

Improved Response of Plaque Psoriasis after Multiple Treatments with Topical 5-Aminolaevulinic Acid Photodynamic Therapy

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We investigated the clinical response of 10 patients with plaque psoriasis to multiple treatments with photodynamic therapy, using topical application of 5-aminolaevulinic acid followed by exposure to broad-band visible radiation. Treatment was performed up to 3 times per week, with a maximum of 12 treatments, using a light dose of 8 Jcm^{-2} delivered at a dose-rate of 15 mWcm^{-2} . Eight patients showed a clinical response. Out of 19 treated sites, 4 cleared, 10 responded but did not clear and 5 showed no improvement. Of the 4 sites that cleared only 1 did so fully, after 7 treatments, 45 days after the start of therapy. Of the 10 sites that responded partially, the greatest reduction in scale, erythema and induration index occurred after a minimum of 3 and a maximum of 8 treatments. The intensity of 5-aminolaevulinic acid-induced protoporphyrin IX fluorescence, recorded prior to the first treatment, varied between sites on the same patient as well as between patients. There was also a variation in fluorescence intensity recorded from the same site immediately prior to subsequent treatments, although the pre-treatment levels generally decreased as the study progressed and then increased as psoriasis relapsed. Biopsies confirmed that fluorescence was localized throughout the epidermis and stratum corneum, but the level was not consistent between sections taken within the same biopsy. We also observed fluorescence at sites distant from the ones that received 5-aminolaevulinic acid, which was not present prior to the start of the treatment programme, but found no evidence of elevated levels of plasma porphyrins. The level of discomfort associated with this therapy increased with increasing values of the calculated photodynamic dose, defined as the product of the initial photosensitizer concentration and the percentage reduction in fluorescence following irradiation. Therefore, although clinical efficacy improved with multiple treatments, unpredictable response and patient discomfort make ALA-PDT unsuitable for the treatment of psoriasis. **Key words:** *protoporphyrin IX; pain; dose.*

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Photodynamic therapy (PDT) using topical application of the photosensitizer precursor 5-aminolaevulinic acid (ALA) has been widely reported for the treatment of non-melanoma skin malignancies (1–5). The accumulation of ALA-induced PpIX in plaque psoriasis was first reported by Kennedy & Pottier (6) and later confirmed by Boehncke et al. (7). The latter group has subsequently reported 2 studies in which patients (3

in each study) with chronic plaque stage psoriasis were treated using alternate-day or twice-weekly topical ALA-PDT. Both studies achieved an outcome comparable to that achieved using dithranol (8, 9). Our own preliminary work confirmed selective localization of PpIX to plaque psoriasis, but showed that photosensitizer levels varied considerably between plaques on the same patient as well as between different patients (10). We also found evidence for the prolonged accumulation of PpIX within plaques distant from the site of ALA application. Recently, Bissonette et al. (11) have reported the detection of a fluorescence component characteristic of PpIX in the autofluorescence spectrum of psoriasis plaques, although the source of the porphyrin was unknown. Microscopic analysis indicated that PpIX was restricted to the stratum corneum.

In our previous therapeutic study (12) we established clinically effective values of light dose (Jcm^{-2}) and dose-rate (mWcm^{-2}) for the treatment of plaque psoriasis by ALA-PDT, although the response to a single treatment was variable. Of a total of 80 test sites, on 20 patients, 14 (17.5%) cleared, 6 (7.5%) showed a partial response and 60 (75%) showed little or no improvement. Defining the photodynamic dose as the product of time-dependant PpIX concentration and light dose, we showed that only for those plaques that cleared was there a relationship between photodynamic dose and clinical response. The aim of this second study was to investigate the efficacy of multiple treatments of ALA-PDT for psoriasis.

MATERIALS AND METHODS

Patients

This study was approved by the Ethics Committee of the hospital. Ten patients with chronic plaque psoriasis were recruited and each gave written informed consent. Three patients were taking systemic therapy (2 acitretin, 1 cyclosporine), which was continued at the same dose throughout the study. Topical therapy (tar, calcipotriol and steroids) was discontinued 2 weeks before PDT, but emollient (aqueous cream) was allowed. Patients were asked to attend up to 3 times a week, if possible, for the supervised application of ALA.

