partial responders initially.

Initial clinical response rates for superficial BCC do not appear to be affected by duration of photosensitizer application nor by the light source used. Most studies applied 5-ALA at a 20% concentration 3-8 hours prior to illumination including that by Warloe et al[113] with 97% clearance after a 3 hour application. Figan et al[92] extended the duration of application to 20 hours with an identical result. Four of these ALA-PDT studies used a laser light source with initial clearance of 88-100% of 487 superficial BCC, whilst 88-97% of 159 superficial BCC cleared using modified slide-projector

tungsten sources. Similar recurrence rates are also reported with 17/487 (3.5%) and 4/159 (2.5%) superficial tumours recurring after PDT with laser and non-laser sources respectively although duration of follow-up was shorter with those studies using tungsten sources (6-36 months and 2-12 months respectively).

Laser-based PDT studies[19,91,113] reported a superior outcome for nodulo-ulcerative BCC in comparison with the two studies using a tungsten incandescent source.[35,92] Initial clinical clearance of 285/381 (75%) treated using a laser (range 64-80%) compares with clearance of only 14/32 (44%) tumours treated using non-laser light. This poor result includes 13/22 tumours treated by Figan et al[92] who combined 5-ALA with desferrioxamine and prolonged application time to 20 hours, two measures anticipated to improve efficacy of ALA-PDT. The study by Wolf et al[35] which cleared only 1/10 nodulo-ulcerative lesions in contrast to 36/37 superficial BCC suggested that inadequate light and/or photosensitizer dose in the deeper tumour tissue might have impaired response.

The poorer efficacy of broadband non-laser sources for thicker noduloulcerative disease may be due to inadequate penetration of light to the deepest section of the tumour. Light dosimetry cannot accurately be compared between studies published to date due to the different wavelength and intensity of light used and the difficulty in determining the actual effective light energy produced by broadband non-laser sources.

Warloe et al[113] enhanced 5-ALA penetration using dimethyl sulphoxide (DMSO) either in combination with 5-ALA or as a pre-treatment in a large open study of over 700 BCC. He also used ethylenediaminetetraacetic acid (EDTA) as iron chelator to promote the intra-cellular accumulation of the active photosensitizer protoporphyrin IX. Tumours were both superficial and nodulo-ulcerative with the latter group sub-divided into lesions estimated to be <2mm or >2mm, although grading of

thickness was approximate, undertaken usually by clinical inspection. He reported clearance without DMSO/EDTA of 92% (130/141) of superficial BCC, 67% (16/24) of 'thin' and 34% (19/56) of 'thick' nodular lesions after one treatment. PDT using 5-ALA (20% in an oil in water emulsion - Unguentum Merck) plus 2-20% DMSO and 2-4% EDTA for 3 hours prior to illumination, achieved clearance rates of 91% (114/125) for superficial BCC, 91% (59/65) of 'thin' and 55% (25/45) of 'thick' nodular lesions. The application of 50-99% DMSO as pre-treatment for 15 minutes before 5-ALA produced virtually identical results to the DMSO/EDTA combination. Repeat treatments in the DMSO/EDTA group achieved clearance rates of 93%, 95% and 60% respectively. Fluorescence microscopy performed on tissue samples excised prior to tumour illumination revealed 5-ALA-induced porphyrin fluorescence to be localized at 3 hours only to the superficial sections of nodulo-ulcerative tumours. The combination of 5-ALA with DMSO/EDTA greatly increased fluorescence in the deeper layers of the tumours although in several thick lesions fluorescence of the tumour remained incomplete. EDTA as a single additive had little effect on fluorescence. The inclusion of a penetration enhancer thus improved efficacy of ALA-PDT in nodular BCC. Recurrence rates of 2-5% are reported with no tendency to late recurrence after a follow-up period of more than 3 years.

Oseroff (personal communication) has also assessed the impact of altering duration of photosensitizer application on clearance. Whilst prolonging application from 4-5 hours to 24 hours had minimal effect on clearing superficial BCC (24 hour treatment group - 69/76 cleared, i.e. 91%), an increase in clearance rate from 78% to 90% was observed for nodular BCC (24 hour treatment group - 18/20 cleared).

Despite the possible inadequate treatment of thicker lesions, surprisingly recurrence rates for nodular BCC do not appear to be higher than for superficial tumours with rates of 0-8%.

Cairnduff et al[90] noted late recurrence of BCC following ALA-PDT, with a median time to recurrence of 11 months with 6/14 tumours recurring during a median follow-up of 17 months. Most other studies have followed patients for shorter intervals although Warloe et al[113] report a low recurrence rate maintained for 3 years.

Warloe et al[113] however did not achieve the same success using ALA-PDT in treating patients with basal cell naevus syndrome as did the Roswell Park Group who used Photofrin.[114] Only 11/18 (61%) of superficial and 3/26 (12%) nodulo-ulcerative BCC cleared in 4 patients. Inadequate photosensitizer penetration in the thicker tumours may partly explain the difference.

2.5 - Cutaneous lymphoma

Cutaneous T-cell lymphoma, characterized by the malignant proliferation of atypical helper T-cells (CD4+ T-lymphocytes) can be treated by topical steroids and UVB phototherapy, PUVA photochemotherapy ± interferon alpha-2a, topical nitrogen mustard, electron beam therapy, oral retinoids, or photophoresis. Efficacy of each therapy depends on the stage of disease and adverse effects can limit use.

Boehncke et al[116] performed PDT using Photosan, a mixture of porphyrins, on cell lines established from patients with CTCL. PDT resulted in a dose-dependant inhibition of transformed T-cell proliferation. They subsequently undertook *in-vivo* fluorescence microscopy during PDT of plaques of CTCL in two patients to demonstrate photobleaching and thus the triggering of photochemical reactions by photodynamic therapy.

Dougherty et al[6] first reported successful PDT for CTCL using haematoporphyrin derivative. However, as with other PDT applications, the opportunity to avoid prolonged photosensitivity by using 5-ALA led to Malik et

al[117] to demonstrate by *in-vitro* studies preferential porphyrin accumulation within the lymphocytic infiltrate of CTCL lesions after 5-ALA photosensitization.

Topical 5-ALA has since been shown to promote the accumulation of protoporhyrin IX within malignant CD4+ T-lymphocytes with preferential uptake by relatively iron deficient cells expressing the transferrin receptor (CD71). PDT of these tumour cells after 5-ALA incubation demonstrated preferential killing compared to normal, unstimulated peripheral blood lymphocytes.[118]

In 1994, Svanberg et al[19] reported the treatment of two patients with 4 plaques of cutaneous T-cell lymphoma (10-50mm in diameter) by topical ALA-PDT using a pulsed frequency doubled Nd:YAG laser (630nm). Laser-induced fluorescence was performed to monitor the accumulation of PPIX and a 5:1 concentration gradient was observed between the lymphomatous plaques and the normal surrounding skin. A complete response, confirmed on histology, was observed in two lesions after 2 treatments. In the same year, Wolf et al[119] successfully used topical ALA-PDT in the treatment of two patients with plaque-stage CTCL, one (patient A) had a solitary 18x9cm plaque and the other (patient B) had 2 plaques (5x3 and 12.5x4cm). Using 20% 5-ALA 4-6 hours prior to illumination with a non-laser source, clinical and histological remission was achieved after 4 treatments in patient A (over 7 weeks) and 5 treatments for both lesions in Patient B (over 18 weeks).

Preliminary data from Oseroff et al[120], indicates that single treatments of ALA-PDT can achieve greater than 50% reductions in tumour mass in lesions up to 1.5cm thick. Repetitive treatments every 1-3 weeks improve response with complete clinical and histological clearance in certain lesions. This data has now been updated and is discussed in Chapter 6.

There are no published reports of PDT use in the treatment of other types of lymphoma.

2.6 - Kaposi's sarcoma

Kaposi's sarcoma (KS), the most common malignancy in patients with AIDS, can present as a spectrum of disease from local cutaneous lesions to disseminated visceral involvement. Therapy for extensive disease is limited to chemotherapy, whilst cutaneous disease may respond to intralesional chemotherapy, cryosurgery or radiotherapy. Kaposi's sarcoma consists of spindle cells with an extensive vascular compartment and thus might respond to PDT using systemically administered photosensitizers with proven vascular photosensitivity.[50-52] PDT may thus be effective in the palliation or cure of mucocutaneous KS lesions. A total of five patients with classical KS have been successfully treated in two centres by PDT, with complete clinical clearance of 17/23 (74%) and 2/2 lesions respectively.[109,121] Four studies[122-125] have reported an efficacy of Photofrin-mediated PDT in AIDS-related Kaposi's sarcoma with control of tumours up to 4cm in diameter.

Dougherty[122] reported tumour clearance in 80% of over 100 cutaneous KS lesions in 5 patients (although the proportion of biopsy proven lesions is not stated) by PDT using either 3mg/kg haematoporphyrin derivative or 1.5-2.0mg/kg Photofrin and 20-70J/cm^2 of 630nm laser light (superficial and interstitial treatments).

Schweitzer et al[123] treated 5 patients each with multiple oral KS lesions by PDT (Photofrin 2mg/kg - intravenous and interstitial) with partial (n=2) or complete clearance (n=3) achieved in all tumours (partial clearance: >50% reduction in measurable size for at least 4 weeks, complete response: clinical disappearance of lesion for at least 8 weeks). No recurrences were observed in the complete responders during 3-7 months of follow-up.

Hebeda et al[124] treated 83 lesions in 8 patients using 2mg/kg Photofrin injected intravenously 48 hours prior to illumination with 70-120J/cm^2 from a 630nm laser source. All patients had biopsy-proven KS restricted to the skin with the diameter of lesions 5-38mm. Size of individual lesions showed a

negative relationship with the probability of complete clearance (p=0.047). A complete response rate (absence of any measurable KS for at least 4 weeks) was achieved in 56/83 (67.5%) lesions with superior clearance for tumours localized to the head. The poor response rate may have been due to the absence of interstitial therapy and no repeat treatments for incomplete responses. The cosmetic result, however, was unsatisfactory due to the high prevalence of scars (41%) and of hyperpigmentation which lasted several months after PDT.

The poor cosmetic result in this study may have been due to the high dose of Photofrin used, with 1mg/kg used by Bernstein et al[125] at Roswell Park Cancer Institute, Buffalo, achieving excellent cosmetic results. This latter study of over 400 lesions in 20 patients cleared 23% (n=92) of lesions (clinically clear for at least 8 weeks) and partly cleared 46% (n=184) using intravenous photosensitizer (and interstitial if tumours over 5mm thick) and 630nm light doses from 100-400J/cm.^{2}

Topical ALA-PDT for KS has not been reported, but due to the apparent absence of vascular damage following ALA-PDT[26] and the relatively poor absorption of 5-ALA through normal overlying epidermis, it is unlikely to be as effective as systemic/intralesional Photofrin for PDT in KS.

2.7 - Cutaneous metastases

PDT has been used for cutaneous metastases, predominantly from breast (Table 2.5) and melanoma (Table 2.6). Interpretation of these small open studies, based on clinical response and typically of patients with advanced disease and failed conventional therapies, can only, however, provide an indication of whether or not PDT is a potential therapy for such tumours.

Table 2.5 Summary of treatment conditions and results of studies using PDT for cutaneous metastases from primary breast adenocarcinoma

Reference	Patient No.	No. of lesions	Drug and Dose (mg/kg)	Interval (hours)	Light dose (J/cm^2)	Light source + wavelength (nm)	CCR - no. (%)	Recurr. - no. (%)	Follow-up (months)
Dougherty *et al,*[109] 1981	35	?	HpD 2.5-5.0	72	18-140	Xe (600-700) ArPDL (635)	?	10/16*	12
Bandieramonte *et al,*[126] 1984	2	18	HpD 3.0	48	60-120	Ar/Dye (630)	0	-	-
Tomio *et al,*[121] 1985	4	8	Haematoporphyrin 5.0	24-48	22.5	He/Ne (630)	0	-	-
Waldow *et al,*[86] 1987	4	146	HpD 3.0/ Photofrin 2.0	24-168	8-60	ArPDL (630)	108 (74%)	'several'	3-8
Schuh *et al,*[127] 1987	14	14	Photofrin 1.0-2.0	2.5-96	36-288	ArPDL (630)	2 (14%)	1 (50%)	6
Gilson *et al,*[128] 1988	1	?	Photofrin 1.0-2.0	48-72	25-100	CuVDL (630)	? (47%)	0	3-5
Buchanan *et al,*[111]1989	12	25	HpD 3.0-4.0/ Photofrin 1.5-2.0	72	25-200	ArPDL (630)/ AuVL (628)	5 (20%)	0	4-12
McCaughan *et al,*[104] 1989	12	29	HpD 3.0/ Photofrin 2.0	48-144	20-30	ArPDL (630)	21 (72%)	13 (62%)	12
Kennedy *et al,*[10] 1990	4	?	5-ALA,20%	3-6	54-540	Tungsten (>600)	0	-	-
Cairnduff *et al,*[90] 1994	5	14	5-ALA,20%	3-4.5	150	CuVDL (630)	5 (36%)	0	6

*No. of patients surviving to 1 year; ? signifies no. not stated in publication.

Table 2.6 Summary of treatment conditions and results of studies using PDT for metastatic melanoma

Reference	Patient No.	No. of lesions	Drug and Dose (mg/kg)	Interval* (hours)	Light dose (J/cm^2)	Light source + wavelength (nm)	CCR - no. (%)	Recurr. - no. (%)	Follow-up (months)
Dougherty *et al*,6 1981	7	7	HpD 5.0	48-120	120	ArPDL (635)	6 (86)	-	-
Tomio *et al*,[121] 1985	2	4	Haematoporphyrin 5.0	24-48	22.5	He/Ne (630)	0	-	-
Gilson *et al*,[128] 1988	1	?	Photofrin 1.0-2.0	48-72	25-100	CuVDL (630)	? (47%)	0	3-5
McCaughan *et al*,[104] 1989	3	7	HPD 3.0/ Photofrin 2.0	48-144	20-30	ArPDL (630)	5 (71%)	3 (60%)	12
Wolf *et al*,[35] 1993	1	8	5-ALA,20%	4-8	90	Tungsten (>570 or unfiltered)	0	-	-

? signifies no. not stated in publication.

Clearance was observed to be complete in only 14-74% of breast adenocarcinoma metastases (Table 2.5), with a high recurrence rate observed in certain studies.

Dougherty[109] observed at least a 50% reduction in size of lesions following PDT in 34/35 patients with cutaneous metastatic breast carcinoma who already had received surgery, radiotherapy and chemotherapy. Patients had lesions ranging from a single 12x12cm lesion to multiple smaller metastases. Recurrence was high in those surviving long enough for 12 month follow-up although retreatment often achieved a further response.

A penetration depth of red light (630nm) of 4mm in human breast cancer has been determined[129] indicating the likely advantage of interstitial photodynamic therapy. Whilst the studies of Dougherty,[109] Tumio et al,[121] Schuh et al[127], and McCaughan et al[104] include interstitial treatment of larger/thicker metastatic breast tumours, clearance rates were not uniformly higher compared with the remaining studies which illuminated lesions only from the surface. Diffusing fibres were used by Lowdell et al[130] to treat 50 breast and squamous cell carcinoma metastases (number of each tumour type not stated) by Photofrin-mediated PDT. A complete clinical response was observed two months after treatment in 26/50 (52%) overall, but with an 81% clearance for the highest light and photosensitizer doses.

Kennedy et al[10] used topical ALA-PDT to treat 4 patients with metastatic breast carcinoma. Although strong protoporphyrin IX fluorescence was induced in percutaneous nodules, there was no effect of PDT, using a tungsten source, on the lesions. Cairnduff et al[90] treated 14 metastatic adenocarcinomas with ALA-PDT using a laser in 5 patients, all derived from primary breast carcinomas. Only 5/6 lesions in one patient demonstrated a complete clinical response which was maintained for 6 months.

Experience of the successful treatment of cutaneous metastatic melanoma deposits by PDT is more limited than with breast metastases

(Table 2.6). Dougherty[6] and McCaughan et al[104] observed no clinical evidence of residual tumour after PDT to over 70% of treated lesions although recurrence rate was not stated in the former study and was 60% at 12 months in the latter study (despite the interstitial illumination of tumours in this study).

Wolf et al[35] failed to clear 8 cutaneous metastases of melanoma after topical ALA-PDT using an incandescent light source with only superficial tumour necrosis achieved in those amelanotic tumours and no response with melanotic metastases. The intensity of pigmentation of the tumours treated in the other studies is not stated although Dougherty[6] comments on the inferior responsiveness of more intensely pigmented tumours. All studies using PDT in melanoma have used photosensitizers optimally activated by light of 410-635nm,[30] within the range of wavelengths normally absorbed by melanin, hence probably limiting the potential for a photodynamic reaction to occur. The future development of photosensitizers with longer activating wavelengths beyond 700nm may thus improve the efficacy of PDT for metastatic melanoma.

Chapter 3 - Topical photodynamic therapy using a non-laser light source

3.1 - Introduction

Photodynamic therapy appears to be an effective therapy for certain cutaneous malignancies. However, the requirement to administer photosensitizers by the systemic route and their potential to induce prolonged generalized photosensitivity limit the ease with which PDT could be performed as a routine procedure. Topical administration of photosensitizer may permit PDT to become a practical out-patient therapy. However, published studies (reported in Chapter 2) have failed to determine the effective depth of action of PDT by this method and there remains no comparison of PDT with existing treatment modalities.

In addition, reliance on laser sources to produce sufficient high intensity light for PDT, restricts the availability of PDT. Whilst lasers can deliver light of sufficient intensity and at an appropriate wavelength for drug activation, their cost, complexity and limited availability has led to a search for alternative cheaper, yet effective, portable, light sources that can be easily used in clinical practice. Cheap incandescent sources used to date, however, have broad emission bandwidths, emit significant amounts of infrared radiation, and are inefficient sources of red light, which is considered to be optimal for porphyrin-based PDT.

The place of PDT in routine clinical dermatological practice thus remains to be established. Clinical studies of PDT have been of open design, often small, with a diverse array of protocols and frequently without histological confirmation of clearance. They often failed to assess dose-response outcomes, compare different wavelengths, and undertook only short-term follow-up of patients.

3.2 - Aims

This thesis seeks to further define the clinical potential of PDT in the treatment of cutaneous malignancy and facilitate its clinical application by assessing a novel light source. The effect on treatment efficacy of alterations in light and photosensitizer delivery will also be assessed. The principal aims of this thesis are to:

1. Evaluate the efficacy for photodynamic therapy of a prototype non-laser source which can emit light of a narrow bandwidth at intensities anticipated to be effective for PDT.
2. Compare, by randomized trial, PDT with cryotherapy, the current treatment of choice, for Bowen's disease.
3. Assess the influence of tumour thickness on the efficacy of ALA-PDT in the treatment of basal cell carcinomas.
4. Determine the influence of alterations in photosensitizer delivery and of light intensity, wavelength, lesion size and total dose on efficacy of ALA-PDT.
5 Assess the prevalence of adverse effects, cosmetic outcome and recurrence rates for ALA-PDT in cutaneous neoplasia.

3.3 - Materials and methods

The overall design of studies described in this chapter are summarized below with particular emphasis on the light source and photosensitizer used. Details specific to the design of individual studies and the treatment protocols of individual case reports are noted under the relevant chapter sub-section.

Patients:

Ethical committee approval was obtained for the treatment of patients with non-melanoma skin cancer and its potential precursor disorders, by

photodynamic therapy. Patients presenting to the Dermatology Department of the Western Infirmary, Glasgow, were invited to participate in the studies. No lesion had been previously treated. Histological confirmation of the diagnosis was acquired by performing a 4mm punch biopsy on all lesions. The consent forms used for these studies are shown in Appendices 1-3.

PDT light source

The light source employed (Figure 3.1) was specifically designed for use in photodynamic therapy by Drs. C. Whitehurst and J.V. Moore of the Paterson Institute, Christie Hospital, Manchester, and funded by the U.K. Cancer Research Campaign. The prototype lamp incorporates a 300W xenon short arc plasma discharge (Laser Lines, Banbury, UK), producing a continuous wave broadband flat spectral output of 50mW/nm across the entire visible spectrum. A dichroic 'hot' mirror (Optical Works, Newquay, UK), for heat rejection, and a toughened, high efficiency filter (Glen Spectra, Stanmore, UK) select the appropriate wavelength and bandwidth before the output is focused into a flexible light guide (Ultrafine Technology, Brentford, UK) via coated aspherical optics (Ealing, Watford, UK), which couples the light to the light guide, and a programmable shutter. The prototype light delivers over 1.0W of photoactivating light directly or up to 0.8W via a light guide within a bandwidth of 30nm and can be filtered to emit any wavelength from 300nm-1.1μm. To broaden the treatment field and produce uniform irradiation of lesions (±2%), 25mm diameter distal optics were attached to the light guide.

Infra-red emission from the source was totally blocked by the wideband dielectric heat filter and the non-infra-red transmitting light guide. Zero infra-red emission up to 35μm wavelength was verified with a calorimeter.

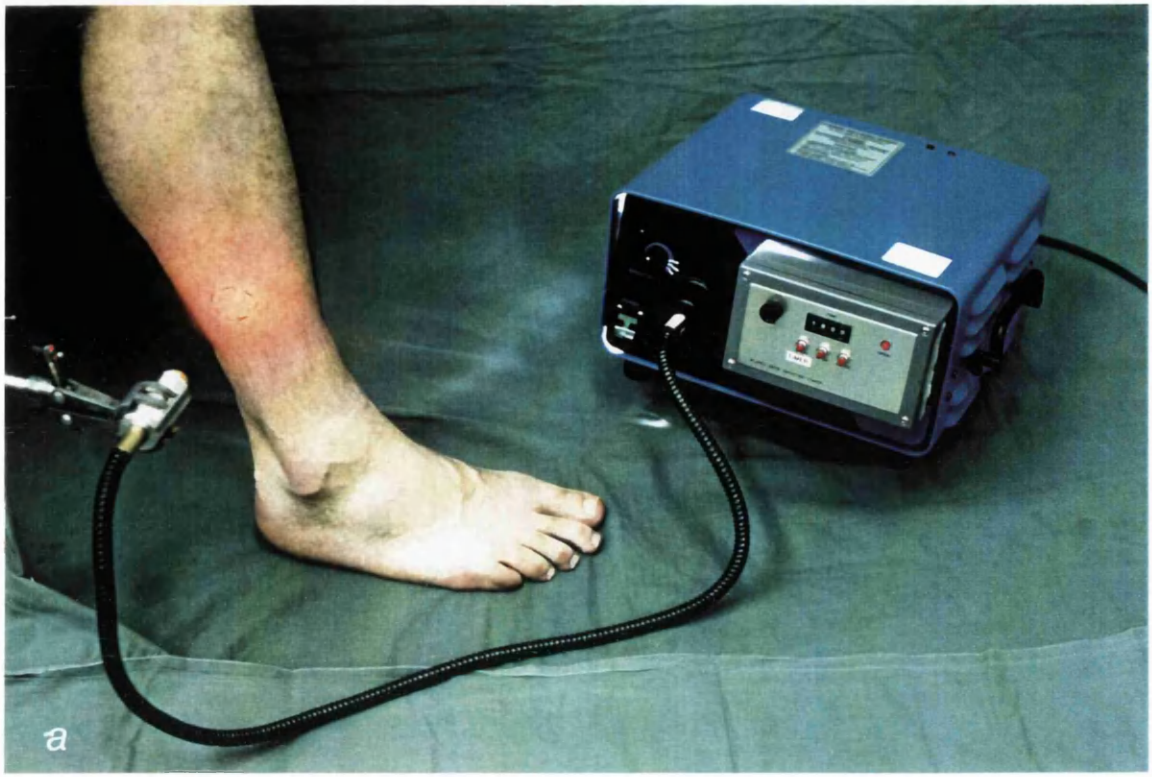


Figure 3.1: Prototype lamp with timer unit and fibre bundle which has a 25mm distal optics apparatus attached. A. First prototype lamp. B. Modified lamp.

Two prototype lamps of identical spectral output were used in the studies described below. They were of identical design except for minor internal modifications to boost power and reduce overall size, with changes in external casing and to the flexible fibre bundle used which in the first lamp delivered 0.5W (5mm guide) and in the second lamp, 0.6W (5mm guide) and 0.8W (8mm guide). The first lamp (measuring 30x30x20cm, Figure 3.1a) was used in the initial open study of actinic keratoses and Bowen's disease and the randomized comparison trial of PDT vs. cryotherapy for Bowen's disease. All remaining studies used the second lamp (Figure 3.1b) which measures 30x15x15cm. As all light dosimetry was calculated from light delivery at the distal end of the fibre bundles, with irradiance individually calculated for each study treatment, no difference in the efficacy of PDT between the two sources was anticipated.

Initial *in-vitro* assessment[131] of the prototype lamp demonstrated an efficiency, using clonogenic cell assays, for HpD-induced cellular photoactivation close to that of an argon pumped dye laser with a relative biological effectiveness (RBE) of 87±3% ($p<0.05$) between 10-100mW/cm^2. The lamp was superior to a copper vapour pumped dye laser with a RBE of 150% at fluence rates above 50mW/cm.^{2}

In a subsequent *in-vivo* study[132] which used tumour growth delay to quantify the relative efficacy of the prototype with an argon pumped dye laser, there was no significant difference in the extent of tumour (mammary carcinoma-T50/80) response between the two light sources.

Dosimetry was calculated at monthly intervals using the same power meter (Coherent 201 - calibrated by the manufacturer against a national standard source). Fluence rates were calculated for specified distances from the fibre-optic tip to the skin surface and a dosage chart derived as exemplified in Table 3.1.

Table 3.1 Example of one fluence table used for the studies using the prototype lamp (figures re-calculated monthly)

630±15nm Filter - Treatment times using 5mm fibre Date:_____				
Field diameter required (cm)	2.5	3	4	5
Distance (Light fibre-skin) (cm)	7	12.5	19	24
Area (cm^2)	4.9	7.1	12.6	19.6
Fluence rate (mW/cm^2)	122	86	48	30
Exposue time for 150J (sec)	1230	1744	3125	5000
Exposue time for 125J (sec)	1021	1448	2594	4150
Exposue time for 100J (sec)	820	1163	2083	3333
Exposue time for 75J (sec)	615	872	1562	2500
Exposue time for 50J (sec)	410	581	1042	1667
630±15nm Filter - Treatment times using 8mm fibre				
Field diameter required (cm)	5	6	7	7.5
Distance (Light fibre-skin) (cm)	14	17	20	22
Area (cm^2)	19.6	28.3	38.5	44.2
Fluence rate (mW/cm^2)	40	28	21	18
Exposue time for 150J (sec)	3750	5357	7143	8333
Exposue time for 125J (sec)	3112	4446	5929	6916
Exposue time for 100J (sec)	2500	3571	4762	5555
Exposue time for 75J (sec)	1875	2678	3572	4166
Exposue time for 50J (sec)	1250	1786	2381	2778

Photosensitizer

1. Preparation: 5-aminolaevulinic acid hydrochloride (Sigma Chemical Co.) was prepared 20% w/w in an oil in water emulsion. The formulation used was 2g 5-ALA, 2ml distilled water and 6g Unguentum Merck (E. Merck Ltd. West Drayton,UK). 5-ALA was mixed with distilled water to achieve partial dissolution. This mixture was then rubbed down into the Unguentum Merck. The finished product was stored in glass jars (2g of finished product) in a refrigerator for use within 48 hours of manufacture. Gloves, goggles and a particulate mask were worn during preparation.

2. Application: Topical 5-ALA was applied to lesions 4 or 6 hours before illumination with the lamp. Surface crusts were removed from the lesions and the surface gently abraded prior to 5-ALA application. Approximately 50mg/ cm^2 of cream was applied to the entire irradiation field, including the clinically disease-free margin. The cream was then kept in place under an occlusive dressing (Tegaderm, 3M) and screened from light for 4-6 hours, after which the 5-ALA cream remaining on the skin surface was carefully removed.

Adverse effects

Patients used visual analogue scales (Appendix 1) to record pain during treatment and over the 10 days following PDT (with subsequent interpretation of $0 < x \leq 3$ as mild, $3< x \leq 7$ as moderate and $7 < x \leq 10$ as severe). Patients were offered local anaesthesic (1% plain lignocaine by intradermal injection) during treatment.

Clearance and recurrence

Lesions were examined on completion of therapy and 24-48 hours later, then at increasing intervals during the following 2 months. Clinical response to the first application of PDT was determined at 2 months and a second treatment administered if lesions persisted. Clearance was defined in

Bowen's disease as clinical clearance with histological confirmation for all lesions where doubt over clinical clearance existed. Clearance was defined in basal cell carcinoma as clinical clearance with histological confirmation for all treated tumours (except where multiple tumours in same patient when a representative post treatment biopsy was performed) two months following either the first or, if required, second treatment. Monthly review of all patients was undertaken for a minimum of 12 months following clearance to observe for recurrence and assess scarring potential of the therapy. Post therapy 3-4mm punch biopsies were performed in all lesions.

