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Fumaric Acid Esters (FAEs) Suppress CD 15- and ODP 4-positive Cells in Psoriasis

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A histological-immunohistological study was conducted to investigate the effect of systemically administered fumaric acid esters (FAEs) on epidermal thickness and composition of the inflammatory infiltrate in psoriatic plaques. 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, pointing to an immunosuppressive effect. This is followed after some delay by a reduction in acanthosis and hyperkeratosis. The reduction in infiltrating T-lymphocytes corresponds to that seen after systemic or intralesional therapy with cyclosporin. However, the normalization of the psoriatic plaques takes longer under the influence of FAEs than under cyclosporin.

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The clinical effects of fumaric acid esters (FAEs) on psoriasis were initially described in 1959 (1). Therapy with FAEs was developed further by the physician Schäfer (2, 3, 4). In the subsequent years FAEs were used effectively to treat psoriasis vulgaris at various clinical centres (5, 6, 7, 8). The effectiveness of FAEs was recently demonstrated again in a double-blind placebo-controlled study in 100 patients, carried out at five German hospitals (14). The toxic side effects referred to in some publications (9, 10, 11, 12, 13) were kept to a minimum by regular monitoring of laboratory parameters.

The mechanism of action of FAEs is not yet fully understood. We therefore conducted a histological-immunohistological study to examine the influence of systemically administered FAEs on the epidermis and the inflammatory infiltrate.

MATERIALS AND METHOD

In 33 patients suffering from severe psoriasis vulgaris (Table I) the histology of the psoriatic plaques was monitored during therapy with increasing dosages of FAEs. The drug consisted of a mixture of dimethylfumarate and monoethylhydrogenfumarate. It was available in two different enteric coated formulations: as a low-strength tablet containing 105 mg of ester mixture (30 mg dimethylfumarate/75 mg monoethylfumarate) and as a forte tablet containing 215 mg of ester mixture (120 mg dimethylfumarate/95 mg monoethylfumarate). The increase in dosage from week to week is shown in Fig. 1.

The biopsies were taken from comparable plaques on the trunk, measuring about 1 to 3 cm in diameter.

33 biopsies taken at the times shown in Table I, were fixed for 12 h in 5% formaldehyde, embedded in paraffin wax and used to assess the following histological criteria: parakeratosis, presence of stratum granulosum, acanthosis, spongiosis.

The intra-epidermal and dermal infiltrates were analysed immunohistologically. Table II shows the antibodies used and their optimal dilutions. The labelled sections (thickness 7 μm) were evaluated semiquantitatively (15, 16, 17) by counting the number of positive-labelled cells per total cell count in a defined area of 0.0324 mm^2 in each section. The infiltrate cells were counted at the intra-epidermal and subepidermal levels and in the mid-dermis in the way shown in Fig. 2, always at the site of densest infiltration. The percentages of positive-labelled cells per area were used to compare the composition of the infiltrate at the different times during treatment.

Statistical analysis

The proportions of stained cells were compared in the Wilcoxon-Mann-Whitney-U-test for independent samples. A significance level of $\alpha = 0.05$ was specified.

RESULTS

Histology

In the course of treatment with FAE the histological changes in psoriasis such as focal parakeratosis, hyperkeratosis, circumscribed spongiosis, mononuclear and granulocytic infiltration in the epidermal layer, focal loss of the stratum granulosum, and subepidermal mononuclear infiltration diminished. Two weeks after initiating treatment, no granulocytes were found at either the intra- or the subepidermal level.

In the sixth week of treatment, parakeratosis was seen in only a few cases. Stratum granulosum was still lacking in 20% of the tissue samples. Subepidermal mononuclear infiltration was present in all samples.

In the eighth week of treatment there was almost complete normalization of the parakeratosis, hyperkeratosis and the stratum granulosum. Focal spongiosis was present in 20% of the biopsies. The subepidermal mononuclear infiltration persisted in 80% of the cases.