Treatment

Nine patients had 2 sites of 20 cm^{-2} circular area within regions of plaque defined for treatment, and 1 patient 1 site only (19 sites in total). Each patient also had a control site defined that remained untreated. On the first day (day 1) of the treatment programme ALA (Department of Colour Chemistry, University of Leeds) dissolved (20% by weight) in a proprietary oil-in-water emulsion (Unguentum Merck, Whitehall Laboratories Ltd, UK) was applied to the treatment sites in a quantity of approximately 25 mgcm^{-2} . Approximately 4 h after ALA application, each site received a single exposure to broad-band visible radiation from a modified slide projector (12), at a fixed dose-rate of 15 mWcm^{-2} . Considering the severe acute

responses we observed in our previous study (12), the first 3 patients in the study received light doses of 2 and 4 Jcm⁻², all subsequent patients were prescribed a light dose of 8 Jcm⁻². Local analgesia was not used during treatment.

Patients returned for repeat treatments at the same sites, for between 7 and 12 treatments delivered at a frequency that varied from 1 to 3 times per week. In 7 patients, for ensuing treatments, ALA was applied (2 under supervision and 5 unsupervised) between 3 and 5 h prior to light exposure. In the other 3 patients ALA was applied immediately after a session of PDT, with no further application until after the next light exposure.

Plaques were located on the upper limb ($n=11$), lower limb ($n=5$) and the trunk ($n=3$). Psoriasis at each site was scored separately for scale, erythema and induration over a range of 0–3 (0=negligible, 1=mild, 2=moderate, 3=severe) at each visit. The total score per visit, for each test site, was recorded before treatment as the scale, erythema and induration (SEI) index. Patients were treated until they were clear or they failed to improve after 3 consecutive treatments, or if they wished to discontinue treatment. Clearing was defined as the treated area being flat and free of scale. The acute response to treatment was graded for erythema, the presence of erosions and change in scaling. Pigmentation was also noted. Patients were asked to describe any sensation during treatment and to estimate its persistence. Pain was assessed during therapy using a visual analogue scale (VAS) over the range 0–10 (0=no pain, 10=unbearable pain). No special measures were used to protect sites after treatment, although patients were advised not to sunbathe or use a sun-bed.

PpIX photo-oxidation and the photodynamic dose

The magnitude of the 635 nm fluorescence emission peak, induced by 488 nm excitation, was used as an indicator of the PpIX concentration at defined points within each plaque. Measurements were made at each visit, before and after PDT, by a method described previously (10). In the same way, the 674 nm peak was used as an indicator of the concentration of PpIX photoproducts and/or protoporphyrin aggregates (13).

In our previous report we calculated the parameter of photodynamic dose by integrating the fluorescence signal recorded during therapy, with respect to light dose. However, as a consequence of a systematic study of PpIX photobleaching in mice (14) we have modified our analysis. Here we define the photodynamic dose as the product of the initial photosensitizer concentration and the percentage reduction in PpIX fluorescence following irradiation (percentage of photobleaching). Therefore, the generation of the dose parameter is not dependant on the delivered light dose, and is based on the assumption that the reduction in PpIX concentration is directly proportional to the concentration of oxidizing species (singlet oxygen) produced during PDT.

Biopsy and blood plasma analysis

Punch biopsies were taken 4 h after the application of ALA, from the centre of plaques on 5 separate patients who were not subsequently irradiated with light and were therefore not included in this therapeutic study. The biopsies were immediately frozen and stored under liquid nitrogen. Frozen sections of 10 µm thickness were cut using a microtome and the distribution of PpIX was investigated using ultra-low light level fluorescence microscopy (15) utilizing 632.8 nm excitation. At least 10 sections were cut from each biopsy, with a minimum of 100 µm between each section.

During the course of the treatment programme, we recorded PpIX fluorescence from plaques distant from the site of ALA application in 2 patients. Blood samples were taken and porphyrins extracted from the plasma by the method of Longas & Poh-Fitzpatrick (16). Porphyrin concentrations were determined by comparative fluorescence analysis against known standards.