Fluorescence/Photography

Fluorescence was detected on the surface of skin/tumour by an ultraviolet lamp (UV56 lamp, UVP, Upland, CA, USA), producing light with peak emission at 365nm. The presence of fluorescence was recorded prior to illumination of treatment sites and the subsequent reduction in intensity was recorded in the fluorescence decay studies (Section 3.7-9). Pre- and post- treatment clinical pictures of lesions were acquired in all studies with photography undertaken by CAM.

3.4 - Actinic keratoses and Bowen's disease - Pilot study

Aim: To assess the efficacy of the prototype light source for photodynamic therapy in the treatment of Bowen's disease and actinic keratoses.

Patients: Patients presenting with lesions of Bowen's disease (in-situ squamous cell carcinoma) or actinic keratoses, 21mm in diameter or less, were invited to participate in the study (for consent form see Appendix 2). Histological confirmation of the diagnosis was acquired by performing a 4mm punch biopsy on all lesions.

Methods: Topical 5-aminolaevulinic acid was applied 4 hours before irradiation. Using appropriate filters, the spectral output of the lamp was adjusted to a 30nm bandwidth centred at 630nm. To broaden the treatment field and produce uniform irradiation of lesions, an 11mm diameter perspex rod or 25mm diameter collimating lens was attached to the 5mm fibre bundle. We included at least a 10% margin around lesions in our field of irradiation permitting us to treat lesions up to 9 mm in diameter using the rod, and 21mm in diameter using the lens with field size diameters of 11mm and 25mm respectively. At fluence rates of 158mW/cm^2 for the rod and 55mW/cm^2 for the lens, lesions received 94-156J/cm^2 The treatment dose was centred on 125J/cm^2 as this dose has been shown previously to be effective in ALA-PDT using laser.[90] Patients were reviewed at monthly intervals and treatment repeated if residual disease was present.

Statistics: Comparison of the size of lesions clearing and of those not clearing after a single PDT treatment, was performed by a Mann-Whitney U test. Comparisons of clearance rates depending on dosage and the apparatus used to deliver the irradiation, were done using a chi-squared test.

Results - Clearance rates: Twenty lesions of Bowen's disease and 4 actinic keratoses (AK), in 12 patients (3M+9F, median age 65yrs., range 43-95yrs.), received photodynamic therapy. Sixteen lesions were sited on the leg and 6 on the forearm or hand, and two on the scalp. The median surface area of all treated lesions was 60mm^2 (range - 9mm^2 - 400mm^2).

Fifteen lesions (12 Bowen's and 3 actinic keratoses) cleared after a single treatment with PDT using this non-laser source. All 9 remaining lesions cleared following a second treatment, 2 months later. The median size of lesions clearing after 1 treatment was 56mm^2 (range 9 - 400mm^2), not significantly different from the size of those lesions requiring a second

treatment (median 60mm^2, range 9 - 380mm^2). The clinical response to PDT of areas of Bowen's disease and actinic keratosis is shown in Figures 3.2 and 3.3 respectively.

The effect of light dose on clearance after a single treatment is shown in Table 3.2. The first five lesions entered into the trial received 94J/cm^2, 75% of our intended treatment dose in order to observe for side-effects. Subsequent lesions received 125J/cm^2 except, as one patient had 7 lesions, two lesions received 94J/cm^2, three 125J/cm^2, and the remaining two, 156J/cm^2. Three lesions in this patient did not clear with a single treatment, one from each of the 3 dose regimens used. Although overall a higher percentage of lesions treated with 94J/cm^2 cleared after 1 treatment, compared with those which received 125J/cm^2, this difference was not statistically significant.

The perspex rod, with the higher fluence rate of 158mW/cm^2, was attached to the fibre bundle for the treatment of 15 lesions, of which 10 cleared (67%) on a single treatment. The collimating lens (fluence rate 55mW/cm^2) was attached to the bundle for the treatment of the 9 larger lesions, with 5 lesions clearing (56%) in this group after a single treatment. This difference in clearance rate, however, was not significant.

Results - Adverse effects: As treatment of our initial lesions (with 94J/cm^2) was well tolerated, the study proceeded, with the increase in dose as described. Side-effects were similar in frequency and severity between the different dosage groups and are therefore listed together.

No pain was experienced by patients during the treatment of 12 lesions. Pain during PDT was described as mild in a further 10, moderate in one, and severe in one lesion which was an area of Bowen's disease that had ulcerated prior to therapy. Only in this latter case was local anaesthesia administered. Over the 10 days following treatment, no pain was associated

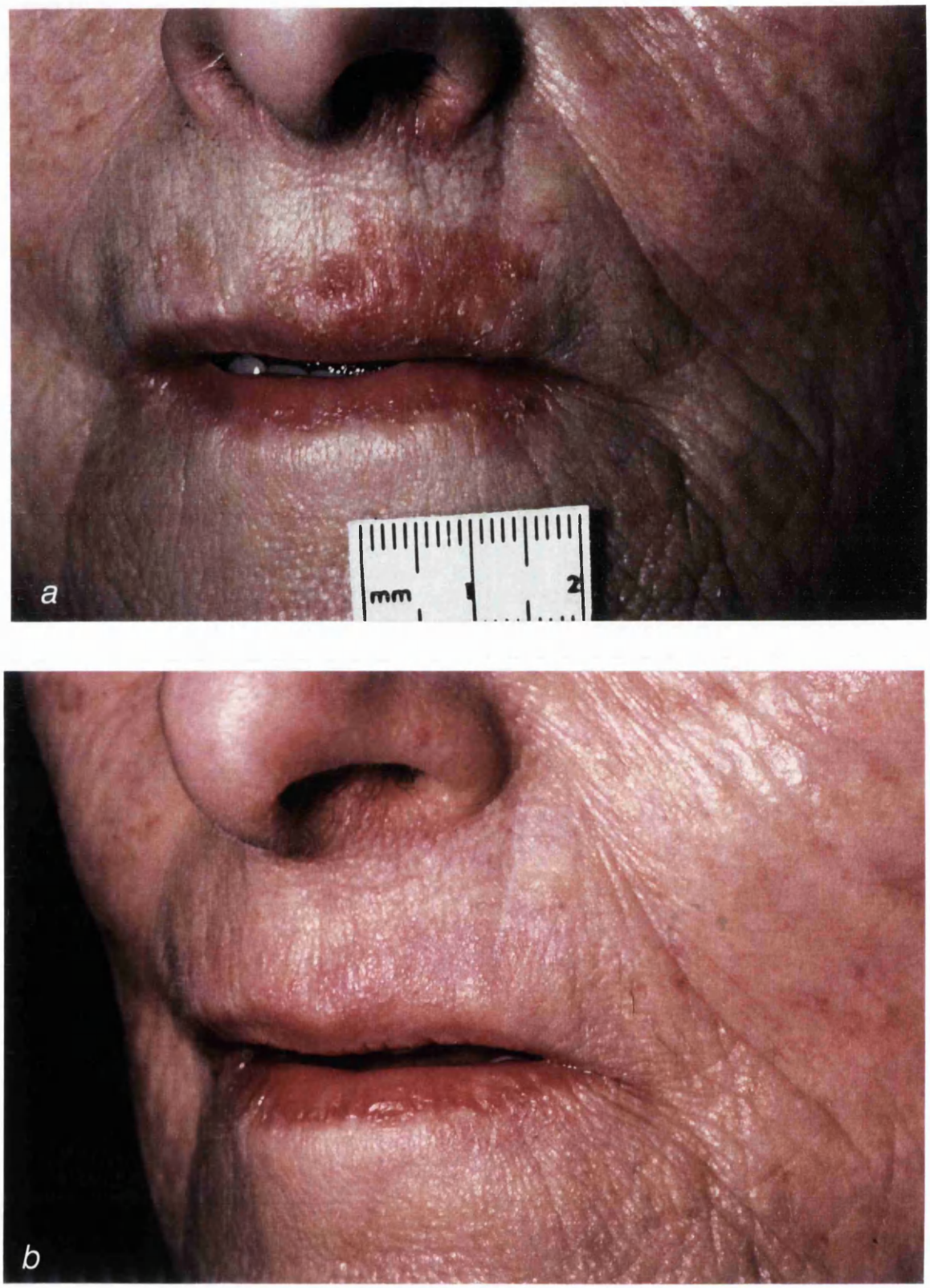


Figure 3.2 Bowen's disease on the upper and lower lip a) before and b) two months after a single treatment with ALA-PDT using the prototype lamp.

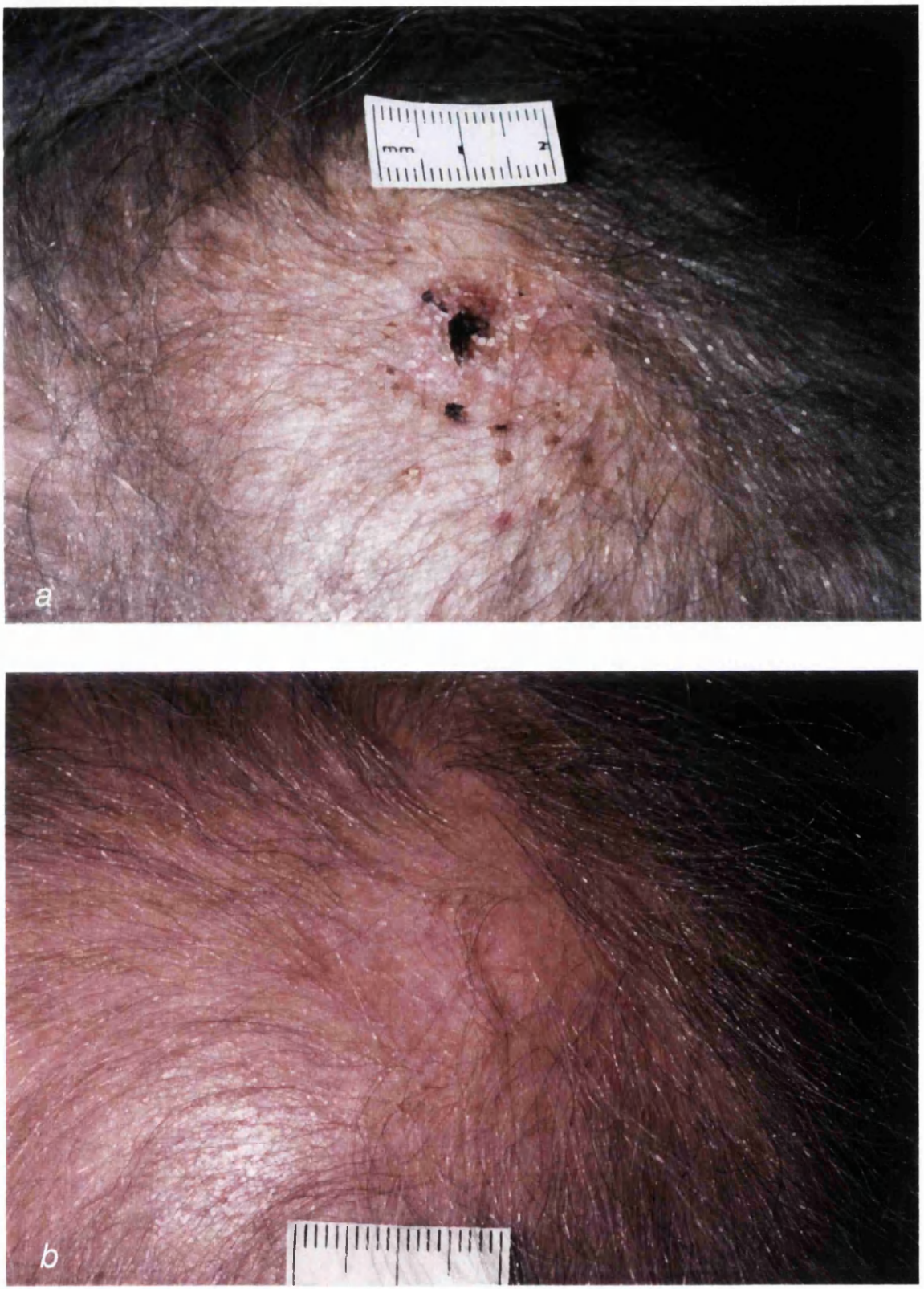


Figure 3.3 An actinic keratosis on scalp a) before and b) two months after a single treatment with ALA-PDT using the prototype lamp.

Table 3.2 Clearance of lesions of Bowen's disease after a single treatment depending on light dose. Treatment times, depending on which end attachment to the fibre bundle was used, are also shown.

Dose (J/cm^2)	Total no. of lesions treated	No. of lesions treated		Treatment time (mins.)		Clear after 1 treatment
		Rod	Lens	Rod	Lens	
94	7	5	2	10	29	5
125	15	8	7	13	39	9
156	2	2	0	16	49	1

with 21 treated lesions, with mild discomfort lasting around 7 days described in the remaining 3 lesions. Whilst erythema and oedematous swelling of the treatment sites were evident on completion of irradiation, only 3 lesions proceeded to blister (by 2 days) with one area of Bowen's disease subsequently ulcerating. No photosensitivity reactions were evident following PDT.

A visible scar in the treatment field, outwith diagnostic biopsy sites, was observed in only three treatment sites, all 3 areas of Bowen's disease treated on the ankle and overlying the achilles tendon.

Results - Recurrence rate: During the 12 months following clinical clearance of the 24 lesions, two areas of Bowen's disease recurred, both at 8 months, in lesions treated with 125J/cm^2. Clearance of these two lesions was achieved with a further treatment session of PDT using the prototype lamp. This gave an overall complete response rate after 1 year of 92%.

Summary: Clearance was achieved with a single treatment in 15 lesions (12 Bowen's and 3 actinic keratoses) and in all of the remaining 9 lesions after a second treatment with ALA-PDT using the prototype light source. The treatment was well tolerated, with pain absent or mild during treatment in 22 lesions, with only 1 lesion requiring local anaesthesia. Over the 10 days following treatment, no pain was associated with 21 treated lesions. During a 12 month follow-up period, 2 Bowen's disease lesions recurred. The overall complete response rate was 92%. Scarring was evident following the PDT in only 3 lesions.

Photodynamic therapy using this portable non-laser light source, appears to be an effective and well tolerated treatment for Bowen's disease and actinic keratoses.

3.5 - PDT vs. cryotherapy in Bowen's disease

Aim: To compare the efficacy and adverse effect profile of photodynamic therapy with that of cryotherapy in the treatment of Bowen's disease.

Patients: Ethical committee approval was obtained for the randomized treatment of patients with Bowen's disease with cryotherapy or PDT. During the 5 month recruitment period, sequential patients attending the department with a clinical diagnosis of Bowen's disease received a diagnostic 4mm punch biopsy. All patients with histological confirmation of the diagnosis and with individual lesions of ≤21mm in diameter, were invited to participate in the study (for information sheet and consent form see Appendices 3+4). No lesion had been previously treated. Individual lesions were then randomized to receive either cryotherapy or PDT.

Methods:

Cryotherapy: Liquid nitrogen was applied to lesions via a hand-held 'Cryac' spray. After initial icefield formation, the freeze was maintained for 20 seconds. A single freeze-thaw cycle technique was employed with a 2-3mm rim of clinically healthy tissue included in the treatment field.

ALA-PDT: 20% 5-ALA was applied to lesions four hours pre-illumination. Using appropriate filters, the spectral output of the lamp was adjusted to a 30nm bandwidth centred at 630nm. To broaden the treatment field and produce uniform irradiation of lesions, a 25mm collimating lens was attached to the 5mm fibre bundle. A minimum of a 10% margin around lesions was included in the field of irradiation permitting the treatment of lesions ≤21mm in diameter using a field size of 25mm. At a fluence rate of 70mW/cm^2 (higher than in the pilot study following internal adjustment of the first prototype lamp), lesions received 125J/cm.^{2}

Clearance and Recurrence: Clinical response was determined after two months and repeat treatments administered if lesions persisted. Following clearance, all patients were reviewed at monthly intervals for 12 months to look for recurrence and late adverse effects. Following therapy, 3mm punch biopsies were performed in lesions where doubt over clinical clearance or recurrence existed.

Statistics: Comparison of clearance rates was performed using a chi-squared test. To permit comparison of the effect of lesion size on success of clearing after a single treatment with each modality, a linear logistic regression analysis was performed. With the probability of a success denoted as p, the logit transform of p was logit (p) = log $(\frac{p}{1-p})$ and this was modelled as a linear function of the log area. Comparison of pain scores in each group was performed by a Mann-Whitney test.

Results - Clearance rates Forty lesions of Bowen's disease in 19 patients (3 male, 16 female, mean age 76yrs., range 62-88yrs.) were randomized to receive treatment. The 20 lesions treated by cryotherapy were located on the legs (n=16), face (n=3) and hand (n=1), and lesions treated by PDT were also located on the legs (n=17), face (n=2) and hand (n=1).

Cryotherapy produced clearance in 10/20 lesions after one treatment, a further six lesions clearing after two treatments and the remaining four lesions required three treatments. PDT resulted in clearance of 15/20 lesions after one treatment and the remaining five lesions after a second treatment. Direct comparison of clearance rates revealed no significant difference between the two treatments (p=0.08).

Analysis of lesion size in each group revealed that, by chance, lesions treated by PDT had larger surface areas (median 150mm^2, range 25-441mm^2) than those treated by cryotherapy (median 82mm^2, range 30-

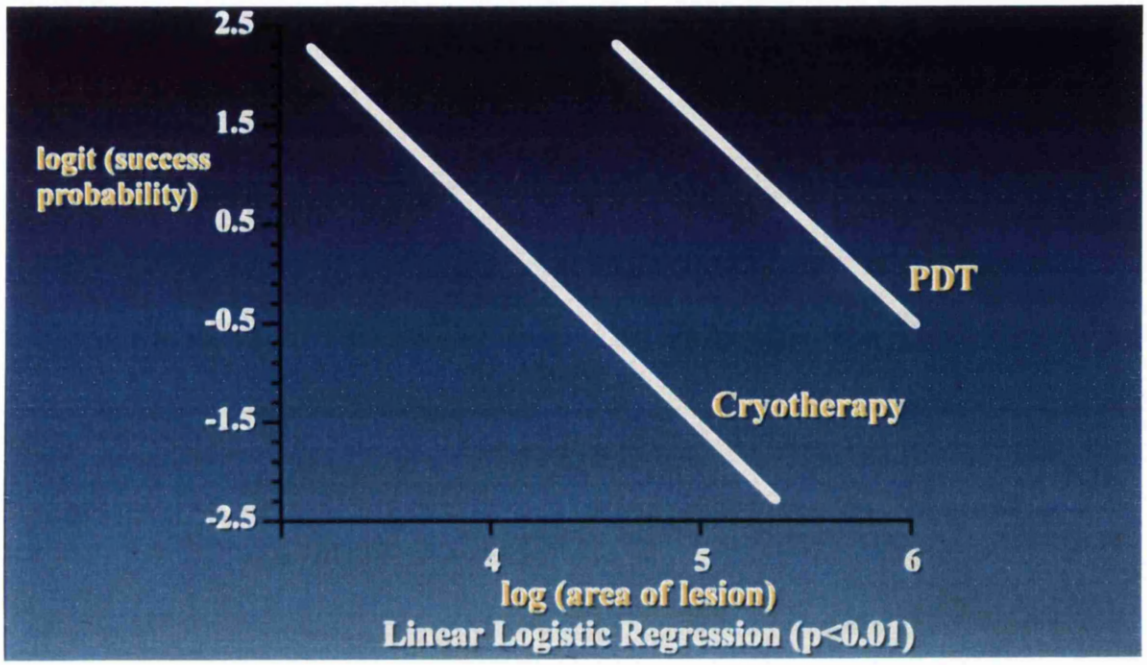
360mm^2). The larger lesions in each group also appeared to be the most likely to require more than one treatment with the lesions persisting after one treatment with PDT, of median size 378mm^2 (range 150-441mm^2) and those persisting after one application of cryotherapy of median size 208mm^2 (range 50-360).

A further comparison of the success of clearing after a single treatment was therefore performed using linear logistic regression to model the effect of lesion size on clearance. As illustrated in Figure 3.4, the probability that a lesion of any size is completely cleared at the first treatment is significantly greater with PDT than with cryotherapy ($p<0.01$). For both treatment modalities it was also confirmed that lesion size affects the probability that it is completely cleared at the first treatment ($p<0.002$).

Results - Adverse effects Pain during cryotherapy was present in 19 lesions and described as mild in 12 and moderate in 7 lesions. Pain during PDT was present in only 11 lesions and was mild in six and moderate in five lesions. This difference was statistically significant ($p=0.01$). No local anaesthesia was requested by patients receiving PDT or cryotherapy. Over the 10 days following treatment, no pain was associated with 10 of the cryotherapy treated lesions and 15 of those that received PDT, with pain otherwise mild or moderate in severity in the remainder in both groups. All six patients who received both treatments due to having multiple lesions, reported PDT as being less painful.

Blister formation occurred after initial cryotherapy in seven lesions with subsequent ulceration in five. Systemic antibiotic was later commenced by the patient's general practitioner for cellulitis arising around two of these ulcerated lesions. In contrast, no blistering nor ulceration occurred following PDT, with no subsequent episodes of secondary infection. No photosensitivity reactions were observed following PDT.

Figure 3.4 Comparison of the success of clearing Bowen's disease after a single treatment with cryotherapy or PDT, using linear logistic regression to model the effect of lesion size on clearance.



At final review 12 months following clearance, a visible scar in the treatment field, outwith diagnostic biopsy sites, was observed in four lesions treated by cryotherapy, whilst visible scarring was absent in all lesions treated by PDT.

Results - Recurrence rate: During the 12 months following clinical clearance, only two areas of Bowen's disease recurred, at 6 and 8 months, both from the cryotherapy group. This gives an overall complete response rate after one year of 90% in the cryotherapy group and 100% in the PDT group.

Summary: In a randomized comparison trial of ALA-PDT vs. cryotherapy in the treatment of 40 lesions of Bowen's disease, cryotherapy produced clearance in 10/20 lesions after one treatment, the remaining 10 lesions requiring two or three treatment applications. PDT resulted in clearance of 15/20 lesions after one treatment and of the remaining five lesions after a second treatment. The probability that a lesion cleared after one treatment was greater with PDT than cryotherapy ($p<0.01$). Cryotherapy was associated with ulceration (5/20), infection (2/20) and recurrent disease (2/20); no such complications occurred following PDT.

PDT, using a non-laser light source and topical 5-ALA, appears to be at least as effective as cryotherapy in the treatment of Bowen's disease with fewer adverse effects.

3.6 - Basal cell carcinoma - Open Study

Aim: To determine the efficacy of topical ALA-PDT using the prototype light source in the treatment of superficial basal cell carcinomas. In particular, this study sought to assess the influence of tumour thickness on the efficacy of

ALA-PDT by comparing tumour response with histological thickness. The effect of altering the duration of photosensitizer application on clearance of BCC was also examined.

Patients and Methods: Patients attending the department with a clinical diagnosis of superficial basal cell carcinoma received a diagnostic 4mm punch biopsy and those with histological confirmation of BCC were invited to participate in the study (for information sheet and consent form see Appendices 5+6). No lesion had been previously treated.

The spectral output of the lamp was adjusted with filters to 630±15nm. A 25mm collimating lens was attached to the light guide to produce uniform irradiation of lesions. A minimum of a 10% margin around lesions was included in the field of irradiation. Alteration of the distance of the collimating lens from the skin surface achieved field diameters of 3-7.5cm (5mm fibre - 3-4cm fields; 8mm fibre - 5-7.5cm field) at fluence rates of 20-86mW/cm.^{2} Individual lesions received 150J/cm.^{2} Patients recruited during Year 1 received 20% 5-ALA application 4 hours pre-illumination, whilst patients entering during Year 2 received photosensitizer 6 hours pre-illumination. The patients were offered local anaesthetic during treatment, if required.

The thickness of the tumour, measured from the granular layer to the deepest tumour cell in the dermis, was determined from examination of the diagnostic 4mm punch biopsy, which was performed by the same clinician (CAM) who sampled the thickest clinically evident part of all lesions. Adverse effects were recorded for all patients, including pain during treatment which was assessed using visual analogue scales.

Statistics: Clearance rates were compared using a two-sided Fisher's exact test. The effects of tumour thickness and lesion size on clearance were modelled through a linear regression analysis.

Results - Clearance rates: Fifty-three basal cell carcinomas in 31 patients (14 male, 17 female, mean age 68yrs., range 29-96yrs.) received PDT. Of these patients, 24 had one BCC, three had two, two had three, one had four and one patient had 13 tumours.

The complete response rate (Table 3.3) after 5-ALA application for 6 hours was significantly higher than for the 4 hour group ($p=0.005$). Although multiple lesions in the same patient were all treated on different occasions, there might be slight departure from independence of the dataset, particularly due to the patient with 13 lesions. Statistical analysis of the remaining 40 lesions continued to demonstrate a significant difference in response ($p=0.008$).

Only 3/6 facial BCC in the 4 hour group cleared , whilst 4/6 limb lesions and 13/15 lesions on the trunk resolved (Figure 3.5). Basal cell carcinomas treated after 6 hours were situated on the face (n=2), limbs (n=7) and trunk (n=17).

The size of lesions treated in each group were similar (median sizes: 4hr., 288mm^2;6hr., 235mm^2). However, the median size of lesions requiring two treatments was larger in both groups (4hr.: 775mm^2, range 288-4480mm^2; 6hr.: 1312mm^2, range 140-4500mm^2) than for lesions clearing after one treatment (4hr.: 399mm^2, range 50-2400mm^2; 6hr.: 150mm^2, range 50-2500mm^2).

Results - Tumour thickness: The effect of tumour thickness on response rate is shown in Table 3.3b. It is important to note that the calculated values of tumour thickness may be an underestimate of the *in-vivo* depth due to tissue shrinkage in biopsy tissue preparation. Whilst all tumours less than

Table 3.3: Complete response (CR) rate following PDT for all 53 basal cell carcinomas (a) depending on duration of photosensitizer application, and (b) depending on tumour thckness. All lesions received 150J/cm$^{2.}$

(a) Photosensitizer duration	4 hour (n=27)	6 hour (n=26)
CR after 1 treatment	16	20
CR after 2 treatments	3	6
Total CR - no./rate	19 (70%)	26 (100%)
Recurrence	3*	0
Follow-up period -mean and range (months)	24 (17-27)	10 (6-16)
Overall response rate	59%	100%
(b) Tumour thickness	CR rate - 4 hour	CR rate - 6 hour
<1mm (n=36)	16/16	20/20
1-2mm (n=13)	3/7	6/6
>2mm (n=4)	0/4	-

* Recurrence at 5, 18, 22 months (Tumour thickness, 1.0, 0.3, 0.4mm respectively).

