

The Effect of Fumaric Acid Esters and Dithranol on Acanthosis and Hyperproliferation in Psoriasis Vulgaris

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In this study we investigated the histological changes, regression of acanthosis and rate of proliferation, that accompany the healing of psoriatic lesions after fumaric acid esters and dithranol treatment. Biopsies were taken before and during therapy as well as from neighbouring untreated, clinically uninvolved skin and healthy, non-psoriatic volunteers. Specimens were assessed using computer-supported image analysis and immunohistology. The parameters primarily examined were the height of the rete pegs and of the epithelium above the papillary body, the rate of proliferation, the actual number of cells in the two epidermal compartments and the cellular density in the epidermis. Both fumaric acid esters and dithranol reduce the degree of acanthosis; however, the mechanism and the rate of the reduction differ. While under fumaric acid esters the reduction is more rapid at first but subsequently slows down, dithranol leads to a slow but steady decrease of epidermal thickness, so that at the end of our study the degree of acanthosis was less under dithranol. As an underlying mechanism of action, we found that fumaric acid esters reduce the rate of proliferation and thereby decrease the number of cells per rete peg as well as the size of the individual keratinocytes. Dithranol in contrast does not reduce cell renewal. The decrease of the number of cells in the rete pegs might be caused by an increased differentiation time. *Key words: histometry; image analysis; proliferation rate; cell size.*

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Histologically, the psoriatic plaque is characterised by an increased height of the rete pegs, whilst the epidermis above the papillary bodies is not thickened to the same extent. Statistical analysis has shown that when healthy, non-psoriatic controls are compared with each other the epidermis above the papillary bodies increases to the same extent as does the epidermis in the area of the rete pegs (1). In contrast, the interindividual variation in active psoriasis is such that the epidermis above the papillary bodies increases markedly more slowly as compared to the epidermis in the area of the rete pegs. In psoriasis, the dependence of minimal epidermal thickness (epmin) on maximal epidermal thickness (epmax) is lost.

The aim of this study was to investigate the histological changes that accompany the healing of psoriatic plaques under fumaric acid esters (FAEs) and dithranol (D), respectively. The mechanism of action of FAEs is thought to be either immunosuppressive or via immunomodulation similar to that of cyclosporine, etretinate or corticosteroids (2). The very first effect of systemic therapy with FAEs is the disappearance of CD 15-positive cells in and beneath the epidermis, accompanied by a significant reduction in T-helper cells beneath the epidermis (3). However, a primary effect on the epidermis via

inhibition of the rate of mitosis and proliferation, as for methotrexate, is also being discussed (4). Dithranol in contrast is thought to act by inhibition of enzyme metabolism or reduction of mitosis and DNA synthesis (5).

MATERIAL AND METHODS

Patients and biopsies

From 1989–1994, 187 psoriatic patients attending our outpatient clinic, as well as inpatients, were recruited for a prospective histological study. Punch biopsies were taken, which were divided into the groups shown in Table I. Patients did not receive any treatment for 1–2 weeks prior to the start of the study. Patients in group 2 were treated with increasing doses of a mixture of dimethylfumarate and monoethylhydrogen-fumarate. In the first week a daily dose of 105 mg of the ester mixture was given, in the second week 210 mg per day up to a maximum of 1,290 mg ester per day, according to the scheme published by some of us (6). Patients in group 3 were treated with increasing concentrations of dithranol, beginning with a 0.03 % preparation in vaseline album. Depending on skin acceptance the concentration of dithranol was increased up to 2% in the fourth week. Every patient was biopsied prior to or during the course of treatment. Biopsies were fixed in formalin 5% for 12 h and embedded in paraffin.

Groups 2 and 3 were subdivided according to the duration of therapy (Table II). We calculated the mean values for all specimens taken up to and including day 14 (F14, D14) and those taken after day 14 until the end of the study, which was day 56 for group 2 (F56) and day 42 for group 3 (D42).