RESULTS

Clinical response

One patient was withdrawn from the study, on unrelated clinical grounds, after 6 treatments. Plaques on this patient showed no improvement, but the data obtained is included here.

Eight patients (80%) responded to topical ALA-PDT. Of 19 treated sites, 4 showed clearing of psoriasis, 1 site (Fig. 1a) cleared completely after 7 once-weekly treatments, 45 days after the onset of the treatment programme and remained clear 4.5 months after the end of the treatment programme (Fig. 1b), despite a general flare of psoriasis. In each of the remaining sites that demonstrated clearance a number of small areas of plaque remained within the treatment field that were surrounded by areas of clearance. Of the remaining 15 sites, 5 showed a reduction in SEI index of greater than 50%, 5 showed a 30–50% reduction in SEI and the remaining 5 showed little or no improvement. Of the 10 sites that responded but did not clear, the greatest reduction in SEI index occurred after a minimum of 3 and a maximum of 8 treatments (median=5). Of these, 3 sites were controlled but did not improve further, whereas 7 sites relapsed between 5 and 20 days after the start of therapy (median=14), with plaque creeping in from the edge. There was no difference in response to ALA-PDT between those sites managed by

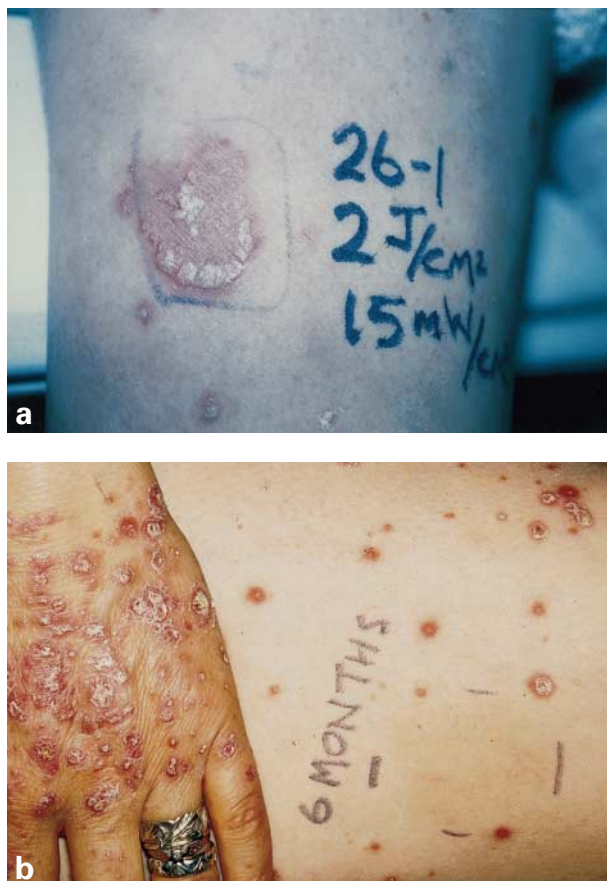


Fig. 1. (a) Psoriasis plaque on the left thigh prior to ALA-PDT before treatment. (b) Treated area 4.5 months after the end of treatment; psoriasis had not recurred within the treated site, which is hyperpigmented, despite a generalized recurrence.

systemic therapy and those on topical therapy before this study.

The acute response to treatment was variable, but the most marked erythema was usually obvious at the second treatment. Scale was the first parameter to improve. Two patients developed erosions. In 5 patients the treated site developed an orange/brown pigmentation during the course of the programme that cleared when therapy was stopped. A persistent light brown pigmentation was still evident 4.5 months later at the treatment site of the plaque that cleared (Fig. 1b).

Pain

Eight patients (80%) reported a sensation during irradiation that ranged in description from tingling through to stinging and burning and which lasted, in most cases, for several hours after therapy. The severity of discomfort, as defined by the VAS value, increased with increasing magnitude of calculated photodynamic dose. Pain was more marked during the first and second treatments, but returned during subsequent treatments as psoriasis relapsed. Five patients (50%) reported a VAS of greater than 5, which persisted for between 6 and 48 h. Three patients (30%) experienced very severe pain during treatment (VAS=9 or 10) and of these, 2 declined the next scheduled treatment, and 1 did not continue the study. All 3

reported tenderness of treated plaques between treatments. There was no significant difference between the level of pain experienced by patients who received ALA 4 h prior to each treatment and those who received ALA immediately after therapy in preparation for the next treatment.