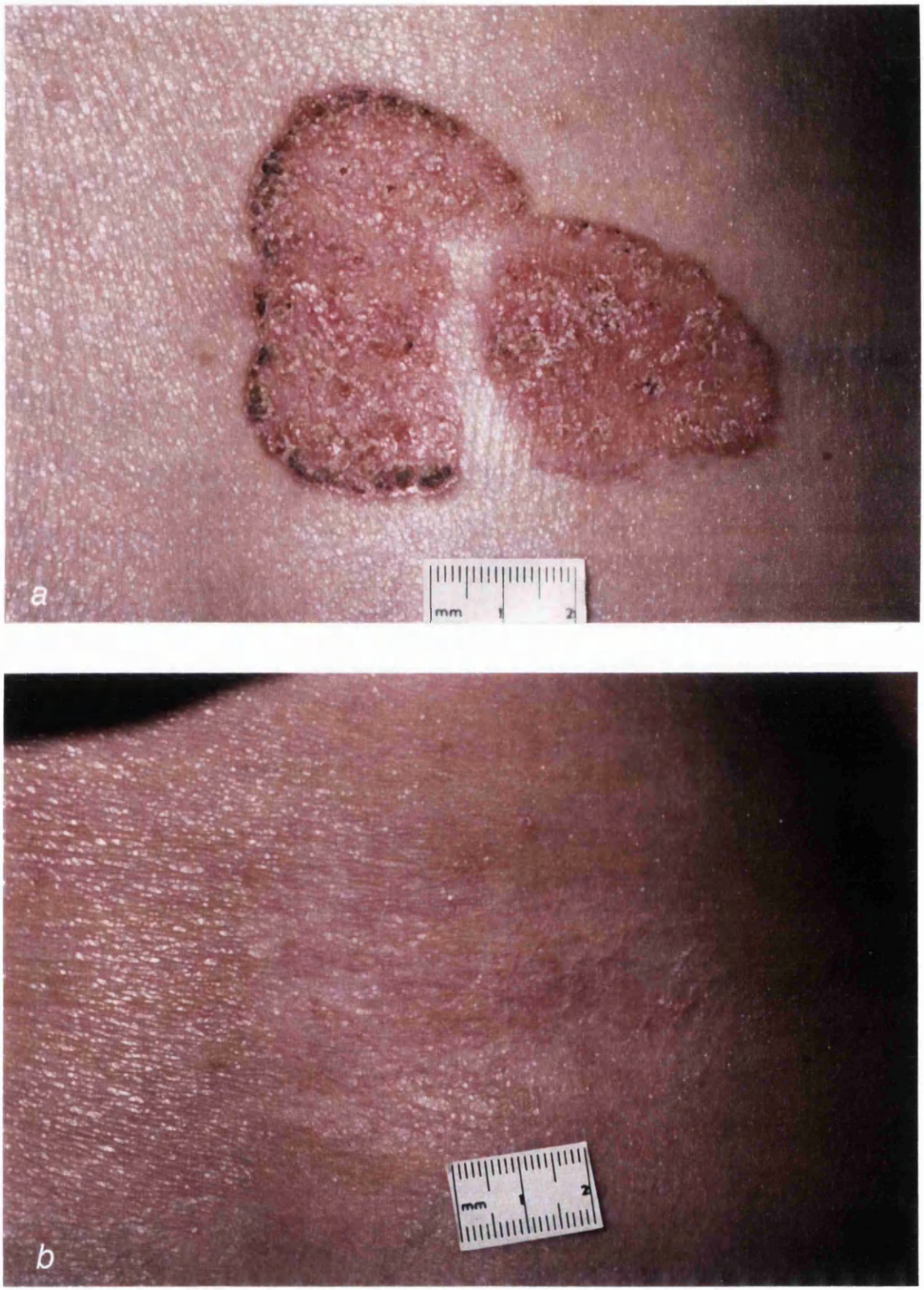


Figure 3.5. Basal cell carcinoma on the back (a) 7.2x5.6cm lesion before photodynamic therapy (0.7mm thick) and (b) the same site 4 months after 2 treatments with ALA-PDT.

1mm thick cleared in each group, only 3/7 tumours 1-2mm in depth cleared in the 4hr. group, in comparison with all 6 tumours in the 6hr. group (p=0.09). No tumour greater than 2mm in thickness cleared, although all 4 such lesions belonged to the 4 hour group.

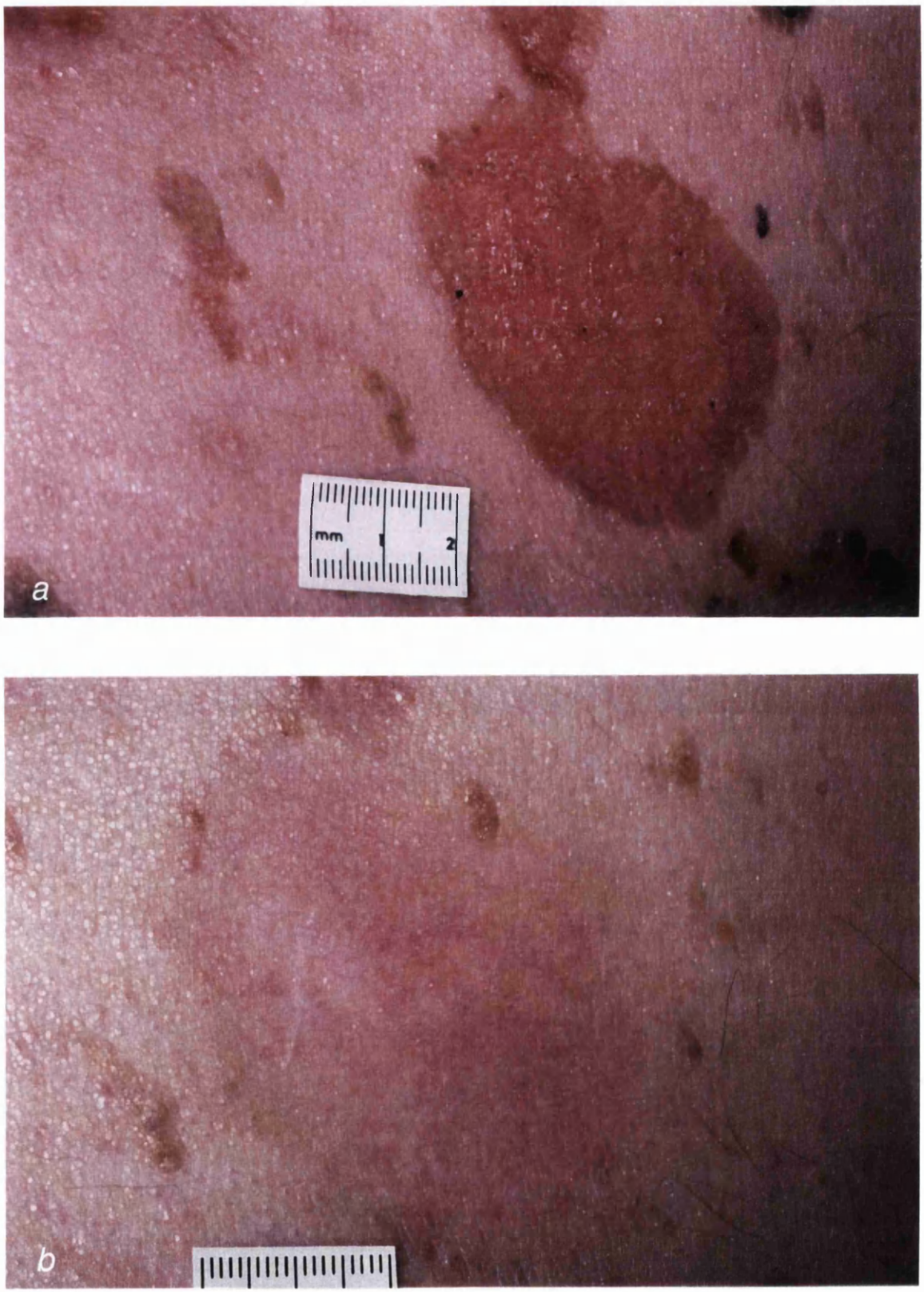
The median thickness of tumours clearing after one treatment was identical for both study groups at 0.4mm (range: 4hr. 0.3-1.0mm, 6hr. 0.3-1.5mm). The median thickness of lesions clearing after two treatments in the 4hr. group was 0.7mm (range 0.5-0.8mm) compared with 0.9mm in the 6hr. group (range 0.6-1.2mm). The 8 lesions in this study which did not clear were overall thicker (median 1.6mm, range 1.0-3.5mm) and included the four lesions that were over 2mm thick.

Basal cell carcinoma thickness was an important determinant of tumour response in the 4 hour group ($p<0.0001$) on linear logistic regression analysis. After allowing for thickness, lesion size did not affect clearance in this group. The 6 hour group was not analysed in view of the 100% CR rate.

Results - Adverse effects: Pain during PDT in each group was absent in 10, mild in 14, moderate in 2 and severe in 1 lesion in the 4hr. group and absent in 5, mild in 16, moderate in 4 and severe in 1 in the 6hr. group. The 2 patients experiencing severe pain on initiation of therapy received local anaesthesia and pain subsided. These were the two largest lesions ($4480mm^2$ and $4500mm^2$) in this study with no other patient requiring local anaesthesia.

Blister formation occurred following PDT in 6 patients which resolved by day 5, but neither ulceration nor infection of treatment sites was observed. No photosensitivity reactions were recorded following PDT. Cosmetic outcome was excellent for all tumours (Figures 3.5 and 3.6) with minimal scar formation. Initial pigmentary changes post-therapy (both hypo- and hyper-

Figure 3.6 Basal cell carcinoma on back (6.7x4.1cm, 0.8mm thick) (a) before and (b) two months after one treatment with photodynamic therapy.



pigmentation) resolved within 6 months. Hair loss was noted to persist in one large BCC treated in the pubic area (Figure 3.7)

Results - Recurrence rate: Three basal cell carcinomas have recurred after 5, 18 and 22 months (tumour thickness 1.0, 0.3 and 0.4mm respectively) in the 4hr. group (Table 3.3), with no recurrences in the 6 hour group. The recurrent lesions were successfully re-treated by PDT. These patients continue on follow-up.

Case report:

Thirteen of the basal cell carcinomas treated in this study were from a 65 year-old male Caucasian. He had received radiotherapy for acne at 26 years of age. He gave no history of arsenic ingestion. His first BCC was diagnosed when 45 years old and he has subsequently developed many further lesions on his trunk and upper limbs, but not face, requiring frequent surgery ± grafting. The extent and multiplicity of BCCs (Figure 3.8) led to his referral from the plastic surgery unit.

The size of the lesions treated ranged from 270mm^2-2795mm^2 (median=1500mm^2). PDT resulted in clearance of 12/13 lesions (Figure 3.6), 6/7 in the 4 hour group and all 6 in the 6 hour group, and reduction in size of the remaining BCC which facilitated direct surgical excision without grafting.

Summary: The efficacy of topical ALA-PDT using the non-laser source was demonstrated in an open study of the treatment of 53 superficial basal cell carcinomas. 5-ALA application 4 hours pre-illumination cleared 19/27 BCC, whilst 6 hour application cleared 26/26 BCC (p=0.005). All 36 tumours <1mm thick cleared following PDT. Four hour application of 5-ALA cleared only 3/7 tumours 1-2mm thick, in comparison with 6 hour application which cleared 6/6 BCC in this category. All 4 BCC >2mm thick and treated at four hours, failed

Figure 3.7 Basal cell carcinoma (7.5x6.0cm, 0.6mm thick) in the groin of a 45 year old female (a) before and (b) 12 months after two treatments with photodynamic therapy.

Figure 3.8 Multiple basal cell carcinomas on the back, several ulcerated.

to clear. Tumour thickness was an important determinant of clearance in the 4 hour group ($p<0.0001$).

Tumour thickness appears to closely predict the therapeutic response of BCC to PDT. Increasing the duration of application of 5-ALA from 4 to 6 hours may improve tumour clearance by increasing the efficacy of PDT for intermediate thickness tumours. PDT may therefore be a useful therapy for superficial basal cell carcinomas <2mm thick.

3.7 - The effects of light dose, fluence rate and lesion size on response of Bowen's disease to ALA-PDT

Aim: To determine the optimal dosage schedule using the prototype lamp for topical ALA-PDT by assessing the response of areas of Bowen's disease to different light dose and fluence rate regimens. Efficacy of ALA-PDT in the treatment of large patches of Bowen's disease was examined to further assess the influence of lesion size on outcome. The rate of decay of photosensitizer during PDT was also assessed via monitoring of surface fluorescence to assist in refining the dosage schedules.

Patients and Methods: Patients with histologically proven areas of Bowen's disease were invited to participate in the dose-response studies, providing no lesion had been previously treated. ALA-PDT used 20% 5-ALA applied 4 hours prior to illumination by the techniques outlined above and clearance was determined after two months in each case with one repeat treatment administered if lesions persisted. Following therapy, 3mm punch biopsies were performed in lesions where doubt over clinical clearance or recurrence existed. Following clearance, all patients were reviewed at monthly intervals for 12 months. Those parameters unique to each part of the dose-response assessment are summarized below:

(a) Light dose/response study - Lamp output was 630 ± 15nm, delivered via a flexible light guide and collimating lens. Irradiance was maintained at 55-86mW/cm^2 permitting a 3cm diameter field size (the variation being due to the fall-off in lamp output during the 2 year period of the study). Lesion size: a maximum diameter of 25mm was permitted to allow for a minimum 10% margin around lesions. A light dose of 50, 75, 100 or 125J/cm^2 was randomly allocated with the intention to recruit 30 lesions in each category. Six further lesions subsequently received 25J/cm^2.

(b) Large patch Bowen's disease study - Patients with patches of Bowen's disease >21mm in maximum diameter were recruited into an open study of ALA-PDT with study design identical to that outlined in Section 3.6 except that 5-ALA was applied 4 hours prior to illumination with a subsequent total light dose of 125J/cm.^{2} In view of earlier experience indicating that larger lesions are more likely to require repeat treatments, up to three treatments were permitted within this study protocol before reversion to conventional therapy. Pain during and over the 10 days following treatment was assessed as described in Section 3.3. Patients were offered local anaesthesia during treatment.

(c) Fluence rate/response study - Lamp output was 630 ± 15nm, delivered via a flexible light guide and either the perspex rod (for lesions up to 9mm in maximum diameter and used in the pilot study - Section 3.4) or the collimating lens apparatus. Irradiance varied between 18-158mW/cm^2 depending on whether the rod (158mW/cm^2) or collimating apparatus was used and the field size with which lesions were treated (2.5-7.5cm - Table 3.1). As data is included from the early pilot and the PDT vs. cryotherapy studies, certain additional irradiance values are included to those listed in

Table 3.1 due to the differences in power output between the prototype lamps used. Only lesions which received 125J/cm^2 were included in this analysis.

(d) Rate of decay of surface fluorescence - Fluorescence at the skin surface due to the presence of protoporphyrin IX was detected using a hand-held ultraviolet lamp with a peak emission at 365nm. This lamp was used throughout the clinical trials of PDT to confirm the initial presence of PpIX and its subsequent surface clearance post-PDT. For a randomly selected group of lesions the fluorescence was also detected at intervals of 10J/cm^2 during PDT to detect changes in the intensity of fluorescence using the scale noted below (all assessments by CAM): Fluorescence intensity: 0 - not detectable; 1 - just visible; 2 - moderate; 3 - strong; 4 - very intense.

Statistics: A stepwise logistic regression procedure was used to assess the influence of dose on the success of PDT. The probability of failure was modelled with success defined as clearance by 2 treatments with no recurrence within one year. As well as lesion size, 3 indicator variables, $\leq$ 100J/cm^2, $\leq$75J/cm^2 and $\leq$50J/cm^2 were included. The same procedure was used to assess the influence of irradiance on clearance with lesion size and fluence rate as explanatory variables. Comparison of fluorescence decay rates was by a Mann-Whitney analysis, adjusted for ties.

Results:

(a) Light dose/response study: A total of 120 lesions in 72 patients initially entered the trial, although 4 lesions (in 3 patients) were subsequently lost to follow-up and not included in the analysis. Clearance rates are summarized in Table 3.4. Whilst 125 and 100J/cm^2 achieved initial complete response rates of 100 and 93% respectively, during the 12 months of follow-up, three recurrences in the 125J/cm^2 group (at 5, 7 and 8 months) and one recurrence

Table 3.4: Dose-response comparison for the treatment of (a) Bowen's disease and (b) basal cell carcinoma by ALA-PDT.

(a)

Dose J/cm^2	No. of lesions	Clear on 1 treatment	Clear on 2 treatments	No. of recurrences over 1 year	Median size (mm^2)	Range of size (mm^2)	Overall clearance rate (%)
125	30	23	7	3	171	36-462	90 (n=27)
100	27	22	3	1	195	25-590	89 (n=24)
75	29	23	3	7	100	25-374	66 (n=19)
50	30	17	10	6	196	25-462	70 (n=21)
25	6	0	0	-	216	64-540	0

(b)

150	16	13	3	1	405	50-2795	94 (n=15)
100	16	12	4	1	400	81-3000	94 (n=15)

in the 100J/cm^2 group (at 7 months) reduced the clearance rates to 90 and 89% respectively. Seven recurrences in the 75J/cm^2 group (after 3,3,6,6,8,9 and 10 months) and five recurrences in the 50J/cm^2 group (after 5,5,6,7,9 and 11 months) reduced overall clearance rates in these groups to 66 and 70% respectively.

Statistical analysis of these results confirmed a significant reduction in the probability of successful clearance at 1 year for lesions receiving less than 100 J/cm^2 ($p<0.001$) with larger lesions overall less likely to clear than smaller ones ($p=0.02$). The odds ratio (95%CI) for this reduction in efficacy after 75 or 50 J/cm^2 was 6.73 (2.33-19.4) and for the influence of size (in mm^2) on clearance was 1.004 (1.001-1.007).

In view of the positive response of the majority of lesions to 50J/cm,^{2} a further six lesions received 25J/cm,^{2} but no complete response was observed although a partial response was noted in three of the lesions.

(b) Large patch Bowen's disease study - 40 patches of Bowen's disease in 36 patients received ALA-PDT, with clearance of 17 after one treatment and a further 13 clearing after a second and 5 after a third, giving an initial complete response rate of 88%. Five lesions (1 ulcerated pre-treatment, 4 clinically thicker plaque than other lesions), failed to clear during three treatments although all showed a partial response. Figure 3.9 shows one lesion that has successfully responded to one treatment and a second area of Bowen's disease demonstrating a partial response after three treatments. During the 12 month follow-up period, 4 patches recurred, three at 3 months and one at 7 months, giving an overall clearance rate of 78%. The median size of lesions clearing within 2 treatments was 623mm^2 (range 405-2236mm^2), on a third treatment was 930mm^2 (range 700-1750mm^2), and was 1600mm^2 (range 868-2530mm^2) for those areas of Bowen's disease which did not clear.

Figure 3.9-a+b Response of large patches of Bowen's disease to PDT. (a) 40x40mm patch on right leg prior to and (b) 2 months following a single treatment.

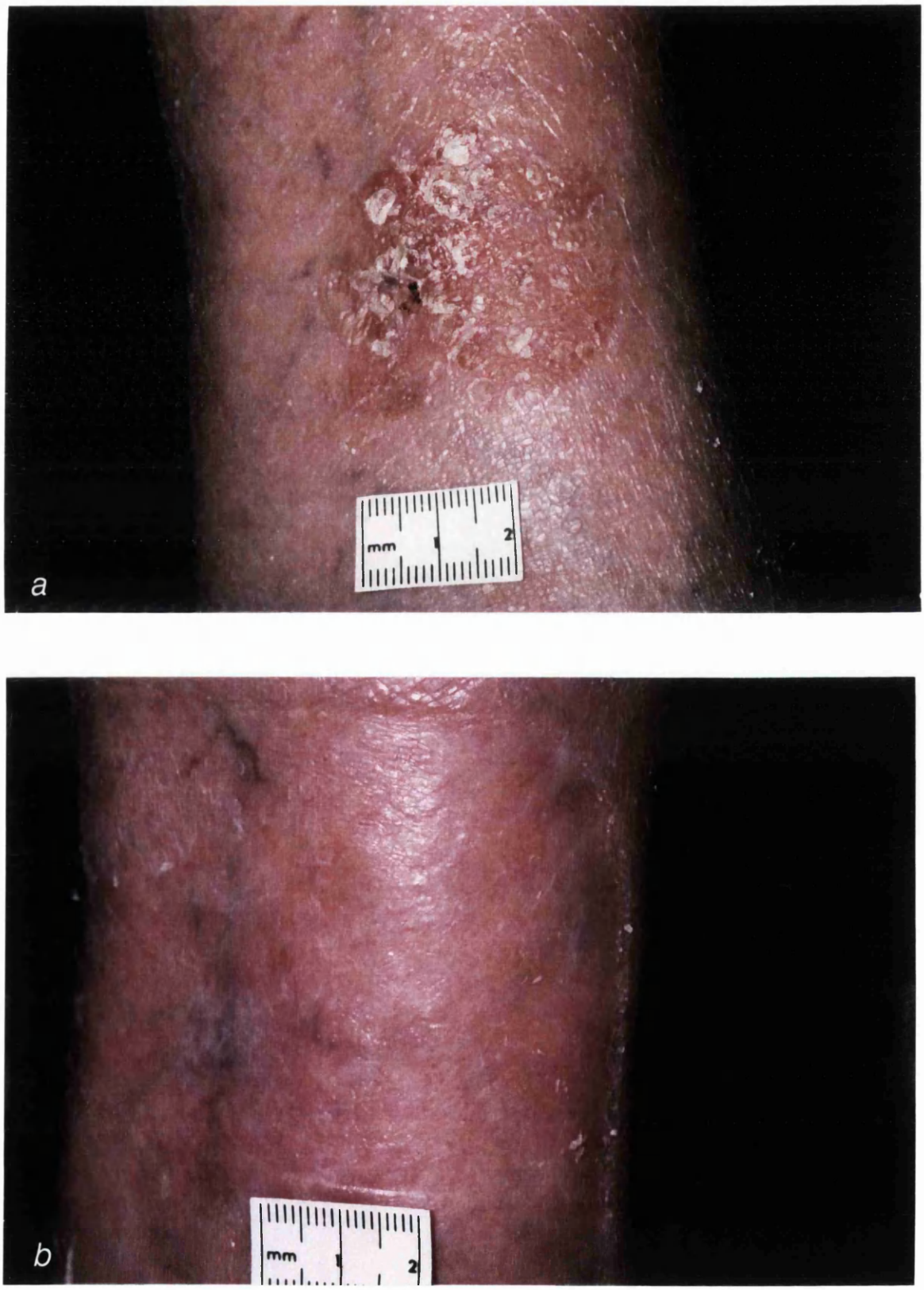


Figure 3.9 c+d Response of large patches of Bowen's disease to PDT. (c) 62x40mm patch on right knee before and (d) 2 months following 3 treatments with PDT.

Treatment was well tolerated with initial oedematous swelling at the lesion site, present on completion of ALA-PDT, and subsiding over 24 hours. Ulceration and infection of treatment sites were not observed. Eschars formed over lesions which separated after 4-6 weeks. During treatment, pain was absent in 14, mild in 12, moderate in intensity in 6, and graded as severe in 8. However, local anaesthesia was requested only in 3 lesions with median size 875 mm^2 (range 460-3850mm^2). Following treatment, pain persisted only in the 8 patients describing pain as severe until day 2 in three, day 7 in three and up to 10 days in the remaining two patients.

(c) Fluence rate/response study: Clearance rates for the range of fluence rates used are summarized in Table 3.5. Logistic regression demonstrated that larger lesions were associated with a lower overall clearance rate ($p=0.007$). After correcting for this influence of size, a significant improvement in response was apparent at fluence rates at or below 48mW/cm^2 ($p=0.0097$). Odds ratios (95%CI) were 1.003 (1.001-1.005) for size and 0.019 (0.0007-0.501) for failure of clearance at fluence rates of $\leq$ 48mW/cm^2.

(d) Rate of decay of surface fluorescence: For all lesions of Bowen's disease which received a dose of 50J/cm^2 or greater, surface fluorescence (grade 3 or 4) was present prior to therapy and was absent on cessation of therapy. This excludes the 6 lesions of Bowen's disease treated with 25J/cm^2 (Table 3.4) in which fluorescence was detectable (grade 1) in 4/6 and not detectable in the remaining 2 lesions post-PDT. The pattern of fluorescence decay at 10J/cm^2 increments is shown in Table 3.6 for 11 randomly selected lesions of Bowen's disease with loss of fluorescence by 30-50J/cm^2 in all lesions.

Table 3.5: Fluence rate-response comparison for the treatment of Bowen's disease by ALA-PDT. All lesions received 125J/cm^2.

Fluence rate mW/cm^2	No. of lesions	Clear on 1 treatment	Clear on 2 treatments	No. of recurrences over 1 year	Median size (mm^2)	Range of size (mm^2)	Overall % clearance rate
158	15	13	2	0	42	9-81	100
122	4	3	1	1	37	16-94	75
86	14	10	2	2	140	9-460	71
70	13	9	4	0	210	110-462	100
55	9	3	6	3	256	195-576	67
48	22	12	6	3	580	351-1122	68
40	8	3	4	0	1225	525-1360	88
30	1	1	0	0	1050	-	100
18	4	1	1	0	2358	1431-2530	50
Total	90	55	26	8	-	-	-

Table 3.6: Fluorescence decay at lesion surface for Bowen's disease and basal cell carcinomas during ALA-PDT.

Case No.	Diagnosis	Fluorescence intensity at incremental light doses (J/cm^2)						
		0	10	20	30	40	50	Loss by
1	Bowen's	4	2	2	1	1	0	50
2	Bowen's	4	3	2	1	0	0	40
3	Bowen's	3	2	1	0	0	0	30
4	Bowen's	4	2	1	1	0	0	40
5	Bowen's	4	3	2	1	0	0	40
6	Bowen's	4	2	1	0	0	0	30
7	Bowen's	4	2	2	1	0	0	40
8	Bowen's	3	2	1	0	0	0	30
9	Bowen's	3	2	1	1	0	0	40
10	Bowen's	4	3	1	0	0	0	30
11	Bowen's	3	2	1	1	0	0	40
							Mean:	37
12	BCC	4	2	2	1	1	0	50
13	BCC	4	3	3	2	1	0	50
14	BCC	3	2	1	1	0	0	40
15	BCC	4	2	1	1	1	0	50
16	BCC	4	2	1	1	0	0	40
17	BCC	4	2	1	1	0	0	40
18	BCC	4	3	1	1	0	0	40
							Mean:	44

Summary: A randomized study of light dose for Bowen's disease using filtered red light and a 5-ALA application time of 4 hours showed a superior efficacy for doses of 100 and 125 J/cm^2 when recurrence rates over a 12 month period were included in the overall clearance data. Overall, larger lesions were associated with a lower clearance rate. Fluence rates of ≤ 48mW/cm^2 were associated with an improved response despite the larger size of the lesions treated. Fluorescence decay assessments support a minimum light dose of 50J/cm^2 to ensure clearance of visible surface protoporphyrin IX in lesions of Bowen's disease.

3.8 - The effects of light dose, fluence rate and lesion size on response of BCC to ALA-PDT

Aim: To determine the influence on outcome of ALA-PDT for BCC of alterations in light dose, fluence rate and lesion size. The rate of decay of photosensitizer during PDT was also assessed to assist in refining the dosage schedules.

Patients and Methods: Patients with histologically proven areas of superficial basal cell carcinoma were invited to participate in the dose-response studies, providing no lesion had been previously treated. ALA-PDT using 20% 5-ALA applied 4 or 6 hours prior to illumination by the techniques outlined above and clearance was determined after two months in each case with one repeat treatment administered if lesions persisted. Following clearance, all patients were reviewed at monthly intervals for at least 12 months.

(a) Light dose/response study - Lamp output was 630±15nm. Alteration of the distance of the collimating lens from the skin surface achieved field diameters of 3-7.5cm at fluence rates of 20-86mW/cm.^{2} Individual lesions from two patients were randomized to receive either 100 or 150J/cm^2 with 5-ALA applied 6 hours pre-illumination. Only lesions with a tumour thickness ≤ 1mm were included.

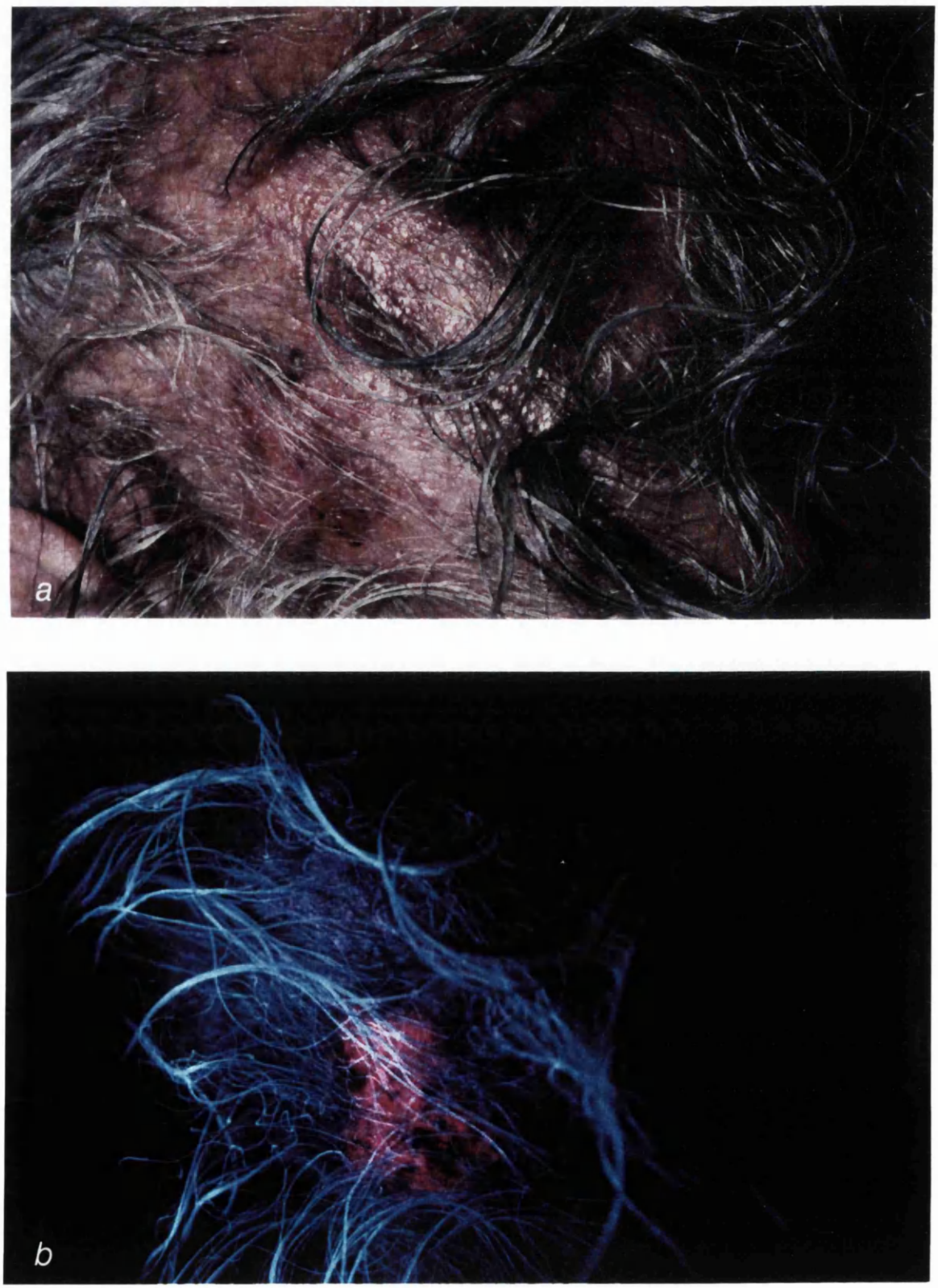
(b) Fluence rate/response study - The lamp characteristics are as described in (a) above. Only lesions treated with 150J/cm^2 with 5-ALA application 6 hours pre-illumination were evaluated.

