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CLINICAL REPORT

Photodynamic Therapy with Topical 5-Aminolevulinic Acid for Mycosis Fungoides: Clinical and Histological Response

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There is no curative treatment for mycosis fungoides (MF), the most common primary cutaneous T-cell lymphoma. The aim of this study was to investigate the response of single lesions to photodynamic therapy (PDT). The study included 10 plaque MF lesions and 2 tumour MF lesions from 10 patients. First, 20% 5-aminolevulinic acid was applied topically to the lesion and adjacent skin for 5–6 h. The lesion was then exposed to red light at around 630 nm. Skin biopsies were taken before treatment, after clinical improvement and after clinical remission. The expression of CD3, CD4, CD7, CD8, CD1a, CD34, CD68, CD71, Ki-67, bcl-2 and p53 was studied immunohistochemically. There was complete clinical clearance in seven of nine plaque lesions. Neither tumour lesion responded to PDT. The biopsies confirmed a regression of the infiltrate after treatment. In the sparse remaining infiltrate a few CD4+ and CD8+ cells were found, most of which showed normal bcl-2. There were also fewer proliferating cells, illustrated by a decrease in Ki-67 and CD71. In conclusion, PDT has good clinical and histological effects in treating local plaque MF lesions. *Key words: mycosis fungoides; photodynamic therapy; ALA; immunohistochemistry*.

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There is no curative treatment for mycosis fungoides (MF). Therapeutic strategies have given various results (1). In early MF, treatment is directed towards the skin lesions, commonly with psoralen and ultraviolet A (PUVA), often with addition of systemic retinoids.

Retinoids reduce the number of PUVA treatments and remission may be prolonged (2). A disadvantage of PUVA is that ultraviolet light has a limited depth of skin penetration (3). In photodynamic therapy (PDT) visible light is used, penetrating more deeply than ultraviolet radiation. In PDT a photosensitizing substance is used which accumulates in the target cells (4). This is followed by irradiation of the lesion with visible light, resulting in selective destruction of the tissue. Precancerous lesions and malignant tumours such as actinic keratoses, basal cell carcinomas and squamous cell carcinomas have been treated with PDT, showing partial and complete clinical response (5–7). Case reports have been published in which PDT has had beneficial effects on MF (5, 8, 9).

In this study, 5-aminolevulinic acid (ALA), a precursor of porphyrins in the biosynthetic pathway of haem, was applied. The addition of exogenous ALA causes an increase in photosensitizing protoporphyrin IX (PpIX) (10) which, after photoactivation by visible light, leads to cell damage and necrosis. The aim of the study was to investigate the clinical response to PDT. Using immunopathological methods, skin biopsies from treated areas were also compared with respect to present cell populations and cell proliferation before and after PDT.

MATERIAL AND METHODS

Patients

Ten patients with MF, six men and four women (mean age 67 years; range 58–81 years) participated (Table I). In two patients, two lesions were treated. Ten lesions were at the plaque stage of MF and two were tumorous MF. The diagnosis was made with routine histopathology and immunophenotyping. The lesions had been present for 3 months to over 20 years. Two of the patients had only one lesion. In all patients the routine blood cell count, the differential count, palpation of lymph nodes and chest X-ray were performed. The investigations showed no signs of internal involvement, except in patient no. 3 who had enlarged lymph nodes on the neck [tumour, node, metastasis (TNM) classification T1, N1, M0]. Six patients had been receiving treatment with 10–25 mg etretinate daily for 6 months to 5 years before starting PDT. Seven patients had previously been treated with PUVA 1–9 months before PDT. Patient no. 4 started with PUVA during the study, with the PDT-treated lesion covered during UVA exposure. Another patient (no. 7) had received 12 X-ray treatments on his only lesion 2.5 years before PDT, with no effect.

Twenty per cent ALA (Porphyrin Products, Utah, USA), dissolved in an oil–water emulsion, was applied topically to the lesion and to adjacent skin under an occlusive and light-shielding dressing for 5–6 h. One patient (no. 6) had had the ALA emulsion applied for 18 h during the last two PDT sessions. The red fluorescence of porphyrins was visualized with Wood's light before treatment. Fluorescence was seen in all cases. The ALA-treated areas were then exposed to incoherent light using a Waldmann PDT 1200 (Waldmann Medical Division, Villingen-Schwenningen, Germany) and emitting light at 600–730 nm with a maximum around 630 nm. The patients were examined with an interval of 1–2 weeks and, depending on the clinical result, the treatment was repeated (Table I). The clinical response was followed by evaluating erythema, infiltrate, squama, pigmentation and ulceration. Immediately after PDT, the patients were asked to evaluate the intensity of pain during treatment using visual analogue scales (VAS) (11).