PpIX photo-oxidation and photodynamic dose

The magnitude of the PpIX fluorescence signal (at 635 nm) recorded prior to the first treatment varied by as much as a factor of 3 between sites upon the same patient and by a factor of 5 among all 19 sites. There was also a wide variation in pre-treatment PpIX fluorescence intensity recorded from the same site at subsequent treatments. However, we noted that the pre-irradiation PpIX levels decreased as the treatment programme progressed and then increased as psoriasis relapsed. Also, we observed a significant increase in fluorescence emission at 675 nm during the treatment programme.

From the second treatment onward the fraction of PpIX photobleaching differed from that observed in the first treatment (having received the same light dose) and from that observed in our previous (single treatment) study. Fig. 2 shows the variations during the treatment programme of SEI index, VAS of pain and photodynamic dose delivered to a single site, from 4 representative patients.

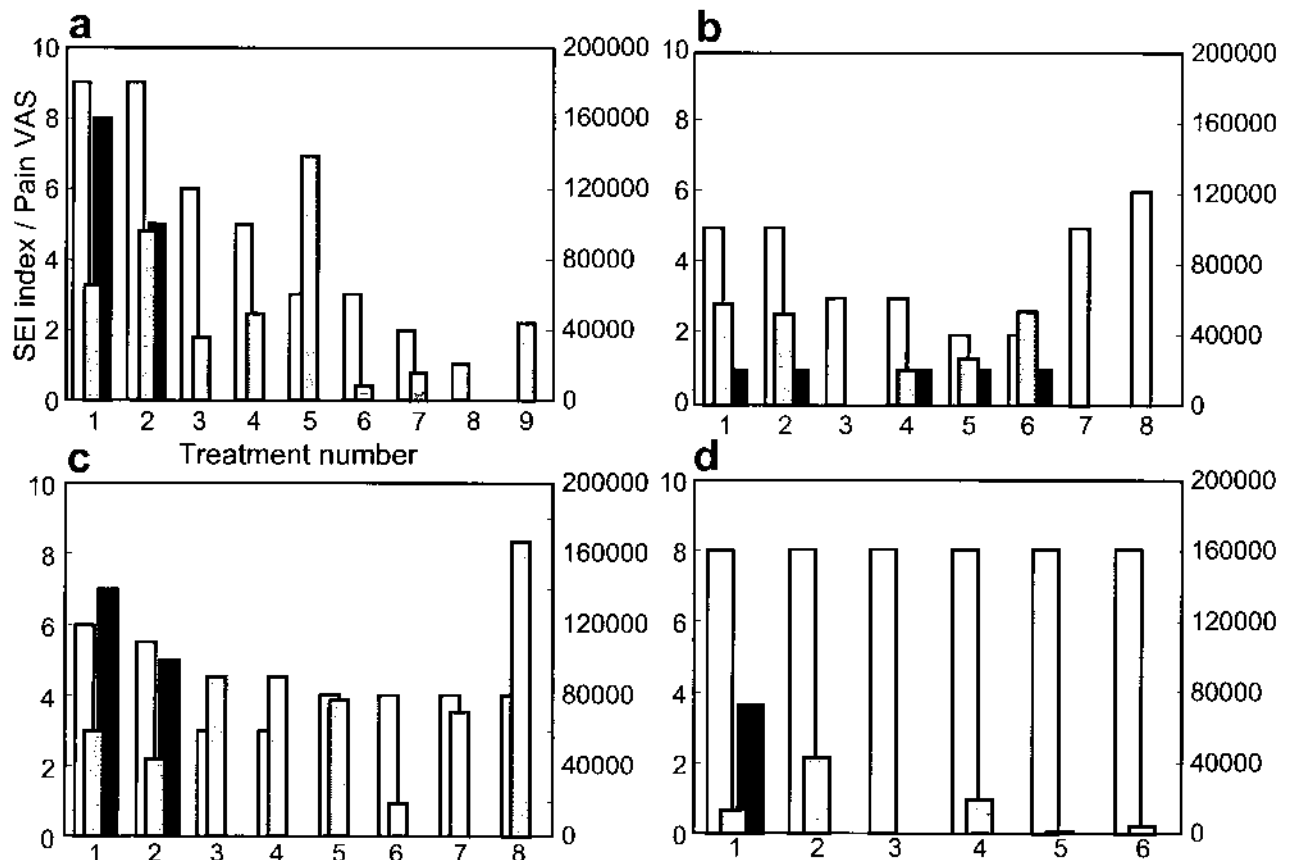


Fig. 2. Variations during the treatment programme of scale, erythema and induration (SEI) index (open bars), VAS of pain (black bars) and photodynamic dose (shaded bars, arbitrary units, right hand scale) delivered to a single site, from 4 representative patients. Site (a) cleared and had not relapsed 4.5 months later, site (b) improved and then relapsed, site (c) improved and was controlled during the treatment programme, but relapsed in the days following the cessation of therapy. Site (d) did not respond.

Biopsy and blood plasma analysis