(c) Lesion size/response study - Data from all lesions treated in Section 3.6 was combined with that derived from BCC treated in this section.

(d) Rate of decay of surface fluorescence - Fluorescence detection at the skin surface due to protoporphyrin IX was as described in Section 3.7(d). The fluorescence noted 6 hours following 5-ALA application is shown in Figure 3.10 which also demonstrates how this technique can assist in delineating tumour margins.

Statistics: Logistic regression analysis was used to assess the influence of lesion size on the success of PDT in BCC, with tumour thickness also entered as an explanatory variable. The probability of failure was modelled, with success defined as clearance by 2 treatments with no recurrence within one year. The same procedure was attempted to compare the two dosage regimens and to assess the influence of irradiance on clearance with lesion size and tumour thickness as explanatory variables. Comparison of fluorescence decay rates for BCC was by a Mann-Whitney analysis, adjusted for ties.

Figure 3.10 (a) A superficial (0.4mm) BCC on the scalp with poorly defined margins. (b) The same lesion demonstrates fluorescence as detected by a UV lamp 6 h. after 5-ALA application.



Results:

(a) Light dose/response study: basal cell carcinomas were treated in two patients with 12 and 20 tumours, who had 6 and 10 lesions respectively treated by each of the light doses studied. The results are summarized in Table 3.4. Although there is insufficient data for formal statistical analysis, no difference in response was noted between the two dosage levels administered.

(b) Fluence rate/response study: Table 3.7 summarizes the results of 33 BCC treated by the same light dose and 5-ALA application duration. Although there was insufficient data for formal analysis, fluence rate had no discernible effect on outcome.

(c) Lesion size/response study - Table 3.8 outlines all 76 BCC which received ALA-PDT by one of three treatment regimens outlined. For BCC lesions treated at 4 hours with 150J/cm^2, size was not significant, although thickness was an important determinant of response ($p<0.0001$). For lesions receiving 150J/cm^2 six hours following 5-ALA application, size was marginally not significant ($p=0.06$), although thickness was again an important determinant of response ($p=0.012$). BCC treated with 100J/cm^2 six hours post-ALA showed no significant effect of size nor tumour thickness on outcome. This latter group was both the smallest and contained thinner tumours in comparison with the other groups.

(d) Rate of decay of surface fluorescence: For all BCC treated by PDT in this thesis, surface fluorescence (grade 3 or 4) was present prior to therapy and was absent on cessation of therapy. The pattern of fluorescence decay at 10J/cm^2 increments for 7 randomly selected BCC is shown in Table 3.6 with loss of all visible fluorescence by 50J/cm^2. The median dose at which

Table 3.7: Fluence rate-response comparison for the treatment of basal cell carcinomas by ALA-PDT. All lesions received 150J/cm^2

Fluence rate mW/cm^2	No. of lesions	Clear on 1 treatment	Clear on 2 treatments	No. of recurrences over 1 year	Median size (mm^2)	Range of size (mm^2)	Overall % clearance rate
122	8	7	1	0	95	70-150	100
86	13	12	1	1 (3 mth)	225	100-495	92
48	2	0	2	0	510	425-594	100
40	3	1	2	0	1050	888-1120	100
30	4	3	1	0	1788	1250-2500	100
18	3	1	2	0	2795	2475-4500	100
Total	33	24	9	1	-	-	-

Table 3.8: Lesion size/tumour thickness-response comparison for the treatment of basal cell carcinomas by ALA-PDT.

Group	Application time (hrs) /Dose (J/cm^2)	No. of lesions	Clear by 2 treatments, no recurrence	Median size (range) (mm^2)	Median thickness (range) (mm)
1	4/150	27	16	288 (50-4480)	0.7 (0.3-3.5)
2	6/150	33	32	405 (50-2795)	0.5 (0.3-1.5)
3	6/100	16	15	400 (80-2475)	0.3 (0.3-1.0)

fluorescence is lost was higher for BCC than Bowen's (p=0.04) possibly due to the 6 hour pre-treatment 5-ALA application time for BCC in comparison with 4 hours for Bowen's disease.

Summary: A light dose of 100J/cm^2 was as effective as 150J/cm^2 in the clearance of BCC, without fluence rate influencing outcome. Tumour thickness is the predominant variable affecting response over size for BCC although neither variable significantly altered outcome in the relatively thin tumour group treated with 100J/cm^2. Fluorescence decay indicated a minimum light dose of 50J/cm^2 to ensure surface PPIX clearance following 5-ALA application 6 hours pre-illumination.

3.9 - Red vs. green light - efficacy and temperature change

Aim: To assess the influence of wavelength on clearance of Bowen's disease by comparing red with green filtered light emitted from the prototype lamp. Differences in decay of surface fluorescence and maximum temperatures during therapy were recorded in addition to clinical clearance and recurrence rates.

Patients and Methods: Sequential patients each with two or more biopsy proven patches of Bowen's disease and with individual lesions of ≤21mm in diameter, were invited to participate in the study. Individual lesions were randomized to receive PDT either with green or red filtered light. 20% 5-ALA was applied to lesions four hours pre-illumination. The spectral output of the lamp was adjusted to 540±15nm (green) or 630±15nm (red) using appropriate filters. A 25mm collimating lens apparatus was attached to the 5mm fibre guide. At a fluence rate of 86mW/cm^2, lesions received 125J/cm^2 of red light

and 62.5J/cm^2 of green light. This dose of green light was chosen as the quantum yield of protoporphyrin IX at 540nm is approximately twice that at 630nm (Figure 3.11).[133]

Fluorescence decay was recorded in a random sample of 5 lesions receiving each wavelength by the method outlined in Section 3.7(d).

Temperature was recorded during PDT by an infrared temperature probe attached to a digital multimeter (80T-IR probe and meter, Fluke, Everett,WA) with recordings before, midway and during the last minute of illumination. Control recordings from the untreated side/limb were also noted.

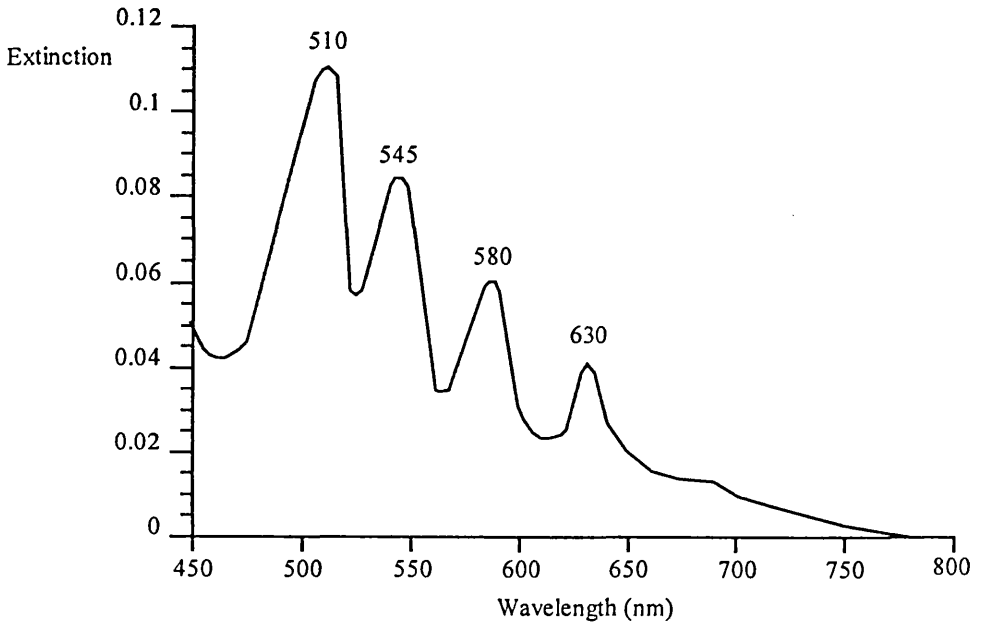
Clinical response was determined after two months and a repeat treatment administered if necessary. Following clearance, all patients were reviewed at monthly intervals for 12 months to look for recurrence.

Statistics: Simple logistic regression was used to compare clearance rates as most patients had only two lesions treated. The explanatory variables were light colour and size. No significant interaction between light, colour and lesion size was evident after fitting a full logistic model and hence the model was fitted without an interaction term.

Comparison of loss of fluorescence between the green and red-light treatment groups was complicated by the identical observations in the green group. The Mann-Whitney test thus failed due to the tied values and a chi-squared test failed due to the small expected numbers in many cells. A Fisher's Exact test was therefore performed by grouping the observations in the 40 and 50 J/cm^2 categories and comparing these observations with those in the 30 J/cm^2 category.

A Mann-Whitney U test was performed to compare the median maximum temperatures and median rise in temperature during PDT.

Figure 3.11 Absorption spectrum for protoporphyrin IX comparing the extinction coefficient with wavelength (adapted from Konig K and Auchter S).[133]



Results: Table 3.9 summarizes the clearance rates during PDT using each filter. A total of 61 patches of Bowen's disease in 26 patients received ALA-PDT with 8/29 lesions treated using the green filter failing to clear after 2 treatments compared with only 2/32 lesions treated by red light. The difference in response was significant (p=0.002) with lesion size not a factor in achieving clearance at 12 months (p=0.403). Further analysis of the effect of size on clearance after one treatment was only marginally not significant (p=0.055) for red-light treated lesions and remained not significant for green-light treated lesions. A high recurrence rate was observed in the group treated by green light, with 7 recurrences, in comparison with only two lesions relapsing after PDT using red-filtered light. Comparing red with green light, the odds ratio is estimated to be 0.13 (95% CI 0.04-0.48) in favour of red light.

Fluorescence decay was assessed in 5 lesions treated by green light with surface fluorescence initially very intense (n=2) or strong (n=3) with a reduction in all cases to moderate intensity by 10J/cm,^{2} to just visible by 20J/cm^2 and to not detectable by 30J/cm^2. No surface fluorescence was evident on completion of PDT (after 62.5J/cm^2) in all remaining lesions treated by green light in this study. Comparison of rate of loss of surface fluorescence with Bowen's lesions treated by red light (Section 3.7 and Table 3.6) suggests an earlier loss of fluorescence in green-light treated lesions although this was marginally non-significant on statistical assessment (p= 0.057).

The temperature during PDT for all lesions and irradiances (28-122mW/cm^2) showed an increase of 3.1^{o}C (1.5-5.6^{o}C) for red light treatments with a maximum value of 34.3^{o}C (29.7-37.1^{o}C). The maximum value was the highest value recorded - either mid-treatment or during the final minute of treatment (usually the higher). For lesions illuminated by green light the median temperature rise was 4.0^{o}C (2.9-5.2) with a maximum value also of

Table 3.9: Comparison of clearance rates for Bowen's disease following ALA-PDT using red or green filtered light

Light	No. of lesions	Clear on 1 treatment	Clear on 2 treatments	No. of recurrences over 1 year	Median size (mm^2)	Range of size (mm^2)	Overall clearance rate (%)
Red	32	24	6	2 (6,7 mths)	125	16-441	88 (n=28)
Green	29	18	3	7 (4,4,4,5,6,7,10 mths)	100	25-400	55 (n=16)

34.3°C (30.4-35.9°C). Subset analysis of those lesions treated at 122mW/cm^2 similarly demonstrated a slightly lower median temperature rise with red light treated lesions (n=6) of 3.8°C (1.9-5.7) than for green light illuminated lesions (n=9) of 4.2°C (2.9-5.4°C), but with an identical mean maximum value of 34.7°C (red: 31.6-37.1°C, green: 33.9-35.9°C). Neither maximum temperatures nor rise in temperature were statistically different between the red and green groups.

Summary: Green light-activated ALA-PDT using the prototype lamp achieved a complete response rate at 12 months of 48% (14/29) in comparison with 88% (28/32) using red light. Green light thus appears less effective than red light at theoretically equivalent doses for protoporphyrin IX production (p=0.002). A more rapid bleaching of PPIX from the skin surface was evident with green compared with red light with a lower minimum dosage, of 30J/cm^2, at which surface fluorescence became undetectable. Tissue temperature rise was slightly, but not significantly, higher with green than red light although no hyperthermic temperatures were recorded in either group.

3.10 - Squamous cell carcinoma, large ulcerated basal cell carcinoma, and metastatic melanoma

The case reports below relate to patients in whom ALA-PDT, using the prototype lamp, was undertaken due to the failure or inappropriateness of existing treatment modalities. Patients were fully informed of the experimental nature of photodynamic therapy.

Case 1: Squamous cell carcinoma

Biopsy proven squamous cell carcinoma on the scalp of a frail 83 year-old female which had continued to grow and ulcerate despite repeated radiotherapy which had reached the limits of tolerance at the time the patient was assessed for ALA-PDT. Examination revealed an ulcer 5x4cm in diameter with an erythematous crusted margin.

PDT was performed using local anaesthesia with 5-ALA applied 4 hours pre-illumination using 125J/cm^2 red-filtered light and a field diameter of 7cm. Although painful to the patient despite local anaesthesia (infiltration made difficult by tethering of the skin to the underlying tissues) treatment was completed and at review 4 weeks later the ulcer diameter had reduced to 4x1.8cm. PDT was repeated with difficulty again in infiltration of anaesthesia. Whilst treatment was completed with only moderate discomfort experienced by the patient, pain became severe during the subsequent 6 hours necessitating opiate analgesia. Examination at this time showed marked localized oedema and erythema. Although by 24 hours pain had settled and erythema and swelling had reduced, the patient declined further treatment.

Case 2: Large ulcerated basal cell carcinoma

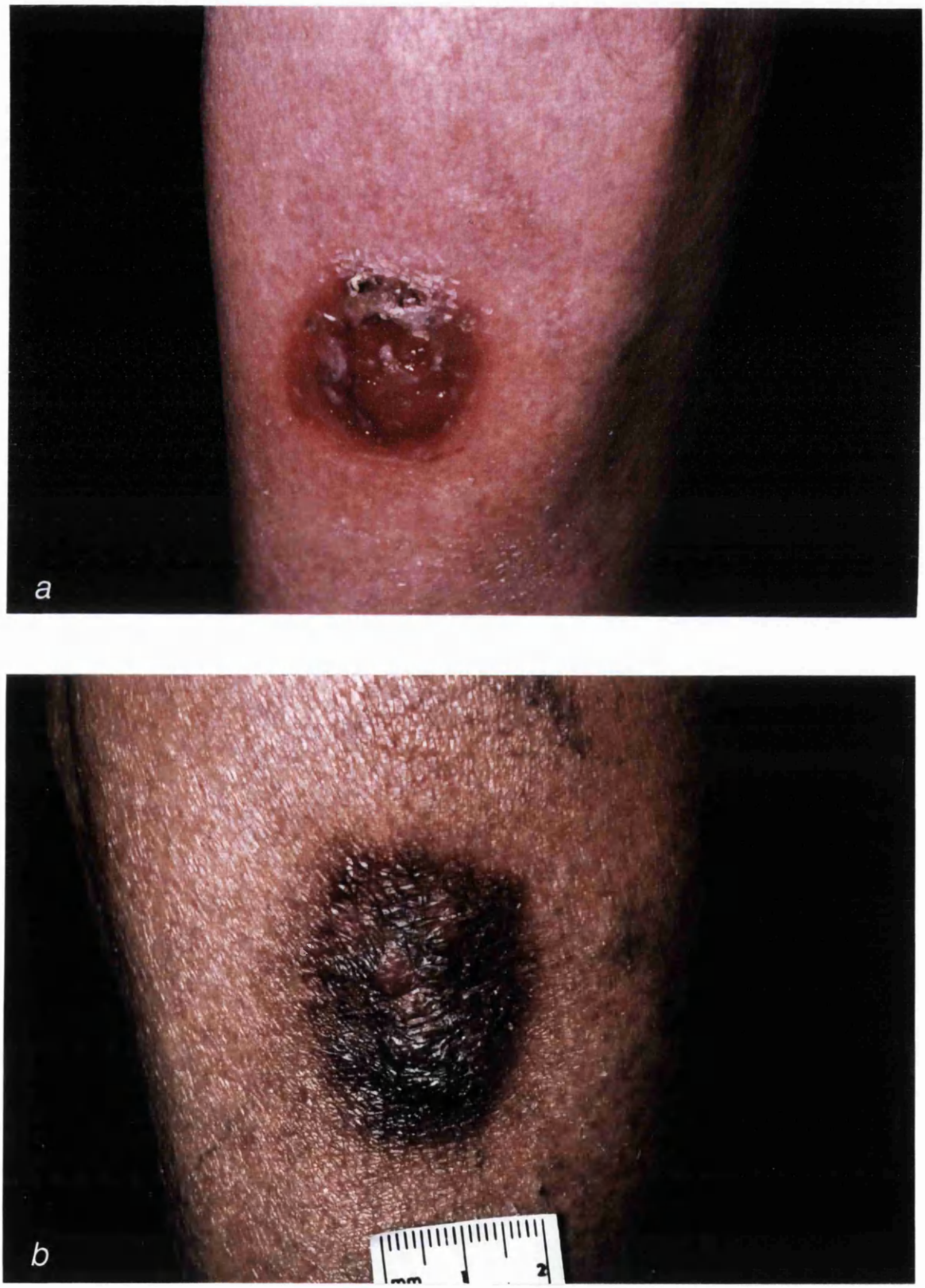
In addition to the partial response of an ulcerated BCC (50x30mm ulcer crater) treated in the patient with multiple lesions described in Section 3.6, with reduction in lesion size (as well as complete re-epithelialization) which facilitated direct surgical closure, a further large ulcerated BCC has been treated by ALA-PDT in an 87 year-old man. This patient had presented with a non-healing ulcer on his right calf of at least 8 months duration. Failure of response to conventional ulcer dressings precipitated his referral with subsequent histological confirmation of a BCC with a tumour thickness of 2.1mm. ALA-PDT with 150J/cm^2 of filtered red light and 5-ALA application 4 hours prior to illumination achieved re-epithelialization of the ulcer within 3

weeks (Figure 3.12). Residual tumour was evident on a biopsy 3 months after initial therapy and PDT was repeated. The ulcer remained healed with no surface evidence of recurrent BCC during the subsequent 3 months until the patient died from a myocardial infarction (known history of ischaemic heart disease). A repeat biopsy had not been undertaken by this time and hence tumour clearance cannot be confirmed. However, this patient had reported his satisfaction with the treatment in relieving him of thrice weekly ulcer dressing changes and bathing restrictions.

Case 3: Metastatic melanoma

A 76-year-old female who had had a superficial spreading melanoma (Breslow = 0.7mm, Clark level 4) excised from her right elbow two years previously, presented with a pigmented lesion on her right shin surrounded by a 2.4x2.0cm erythematous patch. Biopsy of this lesion revealed a partially regressed vertical growth phase melanoma of maximum thickness 0.95mm, Clark level 3. Staging revealed no evidence of other metastatic disease. The patient, who had previously had a deep venous thrombosis in her right leg, was reluctant to receive surgery which would require grafting and a single treatment of PDT ($125J/cm^2$ red light 4 hours following 5-ALA) was administered to this residual, partly melanotic, lesion. No response was evident at 1 month with 4mm punch biopsy confirming persistent disease. The patient was finally persuaded to receive surgical excision of the nodule.

Figure 3.12 Ulcerated basal cell carcinoma on right leg (5.0x3.0cm), tumour thickness 2.1mm, (a) before and (b) 1 month after ALA-PDT.



3.11 - Conclusions

This prototype non-laser source is effective in promoting ALA-PDT in clinical practice in the treatment of pre-malignant non-melanoma skin cancer and superficial basal cell carcinomas.

A 100% initial clinical clearance was achieved in an open pilot study of actinic keratoses and Bowen's disease up to 21mm in maximum diameter, although two treatments with PDT were required to achieve clearance in 37% of lesions. The overall clearance rate at 1 year of 92% compares favourably with results from similar trials using laser light sources (Table 2.1).

In a subsequent open study of large patches of Bowen's disease, 35/40 (88%) lesions >21mm in diameter showed initial complete response after 1-3 treatments, although 4 recurrences during the following 12 months reduced the CR rate to 78%. All remaining lesions showed a partial response. Although the response rate was thus lower for the larger lesions, ALA-PDT may be particularly useful in this group and reduce the requirement for surgery.

ALA-PDT, using the prototype lamp appears to be at least as effective as cryotherapy when compared in a randomized trial for the treatment of Bowen's disease and was associated with fewer adverse effects and a lower recurrence rate. PDT was superior to cryotherapy in clearing lesions following a single treatment after correcting for the effect of lesion size on clearance in each group.

Topical ALA-PDT using the lamp to treat superficial basal cell carcinomas confirmed tumour thickness as an important determinant of clearance for the 27 lesions treated after a 5-ALA application time of 4 hours. Although all 16 tumours <1mm cleared, 3/7 1-2mm and 0/4 >2mm thick cleared. Prolonging application time to 6 hours cleared all 26 BCC although all tumours in this group were <2mm thick and only 6 tumours were 1-2mm in

tumour thickness. ALA-PDT using a 5-ALA application time of 6 hours would thus appear an effective therapy for BCC <2mm in thickness with tumours <1mm thick particularly likely to respond. Tumour thickness also had a significant effect on response when all BCC treated with 150J/cm^2 at six hours were compared. As no lesions >2mm thick were treated at 6 hours, ALA-PDT may even be effective in this thicker group of tumours following the longer photosensitizer application time and requires further study.

An application time of 6 hours was the practical limit to facilitate day treatment of lesions by ALA-PDT. Evidence from fluorescence studies supports 4-6 hour application times for thin tumours, but 5-ALA uptake by thicker tumours may benefit longer application times. Szeimes et al[134] observed that 4 hours after topical application of 10% ALA, protoporphyrin IX flourescence was evident only in skin appendages, but at 12 hours was detectable in tumour cells in deep dermis. Martin et al[135] observed only partial thickness protoporphyrin IX fluorescence in 4/9 nodular BCCs following application of 5-ALA *in-vivo* on average 6 hours prior to imaging. Whilst 6/7 superficial BCCs showed full thickness fluorescence, the absence of reproducible fluorescence marking of the overall thicker nodular lesions implied that ALA-PDT may not be reliable in treating this type of BCC. Roberts et al[9] reported that 4 hours after 20% 5-ALA application using a different vehicle, protoporphyrin IX distribution in BCC did not appear to be affected by tumour depth and was most intense in those regions of tumour immediately adjacent to the dermis.

Superficial BCC lesions can present a particular management problem in view of their size and location. A 95% clearance rate at one year for all 36 tumours <1mm treated suggests that this treatment is at least as effective as conventional therapies. Further study of noduloulcerative tumours is required to determine whether efficacy can be improved by alterations in photosensitizer administration (application time, concentration, lipophilicity,

penetration enhancement) and light delivery (dose, fractionation, interstitial, wavelength).

Fractionation (discontinuous illumination) may improve tumour responsiveness by permitting tissue reoxygenation during 'black' periods although repair of sublethal tumour cell damage may reduce efficacy.[136] Equal on/off times of 30 seconds during treatment using systemic photosensitizers have clinically been associated with improved response although theoretically only 10 seconds at most should be required for oxygen distribution assuming a normal inter-capillary spacing of 100-400 μm. This suggests that vascular occlusion and areas of chronic hypoxia may influence tumour oxygen supply and hence susceptibility to PDT. The importance of fractionation in cutaneous malignancy with topical ALA-PDT is not known.

Interstitial illumination may be useful in thicker nodular lesions as demonstrated with Kaposi's sarcoma studies[122,123] although systemic photosensitizer delivery may also be required, combining to make PDT an invasive therapy reducing its advantages over surgery.

The light source used is unlikely to limit efficacy in thicker tumours providing sufficient energy of appropriate wavelength can be delivered to the tissue. The effective fluence rate for light produced by the prototype lamp used in this thesis has been calculated by the formula described in Chapter 1.4 which incorporates spectral irradiation, optical transmission through tissue and the anticipated absorption of the light by the photosensitizer at the wavelengths used. Previously published graphs of optical transmission[137] and porphyrin absorption[133] (Figure 3.11) were used. Effective fluence rate for the study lamp using the red filter (630±15nm) was almost identical to that of a 630nm dye laser[32] for depths of 0.05mm (18.3W/m^2 for lamp, 18.4W/m^2 for laser) and 2mm (2.69W/m^2 for lamp, 2.81W/m^2 for laser). This supports the study observations that at least for lesions up to 2mm, the lamp achieved

comparable results to previous laser based ALA-PDT studies (Tables 2.1 and 2.4).

Clinical study of light dose used in the treatment of cutaneous malignancy by ALA-PDT appears to have been based on previously reported results rather than dose-response. Following the treatment of 124 confirmed lesions of Bowen's disease each with 1 year follow-up (Section 3.7), a dose of 100-125J/cm^2 is optimal for ALA-PDT using red-filtered light of 630±15nm. Lower doses, although initially as effective, were associated with a higher recurrence rate indicating that insufficient photodynamic damage was being achieved at these doses. Fluorescence decay assessment indicated a minimum dose of 50J/cm^2 to clear surface PPIX in all studied tumours. These observations are supported by calculation of PPIX decomposition which estimates that the concentration of active photosensitizer is reduced to 10% after exposure to an optical dose twice as large as the bleaching fluence. As the bleaching fluence is in the range 20-50J/cm^2 for PPIX, any increase of light dose above 100J/cm^2 is unlikely to be clinically relevant.[138]

Due to ethical considerations, analysis of dose-response was more limited for ALA-PDT in BCC. Although both 100 and 150J/cm^2 proved equally effective, only two patients with multiple tumours were studied and their pattern of response may thus be different due to those undefined factors predisposing them to develop multiple tumours. This initial comparison of dose/response, combined with fluorescence data demonstrating that at least 50J/cm^2 is required before surface PPIX is lost from all tumours, indicates that photodynamic activity can be induced within BCCs at least up to this dose and that the likely optimum therapeutic dose lies between 50-100J/cm^2.

Apart from the initial pilot study, lesion size has been demonstrated to have a significant effect on the probability of clearance on a single treatment with PDT for Bowen's disease in the comparison study with cryotherapy, and was almost significant for red-light treated lesions in the red/green

comparison study. Lesion size also was a significant determinant of overall outcome in the dose/response and fluence rate studies of Bowen's disease. This influence may be a function of lesion volume as size also influenced response with cryotherapy. Tumour thickness appears to have a predominant effect over size for BCC, supporting this hypothesis.

Assessment of the effect of fluence rate on response for both Bowen's disease and BCC revealed no effect on outcome within the rates studied (18-122mW/cm^2) for BCC, but a significant improvement in response was apparent at fluence rates at or below 48mW/cm^2 for lesions of Bowen's. This apparent improvement in clearance at low irradiances despite the larger size of lesions in these categories requires cautious interpretation due to the study design. Fluence rate and lesion size are linked variables as irradiance intensity was reduced as a consequence of the larger fields of illumination required to treat larger lesions. Nevertheless, this effect of low fluence rate was despite the opposing influence of larger lesions which were demonstrated in the same study to overall have a poorer clearance rate than small lesions. The absence of this association for BCC raises further concern that this may not be a re-producible observation.

