

A placebo-controlled randomized study on the clinical effectiveness, immunohistochemical changes and protoporphyrin IX accumulation in fractionated 5-aminolaevulinic acid-photodynamic therapy in patients with psoriasis

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Summary

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Key words

5-aminolaevulinic acid, fluorescence diagnosis, photodynamic therapy, protoporphyrin IX, psoriasis

Conflicts of interest

None declared.

Background Topical 5-aminolaevulinic acid (ALA)-photodynamic therapy (PDT) for the treatment of psoriasis has been evaluated in a few studies. In these studies different treatment parameters were used, there was a variable clinical response, and a nonhomogeneous fluorescence was seen after irradiation with Wood's light.

Objectives To study the clinical effectiveness, immunohistochemical changes and protoporphyrin IX accumulation in ALA-PDT in patients with psoriasis.

Methods Eight patients with stable plaque psoriasis with symmetrical involvement were included in the study. Two symmetrical plaques were randomly allocated to PDT either with 10% ALA or with placebo. Irradiation consisted of 2 and 8 J cm⁻² with a dark interval of 2 h (Waldmann PDT 1200 L, 600–750 nm, 40 mW cm⁻²) once weekly for 4 weeks. Before, during and after irradiation, fluorescence diagnosis was performed. Biopsies were taken at baseline, week 1 and week 6 for immunohistochemical assessment. Psoriatic plaques were clinically assessed using the plaque severity (sum) score. Fluorescence diagnosis was performed and expression of immunohistochemical markers for proliferation, differentiation and T-cell infiltration [Ki67, keratin 10 (K10), CD4, CD8 and CD45RO] was assessed.

Results From week 1 up to week 6, ALA-PDT gave a significant reduction in the number of Ki67+ nuclei, while the K10 expression increased. After 6 weeks significant improvement was observed for CD8 and CD45RO. These changes were absent in the placebo-treated lesions. The sum scores were also significantly lower in the ALA-treated plaques. Heterogeneity of macroscopic fluorescence was observed during treatment despite keratolytic treatment.

Conclusions The present study shows that clinical improvement during fractionated ALA-PDT in psoriasis parallels histological improvement as seen in normalization of epidermal proliferation, differentiation and infiltration of relevant T-cell subsets. Optimizing the current treatment protocol may increase clinical efficacy further.

Over the last few years photodynamic therapy (PDT) with topical 5-aminolaevulinic acid (ALA) has been gaining interest in more and more dermatological centres and its clinical efficacy has convinced many dermatologists to implement this therapy in their daily practice. Although more randomized controlled studies are still needed and 5-year follow-up data are mostly lacking, PDT has been proven to be clinically effective in nonmelanoma skin cancer including actinic kerato-

sis, Bowen's disease and (superficial) basal cell carcinoma. As protoporphyrin IX (PpIX)-accumulating skin disorders in general may be a potential target for PDT, other (nononcological) indications have also been studied or are currently under evaluation. Psoriasis has been shown to accumulate PpIX selectively and its susceptibility to PDT has been previously studied by various authors.^{1–5} In these studies clinical efficacy varied largely but results are difficult to compare because of the

different treatment parameters that were used. Various mechanisms have been proposed for the clinical effect of ALA-PDT in psoriasis. Boehncke *et al.*⁵ reported topical ALA-PDT to have inhibitory effects on tumour necrosis factor- α , interleukin (IL)-1 and IL-6 secretion in a dose-dependent manner, which has also been reported in psoralen + ultraviolet A therapy, whereas Bissonette *et al.*⁶ found PDT with systemic ALA to induce apoptosis in CD3+ cells. Pain during irradiation is one of the main drawbacks in PDT, and this was the case in several reports on PDT of psoriasis. Considering the discomfort of this treatment and the variable clinical efficacy, several authors therefore concluded PDT to be unsuitable for the treatment of psoriasis.^{7,8} On the other hand, there is a permanent need for new treatment modalities in the treatment of psoriasis as topical therapies are not always successful and demand the patient's utmost compliance; moreover, cumulative toxicity and carcinogenicity of many systemic and photo(chemo)therapies are limitations in the long run. Although the long-term safety of PDT remains to be established, current data do not point in the direction of PDT having carcinogenic potential. Taking these factors into account, if efficacy could be increased by optimizing the treatment protocol, PDT of psoriasis would be of additional value in the current treatment arsenal.

