British Journal of Dermatology 2000; **143:** 767–772.

Comparison of red and green light in the treatment of Bowen's disease by photodynamic therapy

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Accepted for publication 19 April 2000

Summary

Background A variety of protocols exist for the treatment of Bowen's disease by photodynamic therapy (PDT) using topical 5-aminolaevulinic acid (5-ALA).
Objective To determine the optimal wavelength (red or green light) for this treatment.
Methods A randomized comparison study of ALA–PDT using red (630 ± 15 nm) or green (540 ± 15 nm) light in the treatment of Bowen's disease.
Results The initial clearance rate for lesions treated by red light was 94% (30 of 32) in comparison with 72% (21 of 29) for those lesions receiving green light ($P = 0{\cdot}002$). Over the following 12 months, there were two recurrences in the red light group and seven in the green light group reducing the clearance rates to 88% and 48%, respectively. The frequency and severity of pain experienced were similar between the two treatment groups. No hyperthermia, nor significant difference in lesional temperatures, was observed between the wavelengths studied.
Conclusion Green light is less effective than red light, at a theoretically equivalent dose, in the treatment of Bowen's disease by topical ALA–PDT.

Key words: 5-aminolaevulinic acid, Bowen's disease, non-laser light source, photodynamic therapy, randomized controlled trial

Topical photodynamic therapy (PDT) with 5-aminolaevulinic acid (5-ALA) is an effective therapy for potentially pre-cancerous lesions, actinic keratoses and Bowen's disease.[1–8] ALA–PDT is at least as effective as the commonly used modality of cryotherapy in Bowen's disease but with fewer adverse reactions.[9] A variety of wavelengths have been used to induce a photodynamic reaction from ALA-induced protoporphyrin IX (PpIX). Red light, from laser[3–5] or a narrow-band non-laser source,[8,9] is usually employed to optimize depth of light penetration in tissue. Certain broad-spectrum sources[1,2,6,7] have also been used and may offer the advantage of utilizing additional larger peaks in the PpIX absorption spectrum.[10] The relative efficacy of using the less penetrating green wavelengths instead of red light has not previously been evaluated in the treatment of Bowen's disease. If effective, the use of green light might reduce treatment-induced pain, as recently observed in a small trial of ALA–PDT for actinic keratoses.[11] We report a randomized comparison study of ALA–PDT in Bowen's disease treated by red or green light. We also assessed adverse treatment reactions along with the rate of decline of surface PpIX fluorescence, and monitored lesional temperatures during treatment.

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Patients, materials and methods

Ethical committee approval was obtained for the treatment of patients with Bowen's disease by ALA–PDT. Sequential patients with biopsy-proven disease and with individual lesions of ≤ 21 mm in diameter were invited to participate. Individual lesions were randomized (via the sealed envelope technique) to receive ALA–PDT either with green or red filtered light. No lesion had been previously treated.

The lamp used ('Paterson lamp'; Photo Therapeutics Ltd, U.K.) incorporates a 300-W xenon short arc plasma discharge.[12] The spectral output of the

lamp was adjusted to 540 ± 15 nm (green) or 630 ± 15 nm (red) using appropriate filters. A 25-mm collimating lens apparatus was attached to the 5-mm fibre guide. At a fluence rate of 86 mW cm^{-2}, lesions received 125 J cm^{-2} of red light or 62·5 J cm^{-2} of green light. This dose of green light was chosen to normalize the quantity of protoporphyrin IX produced because the quantum yield at 540 nm is approximately twice that at 630 nm.[10]

Topical 5-ALA in an oil-in-water emulsion, 20% w/w 5-ALA (Sigma, Poole, U.K.) in Unguentum Merck (E. Merck Ltd, West Drayton, U.K.), was applied to the lesions 4 h pre-illumination. Surface crusts were removed and the surface gently abraded before the application of 5-ALA. Approximately 50 mg cm^{-2} of cream was applied to cover the entire field of illumination, including a clinically disease-free margin of at least 4 mm. The cream was kept in place under an occlusive dressing (Tegaderm, 3M, Loughborough, U.K.) and screened from light. The patient was offered local anaesthetic (1% plain lidocaine by intradermal injection) during treatment.

The clinical response was determined after 2 months and a repeat treatment administered if necessary. Following clearance, all patients were reviewed at monthly intervals for 12 months to look for recurrence. Post-therapy punch biopsies were performed in lesions where doubt over clinical clearance or recurrence existed.