Several *in-vitro* and *in-vivo* studies indicate that high fluence rates of up to 200mW/cm^2 reduce efficacy of PDT[139-142] although others have failed to show this association.[143,144] Whilst the rapid depletion of oxygen at very high irradiances is likely to limit PDT effect, its impact on clinical outcome, at the lower irradiances studied, may only be evident when other parameters (photosensitizer concentration, light wavelength and total dose) are sub-optimal for PDT. Although the PPIX concentration for topical ALA-PDT is presumed to be lower than by systemic administration, a study using systemic photosensitizer has demonstrated that oxygen depletion starts to effect outcome above 50mW/cm^2.[145] This would support our observation that irradiance intensities below this value within the range studied are optimal for

clinical ALA-PDT although further assessment is required in a study designed to avoid linkage between size and fluence rates.

Temperature measurements, using the infra-red probe, revealed an absence of hyperthermia, confirming that at the fluence rates used in this thesis photodynamic, and not hyperthermic damage, is induced.

Green light appears less effective than red light at theoretically equivalent doses for protoporphyrin IX production suggesting that depth of penetration of green light might be inadequate for clearance of Bowen's disease in clinical practice. The rapid loss of fluorescence surface activity in green-light-induced PDT indicates that the PPIX was more susceptible to bleaching with green light than with red. Tissue temperature rise was slightly, but not significantly, higher with green than red light although no hyperthermic temperatures were recorded. The absence of any effect of lesion size for lesions of Bowen's disease treated by green light, suggests that wavelength may have had the predominant effect on outcome in this study.

The light dose using the green filtered light was calculated by comparison of the quantum yield of PPIX at 540nm and 630nm.[133] Using the recently described formula for estimating total effective irradiance[32] (Chapter 1.4) indicates a three-fold higher effective fluence rate for green light at 0.05mm depth (57.0W/m^2) than with red light (18.3W/m^2), but with similar values at 2mm (3.45W/m^2 and 2.69W/m^2 respectively). Thus 62.5J/cm^2 of green-filtered light was probably a relatively higher dose than 125J/cm^2 of red-filtered light. The reduced efficacy of green light in clinical study may thus have been influenced more by light distribution than dose. A model of optical distribution in Caucasian skin indicates that 635nm light peaks in the upper dermis (due to the addition of scattered light to the incident fluence) in comparison with 514nm light which peaks in the stratum corneum.[138] An in-situ fluence equal to the incident unscattered fluence is attained at a depth of

2.5mm at 635nm compared with 0.5mm at 514nm. Despite the superficial nature of Bowen's disease, this difference in penetration appears to be of therapeutic importance when red and green light is compared. The high recurrence rate with green light suggests that the deepest dysplastic cells may have been inadequately treated by green light.

ALA-PDT using the prototype lamp was well tolerated both during irradiation and in the follow-up period. Local anaesthesia was required only where the epidermis was not intact prior to irradiation or for certain larger lesions including 3 large patches of Bowen's disease and the two largest BCCs treated. The majority of patients presenting with Bowen's disease and BCCs were elderly, with Bowen's lesions often on the lower leg, a poor site for healing. Therefore, the avoidance of ulceration (except for 1 lesion in the pilot study) and treatment site infection following treatment was encouraging. The absence of generalized photosensitivity following PDT using topically applied photosensitizer permitted the patients to be easily managed on an out-patient basis.

The low incidence of clinically obvious scar formation is consistent with the good cosmetic results reported following laser induced PDT for Bowen's disease[10,19,90]. Cairnduff[90] and Kennedy[10] have used doses up to 250 and 540 J/cm^2 respectively with no reported scar formation. Scarring was observed in three treatment sites, all overlying the achilles tendon in patients treated in the pilot study of Bowen's disease. Minimal visible scar formation was also observed following ALA-PDT for BCCs although temporary pigmentary change and permanent localized hair loss was noted in certain treatment sites, especially larger lesions on hair-bearing sites.

Individual cases of extensive SCC and metastatic melanoma did not show satisfactory response to ALA-PDT. However, treatment trials were probably inadequate to fully assess efficacy. The patient with a large SCC on her scalp may have continued to respond to PDT if localized 'sectional'

treatments had been commenced which may have permitted more effective local anaesthetic coverage and less severe post therapy oedema and pain. The lack of response of a cutaneous metastatic melanoma deposit is compatible with the poor efficacy of ALA-PDT for this tumour reported by Wolf et al.[35] Absorption of 630nm light by the melanin pigmentation may have been the main reason for the failure of PDT as well as possible inadequate penetration of the nodule by photosensitizer at 4 hours.

Although follow-up intervals remain short for observing tumourogenesis, no tumour appears to have been induced or promoted by PDT during any of the studies outlined above where a total of 263 lesions of Bowen's disease and 77 BCC received ALA-PDT.

This chapter has outlined studies confirming the efficacy of ALA-PDT using the prototype lamp in non-melanoma skin cancer, confirming an efficacy compatible with previous laser and non-laser studies. ALA-PDT may also have a role as an adjunctive/palliative therapy for extensive ulcerated BCC. The influence of duration of photosensitizer application, light dose, fluence rate, tumour thickness and lesion size have been assessed with clearance data in all cases incorporating at least a 12 month follow-up period (up to 24 months for BCCs). ALA-PDT using the prototype lamp was practical as an out-patient therapy and was well tolerated by patients with a low prevalence of adverse events.

PDT in dermatology offers the advantages of being non-invasive, relatively painfree, well tolerated in slow healing sites such as the lower leg, and tissue sparing, leaving the skin surrounding the tumour intact and functional.

Chapter 4 - Morphology of the Skin following topical ALA-PDT

4.1 - Introduction

Although studies have indicated intracellular effects of PDT (Chapter 1), there is little knowledge of the histopathological changes that occur following topical ALA-PDT in clinical practice. Good cosmesis is reported following PDT although these observations have been based on clinical and not histopathological assessment. This chapter assesses the tissue changes following topical ALA-PDT for Bowen's disease and basal cell carcinoma with particular reference to histological evidence of scar formation and the presence of PDT-induced apoptosis.

Tissue response to PDT has been described as a rapidly developing coagulation necrosis and can be observed within hours of treatment. This necrosis is accompanied by an acute inflammatory response and affects normal as well as tumour tissues in the treatment field. These observations have been made *in vivo*[50,146-148] following systemic administration of photosensitizer where PDT response may depend on damage to tumour vasculature as evident from endothelial cell swelling, blood cell aggragation and haemorrhage noted on light microscopy. Wang *et al*[26] recently reported that no direct vascular damage was observed following topical ALA-PDT in the treatment of 40 basal cell carcinomas and 6 patches of Bowen's disease. Laser doppler perfusion imaging was used with care taken to minimize the effects of spatial variation in perfusion by averaging values over the assessment area. Pre-treatment perfusion was higher in the lesion than surrounding skin in all tumours and increased immediately following illumination, falling to the level of normal skin only after healing. All tumours cleared with treatment indicating that direct tumour cell damage and not tissue hypoxia due to vascular impairment was responsible for tumour

destruction. Topical ALA-PDT may thus produce a different pattern of tissue damage from systemic PDT with consequences in respect of healing as well as possibly favouring its use in sites of poor perfusion.

Preservation of the structure of collagen following PDT has been attributed to a relative resistance to photo-dynamic destruction.[58] This may not only preserve the integrity of tissue, but promote a good cosmetic outcome in PDT treated tissue. Structural integrity of rodent colon has been assessed following PDT with aluminium phthalocyanine and compared with changes following thermal treatment.[149] Despite full-thickness necrosis being induced by each therapy, the relative mechanical strength of the tissue, measured by the bursting pressure of colon, was unchanged following PDT, but greatly reduced following thermal treatment. Collagen fibrils were preserved in the submucosal collagen layer with PDT whilst thermally damaged collagen lost its fibrillary architecture. Clinical studies of ALA-PDT in the treatment of non-melanoma skin cancer and its precursor lesions indicate good cosmesis, but without confirmation of an absence of microscopic scar formation.[19,91-5,105,113] Confirmation of a low scarring potential with preservation of tissue function would make topical ALA-PDT particularly useful for lesions on the eyelids, ears and nose where disfigurement can result from conventional treatment modalities.

Apoptosis is an active programmed process of cell death, in contrast to necrosis when cells appear to be passive targets of injury.[150] Malignant cell proliferation is often associated with a reduced frequency of apoptosis and a concomitant loss of response to chemotherapeutic agents, which in a normal cell population, elicit an apoptotic response. The induction of apoptosis by PDT has been demonstrated both *in vitro* (including human squamous cell carcinoma lines[151]) and *in vivo* (in RIF-1 tumours in C3H mice,[152] and HeLa cells in nude mice[153]) and may represent an important method of action of PDT. Evidence of apoptosis has been observed within 1 hour of PDT.[154] The

initiation of apoptosis has been shown to vary with photosensitizer and type of malignant cell. Photofrin-mediated PDT was observed to induce apoptosis in two of three malignant cell lines.[155] The potential of ALA-PDT to induce apoptosis is not known.

4.2 - Morphological changes induced by ALA-PDT

Aims

To determine the macroscopic and microscopic changes in the skin induced by ALA-PDT using the prototype non-laser light source.

Patients and methods

Histopathological specimens were acquired from patients receiving topical ALA-PDT using the prototype non-laser light source by the protocols outlined in Sections 3.4-6. Observed macroscopic changes relate to lesions treated in all clinical studies described in Chapter 3. Post-therapy 4mm punch biopsies were undertaken at intervals up to 2 months following therapy in Bowen's disease and up to 1 year post-PDT for BCC. Post therapy specimens were not taken from the scar sites of earlier punch biopsies. Histopathological specimens in this analysis relate only to lesions of Bowen's disease treated 4 hours post-ALA application by 125J/cm^2 of red light and to BCC's illuminated 4-6 hours post-ALA by 150J/cm^2 of red light. Histopathological specimens were also acquired post-cryotherapy in the comparative study of therapy in Bowen's disease (Section 3.5).

Biopsy specimens underwent routine paraffin processing and were stained with haematoxylin and eosin.

Results

(a) Macroscopic assessment of skin changes following topical ALA-PDT:

Treatment of lesions of Bowen's disease and BCC's treated in the studies described in Sections 3.4-6 resulted in oedema and erythema immediately following ALA-PDT which settled over 48 hours. Blister formation was noted in 3/40 patches of Bowens disease and 6/53 BCC, all resolving within 1 week. Lesions subsequently developed a light crust which could remain for up to 2 months. Ulceration of the skin and cellulitis were not observed following PDT. Post inflammatory hyperpigmentation was evident in a few BCC treatment sites which resolved within 6 months. Hair loss was observed where BCC's were located within scalp hairline (n=1), within pubic hair (n=1)(Figure 3.7b), or on particularly thick body hair (n=4). Whilst lesions of Bowens disease frequently assumed normal skin colour by 12 month review, BCC treatment sites retained a faint erythematous hue in approximately 25% of cases (Figures 3.5 and 3.6). Nevertheless, clearly visible lesion-wide scar formation was only seen on the 3 ankle sites (Section 3.4) and patients otherwise all found the cosmetic result of ALA-PDT acceptable. The small scars left from up to 2 punch biopsies made detailed assessment of PDT-induced scar formation difficult although minimal visible scar formation was evident in certain of the larger BCCs.

(b). Histopathology of the skin after PDT in Bowen's disease:

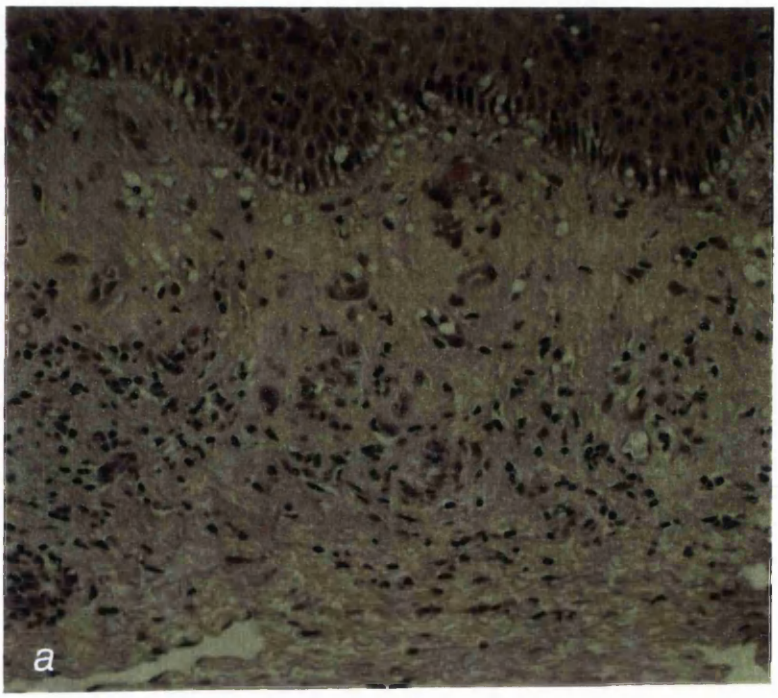
Post-therapy histopathological specimens were available from 20 lesions of Bowen's disease and 4 patches treated with cryotherapy. Immediately following PDT, vacuolation of keratinocytes within dysplastic regions with spongiosis of intervening epidermis and oedema of the superficial dermis was evident in all 3 specimens studied. Multiple apparently apoptotic cells were visible in lesional epidermis in 2 specimens. Capillary dilatation with a mixed acute and chronic inflammatory cell infiltrate

was also present in the dermis with red cell extravasation in the papillary dermis in one specimen. Similar features were noted in 4 biopsies 1 hour post-PDT with evidence of both coagulation necrosis of some superficial dysplastic foci with other nests of cells showing striking nuclear vacuolation. Capillary dilatation with margination of neutrophils was seen in the upper and mid-dermis. At 2 hours following treatment all 3 specimens showed capillary dilatation and red cell extravasation in the upper dermis with full thickness epidermal necrosis in one biopsy with the other specimens showing vacuolation of basal cells (Figure 4.1a).

All seven 1-2 hour samples were assessed for the presence of apoptosis and compared with 7 control biopsy specimens, selected at random from the pre-treatment specimens of lesions in the same study. Apoptotic cells were defined as individual cells exhibiting cytosol and chromatin condensation with significant cell shrinkage.[157] Apoptotic cell counts were made in 10 random high power fields (x100) and a mean value calculated. The mean apoptotic cell counts ranged from 0.7-2.9 per high power field (hpf) in the control samples (mean 1.7) compared with 1.8-12.2/hpf (mean 5.1) for PDT-treated lesions ($p=0.02$ - Mann Whitney test). The presence of apoptosis in a PDT-treated lesion is demonstrated in Figure 4.1b.

By 24-48 hours full thickness coagulative necrosis was seen in 4/6 specimens (Figure 4.2). In the remaining 2 specimens, there was focal necrosis in the superficial epidermis and preservation of the basal layer, but absence of dysplastic keratinocytes. Dilated capillaries in the dermal papillae showed loss of endothelial cells and contained thrombi in 3 specimens and all samples showed a lymphohistiocytic perivascular infiltrate. Patchy hyalanisation and swelling of collagen fibres in the upper and mid-reticular dermis was present in one biopsy at 48 hours (but no scar formation was subequently visible although no further histology is available).

Figure 4.1 Bowen's disease 2 hours following ALA-PDT with (a) a striking basal cell vacuolation and inflammatory infiltrate in the upper dermis and (b) multiple apoptoses present in the epidermis.



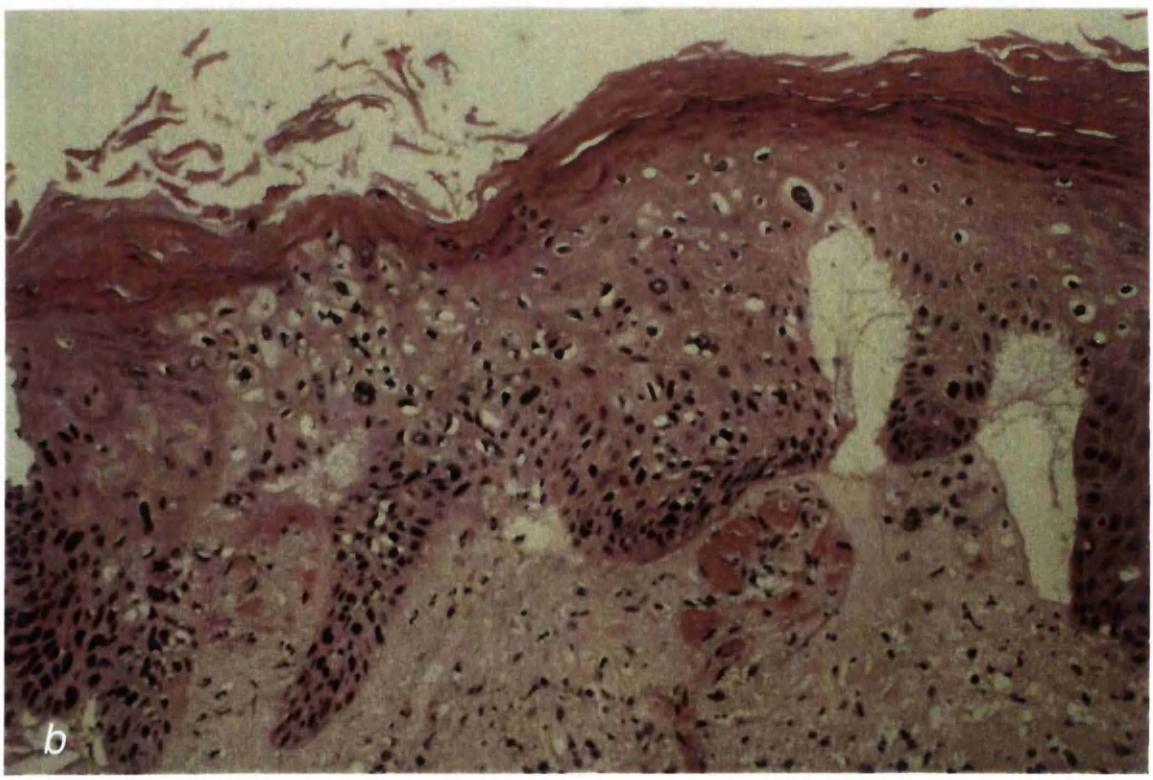


Figure 4.2 48 hours following ALA-PDT to a patch of Bowen's disease. The epidermis is replaced by fibrin coagulum and there is thrombosis of some superficial vessels and a perivascular lymphohistiocytic infiltrate extending through the full thickness of the dermis.

At 24-48 hours post-cryotherapy, full thickness necrosis of the epidermis was evident in all 4 specimens with necrosis of the dermis to 2mm in two lesions. The specimens also showed extensive loss of endothelial cells and red cell extravasation throughout the dermis. Thrombi in vessels to 3mm were noted in two lesions. Although inflammatory infiltrate was less prominant, tissue damage was overall more extensive in the cryotherapy specimens than those samples derived from PDT treated lesions.

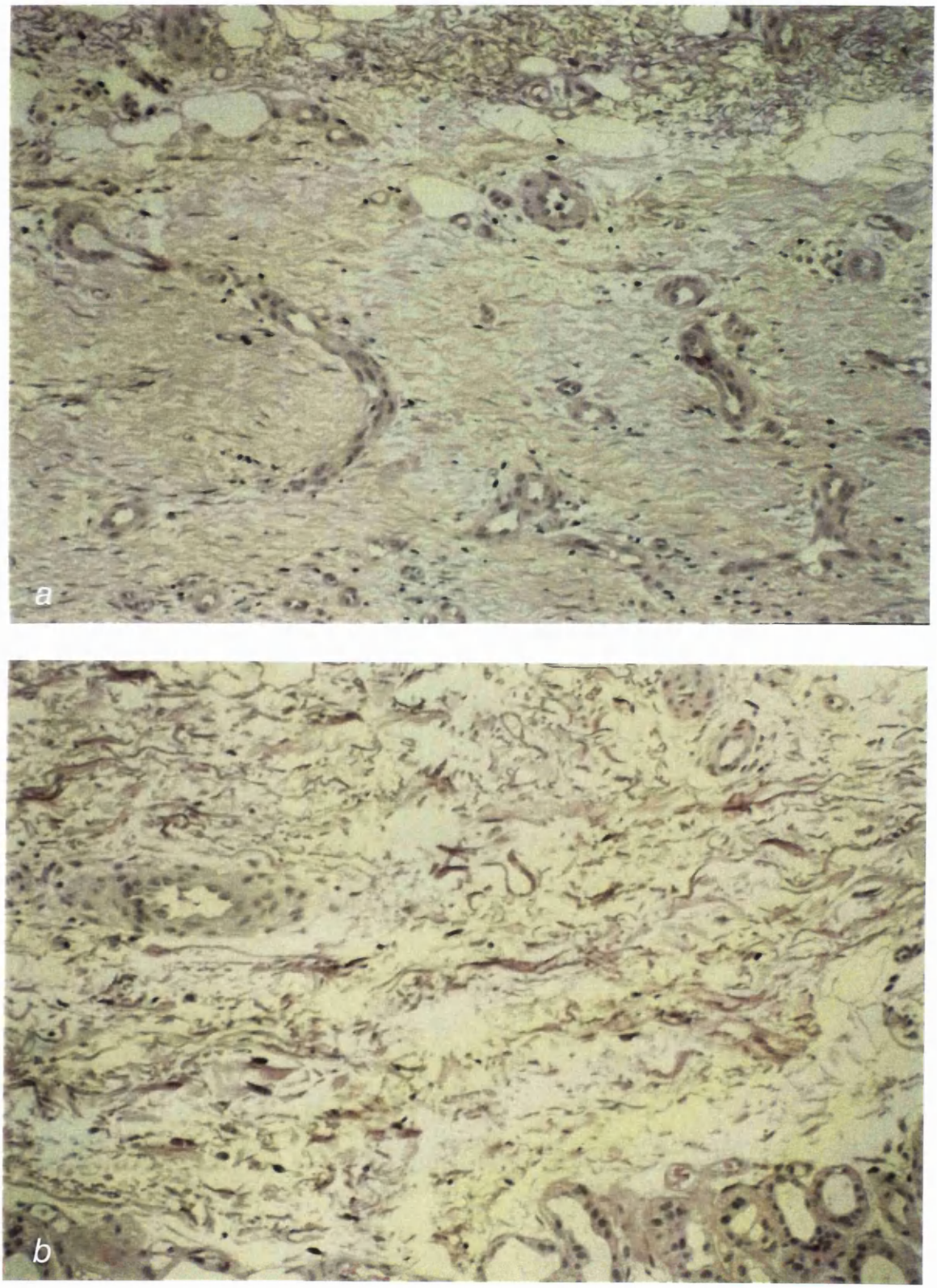
By 4-8 weeks post-PDT, the epidermis appeared normal and without dysplasia although scarring of the dermis was present in 2/4 specimens. This extended to the full thickness of the dermis and represented 2/3 ankle lesions in the open study of ALA-PDT (Section 3.4) where scarring was clinically evident (the remaining lesion was not biopsied post therapy). The remaining 2 specimens showed no evidence of dermal scarring.

(c). Histopathology of the skin after PDT in basal cell carcinoma:

Post-therapy histopathological specimens were available from 29 basal cell carcinomas following PDT. One hour post-therapy vacuolation of the basal cells in the normal epidermis was noted in 2/2 specimens. Vacuolation of tumour cells at the upper border of the tumour was also present with capillary dilatation in the dermis and an acute inflammatory infiltrate extending down 2mm.

Scarring of the dermis was present in 8/22 biopsies acquired 1-4 months post-therapy, extending to 0.5mm in four specimens (Figure 4.3) and 2mm in the remaining lesions. No scar formation was evident beyond 2mm and deeper structures were well preserved. In the remaining 14 biopsies, a chronic inflammatory infiltrate extended to 0.5 - 1mm. No scarring was also present in all 5 biopsies acquired between 6-12 months after PDT although there was persisting evidence of a mild superficial chronic inflammation in all samples.

Figure 4.3 Histology 3 months post-therapy to the site of a BCC shows (a) superficial scar formation and (b) preservation of deep structures.



4.3 - Conclusions

Topical ALA-PDT results in the development of oedema and erythema during therapy with occasional subsequent blister formation. Crusts form over lesions but without epidermal ulceration. Scar formation was site-specific in Bowen's disease occuring in lesions sited at the ankle. Minimal visible scar formation was observed in several BCCs.

The acute histopathological effects of topical ALA-PDT in Bowen's disease were of vacuolation of keratinocytes and their nuclei with capillary dilatation and an acute inflammatory infiltrate. Subsequently, a full thickness coagulative necrosis or focal necrosis developed by 24-48 hours. Scar formation was not evident microscopically other than in those sites where scar formation was obvious clinically. Topical ALA-PDT in BCC resulted in vacuolation of basal epidermal cells and tumour cells. Superficial fibrosis was evident in over a third of treatment sites 1-4 months following PDT, although scar formation was not noted in sites biopsied beyond 6 months from therapy.

The observed effects of topical ALA-PDT indicate that early vacuolation of treated cells is characteristic, but that other features including vascular changes and the development of a coagulative necrosis are not treatment specific. Cryotherapy typically results in homogenisation of the epidermis with loss of cellular outlines and a coagulative necrosis extending into dermis.[156] In comparison with cryotherapy, ALA-PDT may cause less ulceration and bullae formation due to its greater specificity as observed by proportionally less epidermal necrosis and dermal damage. Cryotherapy caused a notably greater degree of tissue damage than PDT.

Although sample numbers were small, photodynamic therapy would appear to induce apoptosis in human keratinocytes *in vivo* in human tissue. This had previously only been shown in animal models.[153-4] The reason

for the development of apoptosis rather than necrosis following PDT remains unknown. As apoptosis requires cell membrane-bound enzymes, PDT using 5-ALA as photosensitiser, which preferentially targets the mitochondria, may facilitate apoptosis. Cryotherapy, as with systemic lipophilic photosensitisers, through the direct destruction of the cell membrane, are instead likely to favour necrosis.

Treatment of an intra-epidermal neoplasm by PDT rarely resulted in either a visible or microscopic scar (although few late histology specimens were acquired). In contrast, destruction of a dermal tumour, whilst resulting in cosmetically acceptable 'wound' sites was associated with mild superficial dermal fibrosis on histopathological examination in over a third of specimens. A contribution of an initial pre-treatment biopsy to inducing scar formation extensively within the treatment field seems unlikely in the absence of this phenomenon with Bowen's disease sites. The absence of fibrosis in those sites sampled beyond 6 months may reflect a spontaneous improvement in early mild fibrosis although sample size is also likely to be relevant as minimal visible scar formation has been observed to remain at 12 months in certain of the larger BCCs.

ALA-PDT thus appears to achieve tumour destruction with less extensive tissue destruction than with cryotherapy, with apoptosis probably contributing to tumour cell kill. Fibrosis following ALA-PDT is reported for the first time, with its superficial distribution possibly reflecting the superficial extent of clinical ALA-PDT activity. The mild extent of fibrosis and the possible improvement with time, associated with the reduced initial extent of necrosis, probably both contribute to the excellent cosmesis observed following ALA-PDT.

Chapter 5 - Photoproducts in ALA-PDT

5.1 - Introduction

Certain porphyrins, including haematoporphyrin, can produce a visible light emitting photoproduct following reaction with singlet oxygen.[158,159] The role of this photoproduct, which has a strong absorbance around 680nm, is not known. The 630/635nm light used in Photofrin-PDT neglects to activate this potentially useful chemical. ALA-induced protoporphyrin IX is a relatively photolabile porphyrin, absorbing throughout the near ultraviolet and visible region[30,133] (Figure 3.9). *In vitro*, PPIX readily forms several oxidized photoproducts, many of which absorb strongly in the same spectral region as PPIX.[160-2] These arise from the action of photosensitized activated oxygen species, predominantly singlet oxygen, upon the surface of the PPIX molecule. Photobleaching of PPIX can give rise to products which do not absorb in the visible region, as observed both *in vitro*[160-2] and *in vivo*.[163,164]

Several PPIX photoproducts possess measured singlet oxygen yields similar to the precursor agent suggesting a possible role for these products in ALA-PDT.[161]

Two main classes of PPIX derived photoproduct have been identified *in vitro*. Chlorin-type isomers, with absorption in the range 640-670nm, are the main product of PDT in simple solvents, with a lower yield of formyl-porphyrin products which have a similar but right-shifted spectrum to PPIX (640-650nm).[165] The medium in which these reactions occur may influence whether other forms of activated oxygen, including superoxide, may be responsible for some photoproducts.