Computer-supported image analysis

All 371 paraffin-embedded specimens were assessed by means of image analysis (17). From each biopsy at least 10 serial sections were obtained. Measurements were performed at two different locations of the 7 µm thick paraffin sections. In this study we examined epmax and epmin (Fig. 1).

Specimens were evaluated by a Laboval (Zeiss) light microscope equipped with a CD-camera connected to an IP-8/AT matrix frame grabber board within an IBM-compatible 486 PC/AT. We used the image analysis program analySIS® (Soft-Imaging Software GmbH). The parameters were assessed by manual outlining of the structures of interest on the digitalized microscopic image displayed on the monitor. Calibration of the video input channel in analySIS® allowed us to measure all distances in their actual length in micrometers. The measured values were further processed and displayed using the program Excel and MS-Windows®. The parameters of group 1 were sorted according to decreasing epmax and location.

Table I. Number of biopsies from patients in groups 1–5, n = 371

Group 1: fully developed lesions prior to therapy	158
Group 2: lesions under therapy with FAE	67
Group 3: lesions under therapy with dithranol	106
Group 4: neighbouring clinically healthy skin from psoriatic patients	18
Group 5: healthy skin from healthy volunteers	22

Table II. Number of biopsies in groups 2 and 3, n=173

Time (days)	0	2	5	10	14	F/D 14	21	24	28	42	56	F56 D42
Group 2		9	1	4	11	25	6		13	2	21	42
Group 3		11			24	35	39	9	17	6		71

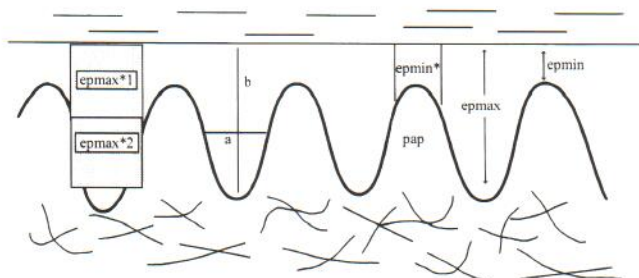


Fig. 1. Parameters assessed by computer-supported image analysis and immunohistology. *epmin* = minimal epidermal thickness; *epmax* = maximal epidermal thickness; *epmax*1* = area of rete pegs in upper part of epidermis, maximal 250 × 250 μm; *epmax*2* = area of rete pegs in lower part of epidermis, maximal 250 × 250 μm; *epmin** = area of epidermis above papillary body; *pap* = papillary body; *a* = width of rete pegs; *b* = total height of rete pegs.

Immunohistology

Additional to the histometric measurements, 84 specimens were stained using the APAAP method with the MIB 1 antibody, detecting the Ki 67 antigen which marks the proliferating cells (in G1-, G2-, M-phase).

The labelled sections were counted semiquantitatively (18), using an eyepiece with a grating that allowed counting of a defined area of maximally 250 μm × 250 μm. In acanthotic epidermis the area of *epmax* was subdivided into an upper (*epmax*1*) and lower (*epmax*2*) part. When the degree of acanthosis was less than or equal to 250 μm there was no *epmax*2* (Fig. 1).

After determining the total number of cells as well as the number of MIB 1 positive cells per 100,000 μm², we calculated the rate of proliferation in an area of 100,000 μm² as positive cells/total number of cells × 100. Information about the size of the cells was derived indirectly from the number of cells per 100,000 μm².

Statistical analysis

The proportions of the parameters were compared by means of the Wilcoxon Mann-Whitney U-test for independent samples (7). A significance level of α=0.05 was specified, unless stated otherwise. Since the measurements had been performed at several different points of time, sample sizes for the individual points of time were often very small. The sample size had to be at least 4.

