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The Response of Psoriatic Epidermis and Microvessels to Treatment with Topical Steroids and Oral Methotrexate

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In a previous paper we showed that the microvessels in a psoriatic plaque as studied by electron microscopy returned to normal before the labeling index of the basal cells did during successful therapy with PUVA or the Goeckerman treatment. In this paper we studied the same parameters in 4 additional psoriatic patients: 2 received oral methotrexate and 2 were treated with a topical steroid under plastic wrap occlusion. The labeling index of the basal cells returned to normal in 3 and near normal in 1. The histologic features of the psoriatic epidermis became normal except for mild to moderate acanthosis, but the capillary loops in the dermal papillae retained their venous capillary ultrastructure and showed no signs of reversion to a normal arterial capillary configuration. The lack of response of the dermal capillaries to the topical steroid and oral methotrexate during the initial clinical improvement raises the possibility that the clinical relapses in psoriasis which may promptly follow discontinuation of topical steroid therapy and oral methotrexate may be related to an inability of these drugs to restore the microvasculature to normal in such situations.

In a previous study of 6 patients we showed that in psoriatic plaques successfully treated by PUVA or Goeckerman therapy, the capillary loops began to shorten and return toward normal 3–8 days before the labeling index (LI) of the epidermal basal cells began to decrease [1]. We also showed that the LI was elevated in the normal-appearing buttock skin of 6 of 11 untreated psoriatics, and in 4 of these 6, the loops were normal arterial capillaries as defined by electron microscopy. These data supported the concept that the initiating factors in psoriasis are in the epidermis, but that epidermal hyperplasia cannot occur without vascular proliferation.

We have extended these studies by investigating the response of the epidermis and capillary loops in psoriatic plaques to the topical application of a corticosteroid under plastic wrap occlusion in 2 patients and to oral methotrexate therapy in 2 others. The rationale was to see whether these two common treatment modalities in psoriasis produce the same effects on the epidermis and microvasculature as PUVA and Goeckerman therapies did. The clinical experience of one of us (IMB) suggested that different effects might be seen since PUVA and Goeckerman therapies *usually* produce remissions of at least a few months off therapy, whereas the use of topical steroids and oral methotrexate produces such sustained remissions less often. Continuous use of topical steroids and oral methotrexate is generally required for control of psoriatic lesions.

These present studies show that within the time limits of the experiments the LI and the psoriatic epidermal histology reverted almost completely to normal without any accompanying changes in the microvasculature.

MATERIALS AND METHODS

We performed in vitro autoradiography with tritiated thymidine, histologic evaluation by light microscopy, and ultrastructural reconstruction of capillary loops on sequential biopsies of psoriatic plaques. Each biopsy was divided in half: one piece for autoradiography and light microscopy and the other for electron microscopy. The details of the biopsy technique and the electron microscopic and in vitro autoradiographic methods including calculation of the LI and definition of normal values are described in the previous paper [1]. Two 65-year-old men (subjects 1 and 2), and 2 women 40 and 52 years old (subjects 3 and 4, respectively) volunteered for these studies which were approved by the Human Investigation Committee at the Yale School of Medicine. Neither subject 1 nor 2 had had systemic or topical therapy for psoriasis for the previous 6 months. Halcinonide cream 0.1% was used in one man and triamcinolone acetonide cream 0.1% was used in the other. The steroids were applied to plaques under occlusion with a plastic wrap for 24 h. The wrap and the steroid cream were changed and reapplied respectively once each day. Subject 3 had not had any therapy for 2 years and had never received methotrexate. Subject 4 had been poorly controlled on oral methotrexate for the previous 3 months. Her regimen, which had been 5 mg p.o. every 12 h for 3 doses once every 3 weeks, was increased to 5 mg every 12 h for 3 doses once weekly at the start of these studies. Psoriasis affected 20% of the body surface in subjects 3 and 4. In each patient, biopsies were taken just inside the edge of a plaque the day before therapy was begun and at similar sites along the edge of the same plaque at intervals during therapy. The sampled plaques were on the mid-axillary line of the chest and lateral thigh in subjects 1 and 2, respectively, and over the lumbar area and the medial calf of subjects 3 and 4, respectively. Subjects 1 and 2 were biopsied daily and subjects 3 and 4 were biopsied at 3- to 7-day intervals. In subject 2 and in subject 3 the last biopsies were obtained 8 and 38 days, respectively, after the preceding ones. The plaques selected for study were large enough so that the sequential areas to be biopsied were not infiltrated by the previous rings of anesthesia. These experiments continued for 4 and 16 days in subjects 1 and 2 and for 17 and 109 days in subjects 3 and 4, until the plaques were completely flat, devoid of scale, and only minimally erythematous.