Figures 3a–b show the distribution of PpIX fluorescence within biopsy sections taken from 2 psoriasis plaques, 4 h after the application of ALA. In 1 (Fig. 3a) PpIX is localized throughout the epidermis with the acanthotic epidermis and the stratum corneum (scale) showing strong fluorescence. In the other, however, (Fig. 3b) PpIX fluorescence is confined to the stratum corneum. Also, the level of PpIX fluorescence was not consistent throughout the set of sections taken from each biopsy, with some sections displaying a much lower PpIX fluorescence intensity.

There was no evidence of elevated porphyrin levels in the plasma of the 2 patients who showed PpIX fluorescence at sites distant from that of ALA application.

DISCUSSION

Multiple treatments with topical ALA-PDT improved the response of plaque psoriasis compared with that previously observed following a single treatment (12). However, the clinical response to multiple treatment ALA-PDT was variable. The site of plaque and the frequency of treatment demonstrated no significance within this small group of patients. Neither was there a clear correlation between clinical response and the delivered photodynamic dose. This suggests that in some patients cytotoxic species may be generated at

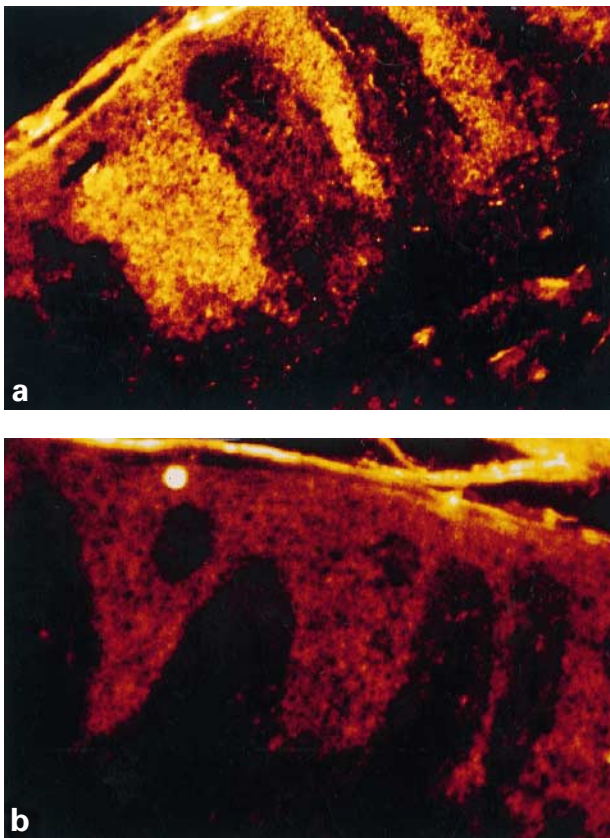


Fig. 3. Microscopic distribution of PpIX fluorescence within 2 psoriasis plaques, 4 h after application of ALA; (a) PpIX is localized throughout the epidermis, the acanthotic epidermis and the stratum corneum (scale) show strong fluorescence, (b) PpIX is confined to the stratum corneum.

tissue sites, or within cells, where they have little or no influence on the pathogenesis of psoriasis.