While previous studies on PDT in psoriasis have focused mainly on the clinical efficacy, knowledge on the histological effects of PDT in psoriasis is crucial, better to understand the mechanism responsible for the clinical improvement in PDT of psoriasis. These data may also be used to optimize the treatment protocol further. Therefore, the aim of the present study was to evaluate the clinical and histological effects of PDT in psoriasis with respect to the histological hallmarks of psoriatic plaques, these being epidermal hyperproliferation, abnormal keratinization and T-cell infiltration. For this purpose, several immunohistochemical markers were chosen including Ki67 (epidermal proliferation), keratin 10 (K10; epidermal keratinization), CD4 (helper T cells), CD8 (cytotoxic T-cells) and CD45RO (memory T-cells). As light fractionation has been proposed as a possible way to improve clinical efficacy,⁹ a twofold fractionated protocol with a dark interval of 2 h was used. As lowering of the fluence rate has also been reported to enhance the clinical response,^{10,11} 40 mW cm⁻² was chosen as a standard fluence rate during the irradiation process. To study PpIX formation and photobleaching in the treated areas, which can also be informative in understanding the photodynamic effects in psoriasis, fluorescence intensity on the skin was assessed using a digital fluorescence imaging system.

Patients and methods

Patients

This study was approved by the local medical ethics committee. The recruitment period lasted from August until September 2004 and the treatment period from September until

December 2004. Eight patients selected out of our research database (age range 39–64 years; five men and three women) with stable plaque psoriasis with symmetrical involvement were included in this study after giving their written informed consent. All the lesions were located on the legs. Lesions were treated with 10% salicylic acid in petrolatum for 1 week prior to the baseline visit to remove excess scales that could potentially interfere with ALA and light penetration. Patients were not allowed to use any systemic psoriatic treatments or topical medication on these lesions for at least 4 weeks. Medication known potentially to aggravate psoriatic lesions, such as β -blockers, was also not allowed. Patients were requested to refrain from sun exposure during the treatment period.

Photodynamic therapy

At the baseline visit two contralateral psoriatic lesions were randomized by means of a special computer algorithm to either PDT with 10% ALA ointment (Medac GmbH, Wedel, Germany) or the vehicle (Medac GmbH). Before the ALA ointment or vehicle was applied lesions were photographed. Subsequently, according to the randomization outcome, ALA ointment and vehicle were applied under an occlusive dressing (Tegaderm; 3M Pharmaceuticals, Zoeterwoude, the Netherlands) to enhance ALA penetration. The occlusive dressing was covered by aluminium foil to avoid premature photobleaching effects. After 4 h the ointments and foils were removed and the skin surface was cleaned using 80% denaturated alcohol v/v. Thereafter the first light fraction of 2 J cm⁻² was delivered [Waldmann PDT 1200 L (Waldmann Medische Technik BV, Tiel, the Netherlands), 600–750 nm, 40 mW cm⁻²]. Lesions were covered again with the occlusive dressing and aluminium foil for 2 h, after which a second light fraction of 8 J cm⁻² was delivered using the same irradiation parameters. The same irradiation protocol was applied once every week for 4 weeks.

To evaluate the clinical response, the sum score, a widely used method to measure the plaque severity, was assessed for both lesions at each visit. In this score erythema, induration and desquamation are each scored on a five-point scale as: 0, absent; 1, minimal (very light pink, hardly any elevation, rare scale); 2, mild (light red/pink, slight elevation, poorly defined scale); 3, moderate (red, moderate elevation, defined scales); or 4, severe (very red, marked ridge, heavy scaling). Finally, a global sum score (range 0–12) was defined as the sum of all three scores together, reflecting plaque severity.

Biopsy procedure

At the baseline visit, week 1 and 2 weeks after the last treatment (week 6), 4-mm punch biopsies from the treated psoriatic lesions were taken under local anaesthesia with 1% xylocaine-adrenaline. Biopsies were embedded in Tissue-Tek OCT compound (Miles Scientific, Naperville, IL, U.S.A.) and directly frozen into liquid nitrogen. Biopsies were stored at – 80 °C until further processing.

Fluorescence diagnosis

Fluorescence intensity on the skin was recorded at every visit before and directly after each irradiation using a digital fluorescence imaging system (DyaDerm; Biocam GmbH, Regensburg, Germany). The DyaDerm digital imaging system consists of a flash light [xenon light source with a custom bandpass filter (370–440 nm)] and a 12-bit Sony CCD camera combined in one adjustable arm coupled to a Pentium IV computer equipped with custom-made image capturing software (Dyaderm Pro v. 1.4; Biocam GmbH). The flash light emits seven light pulses per second to the skin which are recorded by the CCD camera (exposure time 100 μ s) equipped with a special Schott GG 455 longpass filter to filter out the excitation light. As PpIX fluorescence emission consists of light in the red spectrum, the red pixels of the CCD camera were used to generate a fluorescence image. In this way a normal-coloured image and a fluorescence image were processed in real time. Because of the short exposure time to the excitation light, photobleaching of PpIX was minimized. To correct for different lighting environments between pictures, a fluorescence reference standard (Maccal 8044, 738-00; Multifoil, Utrecht, the Netherlands) was included on every image. Images were recorded in 16-bit grey-scale TIFF format.