The autoradiographs were also scored for the presence of histologic abnormalities using the following notations. Ps = parakeratosis, loss of granular layer, acanthosis and in most instances the presence of Munro's microabscesses. Nl = orthokeratosis and a granular layer, but an epidermis that was slightly or moderately acanthotic. Ps/Nl and Nl/Ps = both psoriatic and normal features were present, with the first set of initials indicating the predominant histologic feature. Four to five capillary loops in each biopsy specimen were reconstructed from serial electron microscopic sections.

RESULTS

Tables I and II correlate the LI and light microscopic histology with the ultrastructure of the capillary loops in the sequential biopsies. Table II also indicates the times at which biopsies were performed relative to the third dose of methotrexate given each week.

In subject 1 whose thigh plaque was treated with topical halcinonide cream, the LI fell to 1.7% (normal <4.5%) 48 h after therapy had begun (Table I). At 24 and 48 h, the granular cell layer and orthokeratosis began to appear in the biopsy specimens. By 72 h most of the epidermis exhibited these normal histologic features, and there were only small foci of parakeratosis and an absent granular zone. Acanthosis was still moderate. At 96 h the epidermis was histologically normal

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Abbreviations:
LI: labeling index

except for mild acanthosis. During this 4-day sequence, the capillary loops which exhibited the venous capillary ultrastructure characteristic of psoriasis did not show any change or any reversion toward normal.

In subject 2 whose chest lesion was treated with triamcinolone acetonide cream, the LI fell to 2.7% at 96 h and remained between 1–2% for the rest of the 16-day experimental period (Table I). Serial biopsies showed the focal appearance of orthokeratosis and a granular zone at 24 h. At 48 h, most of the epidermis was orthokeratotic and had a granular zone. Parakeratosis and an absent granular zone were present in only a few small foci. At 96 h the epidermis was histologically normal except for moderate acanthosis. At 7 days and 16 days the acanthosis had decreased but was still present to a mild degree. The venous capillary ultrastructure of the capillary loops did not change or revert toward normal during this 16-day period.

In subject 3 treated with methotrexate, the LI fell to 4.2% 7 days after the drug began to be administered weekly (Table II). Foci of orthokeratosis and granular zone regneration began to appear at day 7 and became predominant at day 11. On day 14, the histology of the epidermis was normal except for moderate acanthosis. However on day 17 the epidermis showed a few focal areas where the granular cell zone was missing. The acanthosis was still moderate. The capillary loops retained their venous capillary ultrastructure, without any signs of reversion toward normal during the 17 days of the experiment. In addition the endothelial cells in the microvessels of the papillary dermis showed a constant, high degree of labeling with tritiated thymidine throughout the experimental period.

Subject 4 responded very slowly to oral methotrexate. The weekly dose had to be increased from 7.5 mg to 25 mg over 109 days before the plaques became flat and were only minimally red (Table II). The initial LI ranged from 15.7–23.3% and fell to levels of 5.3–8.5% when she reached her maximum dose of 25 mg weekly. From days 15–36, after the dose had been increased from 7.5 to 15 mg weekly, the biopsies showed focal areas of orthokeratosis and granular cell zone regeneration. After 3 weeks of therapy with the 25 mg dose (days 64–109) the epidermis became normal (Nl) or nearly so (Nl/Ps) except for the persistence of moderate acanthosis. The ultrastructure of the capillary loops did not revert toward normal and the endothelial cells continually showed a high level of labeling.

In all 4 subjects, the psoriatic plaques first lost their scale and then began to flatten and become less erythematous. The loss of scale was correlated with the development of orthokeratosis and the reappearance of a granular layer. The experiments were discontinued when the plaque under study became a faintly pink macular patch devoid of scale.

