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EFFECTS OF PUVA ON THE EPIDERMAL MELANOCYTE POPULATION IN PSORIATIC PATIENTS

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Abstract. The effect of PUVA treatment on the epidermal melanocyte system was studied in 24 skin biopsies from four male psoriatic patients. The patients had received 40 mg 8-methoxypsoralen and a phototoxic dose of UVA (2–12 joule/cm²), twice a week. Punch biopsies were taken from unaffected and psoriatic skin in the gluteal region before, and after three and nine treatments. The epidermal melanocytes were counted in dopa incubated split-skin preparations and the mitotic index of the keratinocytes was monitored as a control of the PUVA effect. The epidermal melanocyte number was essentially normal in non-treated psoriatic patients. During the PUVA treatment the melanin production and the DOPA activity of the melanocytes increased markedly. However, there was no significant change in the number of DOPA active melanocytes, either in the unaffected or the psoriatic skin. Thus, PUVA treatment is not necessarily associated with an increase in the number of active epidermal melanocytes.

Key words: PUVA; Melanocyte; Psoriasis

Orally administered 8-methoxypsoralen (8-MOP) in combination with long-wave ultraviolet light (UVA) exposure is now a well established technique for the treatment of extensive psoriasis (11, 21, 27). The positive effects of this treatment (PUVA) have been ascribed its antimitotic effect, which seems to depend on the photomediated bindings of psoralens to DNA, forming photoadducts and DNA cross-links (4, 10, 12).

During PUVA treatment there is a marked enhancement of the functional activity of the melanocytes (1). It has also been claimed that the increase in skin pigmentation is associated with an increase in the number of epidermal melanocytes, just as in skin treated with ultra-violet (UV) light alone (7, 8, 12). This would imply that the PUVA treatment has a mitos stimulating effect on the melanocytes (cf. 16, 17), in contrast to its antimitotic effect on the keratinocytes.

As PUVA treatment interferes with the DNA, there has been much concern about the possible carcinogenic risk of long-term PUVA treatment. In view of this, an increased melanocyte proliferation rate might be disquieting, since proliferative stimuli have been found to promote tumour development in other cell systems exposed to mutagens (2, 5, 9, 25). For this reason the effects of PUVA treatment on the epidermal melanocyte population have been reinvestigated in both unaffected and diseased skin of psoriatic patients.

METHODS

Four otherwise healthy male psoriatics 26–30 years of age were studied. The patients were all blond and blue-eyed (skin type II–III) (28) and had a stationary extensive plaque psoriasis. The patients were administered 40 mg 8-methoxypsoralen (body weight 69–78 kg) and were irradiated 2 hours later, twice a week. The light source was a high intensity UVA system (PUVA 4000, Sylvania) with an emission spectrum between 320 and 390 nm and a peak emission of 365 nm. The minimal phototoxic dose (MPD) and first treatment dose was 2–3 J/cm². The light dose was increased to phototoxic levels for each treatment and after 9 sessions the patients had reached 7, 7, 11, 12 J/cm². None of the patients had received PUVA treatment before, and they had been without local or systemic therapy for several months. None had had their gluteal area exposed to sun or UV light for at least 7 months. However, all had at the start of the treatment a noticeable difference between the constitutive pigmentation in the gluteal region and earlier exposed skin above the waist.

Punch biopsies 4 mm in diameter were taken from a psoriatic gluteal plaque and from clinically normal skin about 15 cm from the plaque. The biopsies were taken before commencing the treatment and 3 days after the third and ninth treatment. The same psoriatic plaque was used for all biopsies, which were taken at least 5 cm from each other in order to avoid interference from the wound healing process.

The biopsy specimens were immediately divided in two parts. A larger piece was incubated in 2 N NaBr at 37°C for 1¼ hour and the epidermis was split from the dermis. The epidermal sheet was then incubated in buffered DOPA (L-3, 4-dehydroxyphenylalanine) at pH=7.4 at 37°C for 3 hours in order to visualize the melanocytes (20).

After fixation and dehydration, the epidermal sheet was mounted on glass slides with the basal lamina facing up.