Analysis of fluorescence images

Sixteen-bit grey-scale TIFF fluorescence images were imported in NIH ImageJ software (<http://rsb.info.nih.gov/ij/>). Because the xenon light source used for excitation has the highest intensity in the centre of the illuminated area, shading correction was performed by means of the following algorithm: $S = I/C$, where $C = B/B_{\text{max}}$; B = blank image (image from a white homogeneous background recorded with the Dyaderm system), B_{max} = highest intensity of B , I = (uncorrected) image, C = normalized shading image and S = shading corrected image. Photobleaching in psoriatic lesions was assessed by measuring the mean pixel intensity of highly fluorescing regions within a psoriatic plaque. Measurements were always performed in the same area within a plaque.

Immunohistochemistry

Frozen sections (7 μ m) were cut from the skin samples for immunohistochemical assessment. These were air-dried for 30 min and fixed in cold acetone for 10 min. After blocking for 5 min for endogenous peroxidase, using 0.2% sodium azide (Dako, Copenhagen, Denmark), sections were washed in phosphate-buffered saline (PBS; B. Braun, Melsungen, Germany) for 10 min before incubation with primary antibodies. The following primary antibodies (mouse antihuman) were used, diluted in 1% bovine serum albumin (Sigma, St Louis, MO, U.S.A.)/PBS: anti-CD4 (clone MT310; 1 : 200), anti-CD8 (clone DK25; 1 : 200), anti-CD45RO (clone UCHL1; 1 : 100), Ki67 (clone MIB-1; 1 : 200) (all from Dako) and K10 (clone RKSE60; 1 : 100) (Monosan Laboratories, Uden,

the Netherlands). Sections were washed in PBS for 15 min. Secondary IgG-labelled polymer, horseradish peroxidase anti-mouse EnVision+ (Dako) was added for 30 min. The sections were washed again for 15 min in PBS. To visualize the staining we used AEC+ High Sensitivity Substrate Chromogen for 10 min (Dako). Counterstaining was performed with Mayer's haematoxylin (Sigma). The sections were washed in tap water, dried, and mounted in glycerol gelatine (Sigma).

Furthermore, haematoxylin and eosin (H&E) staining was performed on every biopsy specimen. After dehydration in alcohol and Histosafe, the sections were mounted in Permount. With these H&E sections we verified that the biopsies under study fulfilled the psoriasis-specific histological criteria.

Immunohistochemical analysis

Analysis of digital microscopic images was done using IP-lab software (Scanalytics Inc., Fairfax, VA, U.S.A.). In order to analyse Ki67+ cells, digital photographs were made at $\times 100$ magnification covering the entire epidermis. A line following the basement membrane was drawn and its length was measured. All positive cells above this line were counted and divided by the length of the basement membrane so the number of positive cells per millimetre length of basement membrane could be calculated.

For quantification of K10+ cells, digital photographs were made at $\times 50$ magnification. The surface K10+ (epidermal) cells were measured and divided by the total epidermal surface. Thus epidermal differentiation was defined as percentage K10+ epidermal surface.

T-cell subsets (CD4, CD8 and CD45RO) were analysed by counting all positive cells in the epidermis and dermis separately. These numbers were divided by the length of the stratum corneum. In this way T-cell subsets were expressed as the number of positive cells per millimetre (epi)dermis. All slides were encoded to assure that the analysis was performed blindly.

Statistics

To compare mean sum scores, mean fluorescence intensity and expression of Ki67, K10, CD4, CD8 and CD45RO between the two treatment regimens a two-tailed Student's *t*-test was used. One-way analysis of variance was used to analyse differences between time points within each marker. When significant changes were found, Duncan's *post hoc* test was performed for analysis between time points. $P < 0.05$ was regarded as statistically significant. Microsoft Excel 2000 and Statistica 6.0 software (Statsoft Inc., <http://www.statsoft.com>) were used for statistical calculations.