DISCUSSION

In all 4 subjects, the onset of clinical improvement correlated with normalization of the psoriatic epidermis and return of the LI to normal without a concomitant normalization of the capillary loops. In addition, in the 2 subjects treated with methotrexate the microvascular endothelial cells remained highly labeled throughout the experimental periods even though the epidermis returned to normal (except for slight to moderate acanthosis). Many other investigators who have studied the histologic response of psoriatic lesions to oral, intralesional and topical steroids [2–6], methotrexate [7,8], dithranol [9], coal tar [7] and ultraviolet radiation [9] have also emphasized that the first observable histologic changes in psoriatic lesions that can be correlated with clinical improvement are the reappearance of the granular layer and the development of

TABLE I. *Correlation of labeling index, histology, and ultrastructure of capillary loops in sequential biopsies of patients treated with topical steroids*

Subject	Day										
	0	1	2	3	4	7	8	11	14	15	16
1											
LI (%)	6.3	8.0	1.7	1.9	1.0						
Histo	Ps	Ps/Nl	Ps/Nl	Nl/Ps	Nl						
EM	V.C.	V.C.	V.C.	V.C.	V.C.						
2											
LI	17.6	11.0	6.7		2.7	2.0					1.0
Histo	Ps	Ps/Nl	Nl/Ps		Nl	Nl					Nl
EM	V.C.	V.C.	V.C.		V.C.	V.C.					V.C.

Abbreviations: LI = labeling index in percent; Histo = light microscopic histology; Ps = psoriatic histologic features; Nl = normal histologic features; EM = ultrastructure of loop; V.C. = venous capillary

TABLE II. *Correlation of labeling index, histology, and ultrastructure of capillary loops in sequential biopsies of patients treated with oral methotrexate*

Subject	Day																
	0	3	7	8	11	14	15	17	22	29	36	43	50	57	64	71	109
3																	
LI	8.6	14.0	4.2		4.2	5.4		3.3									
Histo	Ps	Ps	Ps/Nl		Nl/Ps	Nl		Nl/Ps									
EM	V.C.	V.C.	V.C.		V.C.	V.C.		V.C.									
Interval	28	0.5	4.5		2.5	5.5		1.5									
MTX		15	15		15	15		15									
4																	
LI	21.5	15.7		23.3			10.1		8.2	4.9	12.8	5.6	8.5	7.8	7.3	18.0	5.3
Histo	Ps	Ps		Ps			Ps/Nl		Ps/Nl	Ps/Nl	Ps/Nl	Ps/Nl	Ps/Nl	Ps/Nl	Nl	Nl/Ps	Nl
EM	V.C.	V.C.		V.C.			V.C.		V.C.	V.C.	V.C.	V.C.	V.C.	V.C.	V.C.	V.C.	V.C.
Interval		1.5		7			7		7	7	7	7	7	7	8	7	7
MTX		7.5		7.5			15		15	15	15	25	25	25	25	25	25

Abbreviations: LI = labeling index in percent; Histo = light microscopic histology; PS = psoriatic histologic features; Nl = normal histologic features; EM = ultrastructure of loop; V.C. = venous capillary; Interval = interval in days between last dose of methotrexate and biopsy; MTX = dose of methotrexate (mg/week; given in 3 12-h doses)

orthokeratosis within 24–120 h [2–4,7]. The regression of psoriatic acanthosis and papillomatosis appears to be a secondary phenomenon taking place after normalization of epidermal differentiation [9]. Acanthosis can still be found 4 weeks after the skin looks normal [6]. The above studies also confirm our ultrastructural observations on the blood vessels. In studies using steroids and methotrexate, the blood vessels have remained abnormal for up to 5 months as judged by their persistent dilatation and tortuosity [3,7,8]. Since we did not continue the experiments until the psoriatic lesions had *completely* disappeared leaving behind normal-appearing skin, we do not know whether the capillary loops would have eventually returned to a normal arterial capillary ultrastructure or whether the endothelial cell labeling would have decreased to a normal level. Nevertheless our studies and the previous studies cited above seem to indicate that the initial signs of clinical improvement following therapy with topical steroids and oral methotrexate are associated with histologic normalization of the epidermis without any observable effect on the microvasculature.