Surface fluorescence was detected with an ultraviolet lamp (UV56 lamp, UVP, San Gabriel, CA, U.S.A.) with peak emission at 365 nm. The presence of fluorescence was recorded before illumination of all treatment sites and on the completion of each treatment session. Fluorescence was estimated by the same investigator (CAM), who used an arbitrary scale: 0, not detectable; 1, just visible; 2, moderate; 3, strong; 4, very intense. Fluorescence decay at 10 J cm^{-2} intervals was also recorded in a random sample of 10 lesions in each treatment group.

Temperature was recorded during ALA–PDT by an infrared temperature probe attached to a digital multimeter (80T-IR probe and meter, Fluke, Bellevue, WA, U.S.A.) with recordings before, midway and during the last minute of illumination. Control recordings from the untreated side/limb were also noted. Patients scored pain during treatment on a 10-cm visual analogue scale (with subsequent interpretation of $0 < x \leq 3$ as mild, $3 < x \leq 7$ as moderate and $7 < x \leq 10$ as severe).

Statistics

Simple logistic regression was used to compare clearance. The explanatory variables were wavelength and lesion size. No significant interaction between wavelength 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 treatment groups was complicated by the observation that all lesions in the green group lost fluorescence by 30 J cm^{-2}. A Fisher's exact test was performed by grouping observations in the 40 and 50 J cm^{-2} categories and comparing these with those in the 30 J cm^{-2} category. A Mann–Whitney *U*-test was performed to compare the median values for the maximum temperatures and the median rise in temperature during ALA–PDT.

Results

A total of 61 patches of Bowen's disease in 16 patients received ALA–PDT and were followed up for 12 months. Although initially 70 lesions were

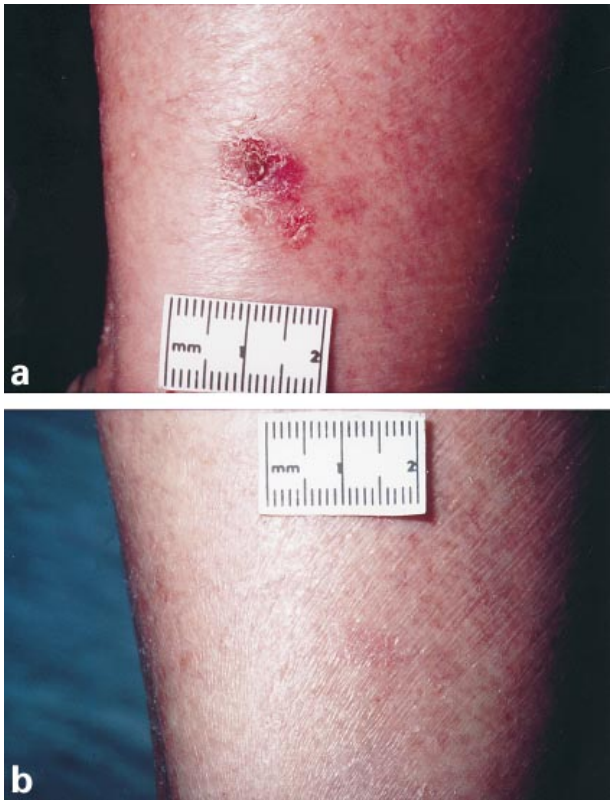

Figure 1. Bowen's disease: (a) 17 × 10 mm lesion on the left calf, before photodynamic therapy using red light. (b) The same area 6 weeks after photodynamic therapy.

randomized in this study, one patient (with three lesions) was lost to follow-up and two patients (with six lesions) died from chronic unrelated disease during the review period. The mean age of patients was 73 years (range: 50–87 years). All lesions were located on the lower limbs and the number of lesions per patient varied between 1 and 6 (median 3). Thirty-two lesions received ALA–PDT using red light and 29 lesions using green light.

In the red light group, 24 lesions cleared following one treatment and a further six after a repeat treatment, achieving an initial response of 94% (Fig. 1). Eighteen lesions treated using green light cleared after one treatment with a further three clearing on repeat ALA–PDT, giving an initial clearance rate of 72% (Fig. 2). The difference in response was significant ($P = 0{\cdot}002$); lesion size was not a factor in achieving clearance as there were similar lesion sizes in the red (median 125 mm^2, range 16–441 mm^2) and green groups (median 100 mm^2, range 25–400 mm^2).