Immunohistology

Before treatment, the intra- and subepidermal infiltrates consisted mainly of T-lymphocytes.

The intra-epidermal infiltrate consisted of 26.4% ODP 4-posi-

Table I. *Patient population, classified according to tissue embedding, sex and biopsy-sampling times (weeks 0, 2, 4, 6 and 8)*

	n	Female	Male	Age (in years)	Psoriasis since (in years)
Total	33	16	17	26–75	0.5–40
Week					
0	8	4	4	26–74	0.5–26
2	6	2	4	27–74	1.5–40
4	6	4	2	28–70	0.5–23
6	6	3	3	36–75	0.5–15
8	7	3	4	35–54	2.0–11

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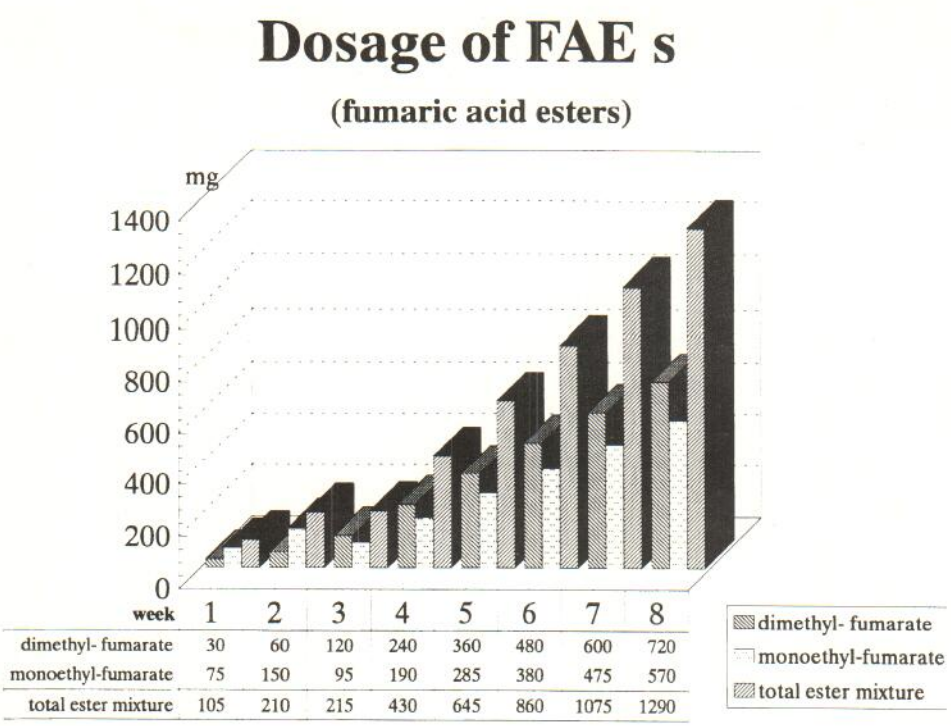


Fig. 1. Dosage schedule of fumaric acid esters from week 0 to week 8.

tive cells, 28.6% CD 45 RO-positive cells, 13% CD45R-positive cells, 23.4% CD 15-positive cells and 12.3% myeloperoxidase-positive cells. 13.3% of the cells stained positive with the HLA-DQ antigen. In the subepidermal infiltrate the total cell count consisted of 38.4% ODP 4-positive cells, 28.9% CD 45 RO-positive cells, 29.6% CD 45 R, 5.2% CD 15- and 1.6% myeloperoxidase-positive cells.

During therapy the intra-epidermal infiltrate diminished by 74.4% on the whole, with a significant decrease ($p = 0.05$) from week 0 to week 2 and a highly significant decrease ($p = 0.001$) from week 0 to week 8. Intra-epidermal ODP4-positive cells were reduced by 51.2%.