Biopsies

Four-millimetre punch biopsies were taken before treatment, after clinical improvement and in clinical remission. The skin samples were formalin-fixed and embedded in paraffin or immediately frozen in liquid nitrogen and kept at −80°C until used. For histological evaluation routine haematoxylin-stained paraffin sections were used.

Immunohistochemistry

The alkaline phosphatase anti-alkaline phosphatase (APAAP) technique was used to investigate expression of the following antigens: CD3, CD4, CD7, CD8, CD1a and CD71. Frozen sections were acetone-fixed for 10 min, air-dried and rinsed with Tris-buffered saline, pH 7.6. The antibodies were used in pretested optimal concentrations (Table II). The sections were incubated for 30 min at room temperature, and then with rabbit anti-mouse immunoglobulins (Dako Z 0259, Glostrup, Denmark) diluted 1:20 for 20 min. After rinsing they were incubated with APAAP (Dako D 651) diluted 1:20 for 20 min.

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Table I. *Characteristics of patients, mycosis fungoides (MF) lesions, photodynamic therapy (PDT) and clinical results*

Patient/ gender	Previous treatment	MF lesion	Location	Size of lesion (cm)	VAS score (median)	Fluence (J/cm^2)	Intensity (mW/cm^2)	No. of treatments	Clinical result	Follow-up (months)
1/M	PUVA	Plaque	Thigh	7 × 4	6.7	90	210 + 220	2	Healed	19
	PUVA	Plaque	Hip	11 × 11	8.4	50 + 33	20 + 40	2	Regression	
2/M	Local steroid	Plaque	Hip	4 × 3	nd	180	320 + 315	2	Healed	19
3/M	PUVA	Tumour	Trunk	8 × 4	nd	180	210–245	3	No response	
	PUVA	Plaque	Back	5.5 × 3	6.3	180	170–240	3	Healed	11
4/F	PUVA	Plaque	Thigh	2.5 × 2.5	2.0	90	230 + 265	2	Healed	4
5/M	None	Plaque	Gluteal	6 × 4.5	0.7	50 + 60	40 + 60	2	Healed	15
6/M	None	Plaque	Gluteal	10 × 6	4.25	90	60–125	8	No response	
7/M	X-ray/ PUVA	Plaque	Arm	10 × 10	4.0	90	70–95	11	Healed	21
8/F	PUVA	Tumour	Abdomen	7.5 × 7.5	2.0	100	40–60	3	No response	
9/F	PUVA	Plaque	Trunk	6 × 5	6.2	90	65–90	3	Healed	19
10/F	PUVA	Plaque	Thigh	10 × 15	8.0	90 + 50	35–55	2	Regression[a]	

[a] Patient no. 10 discontinued PDT.
VAS: visual analogue scale; M: male; F: female; PUVA: psoralen and ultraviolet A.

Table II. *Immunohistochemical staining: average findings in 10 mycosis fungoides infiltrates*

Antibody	Specificity	Dilution	Source	Pretreatment[a]	Post-treatment
Ki-67	Proliferating cell	1:100	Immunotech	+	+[b]
bcl-2	Suppression of apoptosis	1:40	Dako	– to + +	+ +
p53	Tumour suppressor gene	1:100	Dako	– to +	–
CD68	Macrophages	1:50	Dako	+ to + +	+
CD34	Endothelial cell	1:50	Serotec	+	+
CD71	Transferrin receptor	1:20	Dako	+ to + +	+[b]
CD3	Pan T-cell	1:40	BD	+ + to + + +	+ to + +
		1:200	Dako		
CD4	Helper T-cell	1:40	BD	+ + to + + +	+
CD7	Pan T-cell	1:20	Dako	+	+ +
CD8	Suppressor T-cell	1:30	Dako	+	+ +
CD1a	Langerhans' cell	1:40	BD	+ to + +	+

[a] – : negative; + : positive in < 25%; + + : positive in 25–75%; + + + : positive in > 75% of cells.
[b] Only single cells present.
BD: Becton Dickinson.

The staining intensity was enhanced by repeating the last two steps. The reaction was developed with alkaline phosphatase substrate kit 1 (Vector SK-5100, Vector Laboratories, Burlingame, USA). The sections were finally stained with Gill's haematoxylin.