RESULTS

Active psoriatic plaques

Looking at *epmax* and *epmin* in untreated psoriatic lesions (group 1), we found that the *epmax* values of this group were significantly higher than those of group 4 (neighbouring clinically healthy skin) and 5 (healthy skin from healthy volunteers). In contrast, *epmin* values of group 1 were significantly higher than those of group 5 but did not vary significantly from those of group 4.

Concerning the localization the mean value of *epmax* was highest on the back and decreased in the order upper arm, abdomen, lower leg, lower arm, thigh. We observed a large

variance in *epmax* when looking at the biopsies of active psoriasis (group 1) as a whole (Fig. 2) but also when looking at biopsies from the same location. Independent of the location of the biopsy, the height of *epmax* correlated well with *epmin*: *epmin* increased with increasing *epmax*, but, not to the same extent.

Psoriasis under therapy

In the group treated with FAEs, the mean *epmax* measured 120.73 (±27.75) μm at the beginning of the therapy. In contrast, the group treated with dithranol showed a mean *epmax* of 320.75 (±106.82) μm.

Therapy either with FAEs or with dithranol initially caused an increase of *epmax*. Under FAEs *epmax* increased to 389.8 (±116.32 μm) on day 21 in order to drop to 218.29 (±63.07 μm) on day 56 (Fig. 3).

Comparing the values of day 2 with those of day 56, there

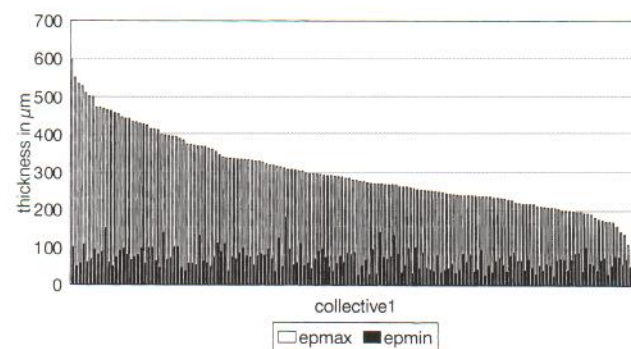


Fig. 2. Epmax and epmin in group 1.

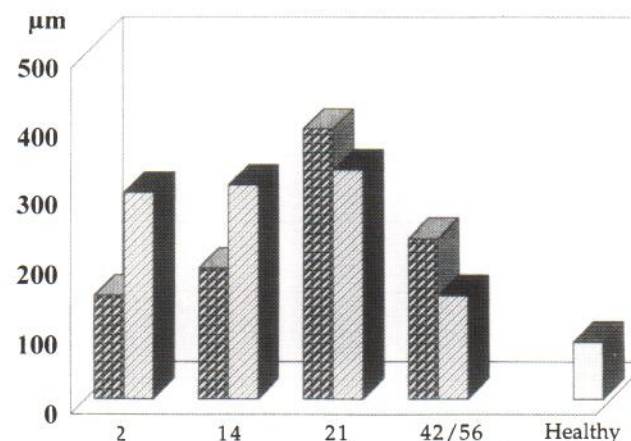


Fig. 3. Epmax (mean values) in patients under FAEs and dithranol in comparison to healthy controls at 2, 14, 21 and 42/56 days, respectively.

was a statistically significant ($p=0.0001$) increase in epmax from the start to the end of observation period.

Under therapy with dithranol (group 3) the mean epmax increased slightly ($380 \pm 102.93 \mu\text{m}$) on day 21 after which it steadily decreased to its lowest value on day 42 ($118.43 \pm 56.13 \mu\text{m}$) (Fig. 3). When comparing the values of day 2 with those of day 42, there was a statistically significant ($p=0.0018$) reduction in epmax.

A reduction down to the value of neighbouring clinically healthy skin (group 4) could be measured only in group 3 (dithranol). The value of healthy skin (group 5) was not reached under either therapy.

Total number of cells in epmax and epmin**

In the active psoriatic plaque, a rete peg (epmax*) contains up to 384 keratinocytes with a mean of 178.9 keratinocytes, while the neighbouring uninvolved skin contains only 71 cells per rete peg. In healthy skin from healthy volunteers (group 5) we found 33.3 cells per rete peg (epmax*).