In vivo, fluorescence emissions have indicated PPIX photoproduct formation from both mouse[163,164] and human tumours.[166] A minor emission at 660-670nm has been shown as a small contribution overlying the PPIX spectrum. Photoproduct formation emitting at 670nm has also been observed

during irradiation of normal murine tissues, such as salivary gland, which accumulate PPIX.[165]

Where the time course of photoproduct formation has been followed in both mouse and human tissue, the relative contribution of the 670nm photoproduct increased relative to the PPIX emission at longer irradiation times.[163,164] This is presumed to be due to the photobleaching of PPIX.

The potential contribution of photoproduct to efficacy in clinical PDT remains to be established. Beyond detection of fluorescence emissions, another method of assessing this contribution is to determine whether the efficacy of PDT is altered by the inclusion of activating wavelengths at 670nm in addition to 635nm.

5.2 - Photosensitization of murine skin by 5-ALA-induced porphyrin

Aim: To compare the phototoxicity *in-vivo* of 5-ALA induced porphyrins following PDT using light of 635nm and/or 670nm. The presence of additional tissue damage by incorporating 670nm light would provide evidence of a potentially useful photoprotoporphyrin with implications for clinical ALA-PDT.

Methods: The skin reactions of 30 female BALB/c mice, all 6-9 weeks old, were studied using the previously described murine whole-foot response model for cutaneous porphyrin photosensitivity.[167] Animals were given 250mg/kg of 5-ALA by intraperitoneal injection and illuminated after 3 hours. The mice were restrained without anaesthesia in specially designed holders to permit illumination of both rear feet. This protocol was derived following review of a previous murine ALA-PDT dose-response study[168] with the intention of optomising the likelihood of visible tissue response, but at a

tolerated dose of photosensitizer. The feet were illuminated by 100J/cm^2 of 635±2nm and/or 670±2nm at 50mW/cm^2 from two argon ion pumped dye lasers with sites receiving both wavelengths illuminated simultaneously.

The study, performed at Roswell Park Cancer Institute, Buffalo, was approved by the local regulatory authority.

The reaction of the murine feet to PDT was scored using the system summarized below:

Score	Physical appearance
0	No apparent difference from normal
0.1	Very slight oedema
0.3	Slight oedema
0.5	Moderate oedema
0.75	Large oedema and/or slight erythema
1	Large oedema and/or erythema, with exudate
1.2	Moderate erythema, with slight crust or scale
1.5	Definite erythema, with scale or crust
1.65	Slight damage to toes
1.75	Definite damage and/or slight fusion of toes
2	Most toes fused, but general shape unchanged
2.5	Foot almost shapeless, but stubs of toes present
2.75	Foot shapeless with no toes
3	Only stub of foot remaining

The reactions were assessed at 24 hour intervals for up to 8 days following treatment until either the reaction had settled or a grade 2 or greater reaction was recorded at which point the mice was sacrificed. Each mouse was coded by a sequence of rings drawn on their tails, but the observer was blinded to the identity of each group during the period of follow-up.

Results: No foot response reaction was noted on either feet of 5 mice which received 5-ALA only and no light. Similarly, 5 mice received to each rear foot 635nm and or 670nm light in the absence of 5-ALA and no cutaneous reaction was observed.. The time course of the cutaneous reactions in each group is shown in Figure 5.1. The peak response was observed from days 4-8 with the greatest response noted in the 635+670nm group. A small response was noted for feet treated by 670nm alone.

The maximum reaction observed in each PDT group is summarized in Table 5.1. As numbers were small, statistical analysis was not performed, but 635nm light appeared to have its phototoxic effects enhanced by the addition of 670nm. Illumination during PDT with 670nm alone elicited a response in 7/10 mice ranging from slight oedema to toe fusion. The absence of a response from either foot in two mice in group 1 suggests that successful intraperitoneal injection of photosensitizer may not have been achieved.

Due to the design of the study, in all feet treated by 670nm alone, the contralateral foot received both wavelengths, providing an opportunity for photoproduct formation. Additional control groups of mice receiving 635nm to both feet or 670nm to both feet were not included due to limitations on the number of mice available in this initial study of the detection of photoproduct phototoxicity.

Summary: This was a pilot study to detect the presence of a 670nm photoproduct using a murine model of cutaneous phototoxicity. A consistent pattern of results indicate that illuminating skin presumed to contain 5-ALA derived protoporhyrin IX achieved a greater phototoxic reaction when 670nm light was added to 635nm light. 670nm light alone also resulted in a small, but discernible reaction in 7/10 sites illuminated. The illumination of contralateral feet with 635 as well as 670nm light is the presumed source of

Figure 5.1 Time course of the cutaneous phototoxicity in murine feet following ALA-PDT using 635 and/or 670nm light. Mean scores for the 10 feet treated in each group are shown with standard error bars.

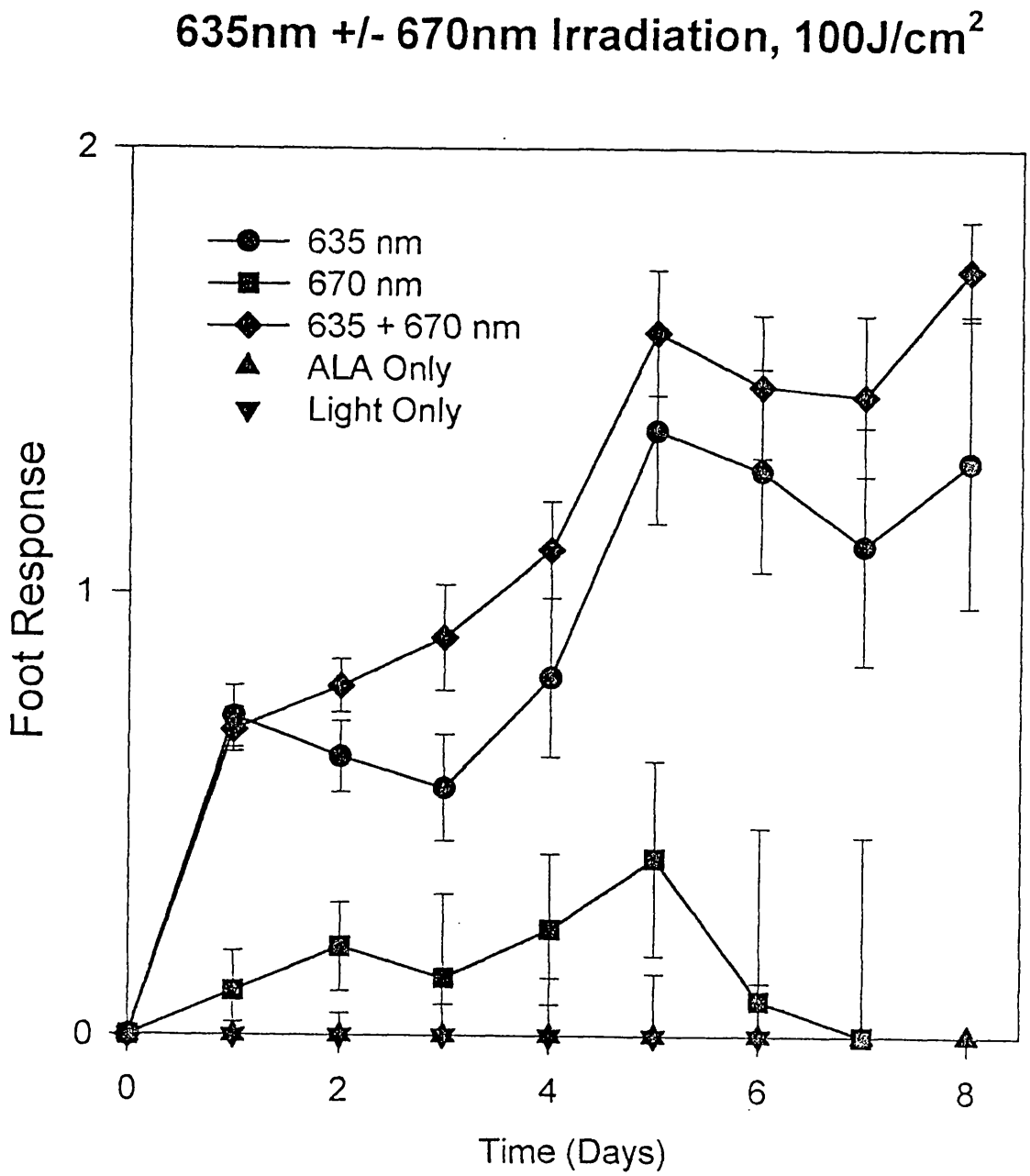


Table 5.1 Maximum scores for reaction of murine feet to ALA-PDT using wavelengths of 635nm and/or 670nm.

Group, left/right foot	Wavelength(s) (nm)	No.	Scores	Maximum response (median)
1L	635	10	0*,0*,0.25,1.2,1.5, 1.5,1.75,2,2,2	1.5
1R	635+670	10	0*,0*,1.5,2,2, 2,2,2,2,2	2
2L	670	10	0,0,0,0.1,0.25,0.25, 0.25,0.5,1.2,2	0.25
2R	635+670	10	0.75,2,2,2,2, 2,2,2,2,2	2

*No reaction on either foot of same 2 mice

photoproduct formation although 635nm light in the ambient laboratory daylight may have contributed. The detection of cutaneous damage by incorporating 670nm light to ALA-PDT would suggest that a clinically useful photoproduct is present at this wavelength.

5.3 - Murine tumour destruction by 5-ALA-induced PDT

Aim: To determine whether the addition of 670nm light to ALA-PDT illumination with 635nm light improves tumour regression *in vivo*. A superior response using both wavelengths would indicate the presence of a therapeutically useful photoproduct of protoporphyrin IX.

Methods: Murine colon carcinoma (Colo 26) was propagated in 6-9 week-old female BALB/c mice. Tumours were obtained by intradermal injection of 1 x 10^6 cells, prepared from established tumours by enzymic digestion, into each flank of the animals. [169] Prior to tumour inoculation, all hair was removed from the region of the injection sites by shaving and depilation. Tumours were used for experimentation when they had reached a surface diameter of 4-6mm. The study, performed at Roswell Park Cancer Institute, Buffalo, was approved by the local regulatory authority.

ALA-PDT was administered to each tumour by the same protocol as outlined in Section 5.1. Mice were sacrificed 7 days following PDT. Tumours were then excised and tumour height was measured with callipers. The observer was blinded to the treatment group of an individual mouse. A ratio of tumour thickness to control thickness was calculated as this has previously been shown to produce a reliable indication of tumour regression in this murine model.[168]

Results: The response of tumours to ALA-PDT using the wavelengths studied is summarized in Table 5.2. A total of 30 mice were available for study. No tumour developed in 2 injection sites and a further 4 tumours did not reach the initial 4mm minimum diameter for entry to the study. Following treatment, 4 mice died within 48 hours, all receiving PDT with combined wavelengths to one tumour and to the contralateral side either 635nm (n=3) or 670nm light (n=1). The combined intensity of photodynamic inflammation/destruction on both flanks was presumed to have contributed. Of the remaining 10 tumours treated with combined wavelengths, the tumour on the contralateral flank was illuminated by 635nm light in 2, by 670nm light in 4 and not illuminated in the remaining 4.

Whilst a reduction in tumour thickness occurred with 635nm and 635+670nm light, 670nm light alone had no apparent beneficial effect. The presence of a PDT reaction with 635nm light on the contralateral flank made no difference to response in the 670nm light treated tumours. The combined wavelengths resulted in an overall 14% greater tumour regression than 635nm alone, although this may not be a significant difference (statistics not performed due to the small study numbers).

Summary:

This pilot study to determine the importance of wavelength in ALA-PDT on tumour regression *in vivo* demonstrated a reduction in tumour thickness with 635nm light-mediated therapy which may have been augmented by the simultaneous illumination with 670nm. 670nm light-mediated PDT had no impact on regression either as monotherapy or when dual illumination was being applied to the contralateral tumour suggesting no circulating photoproduct was available for activation at 670nm. The apparent improvement in response using both wavelengths would indicate the

Table 5.2 Tumour regression induced by ALA-PDT using 635±670nm in murine tumour model. Outcome is indicated by the ratio of mean tumour thickness at 7 days post treatment to mean control thickness (from tumours receiving no intervention).

Wavelength(s) (nm)	No.	Mean thickness (mm) (range)	Tumour/control thickness (%)
635	4	3.2 (1.8-4.5)	89
635+670	10	2.7 (2.4-3.2)	75
670*	4	4.0 (3.6-4.6)	111
670**	6	4.0 (2.9-5.1)	111
light only (635±670nm)	8	3.6 (2.4-4.2)	100
5-ALA only	8	3.4 (2.7-4.2)	94
no intervention	6	3.6 (3.1-3.8)	-

*Contralateral tumour irradiated by 635+670nm.

**Contralateral tumour irradiated by 670nm.

presence of a therapeutically useful photoproduct of protoporphyrin IX for local tumour destruction.

5.4 - Implications for clinical PDT

Two pilot studies are reported in this chapter with the aim of demonstrating the presence of a photoproduct of protoporhyrin IX that is activated by 670nm light. The relevance of this product to clinical ALA-PDT is not known but is thought to be due to the formation of a hydroxyaldehyde chlorin-type photoproduct.[165] Despite discussion of the emission spectra of ALA-PDT induced derivatives, current opinion has been that photoproduct formation does not play a major role in PDT in neoplasia.[170] This has been largely unsubstantiated, based on relative emission peaks without knowledge of singlet oxygen yields and the total quantity of photoproduct formation in a specific PDT protocol. We have not studied those products thought to be derived from PPIX but to have very similar activation wavelengths to the parent photosensitizer. Of potentially greater clinical relevance is the existence of products which would not routinely be activated in clinical PDT due to the narrowness of the bandwidths used.

The initial phototoxicity study demonstrated a greater phototoxic reaction when 670nm light was added to 635nm light in ALA-mediated PDT. Moreover, 670nm light alone had a small, but discernible reaction. A control group with 670nm light to both feet should have been included, but it is presumed that 635nm light was required to initially generate this 670nm photoproduct. Either ambient light or more likely circulating photoproduct was responsible for the tissue effects at 670nm illuminated sites. Apart from confirming these findings by repeating this study with all possible control groups to take account of the effects of treating both feet, an alternative

model of murine ear swelling could be performed to assess cutaneous phototoxicity.[171]

The second study of the importance of wavelength in ALA-PDT demonstrated a reduction in tumour thickness with 635nm light-mediated therapy which was augmented by the simultaneous illumination with 670nm. The regression in tumour thickness to 75% of control tumour depth, 14% greater than the response of 635nm-mediated PDT, suggests that activation of a photosensitizing chemical using 670nm light may be clinically relevant. However, the apparent promoting effect of 670nm light alone in yielding thicker tumours than controls with a ratio of 111%, when either a nil or a minor tumour regression effect is anticipated, suggests that these results be cautiously interpreted in view of the small numbers in each group .

A possible additional complication to the interpretation of these studies is the small absorption that PPIX has at longer wavelengths than 635nm as shown in Figure 3.11. Unfortunately, as the phototoxicity study described above did not contain a 'control' group with 670nm illumination to both feet, the observed effect of unilateral illumination with 670nm (with 635nm applied to the opposite leg), could have arisen due to direct PPIX photodynamic activation rather than via a photoproduct. However, when the same protocol was used in the murine tumour study, no tumour regression was noted when 670nm light was used to both flanks of 6 mice despite the absence of illumination by 635nm light which we presume normally will quench available PPIX. This suggests that 670nm activation of PPIX probably has a negligible effect and that the apparent increase in effect of the combined wavelengths is due to the photoproduct.

Traditional laser-based PDT excludes wavelengths outwith the narrow margin of 635±2nm. The prototype non-laser source assessed in this thesis provides light with its current filter of 615-645nm to ratify its efficacy alongside laser sources. The studies outlined above suggest the

replacement of this by a filter which includes 670nm is justified, at least to enable study of the relative importance of incorporating wavelengths that will activate this photoproduct.

The relatively long irradiation times (average 30 minutes) using the prototype lamp may particularly favour the formation of this photoproduct which has been reported to increase substantially relative to protoporphyrin IX emission at longer irradiation times.[163,164]

Chapter 6 - PDT in cutaneous T-cell lymphoma

6.1 - Introduction

Topical 5-ALA can promote the preferential accumulation of protoporphyrin IX in malignant T-lymphocytes in comparison with normal lymphocytes.[118] Topical ALA-PDT has been used to treat 4 patients with either one or two plaques of cutaneous T-cell lymphoma. Svanberg et al[19] achieved a clinical and histological clearance in 2/4 lesions and Wolf et al[119] also achieved a complete response in all 3 plaques treated. The efficacy of topical ALA-PDT in patients with more extensive CTCL has not been reported, nor the possible differences in response between patch, plaque and tumour stage disease.

An open study and case report in this chapter outline the outcome of topical ALA-PDT, using laser and non-laser light sources, in patients with widespread and multi-stage CTCL.

6.2 - Patch, plaque and tumour stage disease - efficacy of PDT

Aim: This open study, conducted at the Department of Dermatology, Roswell Park Cancer Institute, Buffalo, was undertaken to determine 1. the efficacy of PDT in a group of patients with widespread and multi-stage CTCL, and 2. to determine the optimum treatment parameters.

Methods: Seven patients, each with histologically proven CTCL, were referred for consideration for PDT. Six patients had received previous therapies with either recurrent disease or the development of lesions on new sites precipitating their referral. The previous therapies were; PUVA (n= 2),

topical nitrogen mustard (n= 2), potent topical steroid (n= 3), α-interferon (n= 2), and radiotherapy (n=1, not to PDT sites).

Photosensitizer was administered via the topical application of 5-amino-laevulinic acid (5-30% in M55A cream vehicle - DUSA, NY) 24 hours prior to illumination. An argon pumped dye laser (λ=630nm) and a 1000W experimental quartz halogen lamp (λ= 590-700nm) were the light sources used. Laser light was delivered to tissues through flexible quartz fibres fitted with microlenses. For large lesions (>3cm in diameter), overlapping fields of illumination were employed when using the laser, whilst the non-laser source could illuminate fields up to 17.5 cm in diameter. Individual lesions received 10 - 300J/cm^2 at an irradiance of 10-150mW/cm.^{2} Surface fluorescence was recorded immediately before illumination. Fluorescence was measured at 690nm by dual wavelength excitation at 612nm and 633nm using 2 helium-neon lasers.

Clinical lesions were graded as either patch, plaque or tumour stage with patches defined as a minimally indurated erythematous macular lesions, plaques as indurated, elevated erythematous lesions, and tumours as nodules ± ulceration. All patients thus belonged to T_1, T_2 or T_3 by TNM classification with erythroderma, or lymph node, peripheral blood or visceral involvement, reasons for initial exclusion from this study.

Treatments were performed at 3-4 week intervals with clinical response graded at the patient's return visits. Clinical response was expressed as complete clinical resolution (CCR), sub-complete (>90%) clinical response (sCCR), or partial response (PCR). Clearance was defined as clinical clearance following 1 or 2 treatments, with histological confirmation where doubt over clinical clearance existed. Epidermal toxic response (ETR) was also determined at 6-10 days following therapy by the scale: 0 = no change, 1 = minimal perceptible increase in erythema over baseline, 2 = significant erythema, 3 = scattered areas of desquamation within the lesion, 4

= significant amount of desquamation in the lesion (>90%), 5 = epidermal necrosis with eschar formation. The requirement for local anaesthesia was also recorded. Patients were reviewed monthly for treatment of residual lesions and to observe for recurrent disease.

The data presented represents an interim analysis of the Roswell Park team's research on the response of CTCL. to ALA-PDT. The response of lesions following 1 or 2 treatments was initially assessed with the subsequent analysis of further treatments to non-responding lesions. Whilst all reported patients were examined by CAM, data was pooled from casenote records which detailed every treatment administered. Photographic records of all lesions treated (and clinical response) were viewed as part of the data collection which resulted in computerized records of all treatment episodes.

Results: The seven patients, aged 56-73 years old at enrolment, received ALA-PDT to between 2 and 15 lesions (mean, 7). Two patients received concurrent therapy with topical nitrogen mustard application to sites not treated by PDT. Both patients were observed to obtain a better response with PDT than nitrogen mustard in comparable lesions in similar anatomical locations.

A total of 53 lesions received two treatments; 14 patch stage, 18 plaque and 21 tumour stage lesions. Fluorescence was observed prior to illumination in all lesions treated. The overall clinical outcome of PDT is shown in Table 6.1.

Figure 6.1 demonstrates the response of patch, plaque, and tumour stage lesions to ALA-PDT. In each case depicted, histological confirmation of clearance was acquired with the histopathological clearance of a tumour lesion shown in Figure 6.2.

Table 6.1 Response of CTCL lesions to topical ALA-PDT depending on lesion type and light source used.

Lesion type	PCR no.	sCCR no.	CCR no.	CCR %	CCR - laser (%)	CCR - arc lamp (%)
Patch (n=14)	6	1	7	50	5/8 (62)	2/6 (33)
Plaque (n=18)	8	0	10	56	6/8 (75)	4/10 (40)
Tumour (n=21)	11	3	7	33	7/19 (37)	0/2 -
All (n=53)	25	4	24	45	18/35 (51)	6/18 (33)

CCR = complete clinical resolution

sCCR = sub-complete (>90%) clinical response

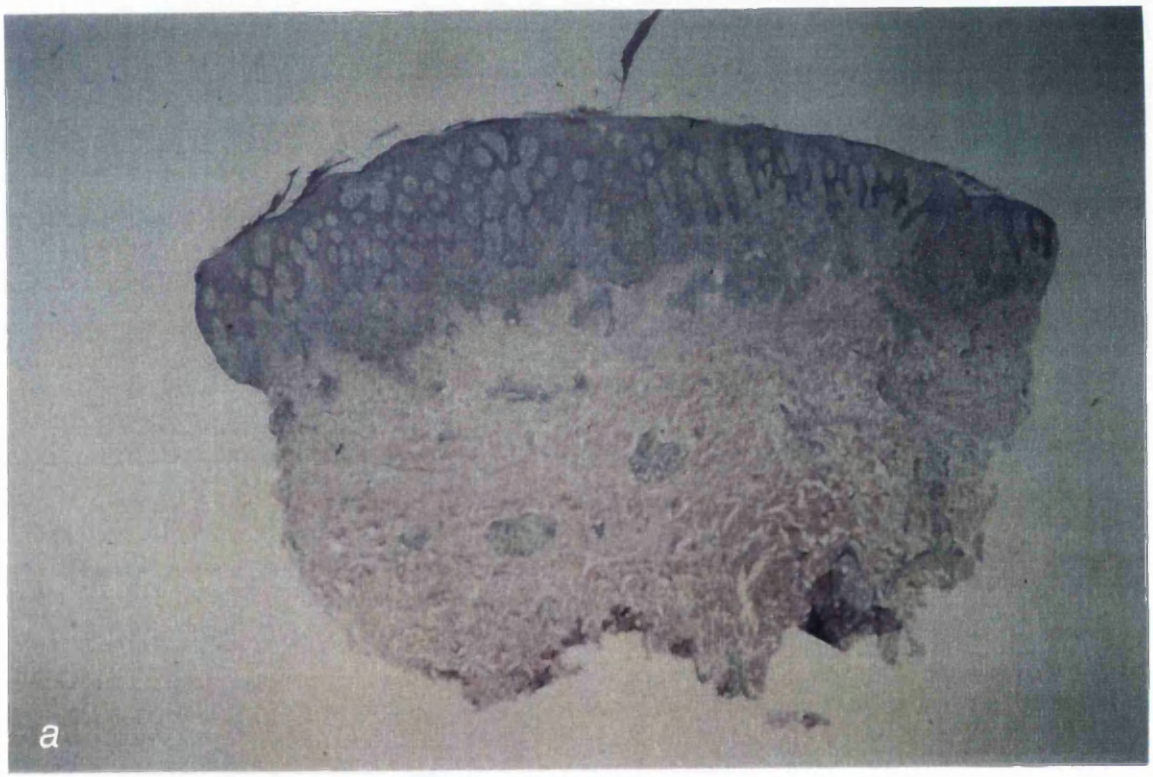
PCR = partial (<90%) clinical response

Figure 6.1- a+b A patch of histologically proven CTCL on the left wrist (a) before and (b) 6 months following topical ALA-PDT.

Figure 6.1- c+d Plaque stage disease on the wrist of another patient (c) before and (d) 1 year following ALA-PDT.

Figure 6.1- e+f Tumour stage disease (maximum depth 1.5cm) on the abdomen (e) before and (f) 6 months following ALA-PDT.

Figure 6.2- a+b Low power views of histopathological sections derived from biopsy of a tumour stage lesion (a) before and (b) 3 months following topical ALA-PDT using the laser source. A densely packed uniform upper dermal basophilic cell infiltrate in section (a) is absent from section (b). Both sections - H+E.



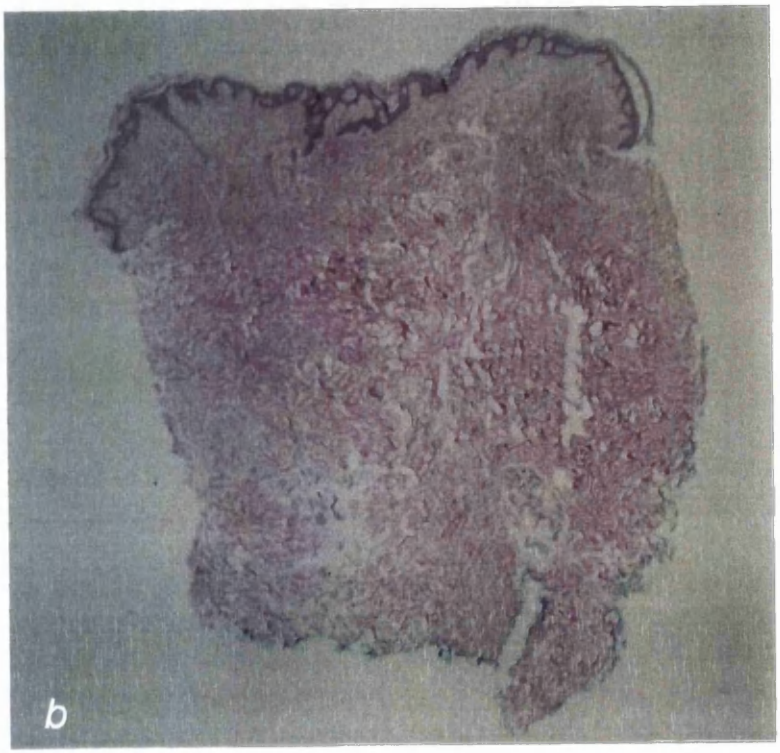
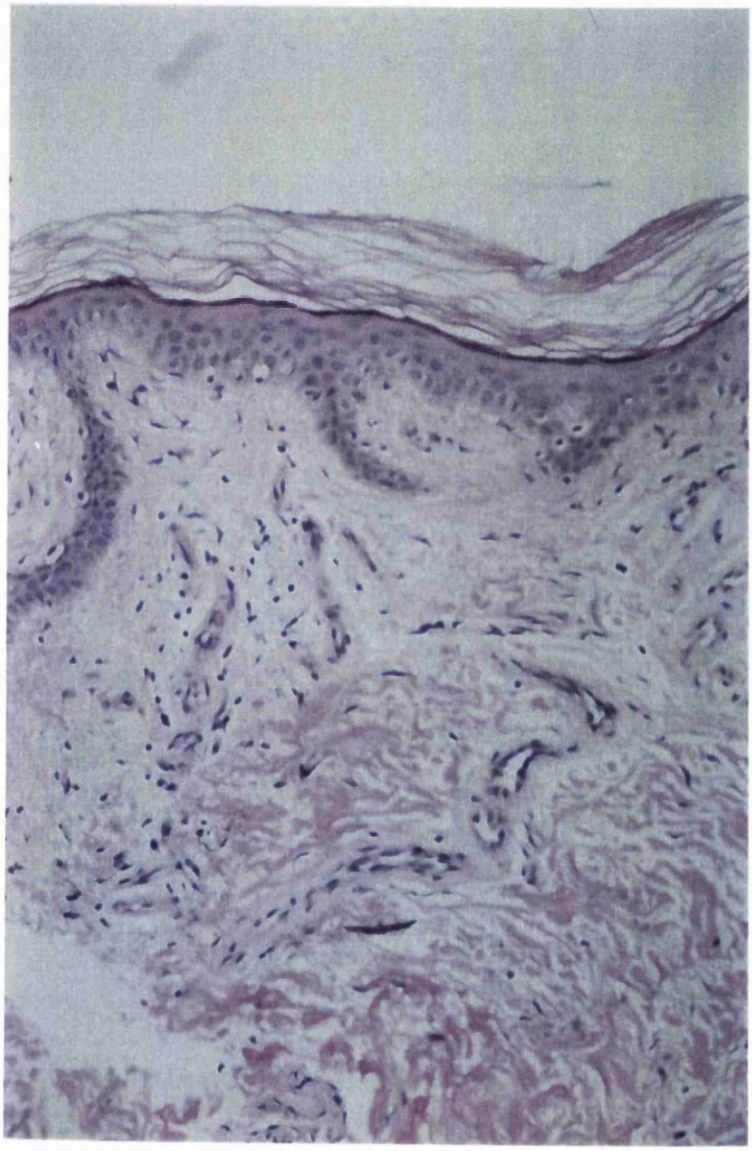


Figure 6.2- c Clearance of tumour infiltrate is evident on high power view of the post treatment biopsy section (H+E).