The avidin and biotinylated horseradish peroxidase macromolecular complex (ABC) technique was used to investigate the expression of CD3, Ki-67, bcl-2, p53, CD34 and CD68 (Table II). The paraffin sections were deparaffinized, rehydrated and incubated twice for 5 min in citric buffer, pH 6.0, in a microwave oven or treated with 0.1% trypsin (Dako S2012) at 37°C for 30 min for CD68. All sections were incubated with the diluted antibodies for 60 min. A 1:200 dilution of the secondary biotinylated antibody was used (Vector Laboratories). The avidin–biotin–immunoperoxidase technique (Vectastain Elite ABC kit, Vector Laboratories) and an aminoethylcarbazole kit (AEC kit; Vector SK-4200, Vector Laboratories) were used to develop the enzymatic reaction. The sections were finally stained with Mayer's haematoxylin.

RESULTS

Clinical results

In 7 of 9 plaque lesions there was clinical complete clearance with no recurrence after a follow-up of 4–19 months (Fig. 1a–c, Table I). Nine of the initial 10 MF lesions in plaque stage were evaluated. Patient no. 10 discontinued the treatment because of pain. Two patients with MF in plaque stages, nos 1 and 10, showed a regression, but did not heal completely. In patient no. 1, one of two lesions healed completely. The other was not fully treated as the patient found the treatment too painful and this lesion regressed partially. Patient no. 6 was the only one of those with plaque MF who did not respond to repeated PDT. Neither tumour lesion responded to PDT.

Side-effects such as erythema, local oedema, scaling and pain were noticed during and after treatment in most patients. One lesion developed an erosion and two lesions ulceration. Five lesions showed hyperpigmentation after completed treatment. The score for pain using 10 cm VAS was measured in nine patients. The median of all measurements was 4, ranging from 0.4 to the maximum, 10. There was large individual variation (Table I).

Histological observations

In 9 of 10 patients biopsies taken before treatment confirmed morphology characteristic of MF, with CD3 + lymphatic

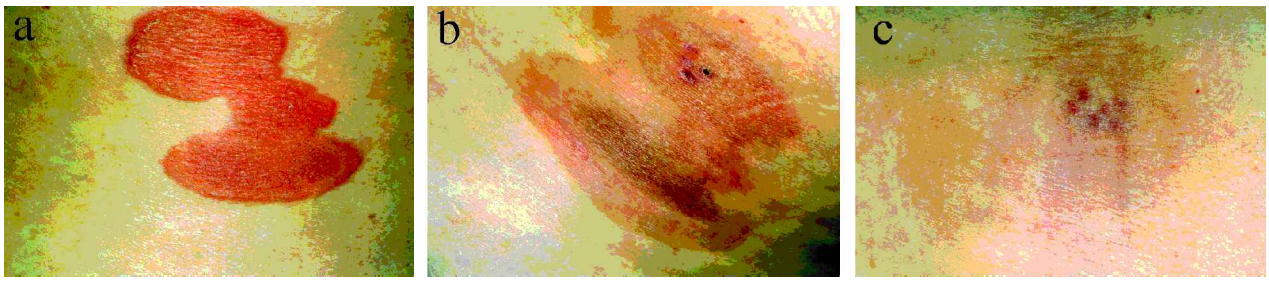

Fig. 1. Mycosis fungoides lesion in the plaque stage on the left trunk in patient no. 9: (a) before photodynamic therapy; (b) after two treatments; and (c) 2 weeks after completed treatment, with scars from biopsies.

infiltrates both in the dermis and subepidermally, together with lymphoepidermotropism and Pautrier's microabcesses (Fig. 2a, c). In one case (no. 5) the morphological aspect of the lesion was of localized pagetoid reticulosis rather than typical MF type (12).

The immunostaining results are summarized in Table II. Immunostaining showed in all cases a predominance of CD3+/CD4+ cells, and in all but case no. 5 relatively few CD8+ cells and weak or negative staining for CD7. Considerable numbers of macrophages positive for CD68 were seen in three cases but in other cases only a few macrophages were present. In three cases increased numbers of CD1a-positive Langerhans' cells were found. In seven cases considerable capillary vessels were observed, as confirmed by staining for CD34. Staining for proliferation markers Ki-67 and CD71 showed fewer than 5% positive cells, which was consistent with low-grade lymphomas. In three patients with tumour and plaque lesions, between 10 and 20% of lymphoma cells were