Table I. *Epidermal melanocyte population density and keratinocyte labelling index in normal and psoriatic skin during PUVA treatment*
For full details, see Methods

	Melanocytes/mm²±S.E.M. (S.D.)		% labelled keratinocytes	
	Normal skin	Psoriatic plaque	Normal skin	Psoriatic plaque
U. L.				
Pretreatment	2 847±96 (304)	1 078±39 (87)*	7	27
3 treatm.	2 886±78 (243)	1 136±27 (81)	5	3
9 treatm.	3 127±170 (539)	1 271±33 (105)**	4	4
D. W.				
Pretreatment	1 457±81 (200)	1 317±76 (226)	2	11
3 treatm.	1 199±39 (122)	1 921±75 (224)	6	10
9 treatm.	1 129±214 (667)	1 424±58 (186)	2	3
T. W.				
Pretreatment	1 516±143 (320)	2 678±112 (275)*	4	14
3 treatm.	1 531±181 (444)	2 477±81 (256)	2	8
9 treatm.	1 641±47 (151)	2 204±29 (76)**	2	2
A. S.				
Pretreatment	1 521±91 (241)	1 442±33 (89)	4	10
3 treatm.	1 698±73 (228)	1 895±82 (272)	1	5
9 treatm.	1 413±96 (303)	1 468±87 (279)	1	2

* Signif. diff. from unaffected control skin ($p<0.05$).
** Signif. diff. from unstimulated psoriatic skin ($p<0.05$).

The number of epidermal melanocytes was estimated as number of cells per mm² skin surface. These estimates were based on counts from 10 ocular fields (0.16 mm² each) using an objective with 25× magnification.

The other part of each biopsy specimen was immediately cut into small pieces, and incubated with tritiated thymidine (^{3}HTdr, New England Nuclear) for 2 hours at 37°C. The incubation medium was Eagle's MEM with glutamine and $NaCO_3$ (20 ml 8% $NaCO_3$ is added to 100 ml Eagle's MEM, and is diluted to 1 000 ml with sterile water) containing 2–10 μCi/ml ^{3}HTdr. After rinsing and fixation, these specimens were embedded in plastic, cut into 5 μm sections and prepared for autoradiography. The percentage of labelled keratinocytes in each of the three basal layers was estimated, counting 2 000 cells in each specimen.

RESULTS

The patients responded well to the treatment, and the psoriatic plaque used for the biopsies improved markedly during the nine treatments. All patients developed a nice sun tan in the initially pale gluteal area, as well as on the rest of the body. The increase in skin pigmentation was first noticed after 3–4 treatments, with some delay in the psoriatic plaques.

Before the treatment, the epidermis from the psoriatic plaque showed a typical histology with pronounced acanthosis, hyper- and parakeratosis. There was also a high labelling index of the keratinocytes in the plaque, compared with clinically normal skin of the same patient (Table I). The initial number of labelled keratinocytes in the plaques varied considerably (10–27%) among the patients (Table I). This variation was not related to the amount of scaling, the thickness of the plaque, or to other clinical differences. Neither was it related to the degree of histological changes. The clinically normal skin showed in some sections a mild hyper- and parakeratosis, and the initial labelling index varied between 2 and 7%.

The improvement in the plaque was apparent in the histological sections as a marked reduction in acanthosis, hyper- and parakeratosis. There was also a reduction in the labelling index to 2–4% (Table I). Furthermore, in the unaffected skin there was a tendency to a reduced number of keratinocytes in S-phase, as compared with the initial values. During the PUVA treatment, there was an increase in the extra- and intramelanocytic melanin, and the melanocytes became more dendritic and more DOPA active. Before the treatment the melanocyte population density in the clinically normal skin was around 1 500 mm² in 3 patients (D. W., T. W. and A. S.; Table I). In one patient (U. L.) 2 800 melanocytes/mm² were found (Table I), which