Results

Clinical efficacy

Although some patients experienced some burning and stinging during the irradiation, generally the treatment was well

tolerated by all patients and no additional analgesics were needed. These sensations were more intense during the second light fraction of 8 J cm^{-2} .

Psoriatic lesions showed some evident clinical improvement during the treatment period which was reflected in a statistically significant decrease in the clinical plaque severity (sum) score ($P = 0.009$) (Figs 1a and 2). Moreover, starting at week 1, the mean sum score of the ALA-treated lesions was significantly lower as compared with the placebo-treated lesions. Classical psoriatic plaques of a few centimetres in size with clinically normal surrounding skin showed the best clinical improvement. This started most frequently in the periphery of the plaque. Areas within a plaque that clinically improved were usually followed by hyperpigmentation. In two patients an increase in the total psoriatic surface area was observed in the ALA-treated sites, highly suggestive for Koebnerization.

Fluorescence intensity on the skin

After a 4-h incubation with ALA, psoriatic plaques showed selectively higher fluorescence compared with the surrounding (nonlesional) skin (Figs 3 and 4). However, fluorescence within most plaques was not homogeneous, despite clinically adequate keratolytic treatment 1 week prior to the baseline visit. Lesional fluorescence intensity after a 4-h incubation with ALA was 2.57 times higher compared with baseline fluorescence (before ALA application). The first light fraction of 2 J cm^{-2} caused no statistically significant photobleaching (2.4%). During the 2-h dark interval thereafter, fluorescence intensity again increased up to 2.99 times baseline fluorescence, indicating new PpIX formation. After the second fraction of 8 J cm^{-2} , a statistically significant decrease in fluorescence intensity (26.69%) was observed.