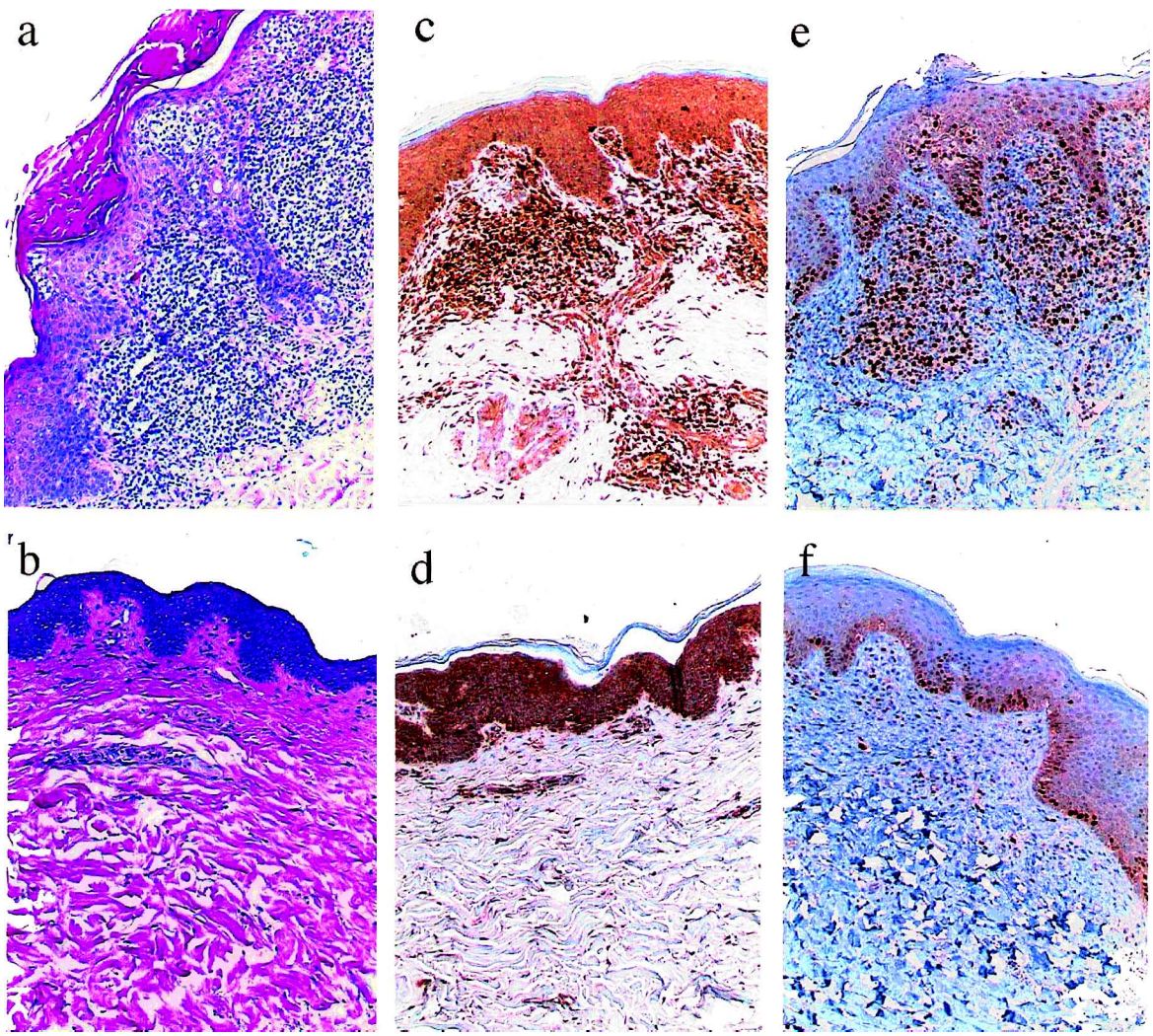

Fig. 2. Histological and immunohistochemical findings of mycosis fungoides lesions in the plaque stage before and after treatment, confirming the significant regress of the infiltrates. Stains: (a, b) haematoxylin; (c, d) CD3/ABC; (e, f) Ki-67/ABC.

in the proliferation phase (Fig. 2e). As expected, a considerable positivity for these markers was also observed in the basal layer of the epidermis. In four of six cases staining for bcl-2 revealed a bcl-2 weak/negative population, together with a population with strong positivity, characteristic for T-cells; while in the remaining two cases all cells were strongly bcl-2 positive. Positivity for p53 in the infiltrate was noted in a relatively sparse cell population in three patients.