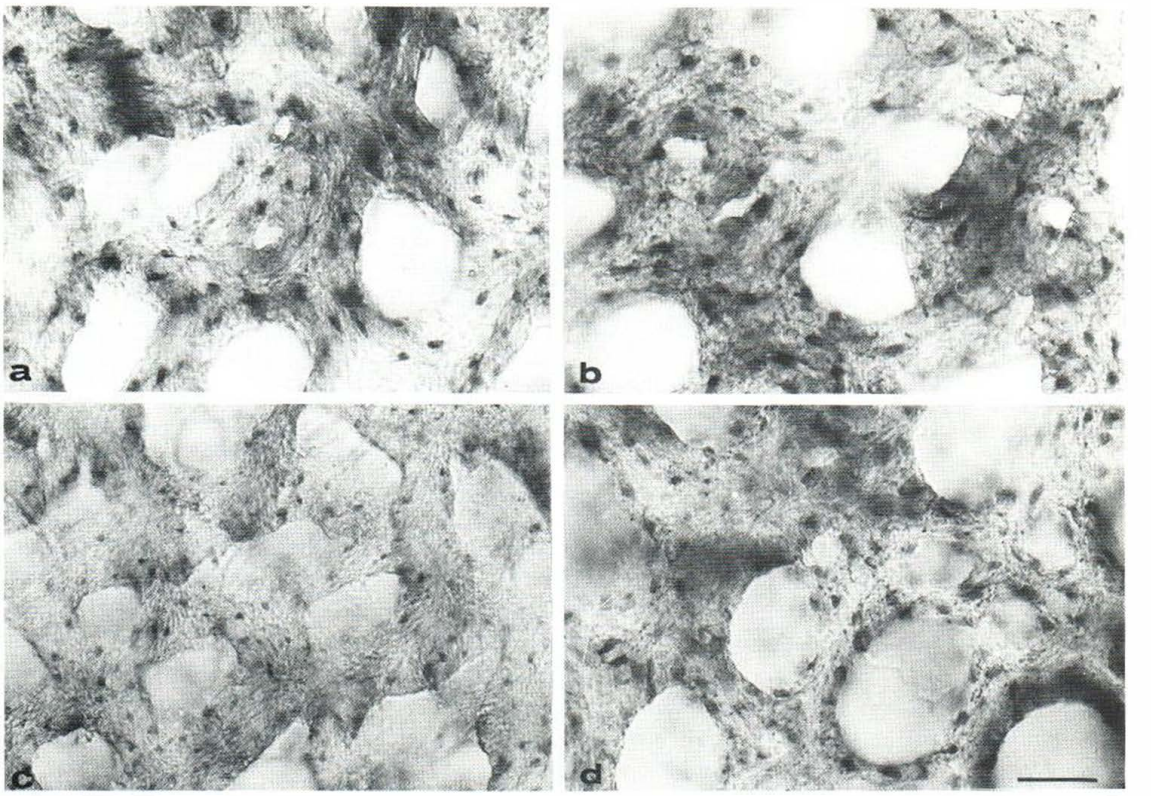

Fig. 1. Photomicrographs of DOPA-incubated split-skin preparations from psoriatic plaques before (*a*, *c*) and after nine PUVA treatments (*b*, *d*). The biopsies were taken from the same gluteal plaque in patients U. L. (*a*, *b*) and D. W. (*c*, *d*). Scale bar 100 μm.

was higher than expected in non-exposed gluteal skin (14, 22, 23). It was rather difficult to count the melanocytes in the split skin preparations from the psoriatic plaque, due to the pronounced acanthosis. To obtain reliable estimates, it was necessary to add counts from several focal planes up and down the ridges. Two patients had about the same number of melanocytes in the psoriatic skin as in the unaffected skin (D. W., A. S.; Table I). The patient with the high melanocyte population density (U. L.; Table I) in clinically normal skin had few melanocytes in the psoriatic plaque, while another patient had almost twice as many melanocytes in the plaque as in the unaffected skin (T. W.; Table I). Similar variations have been found in other patients regarding psoriatic and unaffected skin from other regions of the body (unpublished observations).

There was no systematic change in the number of melanocytes in the psoriatic or unaffected skin, despite a pronounced increase in skin pigmentation during the PUVA treatment. In clinically normal areas, there was a marginal increase in the melanocyte population density in 2 patients (U. L., T. W.) and a corresponding decrease in the other two patients (D. W., A. S.). In no case was this change statistically significant. Similarly, there was no significant change in the number of melanocytes in the psoriatic skin of 2 patients, before and after the nine treatments (D. W., A. S.). However, it is possible that there was a small temporary increase after three treatments in these cases. The patient with the lowest melanocyte population (U. L.) in the psoriatic plaque showed a continuous slight increase, while the patient with the highest melanocyte number (T. W.) showed a gradual decrease during the treatment. It was not possible to obtain a reliable mitotic index of the melanocytes. To differentiate between silver grains and melanosomes, and to ascertain that each nucleus is in close prox-

imity to the emulsion, it is necessary to use semithin sections. Unfortunately, many of the melanocytes could not be recognized in such thin sections, as judged from the split skin data.