A high recurrence rate was observed in the group treated by green light, with seven recurrences (after 4, 4, 4, 5, 6, 7 and 10 months), in comparison with only two lesions relapsing after ALA–PDT using red light (6, 7 months). Comparing red with green light, the odds ratio is estimated to be 0·13 (95% confidence interval 0·04–0·48) in favour of red light.

Treatments were well tolerated in both groups with no ulceration or infection complicating therapy and no photosensitivity reactions following ALA–PDT visits. No significant difference in pain was perceived between the groups with treatment using green light (no pain, nine lesions; mild, 10; moderate, seven; severe: three) comparable with the experience of the red light group (no pain, eight lesions; mild, 10; moderate, 12; severe: two). Two lesions treated by green light in one patient required anaesthesia while it was not required for any lesion treated by red light (including a lesion in the same patient who required anaesthetic for the green light treated sites). Twelve patients with multiple lesions treated by red or green light were able to compare pain directly between the different illumination methods, with four stating that green light was the most painful, six that red light was most painful while two patients could not distinguish between the light used. No clinically obvious scars were evident at 1-year review in either group.

Surface fluorescence was present (grade 3 or 4) before therapy in all lesions. Fluorescence decay during ALA–PDT using green light resulted in a reduction from very intense ($n = 4$) or strong ($n = 6$) before illumination, to moderate intensity by 10 J cm^{-2}, to just visible by 20 J cm^{-2} and to not detectable by 30 J cm^{-1} in all 10 lesions studied. Surface fluorescence during red light illumination

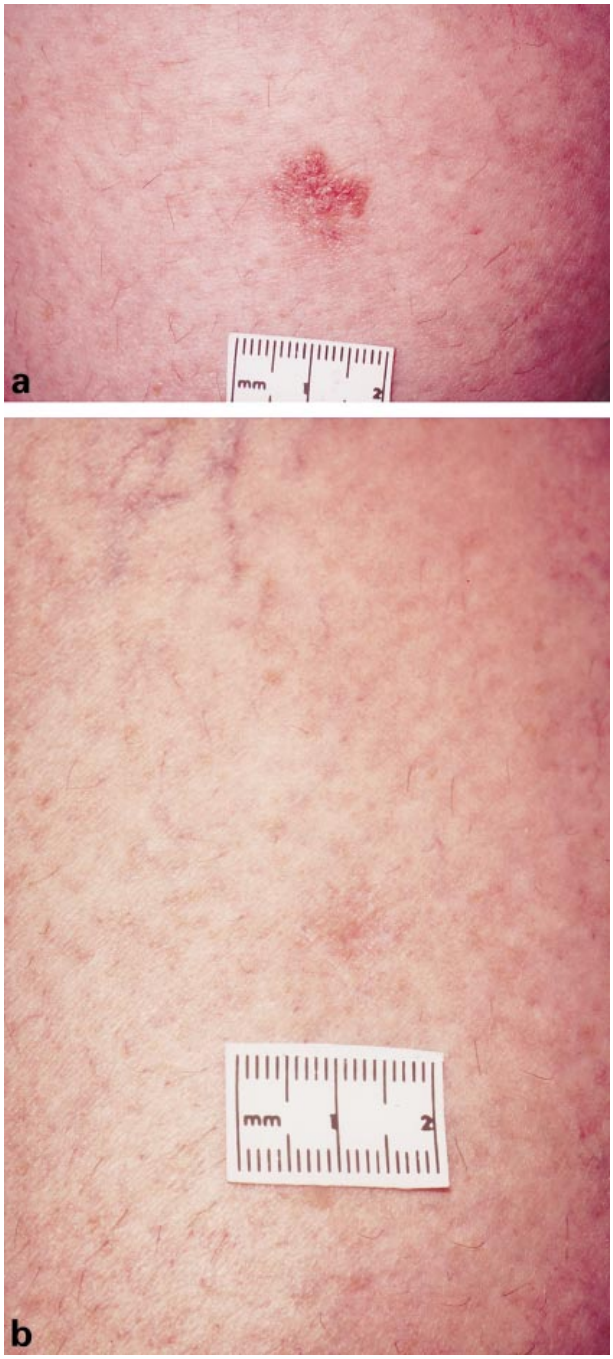

Figure 2. Bowen's disease: (a) 14 × 10 mm lesion on the right shin, before photodynamic therapy using green light. (b) The same area 6 weeks after photodynamic therapy.