The clearance rate of all three lesion types was superior following laser to that for the experimental broadband halogen lamp. Of the 18 lesions clearing following laser alone, 13 cleared following a single treatment. All 6 lesions clearing following illumination with the halogen lamp required only one treatment.

Further ALA-PDT sessions were performed for those 29 lesions which failed to clear after 2 treatments. Six more lesions (1 patch, 1 plaque and 4 tumours) cleared after 3,3,3,4,5 and 6 treatments, whilst the remaining lesions did not clear after a median of 4 treatments (range 3-12). Only 4/23 lesions which failed to clear received 6 or more treatments.

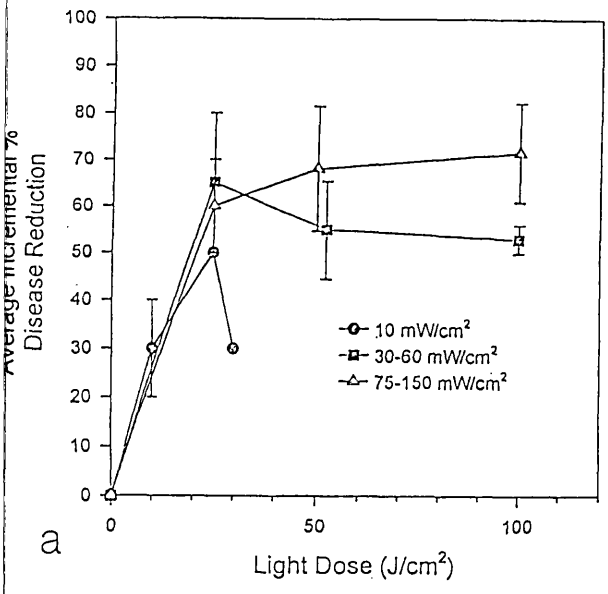
The 8 patch stage lesions cleared after 1 (n=3), 2 (n=4), or 6 (n=1) treatments. The 11 plaques cleared following 1 (n=10) or 3 (n=1) treatments. The 11 tumour stage lesions cleared after 1 (n=6), 2 (n=1), 3 (n=2), 4 (n=1) or 5 (n=1) treatments.

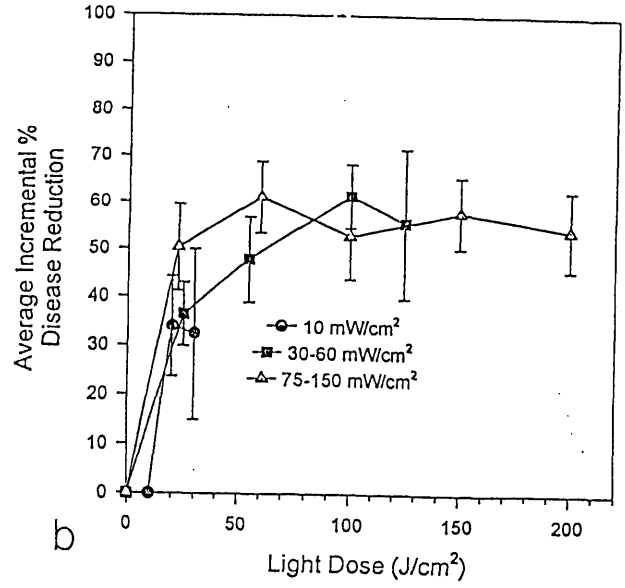
The median size of all lesions clearing was $28cm^2$ (range $2.25cm^2$-$144cm^2$) in comparison with $40mm^2$ (range $9cm^2$-$168cm^2$) for those lesions which did not clear. Pre- and post- treatment biopsies, where available, indicated that tumour lesions up to 1.5cm in thickness had cleared.

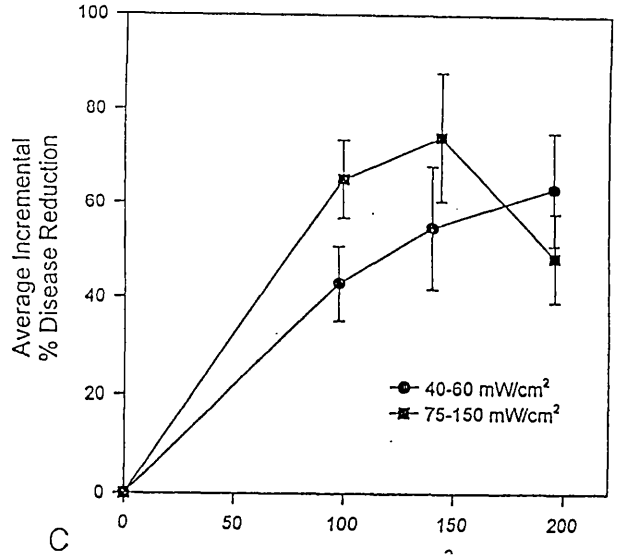
The concentration of 5-ALA used for the 30 lesions which cleared was 5% in 1, 10% in 5, and 20% in 24 lesions. For the 23 lesions not clearing, the majority of lesions also received 20% (n=14) whilst 7 received 10% and 2 lesions, 30% 5-ALA. The irradiance used ranged from 30-150mW/cm^2, median 100mW/cm^2 and the average dose to achieve clearance was 125J/cm^2 (range 50-200J/cm^2).

The clinical response of lesions following individual PDT sessions (Figure 6.3) was included in this study to establish the most favourable treatment parameters. Laser light at 10mW/cm^2 was less effective, for both patch/plaque and tumour stage lesions, than 30-60mW/cm^2 and 75-150mW/cm^2, whilst the latter intensity treatments were broadly similar in

Figure 6.3 Efficacy of ALA-PDT for patch/plaque and tumour stage CTCL using different light dose/intensity regimens and different light sources. (a) Patch and plaque disease treated by laser (36 treatments), (b) Tumour stage disease treated by laser (78 treatments) and (c) All stages treated by halogen light (38 treatments). Light intensities (10-150mW/cm^2) are shown by symbol. Plot of mean ± SEM.





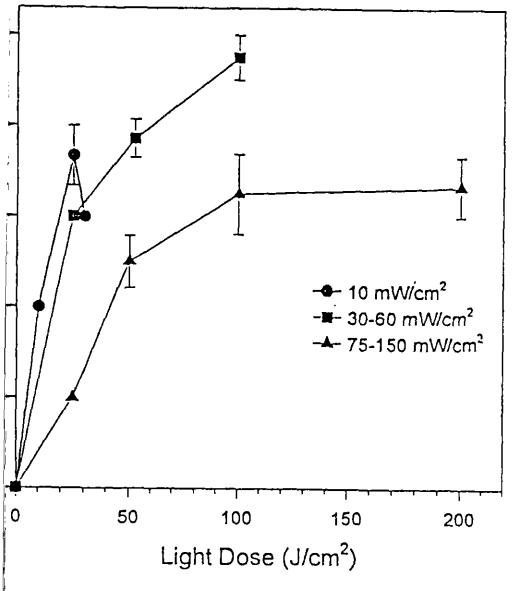


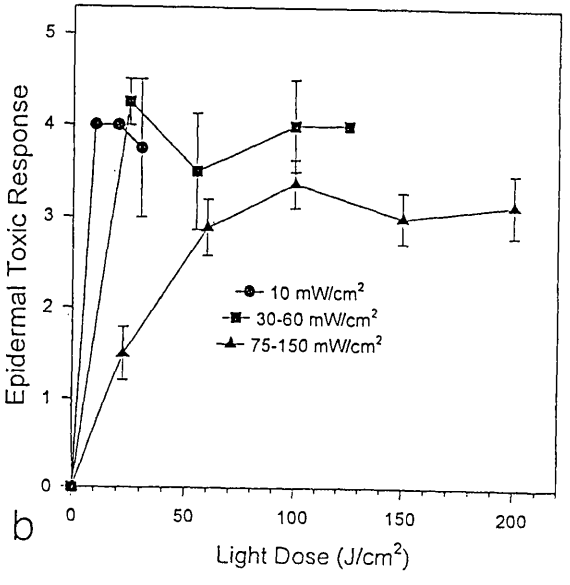
efficacy. A plateau in the laser dose/response, with at least a 50% reduction in lesion size, was apparent from 30J/cm^2 in patch/plaque disease, whilst 50J/cm^2 was required to achieve a similar outcome in tumour stage disease. Limited data from the halogen source (Figure 6.3c) suggests that optimal disease reduction is achieved at 100-150J/cm^2 for treatments at irradiances of 40-150mW/cm^2 although more data using lower light doses is required.

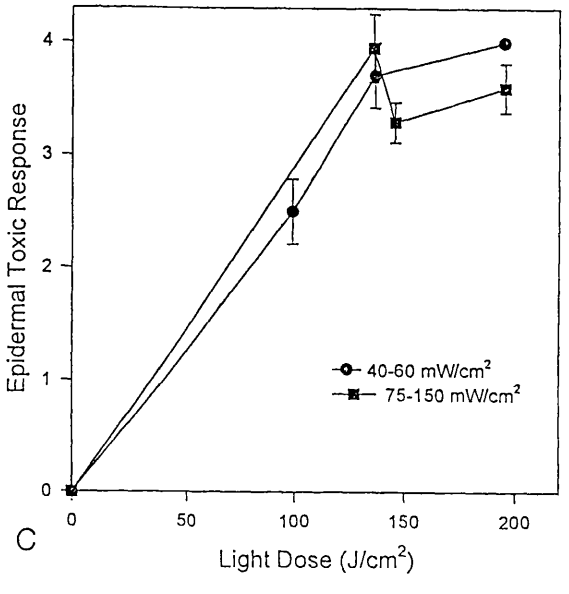
Local anaesthesia was required during the treatment of 23/53 lesions. The size of lesions requiring anaesthesia (36cm^2, range 0.25-252cm^2) differed little from that of lesions which did not (28cm^2, range 4-252cm^2), whilst the intensity of illumination was overall slightly higher for those lesions requiring anaesthesia (anaesthesia:100mW/cm^2, range 10-150mW/cm^2; no anaesthesia: 80mW/cm^2, range 30-150mW/cm^2). The majority of treatments utilizing ≥10% 5-ALA with laser at any dose and power density required anaesthesia. For the halogen source, all treatments >50mW/cm^2 required anaesthesia regardless of 5-ALA concentration or dose. One patient, with 15 lesions, tolerated treatment without anaesthesia regardless of the dosimetry which may suggest that, within the regimens described, the requirement for local anaesthesia was patient rather than dosimetry dependent.

The phototoxic effect on epidermis (ETR) of ALA-PDT was assessed clinically on the scale described above, for individual treatments. Figure 6.4 compares the ETR by lesion type, light source and dose. A similar pattern of response was evident for patch/plaque (Figure 6.4a) and tumour (Figure 6.4b) stage lesions treated by laser, with ETR greater at the same dose for lower irradiance light (10mW/cm^2 and 30-60mW/cm^2). Scattered areas of desquamation (ETR = 3) results from illumination with 40-60J/cm^2 at 75-150mW/cm^2, the lowest plateau dose for maximum efficacy. Higher doses, up to 200J/cm^2, have relatively little effect on ETR, which is much more dependent on irradiance intensity than dose. The non-laser source showed a gradual onset of epidermal damage and parallel response between

Figure 6.4 Epidermal phototoxic response for different light dose and intensity measurements observed during the treatment of (a) Patch and plaque disease by laser (36 treatments), (b) Tumour stage disease by laser (78 treatments), and (c) All stages treated by the halogen light (38 treatments). Light intensities (10-150mW/cm^2) are shown by symbol. Plot of mean ± SEM.







intermediate and high intensity treatments of all disease stages (Figure 6.4c). Widespread desquamation of the epidermis of lesions was noted at doses within the therapeutic window for PDT using this source and a 24 hour photosensitizer application time.

Five of the total of 30 lesions which had initially cleared, recurred during a 12 month follow-up period (range 6-20 months); four lesions at 3 months, one at 10 months. This reduces the clearance rate to 47%.

Summary: Fourteen patch, 18 plaque and 21 tumour stage lesions received topical ALA-PDT with complete clearance in 50%, 56% and 33 % of lesions respectively after two treatments. The clearance rate of all three lesion types was superior following laser to that for an experimental broadband halogen lamp. A further 6 cleared after 3,3,3,4,5 and 6 treatments, whilst the remaining lesions did not clear after a median of 4 treatments (range 3-12). The median size of all lesions clearing was 28cm^2 in comparison with 40mm^2 for those lesions which did not clear. 24/30 lesions which cleared had received 20% 5-ALA 24 hours prior to illumination. Light dose assessment with the laser indicated an optimal response with at least 40-60J/cm^2 at 30-150mW/cm.^{2} The non-laser halogen source showed a plateau in response from 125J/cm^2 for treatments at 40-150mW/cm.^{2} The toxic effect on epidermis of ALA-PDT was greater for lower irradiance light. Five lesions recurred during a 12 month follow-up period.

6.3 - Case report - ALA-PDT, with the prototype lamp, in CTCL

A 47 year-old female with a 10 year history of mycosis fungoides was assessed for possible responsiveness to ALA-PDT during a flaring of her

disease. Previous treatment with PUVA photochemotherapy (2 courses), alpha-interferon, oral retinoids, topical nitrogen mustard, potent topical steroids and radiotherapy had achieved only limited/short-term response to her plaque/tumour stage disease. Examination revealed in excess of 20 plaques on her trunk and limbs. PDT using 125J/cm^2 filtered red light , 4 hours after 5-ALA application, revealed strong surface flourescence prior to treatment in a 6.5x3.2cm plaque. However, no response was clinically evident 4 weeks later when both the treated and a control plaque (measured, but not treated) showed increase in size over the month. The patient declined further treatment and was referred for assessment for radiotherapy.

6.4 - Conclusions

Topical ALA-PDT can achieve clearance of patch, plaque and tumour stage CTCL, with response rates of 50%, 56% and 33% respectively after 2 treatments, and further PDT treatments clearing more lesions.

Careful interpretation of the data presented in this study is required due to the multiple parameters examined. As the study was on-going at the time of this interim analysis, clearance or not after two treatments was chosen as the first index of response to facilitate comparison of CTCL with earlier studies in this thesis. CTCL is less responsive that Bowen's disease or superficial BCC to ALA-PDT over 2 treatments compatible with their primary intradermal pathology and the anticipated greater depth of the majority of lymphoma lesions.

Previous published case reports[19,119] indicate that 2-5 treatments might be expected to clear plaque stage disease with a presumption that thicker tumours would require more treatments. Thus the clearance of 30 lesions after 1-6 treatments may better reflect the response that can be achieved

from ALA-PDT. Only 4/23 lesions which failed to clear received 6 or more treatments suggesting that a superior response might still be achieved by further treatments to the non-responders.

Both laser and non-laser sources can achieve lesion clearance. Laser appears superior in effect to the halogen lamp although fewer lesions were treated using the lamp which was introduced midway through the study and may have included a greater percentage of ineffective dose/irradiance combinations. A superior response of patch and plaque stages over tumour lesions is evident with laser treated lesions and is also suggested in the non-laser group which failed to clear either of the 2 tumours treated.

Optimal response parameters indicate the use of 20% 5-ALA, followed by at least 40-60J/cm^2 of laser light or 125J/cm^2 non-laser light at 30-150mW/cm.^{2} Whilst most patch or plaque stage lesions will clear after 1 or 2 treatments, there is a greater likelihood that more treatments will be required for treating thicker tumour lesions.

Whilst previous case reports[19,119] indicated the efficacy of 4-6 hour topical application of 5-ALA, illumination at 4-5 hour is observed to produce relatively greater epidermal toxicity than when the photosensitizer is left for 24 hours (unpublished data, Roswell Park C. I.).

Greater epidermal toxicity was evident using laser light at low intensities. As the PDT process consumes oxygen at a rate proportional to intensity, higher intensity treatments will deplete oxygen in the papillary dermis and hence the epidermis is left relatively hypoxic, sparing the keratinocytes from damage. In contrast, light intensity will be lower in the dermis preserving high oxygenation levels in the pericapillary zones of the papillary and deeper dermis, facilitating phototoxic reactions within CTCL lesions. A therapeutic window permitting an effective dose (40-60J/cm^2) at an irradiance of 75-150mW/cm^2 avoided maximum epidermal damage.

A rise in tissue temperature with the halogen source, promoting vasodilatation and re-oxygenation of the epidermis, may account for the progressive, dose dependent increase in epidermal damage observed.

These observations suggest that for the treatment of epidermal lesions (Bowen's disease, BCC) where epidermal damage is desirable, lower intensity illumination may be preferable.

Experience in PDT of CTCL using the prototype non-laser source described in this thesis was limited to a single treatment in a patient with extensive and progressive disease, with only a 4 hour application time for 20% 5-ALA. Lack of epidermal toxicity at 4 hours in this patient, combined with the response rates described above from the unique experience of the Buffalo unit, suggests that further evaluation of the lamp's use of ALA-PDT in CTCL in an appropriately designed pilot study is indicated. The prototype lamp, with its spectral output closer to that of the laser than the broadband halogen source, would be expected to achieve a similar response profile.

The request for anaesthesia by patients gives an indication of pain severity, although the tolerance of one patient to all dose and intensity combinations received, demonstrates patient variation in pain perception. Whilst epidermal toxicity is less with an application time for 5-ALA of 24 hours, pain is more likely to be experienced (unpublished data, Roswell Park C.I.). This may result from the accumulation of PPIX in cutaneous nerve endings with prolonged 5-ALA application. Low intensity laser treatments caused less pain, but they were also less effective at clearing lesions. Treatments with the halogen source at a power density $<50 mW/cm^2$ were also minimally painful, but appear to be more effective, possibly due to the broadband illumination of the source although this requires further study.

The mechanism of action of ALA-PDT in CTCL is not fully understood. Direct tumour cell damage from the accumulation of PPIX[117,118] may be complemented by host reactions associated with a PDT-induced inflammatory

response (Chapter 1). This may explain the response of thick lesions to topical ALA-PDT, as adequate light/photosensitizer penetration is unlikely for tumours up to 1.5cm in depth.

The potential to clear CTCL by topical ALA-PDT, in patients with multiple lesions and multi-stage disease, requires further confirmation. An additional non-invasive therapy for CTCL, however, has been demonstrated by one unit, which has treated many lesions in several patients with follow-up times of up to 20 months. Patients receiving ALA-PDT to date have largely been non-responders to existing modalities or patients with rapidly progressing disease. Appropriate patient selection may therefore identify a group more responsive to ALA-PDT, although PDT will require to be compared with existing therapies in this group.

Chapter 7 - Discussion

7.1 - Overview

The primary aim of this thesis was to critically evaluate the usefulness of ALA-PDT in skin cancer. Several open trials, with a variety of protocols, and limited histological/temporal evidence of clearance, were available at its commencement. Most of the published studies focused on systemically administered Photofrin and its precursors, and on the use of laser light to promote PDT. The place of topical administration of 5-ALA and the assessment of the potential for non-laser light in PDT of cutaneous malignancy, however, required further study.

Chapters 3 and 4 report the clinical trials and morphological effects of topical ALA-PDT using a prototype xenon source with an *in vitro* and *in vivo* efficacy comparable to laser. An initial open study in actinic keratoses and Bowens disease confirm the clinical efficacy of ALA-PDT using the prototype lamp. Three randomized, comparison trials focus on dose/wavelength response of Bowen's disease to ALA-PDT, and to its efficacy against cryotherapy for Bowen's disease. A comparison study of the importance of tumour thickness and duration of photosensitizer application on the response of BCC to ALA-PDT is also reported.

To further evaluate the potential of 5-ALA-PDT, in chapter 5, two murine studies report the presence of a photoproduct of PPIX that is activated by 670nm light and may alter therapeutic effectiveness.

In chapter 6, the potential for ALA-PDT in cutaneous lymphoma, previously restricted to 2 case reports, is described in detail for patch, plaque and tumour stage disease in patients with multiple lesions.

Adverse reactions, recurrence rates and the identification of those lesions that may particularly benefit from this new modality are identified from each of the clinical studies.

The implications of the results reported in the preceeding chapters, along with the results of further relevant studies published during the follow-up period of the trials, are summarized below:

7.2 - Prototype non-laser source for PDT

The prototype lamp, incorporating a 300W xenon source, has been shown to be effective in PDT for human skin cancer. The lamp can deliver 100J/cm^2 of 630±15nm light at an intensity of 86mW/cm^2 to a 3cm diameter field in approximately 20 minutes. This provides sufficient power, despite the long wavelength and narrow bandwidth employed, for it to be practical in clinical use. The simplicity of operation and ease by which light can be directed to any body site facilitate its use. Moreover, as a portable, reliable and relatively cheap source (approximately $^1/_{10}$ the cost of a laser system), it has the potential for use outwith a hospital's laser suite.

The comparison of red with green light demonstrated that, with appropriate filters, output can be adjusted to permit evaluation of different wavelength/bandwidths and photosensitizers. No hyperthermic temperatures were recorded during this study supporting pre-clinical tests of the absence of infra-red emission.

7.3 - 5-ALA/PPIX as photosensitizer

5-ALA was used as a precursor for protoporphyrin IX throughout this thesis. Application of 20% 5-ALA in an oil in water emulsion 4 hours prior to illumination was effective in clearing actinic keratoses, Bowen's disease, and superficial BCC up to 1mm thick. A six hour application interval improves clearance of BCC 1-2mm thick. Twenty-four hour application of 20% 5-ALA in another oil in water emulsion promoted clearance of patch, plaque and tumour stage CTCL. Although numbers were limited, 5% and 10% preparations of 5-ALA also achieved clearance of CTCL lesions.

Comparison with previous studies of actinic keratoses/Bowen's disease (Table 2.1) indicates that no benefit may have been attained from prolonging application to 6, 8, or 20 hours with initial 100% clearance rates and comparable recurrence rates achieved after only 4 hour application. In contrast, an increase from 4-6 hours improved BCC response for intermediate thickness lesions, an observation which could not be obtained from previous studies (Table 2.4) which failed to report individual tumour depth and the application times for each lesion. The study by Figan *et al*[92] would suggest that even 20 hours remains insufficient time to clear more than 60% of nodular lesions, although this study had reported a surprisingly low clearance rate for actinic keratoses and Bowen's disease also at 20 hours suggesting their broadband light delivery may have been sub-optimal.

Prolonging application to 24 hours in CTCL treatment, whilst intended to reduce epidermal toxicity, probably improves clearance rates. No response was evident after a single treatment, with a 4 hour 5-ALA application time, of PDT to a plaque of CTCL in one case report in this thesis although 5/7 lesions did clear with this protocol in two other case reports in 4 patients with limited plaque disease.[19,119]

The detection of surface fluorescence prior to illumination in all patients treated confirmed the presence of PPIX formation with the intensity at 4 or 6 hours strongly outlining areas of clinically diseased skin. Only where lesions abutted onto mucous membranes could a strong fluorescence in adjacent normal tissue be noted. The absence of the normal skin barrier from the non-keratinizing epithelium of the mucous membranes will promote uptake, hence care in limiting the field of illumination is required to minimise epidermal toxicity in PDT to these sites.

Fluorescence recording for CTCL lesions after 24 hour 5-ALA application whilst detecting fluorescence prior to illumination, often did not demonstrate a marked differential in intensity at the lesion/normal skin

interface consistent with the expected loss of differential uptake of photosensitizer by the normal/abnormal epidermis by this time.

No generalized phototoxic reactions, locally or generalized were noted throughout the studies performed in this thesis, although all lesions were protected from sunlight for 24 hours and patients were advised to avoid sitting out in the sun for the 24 hours following treatment. Blood and urine samples pre- and post-PDT were monitored in a recent study of actinic keratoses and Bowen's disease with no evidence that topical ALA-PDT alters systemic accumulation of porphyrins or porphyrin precursors.[172]

PPIX may not be the only active photosensitizer produced by exogenous administration of 5-ALA. In a study of explant pieces of normal skin, keratoacanthoma and BCC, porphyrin levels were measured after incubation with 5-ALA. Whilst PPIX preferentially accumulated in the BCC tissues, coproporphyrin accumulated in the keratoacanthomas suggesting that tumours may differ in their 5-ALA metabolism and that other porphyrin products may act as clinically relevant photosensitizers.[173]

7.4 - Light/PDT dose

A wide variation in total light dose, from 30-540J/cm^2 (Table 2.1), exists for Bowen's disease. The variety of different light sources, and hence wavelengths used, in part explains this wide variation. However, even where laser sources were used, dose levels varied between 60-250J/cm.^{2} Within individual studies, small numbers provided insufficient data for statistical interpretation of dose/response. Against this background, a large randomized study of 120 lesions was performed to establish a value for the minimum light dose which could achieve the optimal response. A significant reduction in the probability of clearance at 1 year was evident for lesions which received <100J/cm.^{2}

Dose ranges for BCC are similar, with 30-540J/cm^2 used for non-laser studies and 40-250J/cm^2 for laser trials. Dose/response was more difficult to evaluate with BCC due to smaller numbers of available lesions. A preliminary dose response comparison of two doses and 30 lesions showed no difference in outcome between 100J/cm^2 or 150J/cm.^{2}

Although initially time consuming, establishing that 100J/cm^2 appears optimal for both Bowen's disease and BCC will avoid future unnecessary treatment time.

Total light energy, although the most easily measured, is only one component of the total PDT dose administered to tumours. The total photodynamic impact of a treatment session depends on the dose and wavelength of light reaching the tumour tissue, the concentration and absorption coefficient of the photosensitizer, and for 5-ALA, upon the cells' own preferential ability to synthesize protoporphyrin IX (+ other potentially relevant photosensitizers including photoproducts). Finally, this photodynamic process will be compromised if oxygen supply becomes limited and by self-shielding by superficially placed photosensitizer and photobleaching of the photosensitizer.

The clinical studies reported in this thesis have sought wherever possible to standardize these parameters to achieve a few large comparison trials. Whilst this has been achieved for Bowen's disease and BCC, the limited published experience of ALA-PDT for CTCL has restricted current study to initial efficacy assessment in patients who have failed on at least one previous therapy. ALA-PDT in CTCL will therefore require future randomized comparison of those parameters which appear most critical to lesion clearance.

A formula has also been derived to permit comparison of the output of coherent and non-coherent light sources and of different filter outputs in the same lamp. The total effective fluence (Chapters 1.4 and 3.11), however still

requires estimation of the optical transmission of tissue at a particular wavelength of light through tissue and its absorption by photosensitizer. The output of the prototype lamp evaluated in this thesis, however, was limited to 630±15nm (except for the provision of green light in the red/green comparison study) to permit valid comparison of efficacy with laser studies. An efficacy of this lamp comparable with laser is apparent from these studies (Tables 2.1, 2.4) and implies that its future detailed evaluation in CTCL should yield similar results to the argon laser. The output effective for ALA-PDT from the non-laser source used for a few CTCL lesions, along with that from the tungsten sources reported previously, remain difficult to compare due to their broadband outputs and the possibility that early sources may also have emitted significant amounts of infra-red with its own therapeutic action. The red/geen comparison and photoproduct studies provide evidence for the incorporation of certain wavelengths to optimise ALA-PDT that provide a logical approach to the development of the most therapeutically effective wavelengths required for ALA-PDT.

7.5 - Lesion size/depth and fluence rate

The studies of Bowen's disease established a further, previously unrecognized, variable, lesion size, as having a significant effect on the probability of clearance. Despite restricting lesion size to 25mm in maximum diameter, two randomized trials (PDT vs. cryotherapy, Dose/response) revealed lesion size as significant. The cryotherapy treatment group also demonstrated this observation whilst size for red-light, but not green-light treated lesions in the third comparison study was almost significant. The response of large patches of Bowen's disease and of CTCL lesions also demonstrate this pattern (although with insufficient numbers for statistical analysis once other variables are included). BCC studies did not show this phenomenon, with tumour thickness the major variable influencing outcome.