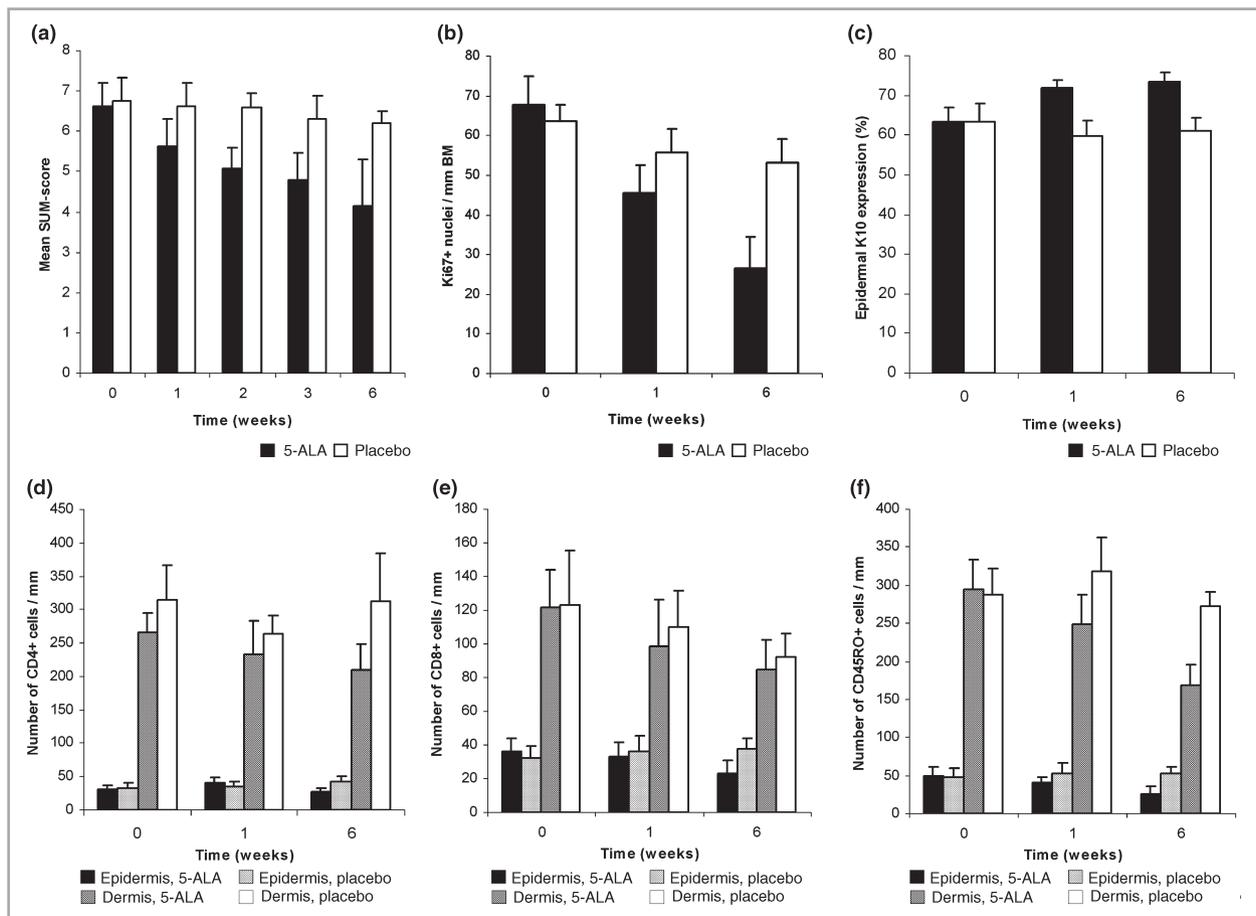


Fig 1. Clinical sum scores and immunohistochemical changes during fractionated 5-aminolaevulinic acid (ALA)- or vehicle-photodynamic therapy. (a) Clinical sum scores at baseline and weeks 1, 2, 3 and 6. The sum scores of the ALA-treated sites significantly decreased ($P = 0.009$), whereas the vehicle-treated sites did not show any significant changes during treatment ($P = 0.239$). (b) Ki67 expression significantly declined in the ALA-treated sites during treatment ($P < 0.001$), but not in the placebo-treated sites ($P = 0.275$). (c) K10 expression significantly increased in the ALA-treated sites during treatment ($P = 0.007$), but not in the placebo-treated sites ($P = 0.651$). (d–f) Epidermal and dermal expression of CD4+, CD8+ and CD45RO+ cells, respectively. Epidermal CD4+ cells in the vehicle-treated sites showed statistically significant increases, whereas CD4+ cells in the ALA-treated sites did not show any statistically significant changes. Dermal CD45RO+ cells showed a statistically significant decrease during ALA treatment. Although the number of epidermal CD45RO+ cells also showed a marked decrease, this was not statistically significant. The opposite was observed with respect to the number of CD8+ cells, where a significant epidermal decrease in the ALA-treated site was seen, vs. a nonsignificant dermal decrease. Results are shown as mean \pm SEM.



Fig 2. Clinical improvement in 5-aminolaevulinic acid-photodynamic therapy-treated psoriatic plaques. After four treatments most psoriatic plaques clinically improved as reflected by a reduction in the plaque severity (sum) score. This was not seen in the placebo and untreated lesions. Note the partial response and the postinflammatory hyperpigmentation.

However, fluorescence was still 2.13 times baseline fluorescence.

Immunohistochemistry

Epidermal proliferation and differentiation

Epidermal proliferation decreased during PDT as shown by a significant reduction in the number of epidermal Ki67+ cells

(−60%; $P = 0.001$) (Figs 1b and 5). Epidermal K10 expression, however, increased significantly, indicating normalization of differentiation (+16%; $P = 0.007$) (Figs 1c and 5). These changes were in parallel with the clinical improvement of the psoriatic lesions and were absent in the placebo-treated lesions.