Under FAEs a slight decrease of the number of keratinocytes per rete peg could be observed in the course of therapy, from a mean of 214 keratinocytes in epmax* in the first two weeks (F14) to 158.4 afterwards (F56).

Under dithranol the number of keratinocytes in epmax* decreased from 310.3 cells per rete peg (D14) to 209.4 cells per rete peg (D42).

Size of keratinocytes in epmax and epmin* under therapy*

In healthy skin from healthy volunteers (group 5) a single keratinocyte of the rete peg has a size of $82.6 \mu\text{m}^2$ (Fig. 4).

In neighbouring clinically uninvolved skin from psoriatic patients (group 4) the cell size of a single keratinocyte in the rete peg measures $192 \mu\text{m}^2$; in the active psoriatic lesion, in contrast a single keratinocyte takes up $206.5 \mu\text{m}^2$ (Fig. 4).

Similarly, in epmin* the cell size in healthy skin from healthy volunteers (group 5) was markedly smaller than that of psoriatic skin: $82.3 \mu\text{m}^2$ in healthy skin in contrast to $164.8 \mu\text{m}^2$ in psoriatic skin. In neighbouring skin a single keratinocyte takes up about $124.2 \mu\text{m}^2$.

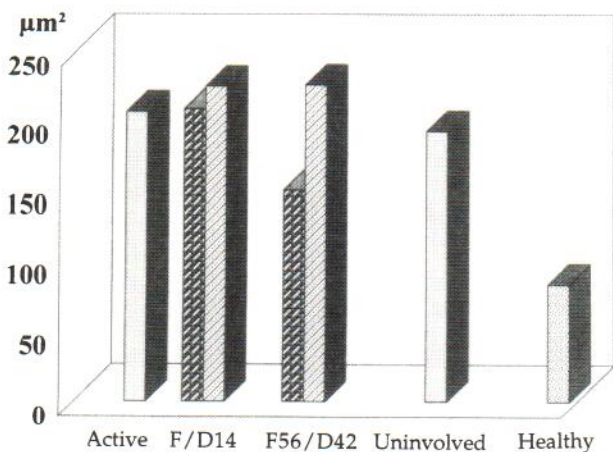


Fig. 4. Size of keratinocytes in epmax* in patients under FAEs at 14 and 56 days, and dithranol at 14 and 42 days in comparison to active psoriatic plaques, uninvolved skin of psoriatic and healthy controls.

In group 2 the cell size in the rete peg (epmax*) diminished from initially $208.1 \mu\text{m}^2$ (F14) to $149 \mu\text{m}^2$ on day 56 (Fig. 4). This reduction of cell size was not significant from F14 to F56.

In group 3 the size of keratinocytes in epmax* hardly changed from D14 with $224.5 \mu\text{m}^2$ to D42 with $225.6 \mu\text{m}^2$ (Fig. 4). In epmin* the size of keratinocytes showed no significant difference either under FAEs or under dithranol.

Rate of proliferation per 100,000 μm^2 in epmax and epmin**

In epmax* the rate of proliferation was highest in active psoriasis, 14.9%, while that for uninvolved neighbouring skin and healthy skin was lower, 4.9% and 5.5%, respectively (Fig. 5). Proliferation was extremely high in the lower part of the rete pegs (epmax*2) of group 1 with a rate of 29.5%. In epmin* the proliferation rate was 3.4% for group 1, 1.3% for group 4 and 5.1% for group 5.

In group 2 the rate of proliferation decreased from 28.9% at F14 to 6.8% at F56 (Fig. 5). This decrease was significant by $\alpha=0.1$, but not significant by $\alpha=0.002$.

