

## A Novel Dithranol Formulation (Micanol): The Effects of Monotherapy and UVB Combination Therapy on Epidermal Differentiation, Proliferation and Cutaneous Inflammation in Psoriasis Vulgaris

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Micanol, in which dithranol is micro-encapsulated in crystalline monoglycerides, is easy to wash off and staining and irritation are inconspicuous. These features make it appropriate to use in an out-patient setting. In this study the immunohistochemical effects of this new dithranol formulation were studied and compared with UVB and the combination of these therapies in skin biopsies of 8 patients with psoriasis. Markers for epidermal differentiation, proliferation and cutaneous inflammation were assessed. The present study suggests that Micanol predominantly had diminishing effects on inflammation markers, hardly affecting the epidermis. UVB had a broad spectrum of reductions. It is feasible that the combination resulted in various synergistic effects. Previous studies, however, revealed a relative persistence of the inflammatory infiltrate with more effects on epidermal processes following dithranol treatment. Based on the present study and on previous studies, it is hypothesised that Micanol delivers the active substance more directly in the dermal infiltrate, leaving the epidermis relatively unaffected. This might explain the low irritancy of Micanol treatment. *Key words: immunohistochemistry; crystalline monoglycerides; low irritancy; synergistic effects.*

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Topical treatment of psoriasis with dithranol, either or not combined with UVB phototherapy, is a safe and effective approach for those patients who do not respond to first line treatments like vitamin D<sub>3</sub> analogues and topical steroids. Short contact schedules and new wash-off formulations have popularised the dithranol treatment of psoriasis vulgaris. Recently a new principle was introduced: dithranol micro-encapsulated in crystalline monoglycerides (Micanol) (1). The advantage of this new dithranol formulation is that it is very easy to wash off. In view of the relatively high melting point of the formulation, the cream has to be massaged into the skin. In our centre, a three-way comparative study was carried out in 36 patients, showing that Micanol has substantial clinical efficacy without significant irritation and staining of the skin and the patients' environment. Further it was shown that the efficacy of dithranol in this formulation was comparable with UVB phototherapy; the combination of the two therapies tended to be more effective than either monotherapy (Gerritsen et al., manuscript in preparation).

The aim of the present study was to compare the immunohistochemical effects of dithranol in this new formulation with

the changes due to UVB phototherapy and the combination of Micanol and UVB. These effects were compared and contrasted with studies previously carried out on the effects of dithranol in other formulations. Before and at the end of treatment, punch biopsies were taken from a group of 8 patients who participated in a larger clinical study on the efficacy and safety of these treatments. Assessments were carried out of immunohistochemical stainings with monoclonal antibodies against transglutaminase, involucrin, filaggrin, Ki-67, T-lymphocytes and polymorphonuclear leukocytes (PMN).

### MATERIAL AND METHODS

#### Study design

Eight patients with extensive chronic plaque and/or guttate psoriasis were included in a partly open, partly double blind, placebo-controlled comparative study. The body of a patient was divided into two body-halves. Each body-half received one of the following treatments:

- (a) Micanol cream (Zyma SA, Switzerland) only.
- (b) Placebo cream combined with UVB.
- (c) Micanol cream combined with UVB.

The patients were treated during a maximum of 8 weeks. The UVB treatment was the open part of the study. UVB was given three times a week; short contact dithranol therapy was given daily. Clinical assessments were carried out three times a week in the first 2 weeks and once a week during the following 6 weeks.

#### Patients

The group of 8 patients consisted of 2 males and 6 females; their age varied from 24 to 65 years. These patients participated in a larger study on the clinical efficacy of Micanol, UVB phototherapy and the combination of UVB and Micanol. The psoriatic lesions were symmetrically distributed, chronic and in a reasonably stable phase. The percentage body involvement with psoriatic lesions was 5-35%. Apart from psoriasis, the patients did not have other dermatological or internal diseases. Concomitant treatment was maintained only when it was not expected to interfere with the test medications. Before study initiation, no local anti-psoriatic treatment had been administered for 2 weeks and the patients had not used systemic treatment for at least 4 weeks. Permission of the local Ethics Committee and written informed consent from all patients were obtained.

#### Treatments

*Dithranol/placebo treatment.* The Micanol cream (Zyma SA Switzerland) and the placebo cream were supplied in concentrations of 0.25%, 0.5%, 1%, 2% and 3%. The placebo cream consisted of the vehiculum of the Micanol without the active substance.