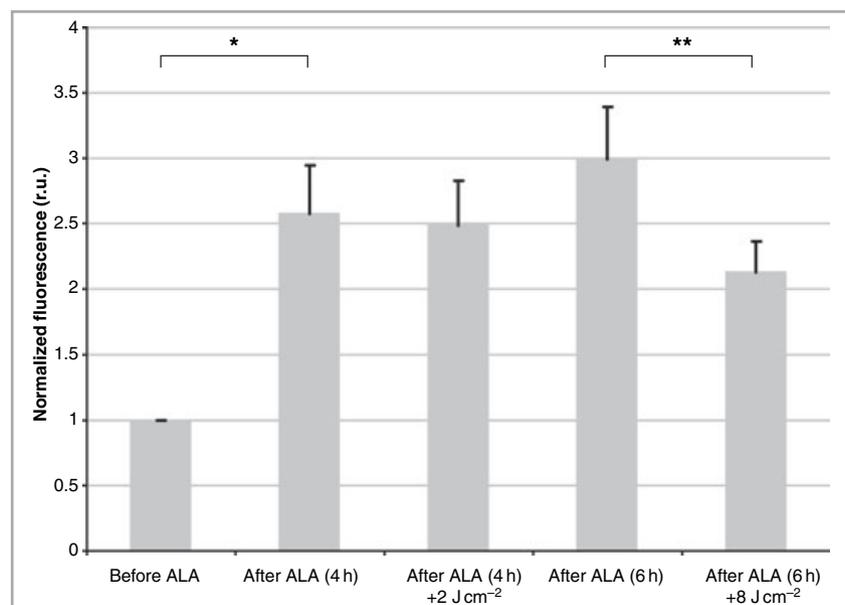
T-cell infiltration

CD8+ cells were mainly seen in the papillary dermis and to a lesser extent in the epidermis (Figs 1e and 5). From baseline to week 6 the number of epidermal CD8+ cells showed a marked decrease (−36%; $P = 0.036$) in the ALA-treated lesions. In the dermis a decrease was also noted, but this was not statistically significant (−31%; $P = 0.232$). The number of CD45RO+ cells, however, decreased in both epidermis (−48%; $P = 0.034$) and dermis (−43%; $P = 0.013$) (Figs 1f and 5). CD4+ cells in the ALA-treated lesions did not show any significant changes in either dermis (−21%; $P = 0.429$) or epidermis (−13%; $P = 0.112$) (Fig. 1d). The placebo-treated lesions did not show any significant changes in the studied T-cell subsets except for a significant increase in the number of epidermal CD4+ cells (+34%; $P = 0.029$).

Fluorescence patterns

Although the studied psoriatic lesions were pretreated with a keratolytic agent, most lesions showed inhomogenous fluorescence, mostly in a follicular pattern and different at the different days the treatment took place (Fig. 4). From the clinical appearance of the lesions the distribution of fluorescence within the plaque was not always predictable. Fluorescence values at some sites within the plaques were lower than normal surrounding skin fluorescence. Epidermal skin barrier also appeared to influence PpIX formation and distribution within the plaques as high fluorescence values were always seen around the sites of biopsy.

Fig 3. Photobleaching of protoporphyrin IX during fractionated 5-aminolaevulinic acid (ALA)-photodynamic therapy (PDT). Macroscopic fluorescence was measured during fractionated ALA-PDT at several time points using the DyaDerm imaging system. Statistically significant changes in fluorescence were seen at 4 h incubation and after illumination with the second light dose of 8 J cm^{-2} , while the first light dose did not induce statistically significant photobleaching. Fluorescence values are depicted in relative units (r.u.) mean \pm SEM with baseline fluorescence set to 1.0. * $P < 0.001$, ** $P < 0.01$.



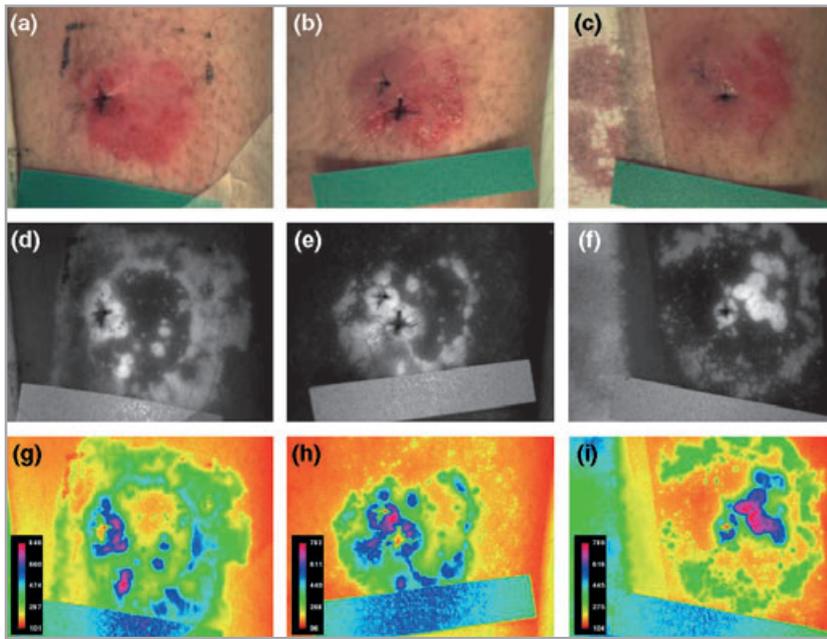


Fig 4. Clinical (a–c), fluorescence (d–f) and pseudocolour images (g–i) from the same patient after 5-aminolaevulinic acid application (4 h) at baseline (a, d, g), week 1 (b, e, h) and week 2 (c, f, i). The distribution of fluorescence varied largely at the different time points and was generally in a follicular pattern. Note that at the sites of biopsy fluorescence was always present.