reduced in intensity from very intense ($n = 7$) or strong ($n = 3$) before illumination, to strong ($n = 3$) or moderate ($n = 7$) by 10 J cm^{-2}, moderate ($n = 4$) or just visible ($n = 6$) by 20 J cm^{-2}, to just visible ($n = 6$) or not detectable ($n = 4$) by 30 J cm^{-2}, with loss of fluorescence in all by 40 J cm^{-2} in all 10 lesions studied. No surface fluorescence was evident on completion of ALA–PDT in all remaining lesions treated. Comparison of rate of loss of surface fluorescence with Bowen's lesions treated by red light suggests an earlier loss of fluorescence in green light treated lesions, although this did not reach significance for the number of lesions studied ($P = 0{\cdot}06$).

The temperature during ALA–PDT for all lesions (median, range) showed an increase of 3·1 °C (1·5–5·6 °C) for red light treatments with a maximum value of 34·3 °C (29·7–37·1 °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 °C (2·9–5·2 °C) with a maximum value also of 34·3 °C (30·4–35·9 °C). Neither maximum temperatures nor rise in temperature were statistically different between the red and green groups.

Discussion

ALA–PDT using green light is less effective in treating Bowen's disease than ALA–PDT using red light; the latter demonstrates an efficacy comparable with previous studies (complete clearance rates at 1 year: 90–100%).[8,9] This difference is presumed to be due to the reduced depth of tissue penetration by green light as the total light dose was calculated to provide theoretically equivalent yields of photosensitizer in each group.

5-ALA-induced photosensitivity has a porphyrin-like spectrum with maximum excitation at 410 nm and four smaller peaks at 510, 540, 580 and 630 nm.[13] Calculation of the equivalent light dose was based on comparison of the quantum yield of PpIX, which shows absorption at the 540 nm peak approximately twice that at 630 nm.[10] This is supported by the estimation, using a recently described formula, of total effective fluence at a given tissue depth, depending on the wavelength used, and incorporates measurements of incident spectral irradiance, optical transmission and absorption by the photosensitizer.[14] This indicates a threefold higher effective fluence rate for green light at 0·05 mm depth (57·0 W m^{-2}) than for red light (18·3 W m^{-2}), but with similar values at 2 mm (3·45 W m^{-2} and 2·69 W m^{-2}, respectively). As the epidermis is typically 0·1–0·15 mm thick, 62·5 J cm^{-2} of green-filtered light was probably a relatively higher dose than 125 J m^{-2} of red-filtered light for the epidermal lesions treated. The complex nature of tissue optical properties *in vivo*, particularly in the presence of a photosensitizer, makes accurate derivation of the relative total effective light doses difficult. However, our calculations were made in the context of an otherwise identical protocol with probably fewer structural differences between lesions of Bowen's disease than in other tumours.

Although UV-lamp estimation of fluorescence was subjective, comparison of the same investigator's observations demonstrated a more rapid loss in green light-mediated ALA–PDT consistent with accelerated photobleaching of PpIX, with all lesions treated using green light bleached by 30 J cm^{-2} compared with 40 J m^{-2} for lesions receiving therapy with red light. The observations also suggest adequate dosimetry in each group as it has been estimated that only 10% of active photosensitizer remains after an optical dose twice as large as the bleaching fluence.[15]

The reduced efficacy of green light in clinical study is thus likely to have been influenced more by light distribution than incident dose. A model of optical distribution in Caucasian skin indicates that 635 nm light peaks in the upper dermis (due to the addition of scattered light to the incident fluence) in comparison with 514 nm light that peaks in the stratum corneum.[15] Fritsch *et al.*[11] recently reported a half-side comparison study of ALA–PDT for extensive facial actinic keratoses using either red or green light. In the six patients studied, green light appeared to be as effective as red in clearing all visible lesions. The difference in outcome between the studies is likely to reflect the pathological differences between actinic keratoses, characteristically a focal disease sparing adnexal structures, and Bowen's disease, which commonly involves the appendages. Thus, sleeves of dysplastic cells growing down appendage ducts, which can extend to 3 mm in depth, may survive ALA–PDT that uses less penetrating wavelengths. This hypothesis is supported by the higher recurrence rate of lesions treated by green light.