Daily applications of the dithranol and/or placebo cream were carried out on one or both halves of the body by the patient at home. The starting concentration was 0.25% for 30 min. The cream was subsequently removed with water and detergents. After 2 days the

dithranol concentration was increased if no stinging or burning occurred. At the highest dithranol concentration of 3% the application period was lengthened at each visit with 30 min up to a maximum of 120 min.

**UVB treatment.** All patients received UVB treatment on one or both body-halves, three times a week during 8 weeks. UVB exposure was carried out in a Waldman UV 1000 cabin (Waldman AG, Schweningen, Germany), equipped with 26 Voltarc USA F71 T12/2072 bulbs with an irradiance of 1.9 mW/cm<sup>2</sup>. The lamps had an emission spectrum of 285–350 nm, maximal at 310–315 nm. Before starting the minimal erythema dose (MED) was assessed. Half this dose was given to start UVB treatment. The doses were individually adjusted to cause suberythematous to slight erythematous reactions without burning.

#### Assessment of clinical efficacy

The patients were treated for 8 weeks or shorter if lesions were cleared earlier. If only one body-half was cleared, all treatment of this side was stopped while treatment on the other half was continued. Clinical improvement was assessed using the PASI-score. The PASI-scores were calculated per body-side as half PASI-scores.

#### Biopsy procedure and immunohistochemical staining

In all 8 patients punch biopsies of 3 mm were taken from a representative lesion before and after treatment. The biopsy procedure has been described before (2). For immunohistochemical staining a panel of monoclonal antibodies was used.

To assess epidermal differentiation, monoclonal antibodies against involucrin (MON-150 (3), 1:25), anti-human keratinocyte transglutaminase (1:100, Mouse Monoclonal Antibody, BT621, IgG<sub>2a</sub> Biomedical Technologies Inc.) and against filaggrin and profilaggrin (anti-filaggrin, 1:500, BT576, Biomedical Technologies Inc.) were used.

To approximate the number of cycling epidermal cells in the basal layer, an antibody directed against the Ki-67 antigen was used (MIB-1, 1:50, Immunotech, SA, Marseilles, France).

Analysis of the inflammatory infiltrate was made by assessment of T-lymphocytes and PMN, respectively, using the monoclonal antibodies DAKO-T11 (1:100, Dakopatts, Copenhagen, Denmark) and DAKO-elastase (1:100, Dakopatts, Copenhagen, Denmark).

#### Staining procedure

For all monoclonal antibodies, except for T11, an indirect immunoperoxidase technique was used. Staining with T11 was performed with an indirect peroxidase-anti-peroxidase technique (PAP). The staining techniques have been described previously (2). The PAP procedure was carried out using the microwave method (4).

#### Histological examination

The histological examination was performed blinded. These scoring methods have been performed and published before (2, 5).

Involucrin and transglutaminase expression was assessed by calculation of the ratio positive cell layers/total cell layers of the viable epidermis. This was done at two sites: above the dermal papilla and between two dermal papillae. Filaggrin expression was assessed by measuring the percentage of the length of the stratum corneum and stratum granulosum which was stained.

Epidermal proliferation was measured by counting the number of MIB-1 positive nuclei per mm length of the section.

Inflammation (PMN and T-lymphocytes) was assessed separately for dermis and epidermis. Dermal inflammation was semi-quantitatively enumerated by expressing the number of positively stained cells as a percentage of the total number of infiltrate cells: 0, no positive cells; 1, sporadic; 2, 1–25%; 3, 26–50%; 4, 51–75%; 5, 76–99%; 6, 100%. Epidermal inflammation was assessed using a five-point scale: 0, no staining; 1, sporadic staining; 2, minimal staining; 3, moderate staining; 4, pronounced staining.

#### Statistical evaluation

Data are reported as means  $\pm$  SEM. Changes in PASI and immunohistochemical markers, due to therapy, were evaluated with the Student *t*-test for paired values, in Figs. shown as \*:  $p \leq 0.05$ ; \*\*:  $p \leq 0.005$ . A two-tailed hypothesis was employed to interpret data. A synergistic effect of two treatments was defined as an effect which proved to significantly exceed the sum of the effects of two individual treatments. In order to find out whether a synergistic effect can be validated in statistical terms we performed a two-way analysis of variance (2-way ANOVA).