While the primary intracellular site of ALA-induced PpIX localization and phototoxicity is within the cellular mitochondria, the distribution of PpIX is dependant on the incubation time, and at long time intervals (> 5 h) PpIX is also present in the cytoplasm and the plasma membrane. Sustained ALA incubation or the application of high ALA concentrations has been shown to lead to the formation of aggregated species (17) that are less efficient at producing cytotoxic species during irradiation. We note that our treatment regime involves exposure of the plaque to relatively large quantities of ALA over an extended period of time and that PpIX fluorescence was still present in plaques prior to the application of further ALA. The variation in the distribution of PpIX localization and the aggregation state of the sensitizer during the treatment programme must therefore be considered. It is interesting to note that from the second treatment onwards we observed an increase in fluorescence emission centred around 675 nm as well as a reduction in the extent of PpIX photobleaching during each irradiation. We suggest that this may be due to the formation of aggregated PpIX.

The distribution of PpIX within plaque psoriasis after the superficial application of ALA is determined by the rate of diffusion of ALA through the plaque, the rate of synthesis of PpIX within the plaque and the rate at which it is cleared. Figs. 3a and b show the distribution of PpIX within a section of plaque, 4 h after the application of ALA. PpIX is restricted to the stratum corneum and epidermis, involving the basal layers and the pilosebaceous units. The level of PpIX fluorescence within the dermis is much lower. In terms of the response of proliferating keratinocytes to irradiation at this time point, this seems encouraging. However, in terms of targeting T-cells (concentrated within the dermis) this is somewhat disappointing. Also, we have observed a marked variation in the level of epidermal PpIX fluorescence between sections from the same biopsy sample, as well as between biopsies from different patients. While our measurements of initial PpIX surface fluorescence could be correlated with the trend in observed fluorescence signal from corresponding biopsy samples, we were unable to resolve any of the macroscopic variations in PpIX fluorescence implied by the results obtained from analysis of the biopsy samples. It is possible that either the inhomogeneous distribution of PpIX within the epidermis and/or the lower concentration of PpIX in the dermis may explain the variation in clinical response observed. The variance of our experience to that of Boehncke et al. (8), who cleared 3 patients with multiple treatments of ALA-PDT, is difficult to explain; we have, however, been able to reproduce the variable efficacy of ALA-PDT for psoriasis, both for single and multiple treatments.

Following topical ALA, PpIX is removed from most normal tissues within 24 h (1). We have, however, confirmed our previous findings (10, 12) that PpIX fluorescence emission persists in plaque psoriasis for up to 1 week following the application of ALA. We have also observed PpIX at distant sites, that was not present prior to the start of the treatment programme. While we cannot rule out the possibility of surface re-distribution of ALA between measurements, and despite our failure to measure elevated levels of plasma porphyrins between treatments, this could indicate a systemic

effect. It is possible that over long periods of ALA incubation lipophilic PpIX is concentrated within the scale associated with psoriasis and not metabolized as normal.

While the clinical results from this study may appear promising, the pain experienced by patients during treatment, which often persisted until the following treatment session, presents an obstacle to acceptability. A number of authors have previously reported patient discomfort during topical ALA-PDT (18, 19), a phenomenon that appears specific to PpIX photosensitization. In this study some sensation during treatment was reported by 80% of the patients, and 30% of patients classified this as very severe pain. Furthermore, 50% experienced discomfort during and between treatments. Two patients declined 1 or more individual treatments. All patients who reported pain during treatment stated that they would be unwilling to undergo treatment of larger areas of disease. We therefore conclude that the variability of therapeutic effect and the associated discomfort makes ALA-PDT, using current treatment methods, unsuitable for the treatment of extensive psoriasis. A number of other centres are currently investigating the use of different photosensitizers (9, 20) for topical PDT.

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