The subepidermal infiltrate decreased by 52.1% (Fig. 3), but due to the high standard deviation this decrease was not significant either between week 0 and week 2 or between week 0 and week 8. The number of ODP 4-positive cells at the subepidermal level decreased by 84.4% within the first eight weeks of treatment (Fig. 3). This reduction was even significant ($p < 0.05$) between week 0 and week 2.

CD 15-positive cells (granulocytes) which accounted before treatment for 23.4% of the intra-epidermal infiltrate, 5.2% at the immediately subepidermal level and 3.2% of the infiltrate deeper in the dermis, had disappeared completely at all localizations and in all sections by the second week (Fig. 3).

The proportion of CD 45 RO-positive cells showed a discrete increase from 28.6% to 33.0% at the intra-epidermal level and from 28.9% to 33.6% at the subepidermal level. Deeper in the dermis a reduction from 20.3% to 11.9% was observed. There was a slight increase in CD 45 R-positive cells during the treatment with fumaric acid esters. The percentages of HLA-DQ-positive, Ki 1-antigen-positive and myeloperoxidase-positive cells were largely unaffected by the treatment.

Table II. *List of antibodies, their specificity and optimal dilution, and suppliers*

Antibody	Identified cells	Optimal dilution	Supplier
ODP4	T-helper cells	1:50	Dako
CD45RO UCHL 1	activated T-cells	1:200	Dako
HLA-DQ	activated cells	1:10	Becton-Dickinson
CD15	mature granulocytes	1:50	Dako
CD45R	B-cells	1:100	Dako
Ki1	Hodgin cells	1:50	Dako
myeloperoxidase	granulocytes	1:200	Dako

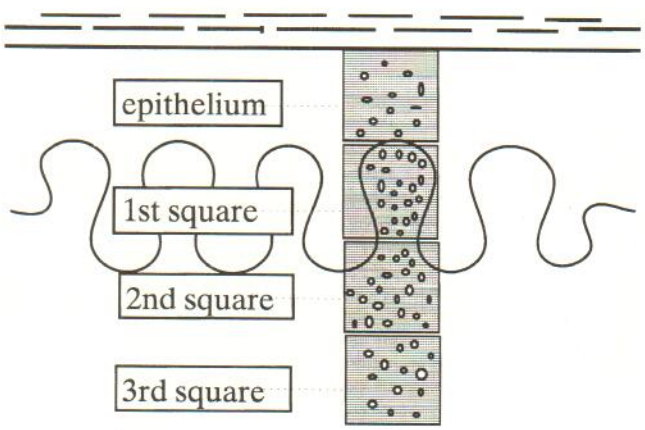
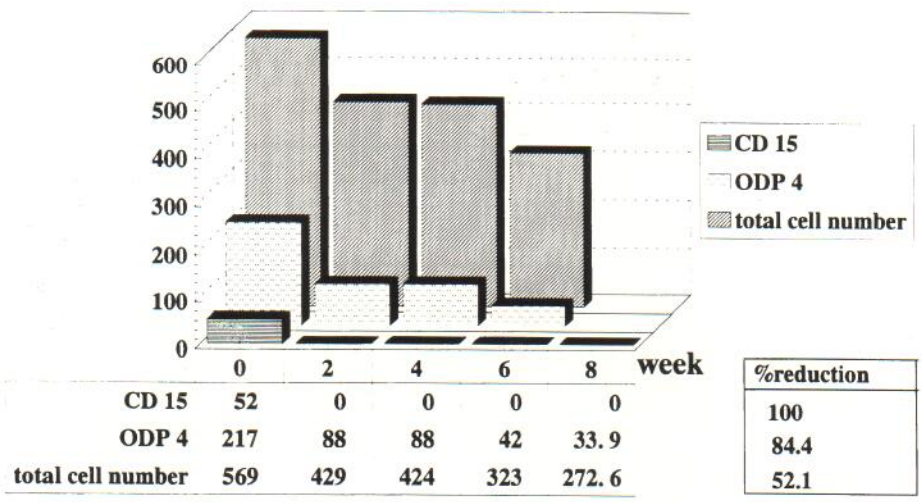


Fig. 2. System for the evaluation of labelled infiltrate cells in the epithelium and the subepidermal areas in squares measuring 0.90 μm × 0.90 μm.