## RESULTS

#### Clinical response

All patients showed a marked improvement on both body-halves. Two patients cured within 8 weeks on one or both body-halves. The mean total UVB dose was  $20 \pm 3.0$  J/cm<sup>2</sup> for the body-halves that were treated with UVB monotherapy and  $18 \pm 5.0$  J/cm<sup>2</sup> (mean  $\pm$  SEM) for the combination therapy treated body-halves. No statistically significant difference in UVB dose was seen between the body-halves that were treated with UVB monotherapy or combination therapy. The PASI-score decreased significantly for all treatments. The relative improvement with Micanol was 69% ( $p = 0.006$ ), UVB therapy resulted in a relative improvement of 71% ( $p = 0.04$ ) and the combination of both resulted in a relative improvement of 78% ( $p = 0.003$ ). The combination of Micanol and UVB revealed a significantly synergistic effect on the PASI-score ( $p \leq 0.05$ ).

#### Histological results

In all the biopsies taken before treatment, psoriatic histological features were present: hyperkeratosis, acanthosis with thinning of the suprapapillary layer, pronounced elongation of the rete-ridges and in the dermis a marked cellular infiltrate. After treatment a diminution in epidermal thickness and dermal cellular infiltrate was seen in all biopsies, although hyperkeratosis and acanthosis were still present, also in the biopsies taken from the clinically cured body-halves.

The results of the immunohistochemical stainings are shown in Figs. 1–3. Micanol had mainly decreasing effects on inflammation markers: a highly significant reduction of the number of PMN ( $p \leq 0.005$ ) and on the number of T-lymphocytes ( $p \leq 0.005$ ) was observed. The epidermis was not significantly affected by Micanol. UVB had an extensive profile of immunohistochemical reductions: highly significant reductions ( $p \leq 0.005$ ) of the number of transglutaminase and involucrin positive cell layers, the number of Ki-67-positive basal keratinocytes and T-cells. The combination of dithranol and UVB resulted in various synergistic effects. Statistically significant synergy was shown for the Ki-67-staining ( $p \leq 0.05$ ), filaggrin-staining in the stratum corneum ( $p \leq 0.05$ ), PMN in the dermis ( $p \leq 0.05$ ) and in particular, of T-cells in dermis and epidermis ( $p \leq 0.005$ ).

## DISCUSSION

The design of the study was partly a left/right comparison, partly a parallel group study. The left/right analysis has the drawback that systemic effects of topical treatments might occur, resulting in a contralateral effect. Therefore it is remotely possible that the difference between the three regimes might