In group 3 it remained nearly the same for D14 with 12.7% to D42 with 14.9%, without any statistical significance (Fig. 5). The high value for D14, however, obscures the fact that on day 2 the rate of proliferation in our small group was only 6.9%, with the rate of proliferation increasing under therapy.

DISCUSSION

Only a few authors have studied the cell density in human epidermis in dependence of the degree of acanthosis (9, 10). Pinkus (9) as well as Weinstein et al. (10) observed that the size of the single keratinocyte increases with increasing acanthosis. Indeed, in our study the individual cells increased in size with increasing acanthosis. Acanthosis in the psoriatic plaque is therefore not only due to an increase in the number of cells but also due to an increase in the size of the cells. Acanthosis only takes place in the region of the rete pegs (1, 11). Regarding the epidermal thickness in the area above the papillary body, there is only a slight difference between the epmin of active lesions and uninvolved skin. In the literature,

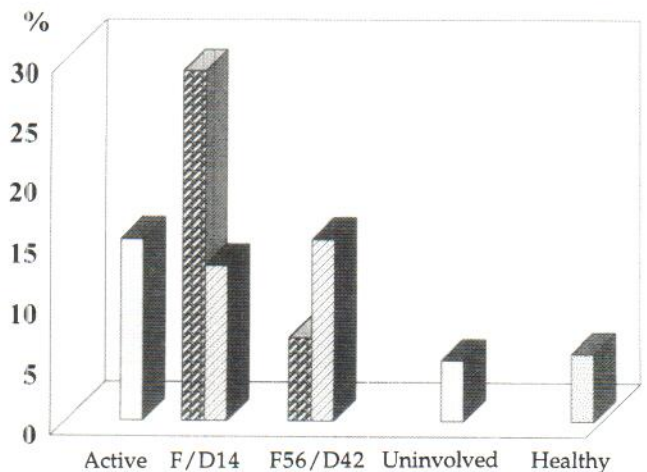


Fig. 5. Rate of proliferation per 100,000 μm^2 in % in epmax* in patients under FAEs at 14 and 56 days, and dithranol at 14 and 42 days in comparison to active psoriatic plaques, uninvolved skin of psoriatic and healthy controls.

reflecting the diversity of results (8, 12), varying values can be found.

Different therapeutic agents cause different effects on psoriatic epidermis. We found that FAEs reduce the size of keratinocytes and the cell renewal, while the number of keratinocytes per rete peg is not reduced to the same extent. Under therapy with dithranol in contrast, neither the cell size nor the proliferation rate really change, while the total number of keratinocytes per rete peg decreases, so that after therapy for at least 42 days the height of the rete pegs has normalized.

The rate of proliferation in an epithelium correlates with the degree of acanthosis (8, 13, 14). Nevertheless, one must consider the possibility of a time lag between the increased rate of proliferation and the formation of acanthosis. After reaching the maximal degree of acanthosis, the rate of proliferation returns to its normal value (15). In chronic stationary plaques the rate of proliferation before therapy is only slightly higher than in healthy skin. The rate of proliferation reflects the proportion between positively stained cells and the total number of cells. The extreme decrease of the proliferation rate under FAE is merely due to the increase of the total number of cells per 100,000 μm^2 .

Under FAEs acanthosis initially regresses more quickly than under dithranol. FAEs work by decreasing the proliferation of keratinocytes and thus the number of cells per rete peg. Thus, regression of acanthosis under FAEs is caused by a combination of the reduction of number and size of cells.

Under dithranol the rate of proliferation does not change, except for a short temporary increase. The size of cells is unchanged and the reduction of acanthosis is solely caused by a decrease in the number of cells per rete peg. This decrease in the number of cells per rete peg under dithranol must be due to an increased scaling of the epidermis, which in turn might be related to an increase in cell differentiation under dithranol. Clinically, we observed an irritation with a visible desquamation under dithranol.

In order to shed more light on the mechanisms of anti-psoriatic therapy, further studies investigating the degree of acanthosis, rate of proliferation and size of keratinocytes need to be performed.

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