Biopsies were taken after treatment in nine patients. Histological evaluation and CD3 staining confirmed a significant regression of the infiltrates in all cases studied (Fig. 2b, d). The regression depended on a considerable decrease in the numbers of CD3+/CD4+/CD7-weak or negative cells. In clinically responding lesions, the biopsies showed only a few remaining infiltrates. Small numbers of CD4+ and CD8+ cells were found, most showing normal bcl-2 positivity. The expression of Ki-67 and CD 71 decreased in the responding infiltrates (Fig. 2f). There were no differences in CD 71 positivity between responders and non-responders. Capillary vessels were still present. In three lesions a relative increase in macrophage numbers was observed. CD1a-positive Langerhans' cells diminished significantly in biopsies taken during treatment, returning to normal in biopsies taken later after treatment in three lesions but remaining low in the other biopsies. A decrease in CD71 positivity was shown in the epidermis. There were more p53-positive cells within the epidermis.

DISCUSSION

A good clinical response to PDT was achieved in 7 of 9 MF plaque lesions with no recurrence in the treated area after 4–19 months of follow-up (Table I). Two of the non-healed lesions of MF at the plaque stage had not received optimal therapy because of pain during treatment. No systematic controls were used in this study. Two patients had only one lesion, one patient received PUVA, but four patients had other lesions that did not change during PDT. Previous reports by Svanberg et al. (5) showed a complete response in 2 patients with 4 lesions treated once or twice with PDT with a follow-up time between 6 and 14 months. Wolf et al. (8) reported complete remission in two patients with plaque-stage MF after 4 or 5 PDT sessions with a follow-up time of 3 and 6 months. Orenstein et al. (9) treated 2 patients with MF, 1 with a patch lesion and one with 5 tumour lesions, with a complete response and follow-up of 27 and 24 months, respectively.

Earlier reports showed that malignant CD71+ lymphocytes from a patient with cutaneous T-cell lymphoma/Sezary syndrome accumulated greater PpIX levels than normal lymphocytes (4). A decrease in CD71+ lymphocytes was noticed in the present material after treatment, which is related to lower proliferation. It might also indicate that ALA-based PDT destroys the malignant T-cells more selectively. However, there was no relationship between clinical outcome and the degree of CD71 positivity in the infiltrate before treatment.

Little is known about the optimal fluence of irradiation and application time for ALA in MF. This study was started in 1995. At that time most studies were done with PDT for basal cell carcinoma, solar keratosis and Bowen's disease with an ALA application time of 4–6 h and a fluence of 150–180 J/cm^2 (13–15). An application time of 5 h was chosen. Initially, a fluence of 180 J/cm^2 was given, but this had to be halved owing to troublesome pain. Initially, a short treatment time and high intensity were chosen, since the authors did not realize that high intensity caused so much pain. Pain meant that the intensity had to be decreased in some patients, giving a wide range of values. However, even patients treated with low intensity healed, which suggests that high intensity should be avoided as it causes more pain. There seems to be large individual variation in susceptibility to pain during PDT. The median VAS value for pain was 4, but it varied greatly from 0.4 to 10. The impression gained was that the larger area treated the greater the pain, but pain is a subjective experience that varies between individuals. The second lesion on patient nos 1 and 10 showed only a minor regression (Table II). However, the treatment had to be interrupted because of pain, resulting in incomplete PDT, which may have caused treatment failure.

Peng et al. (16) showed that the penetration of ALA with 4% EDTA could be increased in basal cell carcinoma by prolonging the application time (29–48 h). Orenstein et al. (9) used 16 h of ALA cream application to treat MF. In the present study, the cream was applied for 18 h during the last two treatment sessions in patient no. 6, who had been unresponsive to six previous treatments, but this did not improve the clinical or histological response. Patient no. 7 received 11 treatments of PDT before his plaque-stage MF healed. A fibrosis was noticed in the histological evaluation before PDT, which could be partially explained by earlier X-ray therapy and may have slowed the response.

Seven of the patients had been treated with PUVA 6 weeks to 2 years before PDT. None of the MF lesions included in the study had responded to PUVA before starting PDT.

Six patients were treated with etretinate daily. Retinoids appear to have an immunoregulatory effect on skin mononuclear infiltrates (2). This may have affected the clinical response to PDT, although the same clinical and histological response was seen in the patient not taking retinoids.

In all responding lesions almost complete or complete regression of MF infiltrates was observed.

Increased p53 expression was seen in the epidermis after PDT, possibly caused by the phototoxic reaction or the heat during treatment.

In conclusion, this study shows good clinical and histological effects from treating plaque-stage MF with PDT. Most patients responded to two or three treatments, which is an advantage over PUVA, especially in patients with few lesions. PDT is a useful tool for treating local plaque MF lesions.

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