DISCUSSION

The epidermal melanocyte population seems to be essentially the same in psoriatic patients as in nonpsoriatic individuals. Healthy Caucasian men in the age group 25–35 years have about 1 500 melanocytes per mm^2 in unirradiated skin on the buttocks (14, 22, 23). Similar values were found for unaffected skin in three of the present patients.

The number of melanocytes in psoriatic plaques seems to vary considerably between individuals, possibly also between different plaques in the same individual (unpublished data). This variation might be due to a variety of factors, one being the degree of inflammatory reaction in the plaque. In 2 of the present patients, the melanocyte number was the same in the psoriatic plaque as in the unaffected skin. In psoriasis there is a marked increase in the proliferation rate of the keratinocytes. It has not been possible to measure the proliferation rate of the melanocytes in psoriatic skin. However, the finding that the melanocyte population may be about the same in psoriatic as in unaffected skin suggests that the proliferation rate of the melanocytes is not necessarily increased in psoriatic plaques. The high proliferation rate of the keratinocytes in psoriasis is combined with a folding of the basal layer, acanthosis, and an increased shedding of differentiated cells, scaling. So far no cytokinetic information on normal human melanocytes in vivo is available. In an experimental study it was found that an increased melanocyte proliferation rate was followed by a larger number of melanocytes per unit surface area of the skin (16, 17, 18). In the following discussion it will therefore be assumed that an increased melanocyte population density reflects an increased proliferation rate of the melanocytes.

In our patients the PUVA treatment did not induce any systematic change in the melanocyte number. This was true for skin both from clinically normal areas and from psoriatic plaques. The patients received PUVA treatments twice a week, with a UVA dose sufficient to induce an erythema of the skin, which is the standard PUVA treatment schedule of our clinic. For practical reasons the patients were not followed with biopsies for more than 9 treatments. At this stage the psoriatic plaques were clearly improved in all cases, both clinically and histologically. There was also a pronounced decrease in the mitotic activity of the keratinocytes, as would be expected with an adequate PUVA dose. Nine treatments are of course very few, in comparison with the total number of treatments a psoriatic patient will undergo. However, the melanocytes respond rapidly to proliferative stimuli (16). Therefore, the time interval studied should be more than enough to demonstrate any lasting PUVA-induced increase in the melanocyte population.

It is difficult to explain the difference between the present results and earlier reports that PUVA may induce an increase in the number of melanocytes in normal skin (8, 12, 13). Psoralen in combination with UVA has an antimitotic effect on several different cell types, and there is no reason to assume that melanocytes should react differently (3). However, the PUVA effect is probably dose dependent and might well be affected by differences in the treatment schedule. For keratinocytes it has been demonstrated that after a single PUVA treatment there is an initial decrease in the mitotic activity followed by an increase above normal levels (24). In normal use of the PUVA treatment this late overshoot in the mitotic activity is curtailed by a second PUVA dose. If the melanocytes react similarly, an increased number of melanocytes may be obtained with a different dose and treatment schedule. It is also possible that there is a difference in the PUVA sensitivity of the melanocytes in skin of healthy and psoriatic individuals (cf. 6).

Earlier observations on the melanocyte reaction to PUVA treatment were made on skin from healthy individuals (8, 12, 13). Kaidbey & Kligman intentionally used suberythemal doses, while Pathak et al. used treatment doses corresponding to those in the present study. None of these investigators has presented controls for the antimitotic effect of the treatment on the keratinocytes. This might be required, since the melanocyte reaction to PUVA may depend on many variables. For instance, the same investigators reported both a marked increase and no change in the melanocyte number after a single PUVA treatment in separate studies (7, 12, 13). The present finding that an effective PUVA treatment is not necessarily associated with an increase in the number of functioning melanocytes indicates that the melanocyte reaction

to PUVA may be more complicated than assumed previously.

It has been suggested many times in the literature that prolonged UV exposure might result in the development of skin cancer (26). Therefore, there has been much concern about a possible carcinogenic effect of long-term PUVA treatment. The most serious skin tumour to be considered is the malignant melanoma. If PUVA were to interfere with the DNA molecules of the melanocytes, there would be an increased risk of malignant transformation of these cells. This risk would be even higher if PUVA in addition had a mitos stimulating effect on the melanocytes (7, 12, 13, 18). It is known from other cell types exposed to mutagens that a proliferative stimulus has a promoting action on tumour development (2, 5, 9, 15, 19, 25). In view of this it may be felt reassuring that an effective PUVA treatment does not necessarily induce an increased formation of new melanocytes.

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