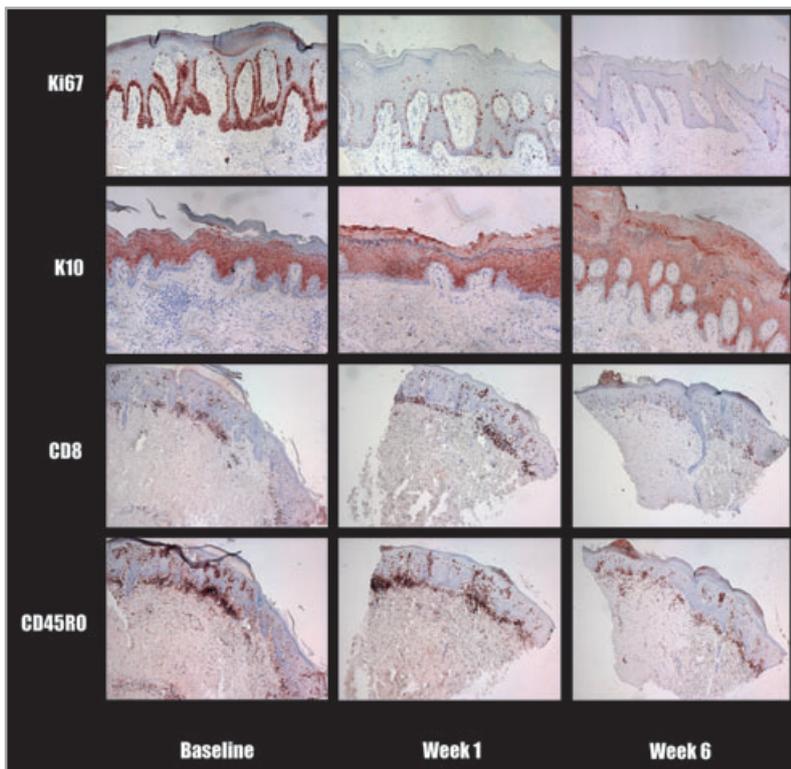


Fig 5. Immunohistochemistry. Ki67, keratin 10 (K10), CD8 and CD45RO stainings at baseline, week 1 and week 6. Ki67 and K10 are displayed at ×100 magnification; T-cell subsets are displayed at ×50 magnification.

Discussion

Four fractionated PDT sessions of $2 + 8 \text{ J cm}^{-2}$ (2-h dark interval) with 10% ALA ointment induced clinical improvement in psoriatic plaque severity as measured by a statistically significant lower sum score compared with the sites that were treated with vehicle-PDT. Several authors have described clinical improvement of psoriasis after multiple PDT sessions but an optimal

treatment protocol has not yet been established.^{1–4,7,12} Most authors agree that the dose per treatment session required for a clinical improvement must not be too high, as Koebnerization and severe pain during irradiation have been reported.⁸ In our study in two patients an increase in psoriatic activity was observed in the ALA-treated sites, but not in the placebo-treated sites. In these sites new psoriatic lesions arose, which is highly suggestive for the Koebner phenomenon as nonlesional psoriatic

skin is also affected by photodynamic effects. Although various factors have been reported to modulate the Koebner response,¹³ in our patients this was unpredictable. In this light, it would be interesting to study the photodynamic effects of methylaminolaevulinate (MAL)-PDT which is now licensed in almost every European country. As MAL has been reported to accumulate more selectively than ALA,¹⁴ the risk of Koebnerization could theoretically be reduced. To the best of our knowledge, the exact differences in PpIX formation in psoriasis between MAL and ALA have not been studied yet. However, when PpIX is regarded to accumulate in psoriasis and nonmelanoma skin cancer by similar mechanisms, with MAL (as Metvix®; Galderma, Fort Worth, TX, U.S.A.) one might expect less pronounced photodynamic effects in nonlesional skin (e.g. Koebnerization) compared with lesional skin. It is unknown, however, how PpIX levels between 10% ALA cream and Metvix relate, as Metvix contains a relatively high concentration (160 mg g⁻¹) of MAL compared with the 100 mg g⁻¹ ALA that was used in our study, although in a study of Fritsch *et al.*¹⁴ MAL appeared to be less potent than ALA with respect to PpIX formation in actinic keratosis. Considering the fact that 1% ALA cream in a study of Radakovic-Fijan *et al.*⁷ was able to exert pronounced photodynamic effects in the treatment of psoriasis, the concentration of MAL in Metvix cream might be too high for this indication.