Our previous studies showed that PUVA and Goeckerman treatments restored both the microvasculature and epidermis to normal [1]. The vessels began to return to normal 3–8 days before the LI did and the appearance of orthokeratosis and granular layer regeneration coincided with the beginning of capillary loop normalization. We had hypothesized that the therapeutic success of PUVA and Goeckerman therapies was related to the inhibition of endothelial cell proliferation which therapy deprived proliferating basal cells of an adequate blood supply necessary to support epidermal hyperplasia. In these current experiments with halcinonide and triamcinalone creams and oral methotrexate, the initial clinical improvement appears to have been associated with a normalization of the epidermis without a concomitant morphologic improvement in the microvasculature. These observations supported by the other reports cited above raise the possibility that the clinical relapses in psoriasis which may promptly follow discontinuation of topical steroid therapy and methotrexate and the less frequent sustained remissions following discontinuation of methotrexate therapy may be related to an inability of these drugs to restore the microvasculature to normal in such situations. A reduction of the LI may be all that is necessary to allow normal epidermal differentiation and temporary healing of psoriatic lesions as suggested by Fry and McMinn [8]. In our previous studies [1] the normal-appearing skin in 2 psoriatic patients exhibited an elevated LI, normal epidermal histology except for focal basal cell hyperplasia, and venous capillary loops in the dermal papillae. Thus, it is possible that whenever the temporary restraining influences of topical steroids or methotrexate are removed, the microvasculature would be ready to provide the nutritional support for an epidermis ready to proliferate. The venous capillaries are able to provide the necessary nutrients in sufficient amounts through a highly developed system of bridged fenestrations in the endothelial cells. The endothelial cells of arterial capillaries lack these structures.

Fry and McMinn believed that the steroid-induced normalization of epidermal histology was independent of direct mitotic inhibition of the basal cells [2]. Our data also show that the granular cell layer begins to reappear at 24 h while the LI is still elevated. Thus the possibility that topical steroids have an action other than, or in addition to, direct mitotic inhibition needs to be considered. Although there may be a pharmacologic effect of topical steroids on basal cells that produces an inhibition of thymidine uptake, this would not explain why there is such a prompt return of the granular layer with overlying orthokeratosis while the LI is still elevated. Since topical steroids produce vasoconstriction, it is conceivable that a decreased blood flow resulting in less nutrients being supplied to the epidermis might play a role in initiating epidermal normalization. We would not be able to detect such a mechanism with these morphologic studies.

The present experiments provide additional evidence that in the development of therapy for psoriasis one needs to consider the contribution of the microcirculation to the development and regression of the psoriatic lesion.

REFERENCES

1. Braverman IM, Sibley J: Role of the microcirculation in the treatment and pathogenesis of psoriasis. J Invest Dermatol 78:12–17, 1982
2. Fry L, McMinn RMH: The action of chemotherapeutic agents on psoriatic epidermis. Br J Dermatol 80:373–383, 1968
3. Komisaruk E, Kosek JC, Schuster DS: Histology of psoriasis injected with triamcinolone. Arch Dermatol 86:422–425, 1962
4. Braun-Falco O: Histologische und histochemische Veränderungen in Psoriasisherden unter enteraler Triamcinolon-Behandlung. Acta Histochem 8:350–370, 1959
5. Freedman RI, Reed WB, Becker SW: Effect of local corticosteroids on psoriasis. A histopathologic study. Arch Dermatol 87:701–705, 1963
6. Frank L, Steiner K, Bender B, Winston M: Flurandrenolone and occlusion in psoriasis. Arch Dermatol 89:404–410, 1964
7. Van Scott EJ, Reinertson RP: Morphologic and physiologic effects of chemotherapeutic agents in psoriasis. J Invest Dermatol 33:357–369, 1959
8. Fry L, McMinn RMH: Action of methotrexate on skin and intestinal epithelium in psoriasis. Arch Dermatol 93:726–730, 1966
9. Braun-Falco O: Dynamics of growth and regression in psoriatic lesions: alterations in the skin from normal into a psoriatic lesion, and during regression of psoriatic lesions, in Psoriasis. Proceedings of the International Symposium, Stanford University, 1971. Edited by EM Farber, AJ Cox, Stanford, Stanford Univ Press, 1971, pp 215–237