It is anticipated that shorter wavelength light would also be inferior for the treatment of basal cell carcinomas by ALA–PDT. Tumour depth influenced clearance in a previous study we conducted (using red

light) where all lesions < 1 mm in depth cleared while thicker lesions demonstrated a progressively poorer response.[16]

As hyperthermic tissue injury can contribute to therapeutic effect in Bowen's disease,[17] we recorded temperature change during therapy with an average increment of 3–4 °C, with no hyperthermia and no significant differences between red or green light mediated therapy.

The ability of green light to clear 48% of lesions of Bowen's disease, although inferior to that of red light, suggests that combining these wavelengths may permit the maintenance or improvement of the already high clearance rates reported for red light, while shortening treatment times. One would anticipate that green light acts superficially, while proportionally more red light could react with PpIX in the deeper tissues. Our group is currently studying the use of an appropriate filter in the xenon short arc light source described.

Studies using broadband sources for ALA–PDT have reported success, although follow-up intervals were short (3–7 months) and the most relevant bandwidths emitted from these sources are not known.[1,2,6,7] Initial studies of ALA–PDT used modified slide projectors, with light filtered out below 600 nm, leaving relatively inefficient sources of light.[1,2,6] Clearance rates of 90–100% for actinic keratoses/Bowen's disease were achieved by Kennedy and Pottier,[1] although irradiances of 150–300 mW cm^{-2} suggest that hyperthermic tissue injury may have contributed. Wolf *et al.*[2] also used 5-ALA, a slide projector, and filtered out wavelengths less than 570 nm. At irradiances of 50–100 mW cm^{-2}, all nine actinic keratoses and five of six early invasive squamous cell carcinomas cleared. Sziemes *et al.*[7] used a 1200-W metal halogen lamp source, emitting 570–750 nm light at 160 mW cm^{-2}. They cleared only 12 of 17 facial actinic keratoses and 0 of 19 lesions on the upper limbs. Recently, Varma *et al.*[18] used the same light source to treat 50 lesions of Bowen's disease with initial clearance of 90%, but with 14 recurrences during 12 months of follow-up.

Fijan *et al.*,[6] while including green light from an incandescent source (540–720 nm, 300 J cm^{-2}, 150–250 mW cm^{-2}), used desferrioxamine and applied 5-ALA 20 h prior to illumination. Repeated treatments cleared only five of 10 sites of Bowen's disease with two recurrences. With several differences in protocol, it is difficult to interpret the influence of the bandwidth in the surprisingly poor efficacy of ALA–PDT in this study.

Cairnduff *et al.*,[3] Calzavara-Pinton[4] and Svanberg *et al.*[5] all treated Bowen's disease with ALA–PDT, using 630 nm laser light. Dosimetry differed, although irradiance was less than 150 mW cm^{-2} in each study. High clearance rates on clinical examination were generally observed (97%, 100%, and 90%, respectively) and follow-up periods of 6–29 months indicated a recurrence rate of up to 10%. Successful clearance of Bowen's disease would thus appear to benefit from targeting the 630 nm PpIX absorption peak. Although the inclusion of longer wavelength light may activate PpIX photoproducts (650 nm and 670 nm), their clinical importance remains to be established.[19] Our current study confirms that narrow-band red light from a non-laser source is as effective as monochromatic 630 nm laser light.

The majority of patients presenting with Bowen's disease are elderly, with lesions often on the lower leg, a poor site for healing. This study confirmed the absence of ulceration and secondary infection following ALA–PDT, with reduced morbidity in comparison with the standard therapy.[9] Pain during ALA–PDT may prove sufficiently severe as to require local anaesthesia.[3,5,6,8,16] The treatment of actinic keratoses by green light was less painful than red light-mediated therapy over large areas of forehead in the study of six patients by Fritsch *et al.*[11] We did not observe any significant difference in pain experienced—a trend towards red light being more painful was noted, although the only anaesthesia required was for two lesions treated by green light. In addition to the effects of larger study size, the similarity in severity of pain that we report may reflect differences in the diseases treated. Compared with the smaller foci of actinic keratoses, a relatively greater area of dysplastic tissue may permit a higher intensity of photodynamic activity with green light in Bowen's disease, albeit at a more superficial level than red, cancelling the anticipated reduction in pain with green light mediated therapy.

Acknowledgments

The development of the lamp was supported by the Cancer Research Campaign (U.K.). The authors acknowledge the statistical advice provided by Mr J.H.McColl, Senior Lecturer, Department of Statistics, University of Glasgow, U.K.

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