Koebnerization thus appears to be a significant adverse event that is most likely to be dependent on the photodynamic dose, although an exact dose–effect relationship has not yet been established. On the other hand, photodynamic doses that are too low do not appear to be therapeutically effective.^{1–3,8} In a recent study Radakovic-Fijan *et al.*⁷ reported no significant differences in the clinical response between 5 and 10 J cm⁻², whereas 20 J cm⁻² appeared to be more effective. In this study 1% ALA ointment was used. As psoriasis has been reported to accumulate PpIX strongly, lower concentrations of ALA than used for dermatological indications may be sufficient to exert a beneficial clinical effect. In addition, the primary goal of PDT in psoriasis is not likely to be the cytotoxic effect, for which a few higher photodynamic doses are required, but rather the immunomodulatory effect in which exposure to multiple lower photodynamic doses over a longer period of time is thought to be required. Considering the fact that at the end of the second illumination macroscopic fluorescence was still 2.13 times above baseline fluorescence, in our study depletion of PpIX cannot be held responsible for the mediocre clinical efficacy.

Fractionation of the light dose in two fractions with a dark interval in between has been reported as a way to enhance PpIX formation as resynthesis of PpIX is thought to take place in the dark period. Moreover, during the dark period reoxygenation of the tissue may lead to additional singlet oxygen and reactive oxygen species formation.¹⁵ These events may account for the increased PDT-induced photodamage. Lowering of the fluence rate has also been reported to result in more and deeper PDT-induced photodamage by a similar mechanism.¹⁰ As mean fluorescence directly after the first illumination did not show a statisti-

cally significant decrease, it is likely that if the amount of photobleaching is regarded to be related to photodynamic effect, then the first dose of 2 J cm⁻² was too low to exert a significant clinical effect. The second illumination, however, did induce statistically significant photobleaching. In this light, a twofold illumination scheme of 5 + 5 J cm⁻² might be more effective, but the risk of Koebnerization may theoretically be increased. As penetration of ALA cream is one of the main problems in the treatment of dermal processes, lowering of the fluence rate may be more useful than light fractionation.

Increased epidermal proliferation, abnormal keratinization and dermal infiltration of T-cells and neutrophils are the histological hallmarks of plaque psoriasis, whereas normalization of these parameters is associated with clinical improvement. In our biopsies normalization of epidermal proliferation was seen, as reflected by a significant decrease in Ki67 expression, which is a cell cycle-associated antigen expressed in all parts of the cell cycle except G₀ and early G₁, thus representing the epidermal proliferative compartment.¹⁶ Normalization of keratinization was also observed, as seen in an increase in expression of epidermal K10, a marker for terminal differentiation which is generally decreased in psoriasis.¹⁷

Analysis of T-cell infiltration also showed statistically significant increases in epidermal CD4+ cells in the vehicle-treated sites, whereas CD4+ cells in the ALA-treated sites did not show any statistically significant changes. The photodynamic effect might be responsible for the stabilization of the CD4 influx into the skin. Dermal and epidermal CD45RO+ cells showed a statistically significant decrease during ALA treatment. With respect to the number of CD8+ cells, a significant epidermal decrease in the ALA-treated site was seen, as opposed to a nonsignificant dermal decrease.

Thus, the current treatment protocol as described in our study demonstrated pronounced antipsoriatic effects on epidermal keratinocytes with respect to proliferation and differentiation. A decrease in the number of CD45RO+ cells was the only statistically significant dermal effect that was observed. This is not surprising when the penetration depth of the light source and ALA cream used are considered. PDT with topical ALA exerts its main effects on the surface of the skin where the amount of light energy and photosensitizer concentration are the highest. Hence its effects are expected to be found mainly in the epidermis.

Heterogeneity of fluorescence on the skin as well as histologically within psoriatic plaques has been described by various authors.^{2,7,18} A part of this seems to be related to the amount of hyperkeratosis within a psoriatic plaque, as pretreatment with a keratolytic agent improves fluorescence homogeneity.¹⁸ It is not known what causes the remaining heterogeneity of fluorescence after clinically efficient desquamation, as observed in our patients. These variations in fluorescence might explain the variable and mostly partial clinical response seen after PDT.

The present study reconfirms that PDT has an antipsoriatic efficacy and is well tolerated. Because of the variable clinical

effect and mediocre clinical efficacy compared with other established topical treatments, PDT of psoriasis using the current protocols would not be recommended as a first-line treatment option. However, in certain patients with limited chronic plaque psoriasis where conventional treatments have failed, PDT can be an alternative. Perhaps the addition of topical corticosteroids to PDT might enhance clinical efficacy further and reduce the risk of Koebnerization. More studies are needed, better to understand PpIX formation and its photodynamic effects in psoriasis so that an optimized treatment protocol can be developed.

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