Fig. 3. Number of CD 15- and ODP4-positive cells in comparison with the total cell number in an area 270 μm beneath the epidermis.



	0	2	4	6	8	%reduction
CD 15	52	0	0	0	0	100
ODP 4	217	88	88	42	33. 9	84.4
total cell number	569	429	424	323	272. 6	52.1

DISCUSSION

During therapy with FAEs, psoriatic lesions heal during a period of 6–8 weeks (14). The very first effect of systemic therapy with FAEs is disappearance of the intra-epidermal infiltrate.

This is followed with some delay by a decrease in acanthosis and hyperkeratosis, while mononuclear infiltrates beneath the epidermis diminish slowly up to week 6–8.

Looking at the individual fractions of the infiltrate, the first and most striking changes are observed with respect to the granulocytes. They disappeared completely in the epidermis and the upper dermis only 2 weeks after starting therapy.

Parallel with the disappearance of the granulocytes the ODP 4-positive cells (T-helper cells) exhibit a significant reduction from week 0 to 2, while surprisingly the decrease in the total cell count is not significant at either time point.

The histological and immunohistological changes during therapy with fumaric acid esters point to an inhibitory effect on the granulocytes (CD 15-positive cells) in the first few days after starting treatment. There is also a disproportionately large reduction in the ODP 4-positive cells in comparison with the total cell count in the subepidermal infiltrate during the first 2 weeks. These results suggest an immunosuppressive effect of fumaric acid esters. Nieboer noted lymphopenia, especially a decrease in suppressor cells, and leukopenia during FAE treatment (6).

Hagedorn et al. (18) and Petres et al. (19) were able to demonstrate a dose-dependent reduction in the incorporation of [^{14}C]thymidine into the DNA of human lymphocytes. The authors discussed the influence of FAEs on the enzymes of nucleic acid synthesis, the citric acid cycle, or a defective synthesis of enzymes (19). Kuroda et al. reported that FAEs inhibit the growth of Ehrlich tumour cells in vivo (20), while mouse and chick embryo cells were resistant to fumaric acid (21).

The reduction in infiltrating T-lymphocytes corresponds to that produced by other systemic antipsoriatic drugs. A reduction in both T-helper cells (CD 4) and T-suppressor cells (CD 8) in psoriatic lesions is described during systemic or intralesional therapy with cyclosporin (22, 23). During intralesional therapy with cyclosporin, Baker et al. (24) found a significant reduction in CD 4-positive cells as well as in CD 8-positive cells in the epidermis within 12 days, while in the dermis only the reduction in CD 4-positive cells was significant. In the second week of treatment with FAEs, the T-helper cells in the tissue have decreased significantly. They then continue to fall, although no longer significantly, during the following weeks of treatment.

The reduction of granulocytes (CD 15) and T-helper cells (ODP4) during treatment with FAEs corresponds to the healing pattern found during intralesional or systemic treatment of psoriasis with cyclosporin (25).

There are, however, differences in the time course of healing of the psoriatic plaques. In contrast to therapy with cyclosporin, in our patients the reduction in the T-helper cells within the first 2 weeks did not correlate with the clinical healing of the lesion, which followed 4–6 weeks later, i.e. between the sixth and eighth week. The clinical healing of psoriasis under the influence of FAEs corresponds to the regression of epidermis hyperplasia and the extent of inflammatory subepidermal infiltrate.

FAEs induce exactly the same histological healing pattern as cyclosporin, although the time course differs considerably. Instead of hours or days, FAEs require weeks to achieve regression of a psoriatic lesion.

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