This implies that, providing other factors are near optimal, size will influence outcome, and requires consideration in future study designs.

Fluence rate and lesion size were linked variables in the Bowen's/BCC studies and hence assessment of fluence rate on response was confounded. An apparent improvement in response of Bowen's disease at or below 48mW/cm,^{2} however, although not supported in the BCC study, is similar to the observed improvement in the response of CTCL lesions treated by the halogen (but not laser) source at <50 mW/cm.^{2} This further supports the observation, from study of systemic ALA-PDT[145], that oxygen depletion starts to affect outcome at fluence rates above 50mW/cm.^{2} This variable may also only become apparent where treatment conditions are sub-optimal and fluence rate, rather than another variable, e.g. thickness or wavelength, become critical to maximizing photo-dynamic reactions within the tumour.

7.6 - Tumour thickness vs. response

Tumour thickness probably has a strong influence on the outcome of ALA-PDT in CTCL as well as BCC. Although few lesion depths are known, clearance rates for patch and plaque stage lesions were similar, whilst the rate dropped for tumour stage disease. However, documented clearance of tumours up to 1.5cm, with laser-PDT and 24 hour 5-ALA application, suggests a difference in achievable depth of treatment with CTCL compared with BCC. Whilst prolonging ALA may increase clearance rates, it is possible that the difference in cell type and/or tumour structure greatly affects outcome.

Malignant lymphocytes preferentially accumulate PPIX over normal, unstimulated lymphocytes.[118] Most superficial, but few nodular BCC, demonstrate full thickness PPIX fluorescence by 6 hours. Svanberg *et al*[19] demonstrated PPIX distribution following 4-6 hour 5-ALA application, by laser induced fluorescence, to show a demarcation between tumour and normal

skin of 15:1 for BCC (and Bowen's disease), and 5:1 for CTCL. However, the tumour:normal tissue ratio at 24 hours is not known for either tumour. Doubts also remain over the potential of BCC cells to adequately absorb 5-ALA in the current oil in water emulsions used. Whilst Szeimes *et al*[134] proposed that increasing from 4-12 hours would promote uptake of 5-ALA to include deep dermal tumour cells, Roberts *et al*[9] indicated, although after only 4 hour 5-ALA application, that it was not depth but uptake beyond those tumour cells immediately adjacent to 'normal' dermis was limited. Thus adequate penetration into the tumour mass, when a topical route of photosensitizer delivery is used, may be more important to response and may not be accurately reflected by an intense surface fluorescence which may only represent the presence of PPIX in an outer rim of tumour cells.

Fluorescence measurement unfortunately is limited both by the depth of penetration of the wavelength used, permitting often only very superficial detection (e.g. UV light), and the inability if using a single wavelength of distinguishing its precise location in a tissue (e.g superficial vs. deep dermis). As knowledge of tumour depth is usually not known, *in vivo* fluorescence detection is thus at best only an estimation of PPIX distribution. Quantitative fluorescence microscopy of tissue samples is therefore required to achieve a better understanding of photosensitizer uptake by cells, but necessitates excision of tissue.

Current interpretation of the literature and above results thus suggest that thick tumours (nodular BCC and CTCL) benefit from longer photosensitizer application times thus making tumour depth a critical factor in response, but that additional factors, e.g. lipophilicity of 5-ALA vehicle, tumour cell mitotic rate, relative cellular iron deficiency, cell-to-cell adhesion, are likely to influence the preferential accumulation of PPIX between different tumour types and sub-types.

7.7 - Mechanisms of action of ALA-PDT

Studies in this thesis have demonstrated: 1. The presence of both necrosis and apoptosis following ALA-PDT 2. That direct cell damage is the predominant route of tumour destruction 3. That the response of CTCL lesions may in part be immune-mediated 4. Microscopic scar formation is common following ALA-PDT to BCC.

A full thickness or focal coagulative necrosis was observed following ALA-PDT to Bowen's disease and BCC. Apoptosis were observed in high number in several sections 1 hour following therapy suggesting that both modalities of cell death are promoted by ALA-PDT.

The subcellular localization of PDT-induced photodamage may influence the rapid development of apoptosis, with cell membrane photodamage preventing apoptosis. When a photosensitizer (tin etiopurpurin) which targets mitochondria, was substituted by a drug analogue (tin octaethylpurpurin amidine) which also targets cell membranes, apoptoses were not observed until 24 hours after PDT, in comparison with <1hour with the original agent.[174] PPIX also accumulates preferentially in the mitochondria, but also plasma and nuclear membranes. Apoptosis may thus occur if sufficient mitochondrial damage has occurred early on during PDT, but less specific mechanisms, including inflammation and hypoxia, may promote coagulative necrosis.

The importance of apoptosis to tumour cell death in PDT has recently been highlighted by a study of cells expressing the mitochondrial protooncogene Bcl-2, which inhibited not only apoptosis but overall PDT-induced cell kill *in vitro*.[175] Bcl-2 is thought to promote cell survival by inhibition of apoptosis.[176] Thus cells expressing Bcl-2, which appear restricted to long-lived or proliferating cell populations,[177] may be relatively resistant to PDT.

Direct tumour damage appeared to be the primary route of cell kill from samples examined as endothelial damage to adjacent vessels was not noted to precede tissue damage. Assessment of histology following PDT remains a crude method of assessment, but is consistent with the observations of Wang *et al*[26] that superficial blood flow increased post ALA-PDT to Bowen's disease and BCC, only returning towards pre-treatment levels on healing. However, a recent study demonstrated that endothelial cells *in vitro* accumulate 1.5-4 times more PPIX when proliferating than when quiescent.[178] Current evidence would suggest that this accumulation does not result in therapeutically significant vascular damage.

The hypothesis that clearance of deep tumour tissue is promoted in CTCL by a PDT-induced immune response suggests effects of PDT on tissue that extend beyond a non-specific inflammation. Evidence of a complex response is emerging of potentiation of antitumor immunity, but with inhibition of skin contact hypersensitivity and increased skin graft survival. A BALB/c mouse model has been used to demonstrate that PDT induces, in both normal and tumour tissue, interleukin (IL)-6 and IL-10 but not tumour necrosis factor-alpha.[179] This suggests that the general inflammatory response induced by PDT may be mediated, at least in part, by IL-6. IL-6 may also modulate the local anti-tumour response. By contrast, the enhanced IL-10 expression may promote the suppression of cell-mediated responses seen following PDT.

Clinical studies of ALA-PDT in the treatment of non-melanoma skin cancer and its precursor lesions report a good cosmetic outcome.[19,91-5,105,113] Whilst the studies reported in this thesis show similar cosmetically acceptable results, there was microscopic evidence of scar formation in 36% of treated BCCs after 1-4 months, although virtually absent following PDT to Bowen's disease. Nevertheless, scarring would appear less severe than might be expected from other therapies with scar formation clinically evident in 20% of

sites of Bowen's disease treated by cryotherapy, another modality considered to have a relatively low scarring potential. Moreover, the absence of fibrosis from BCC sites biopsied at 6-12 months post-PDT may reflect spontaneous improvement when tissue repair is complete.

Recent evidence supports the a reduced potential for scarring by PDT. Heat shock protein 47, associated with collagen type I metabolism, increased following hyperthermia, but not PDT, in a study of normal murine fibroblasts.[180] Preservation of the structural integrity of collagen has also been demonstrated in comparison with hyperthermia in rodent colon.[149] This may not only preserve the integrity of tissue, but promote a good cosmetic outcome in PDT treated tissue.

7.8 - Is ALA-PDT carcinogenic?

Following over 20 years of experimentation of PDT, and 8 years of ALA-PDT, only one tumour has been reported to have possibly been induced by this therapy.[60] This case report concerns an 82 year-old man who had received, over 4 years, 7 sessions of ALA-PDT to treat 28 actinic keratoses and 3 squamous cell carcinomas. Six months following his last treatment, a new pigmented lesion was noted and excised, to confirm a melanoma (Clark level 2, Breslow: 0.4mm). The site of the melanoma had been included in the treatment field of 4 of the PDT sessions. Whilst there is no proof of a causal association in this patient with a long history of cutaneous malignancy, concern remains that the PDT may have promoted, if not induced, the tumour, possibly by repeated Inflammation following each PDT session.

No tumours are known to have developed/metastasized following ALA-PDT administered in trials in this thesis, although follow-up intervals remain short (maximum 3 years) for the detection of malignancy.

PDT with systemic photosensitizers has been shown to be immunosuppressive.[181] However, *In-vitro* studies with haematoporphyrin

derivative and phthalocyanines, whilst demonstrating nuclear damage, have shown no mutagenic activity above background levels.[59] Cell death is presumed to occur rather than the induction of mutagenic or carcinogenic phenotypes.

7.9 - Resistance to ALA-PDT

Certain cell populations and even certain people may be less sensitive to ALA-PDT than others, but evidence of precise explanations are still lacking. Patients may differ genetically in their response to PDT-mediated oxidative stress or following additional ingestion of anti-oxidants , e.g. vitamins C or E, which may render individuals temporarily less susceptible to PDT-induced intracellular reactions. As discussed above, cells expressing the proto-oncogene Bcl-2, may be relatively resistant to PDT. 5-ALA induced PPIX is known to accumulate primarily in epithelia and glands, but not mesenchymal tissues.[10] Differences in the capacity of certain cell types to stimulate the haem cycle may be an important factor, with cells relatively low in intracellular iron able to preferentially accumulate PPIX, such as transferrin receptor positive activated lymphocytes.[20]

7.10 - Clinical indications for ALA-PDT in skin cancer

Indications for the use of topical ALA-PDT in clinical practice can now include Bowen's disease, following the addition of the first randomized comparison trial with established therapy, to the multiple open studies published. Other indications are less thoroughly evaluated, but may be of greater clinical use, offering advantages over existing therapies.

The potential indications for topical ALA-PDT are:

1. Actinic keratoses, especially widespread disease, not readily cleared by cryotherapy or topical 5-fluorouracil without great discomfort/multiple applications.

2. Superficial (<1mm) BCC - especially multiple/large lesions.

3. Large patch Bowen's disease.

4. Patch/plaque stage CTCL, especially limited to a few lesions, where whole body photochemotherapy/systemic therapy may be inappropriate and local topical therapies have failed or are contra-indicated.

The number of treatment sessions of PDT required for certain lesion types will influence the viability of PDT in clinical practice. Although 3 cryotherapy sessions may be required for small lesions of Bowen's disease in comparison with 1-2 PDT treatments, in view of longer treatment time and patient inconvenience, it is the superior adverse effect profile and the advantages of use in certain anatomical sites, that will cause clinicians to use ALA-PDT for these lesions. However, even the requirement of 3 or more treatments to certain lesions may still make PDT attractive if large patches of Bowens disease or superficial BCC can be cleared without surgery±grafting.

The clearance of 24/30 CTCL lesions in 1-2 treatments may also be practical for routine clinical therapy, but comparison with existing modalities and either improved response rates or early selection of lesions likely to respond, are still required before the full potential of ALA-PDT in CTCL can be correctly assessed.

Reproducible high clearance rates for BCC >1mm are also required before ALA-PDT can be introduced in practice for this indication.

The incidence of adverse events will also influence the introduction of ALA-PDT into wider clinical practice. Despite histological evidence of extensive epidermal damage, PDT was associated with no extensive epidermal ulceration as witnessed following 25% of lesions which received cryotherapy.

The requirement for anaesthesia was limited in the studies reported to large or ulcerated lesions of Bowen's disease or BCC, but was requested for the majority of lesions (excluding all lesions in one patient) receiving PDT for

CTCL with a 5-ALA application time of 24 hours. Thus, PDT can be tolerated without anaesthesia for small lesions (<3cm diameter) where 4-6 hour application times for 5-ALA are employed. Even the current requirement for anaesthesia for large lesions is possibly not a limitation as conventional therapy would likely also require anaesthesia or consist of several painful cryotherapy applications.

Pain associated with topical ALA-PDT may limit its therapeutic applications for multiple lesions. Uptake of 5-ALA by nerve endings may contribute to the requirement for anaesthesia, particularly following prolonged 5-ALA application. In the absence of infrared emissions, however, pain may in part reflect the overall intensity of photodynamic activity as suggested by the requirement for anaesthesia in large lesions. Future improvements in the efficacy of PDT may unfortunately result in shorter, more successful, but more painful treatments. The application of topical local anaesthesia, possibly combined with the photosensitizer, may diminish this problem, although a vasodilatory agent, rather than a vasoconstrictive compound, should be used to minimise tissue hypoxia impeding photodynamic activity.

The low prevalence of clinically obvious scars, with preservation of tissue function, makes topical ALA-PDT particularly useful for lesions on the eyelids, ears and nose where disfigurement can result from conventional treatment modalities.

ALA-PDT may also be useful as an adjunctive/palliative therapy in extensive/ulcerated BCC or SCC. The use of ALA-PDT alone for squamous cell carcinoma, in view of the potential for metastases, should remain restricted to closely monitored research until efficacy has been proven in larger trials as only 2-6 lesions have been treated in each previous study (Table 2.2) and the case reported above (Chapter 3.10) indicated only a partial response of a large ulcerated tumour.

Pigmented tumours also have the potential to respond to PDT, but 5-ALA, activated at 630/635nm is not an effective photosensitizer due to light absorption by melanin. Zinc napthalocyanine, illuminated by 774nm light can reduce the size of B16 pigmented melanomas subcutaneously transplanted into mice by up to 40% in a pilot study.[182]

7.11 - Future Improvements - Light

Future improvements in the efficacy of ALA-PDT using the prototype source may be achieved by:

1. Combination of red and green light illumination,
2. Incorporation of 670nm light for photoproduct activation, or
3. Light fractionation.

The ability of green light to clear 48% of Bowen's disease (72% initial clearance), although inferior to red, suggests that combining these wavelengths may permit the maintenance/improvement of the 88%-100% clearance rates reported in this thesis for red light, whilst shortening treatment times. In theory, one would anticipate green light acting superficially, whilst proportionally more red could react with PPIX in the deeper tissues.

Green light may allow higher light doses to be more safely administered in tissues where tissue perforation could be catastrophic (e.g. intra-peritoneal PDT). However, light distribution within a cavity will also be altered by the wavelength, with experience of intra-peritoneal PDT indicating that green was less favourable than red in achieving whole peritoneal illumination using minimal access techniques.[183]

The reduced penetration of green light may make its use less painful than red-light ALA-PDT facilitating its use in large (but superficial) areas, such as for the treatment of diffuse 'field-change' actinic keratoses.[184] Fritsch *et al* describe the improvement to the temples/foreheads of 6 patients with

widespread facial actinic damage who received ALA-PDT with 30J/cm^2 of green (543-8nm) light to one side and the same dose of 'red' (570-750nm) light to the other side of their faces. Each protocol appeared equally effective, but with less pain attributed to green-only treated lesions. It is not possible to compare the photodynamic activity of these two light energies in view of the broadband nature of the red light used. Nevertheless, this study supports a role for shorter wavelength light in superficial disease.

The relative absorptions of 540nm and 630nm light by PPIX would suggest that the doses used in this thesis, of 62.5 and 125J/cm^2 respectively, are biologically equivalent, supported by the observed earlier loss of surface fluorescence from a sample of green and red treated lesions by, on average of 30 and 37J/cm^2 respectively. Whilst green light alone may be effective for actinic keratoses, the higher recurrence rate of Bowen's disease and the critical importance of tumour depth in BCC clearance would suggest that it has limited application alone in ALA-PDT for use where either adnexal structures and/or dermis/subcutis are affected by dysplasia/tumour.

The clinical relevance of PPIX photoproducts remains disputed. Long irradiation times, as encurred using non-laser sources at low fluence rates, may favour the formation of 670nm photoproduct.[163,164] The two murine studies presented, add to a limited literature (all based on laser studies), to suggest that further studies incorporating activation of this photoproduct are indicated.

At present the potential of light fractionation, with brief 'dark' intervals during illumination, in topical ALA-PDT for cutaneous malignancy remains unknown. Intercapillary spacing is theoretically the most important factor[136] although, in practice, longer intervals are probably required because of sub-optimal PDT activity due to low fluence rate or photosensitizer concentration. Fractionation may permit shorter overall treatment times by achieving a more efficient photodynamic reaction.

7.12 - Future Developments - Photosensitizer

The relatively low lipophilicity of 5-ALA impedes its diffusion through biological membranes and hence high concentrations of the agent are required. Modification of 5-ALA by esterification with long chain (C6-C8) alcohols can reduce up to 150-fold the amount of ALA required to achieve the same level of PPIX (which itself is lipophilic).[185] Clinical trials are required to confirm these *in vitro* observations.

Improved delivery of photosensitizer via iontophoresis may also facilitate improved tissue levels with lower concentrations of 5-ALA required although this is still to be verified as effective for application to the skin overlying lesions and may not be practical for routine use.[186] Iron chelation continues to be evaluated to potentiate PPIX production with a reduced dose of 5-ALA , with a new agent, 1,2-diethyl-3-hydroxypyridin-4, recently confirmed *in vivo* to double PPIX production.[187]

Local injection of photosensitizer into tumour might improve delivery, but would make ALA-PDT an invasive therapy. Nevertheless, if a high efficacy could be established for nodular BCC by this method, there may still be a therapeutic role for this non-surgical approach. A study to assess the viability of this approach has now been commenced.

Several new agents under evaluation for photodynamic therapy, BpD-MA, mTHPC, NPe_6, $SnET_2$, $TPPS_4$, ZnPc, sulfonated aluminium phthalocyanines ($AlPcS_n$), are more potent than current agents, with peak absorption wavelengths beyond 650nm which should facilitate the treatment of thicker tumours.[22] The potential for topical application of these agents remains unknown.

7.13 - Summary

Topical ALA-PDT is confirmed as an effective therapy for certain non-melanoma skin cancers. PDT offers the advantages of being non-invasive, well tolerated in slow healing sites, and tissue sparing, leaving the skin surrounding the tumour intact and functional. ALA-PDT may be particularly useful for large superficial tumours and for lesions in anatomical sites where disfigurement from conventional therapies may be a particular risk. The novel non-laser source used, along with the topical application of photosensitizer, makes ALA-PDT a more easily accessible therapy. The protocols evaluated in this thesis also permit ALA-PDT to be a day/out-patient procedure.

The adequacy of treatment depth remains a major limitation with nodular BCC inappropriate for ALA-PDT. Until it is possible to predict those thicker lesions which will respond to PDT, its practical role for such tumours, and particularly potentially metastatic cancers, such as squamous cell carcinoma, must be restricted to carefully monitored studies. New photosensitizers and refinement of current protocols may permit this development. A proportion of lesions are likely to remain resistant to PDT either through genetic or environmental factors.

This thesis confirms, in large open studies and where possible, in randomized comparison studies, certain applications for ALA-PDT delivered by a non-laser source. Modifications to photosensitizer application times and bandwidth/wavelength used should optimize treatment. An interim assessment of ALA-PDT in CTCL also suggests that this may be a useful additional therapy.

7.14 - Principal observations

The principal observations derived from the studies in this thesis are:

1. A novel non-laser lamp is confirmed as an effective and practical light source for ALA-PDT.

2. Topical ALA-PDT is at least as effective as cryotherapy in Bowen's disease with fewer adverse reactions.

3. Topical ALA-PDT is most effective for the treatment of BCC up to 1mm in depth and efficacy for thicker lesions may be increased by prolonging photosensitizer application from 4-6 hours.

4. ALA-PDT can clear large lesions of Bowen's disease and BCC. Lesion size influenced outcome for Bowen's disease, whilst tumour thickness was the predominant influence in BCC clearance.

5. 100-125J/cm^2 of red light (630±15nm) is more effective than lower doses at clearing Bowen's disease and minimising recurrence.

6. Red light (630±15nm) is more effective than green light (540±15nm) in the clearance of Bowen's disease.

7. Whilst there are no observed specific histological changes of ALA-PDT, both necrosis and apoptosis occur. Microscopic scar formation was observed in a few basal cell carcinomas, but rarely occurred after PDT to other lesions.

8. Evidence to support the existence of a photoproduct of PPIX, activated by 670nm light, is complemented by a preliminary study that suggests that this may be a therapeutically useful agent.

9. Topical ALA-PDT can clear patch, plaque, and tumour stage CTCL lesions, although an overall response rate of 45% requires its comparison with existing modalities to determine the place of PDT in CTCL management.

10. ALA-PDT is a well tolerated non-invasive therapy. Anaesthesia was usually required only for large lesions or for prolonged photosensitizer application. A cosmetic outcome acceptable to patients was universal with only minimal scar formation clinically apparent.

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Appendix 1: Visual Analogue Score Sheet

Patient Assessment of Treatment No.___ Name_____

We would be grateful for your time in completing the questions below to help us assess whether or not you experienced discomfort following your treatment today in the Dermatology Department.

Please place a cross on each of the scale barrs below to reflect the SEVERITY of pain experienced following treatment (0 indicates no pain, 10 indicates very severe pain).

PAIN SEVERITY SCALE

0_____10

First 24 Hours:
0_____10

Day 2 after treatment:
0_____10

Day 3 after treatment:
0_____10

Day 4 after treatment:
0_____10

Day 5 after treatment:
0_____10

Day 6 after treatment:
0_____10

Day 7 after treatment:
0_____10

Day 8 after treatment:
0_____10

Day 9 after treatment:
0_____10

Day 10 after treatment:
0_____10

Please comment on days when blistering was also noted.

Thank you for your help in completing this assessment.

Appendix 2: Consent Form for Bowen's Disease/ Actinic Keratoses Study

Your Doctor will have told you that your recent skin biopsy shows that you have an early, mild form of skin cancer which is normally easily cured by existing methods such as surgery or freezing with liquid nitrogen. You are invited to participate in a study assessing the value of a new alternative treatment called photodynamic therapy (PDT). PDT involves the application of a cream to the skin cancer followed 4 hours later by application of a strong light from an appropriate source. The light may cause a slight stinging sensation after treatment and swelling may occur. Rarely, it may be followed by blistering and crusting.

While preliminary results suggest that PDT is effective, it is also possible that it may not work but your participation in the trial may be of benefit to future patients who may receive PDT. If there is evidence that PDT is not working, you will , of course, have further treatment with freezing or surgery which is very likely to be effective.

If you wish to take part in this trial, your general practitioner will be advised of this and the clinical management that you will undergo. If you do participate in the trial, you will be asked to keep a chart assessing any discomfort in the 10 days following the new treatment and follow-up would be required for at least 1 year. You may be asked to donate a small further sample of skin from the treated area so that the response to treatment can be assessed accurately. This would involve a small skin biopsy similar to the one you had to make the diagnosis. As you know this might be slightly uncomfortable for a few days afterwards.

If you do not wish to participate in the trial or wish to withdraw at any time after commencing the trial, your care will in no way be affected. If you are pregnant or likely to become so, you should not participate in this trial.

Consent:

I, _____ of _____

give my consent to the research procedures described above, the nature, purpose and possible consequences of which have been described to me by _____

Signed _____ Date:_____

Witness:_____

Appendix 3: Patient Information sheet - Bowen's Disease (Comparison Study)

Your Doctor will have told you that your recent skin biopsy shows that you have a skin condition called Bowens disease, in which a red/scaly patch develops on the skin which may become cancerous. You are invited to participate in a study assessing the value of a new alternative treatment called photodynamic therapy (PDT) comparing it to freezing with liquid nitrogen. If you participate, you will be randomised to receive either PDT or freezing. PDT involves the application of a cream to the skin lesion followed 4 hours later by application of a strong light from an appropriate source. The light may cause a slight stinging sensation after treatment and swelling may occur. Rarely, it may be followed by blistering and crusting. We expect PDT to cause less pain and discomfort than freezing.

While preliminary results suggest that PDT is effective, further trials are needed. Your participation in the trial may be of benefit to future patients who may receive PDT. If there is evidence that PDT is not working after 2 treatments, you will, of course, have further freezing treatment.

If you wish to take part in this trial, your general practitioner will be informed. If you do participate in the trial, you will be asked to keep a chart assessing any discomfort in the 10 days following the new treatment and follow-up would be required for at least 1 year. You may be asked for a small further sample of skin from the treated area so that the response to treatment can be assessed accurately. This would involve a small skin biopsy similar to the one you had to make the diagnosis.

Appendix 4: Comparison Study - Patient Consent Form

Your Doctor will have told you that your recent skin biopsy shows that you have an early, mild form of skin cancer called Bowen's disease which is normally easily cured by existing methods such as freezing or surgery. You are invited to participate in a study assessing the value of a new alternative treatment called photodynamic therapy (PDT) comparing it to comparing it to freezing with liquid nitrogen. If you participate, you will be randomised to receive either PDT or freezing. PDT involves the application of a cream to the skin cancer followed 4 hours later by application of a strong light from an appropriate source. The light may cause a slight stinging sensation after treatment and swelling may occur. Rarely, it may be followed by blistering and crusting.

While preliminary results suggest that PDT is effective, it is also possible that it may not work but your participation in the trial may be of benefit to future patients who may receive PDT. If there is evidence that PDT is not working after two treatments, you will , of course, have further treatment with cryotherapy or surgery, which is likely to be effective.

If you wish to take part in this trial, your general practitioner will be advised of this and the clinical management that you will undergo. If you do participate in the trial, you will be asked to keep a chart assessing any discomfort in the 10 days following the new treatment. Follow-up will be required for at least 1 year. This would also happen if you are allocated to cryotherapy or surgery. You may be asked to donate a small further sample of skin from the treated area so that the response to treatment can be assessed accurately. This would involve a small skin biopsy similar to the one you had to make the diagnosis. As you know this might be slightly uncomfortable for a few days afterwards.

If you do not wish to participate in the trial or wish to withdraw at any time after commencing the trial, your care will in no way be affected. If you are pregnant or likely to become so, you should not participate in this trial.

Consent:

I, _____ of _____

give my consent to the research procedures described above, the nature, purpose and possible consequences of which have been described to me by _____

Signed _____ Date:_____

Witness:_____

Appendix 5: Patient Information sheet for Basal Cell Carcinoma Studies

Your Doctor will have told you that your recent skin biopsy shows that you have a skin condition called a rodent ulcer or basal cell carcinoma, which is the most common form of skin cancer in the U.K. and is normally easily cured by existing methods such as surgery. You are invited to participate in a study assessing the value of a new alternative treatment called photodynamic therapy (PDT). If you participate, you will be randomised to receive either PDT or freezing. PDT involves the application of a cream to the skin lesion followed a few hours later by application of a strong light from an appropriate source. The light may cause a slight stinging sensation after treatment and swelling may occur. Rarely, it may be followed by blistering and crusting. We expect PDT to cause less pain and discomfort than freezing.

While preliminary results suggest that PDT is effective, further trials are needed. Your participation in the trial may be of benefit to future patients who may receive PDT. If there is evidence that PDT is not working after 2 treatments, you will, of course, have further freezing treatment.

If you wish to take part in this trial, your general practitioner will be informed. If you do participate in the trial, you will be asked to keep a chart assessing any discomfort in the 10 days following the new treatment and follow-up would be required for at least 1 year. You may be asked for a small further sample of skin from the treated area so that the response to treatment can be assessed accurately. This would involve a small skin biopsy similar to the one you had to make the diagnosis.

Appendix 6: Consent Form for Basal Cell Carcinoma Studies

Your Doctor will have told you that your recent skin biopsy shows that you have a common form of skin cancer, called basal cell carcinoma (or a rodent ulcer) which is normally easily cured by existing methods such as surgery. You are invited to participate in a study assessing the value of a new alternative treatment called photodynamic therapy (PDT). PDT involves the application of a cream to the skin cancer followed 4 or 6 hours later by application of a strong light from an appropriate source. The light may cause a slight stinging sensation after treatment and swelling may occur. Rarely, it may be followed by blistering and crusting.

While preliminary results suggest that PDT is effective, it is also possible that it may not work but your participation in the trial may be of benefit to future patients who may receive PDT. If there is evidence that PDT is not working, you will , of course, have further treatment with surgery which is very likely to be effective.

If you wish to take part in this trial, your general practitioner will be advised of this and the clinical management that you will undergo. If you do participate in the trial, you will be asked to keep a chart assessing any discomfort in the 10 days following the new treatment and follow-up would be required for at least 1 year. You may be asked to donate a small further sample of skin from the treated area so that the response to treatment can be assessed accurately. This would involve a small skin biopsy similar to the one you had to make the diagnosis. As you know this might be slightly uncomfortable for a few days afterwards.

If you do not wish to participate in the trial or wish to withdraw at any time after commencing the trial, your care will in no way be affected. If you are pregnant or likely to become so, you should not participate in this trial.

Consent:

I, _____ of _____

give my consent to the research procedures described above, the nature, purpose and possible consequences of which have been described to me by _____

Signed _____ Date:_____

Witness:_____