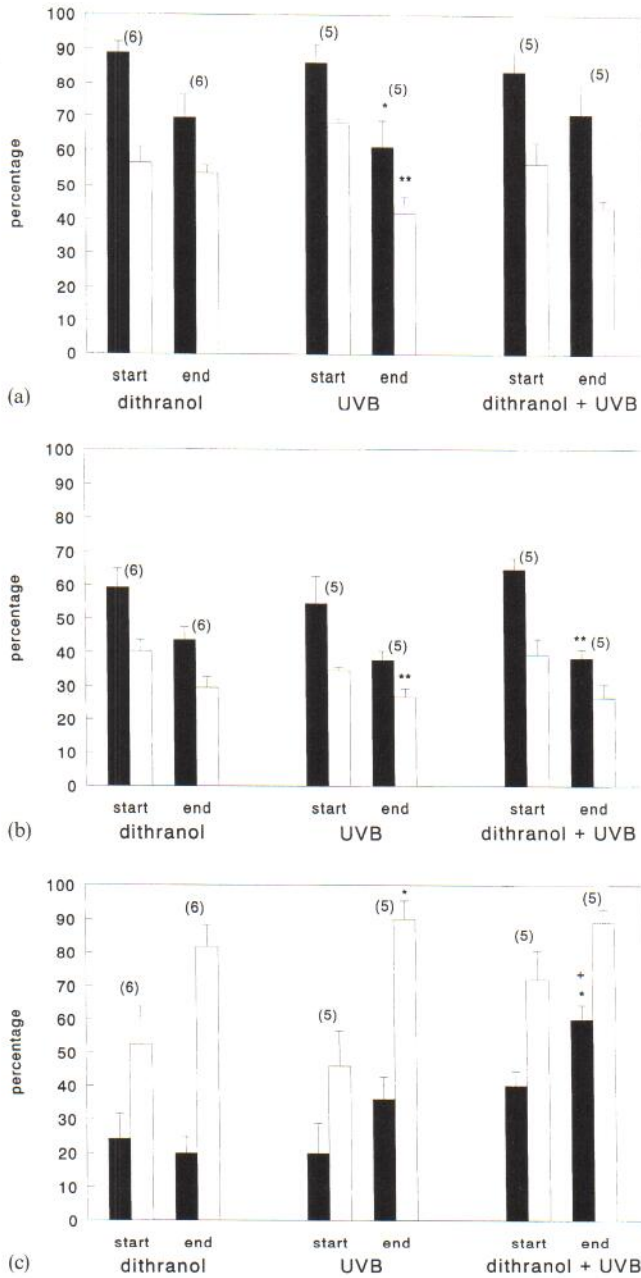


Fig. 1. Differentiation markers: a) transglutaminase above the dermal papilla (■) and interpapillar (□) before and after therapy; b) involucrin above the dermal papilla (■) and interpapillar (□) before and after therapy; c) filaggrin in the stratum corneum (■) and stratum granulosum (□) before and after therapy; (Group comparison: \* =  $p \leq 0.05$ ; \*\* =  $p \leq 0.005$ ; synergism: + =  $p \leq 0.05$ ; ++ =  $p \leq 0.005$ .)

have been expressed more evidently in a parallel group design. The clinical results observed in the present study are comparable with the results observed in the plenary group (Gerritsen et al., manuscript in preparation). In the present study of 8 patients, a statistically significant synergistic effect of the combination therapy was seen with respect to the PASI, indicating a more pronounced clinical effect of the combination therapy.

Our immunohistochemical data suggest that Micanol monotherapy had significant decreasing effects on dermal accumulation of PMN and dermal and epidermal accumulation of

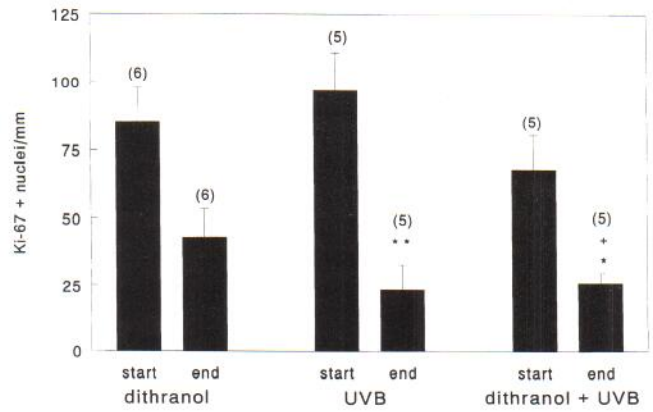


Fig. 2. Epidermal proliferation: Ki-67 staining before and after therapy. (Group comparison: \* =  $p \leq 0.05$ ; \*\* =  $p \leq 0.005$ ; synergism: + =  $p \leq 0.05$ ; ++ =  $p \leq 0.005$ .)

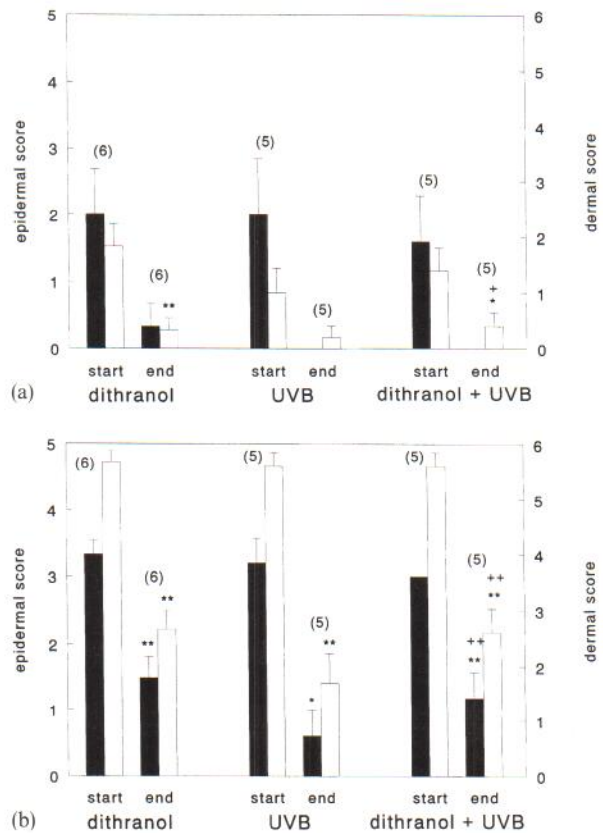


Fig. 3. Inflammation: a) PMN in the epidermis (■) and dermis (□) before and after therapy; b) T11 in the epidermis (■) and dermis (□) before and after therapy. (Group comparison: \* =  $p \leq 0.05$ ; \*\* =  $p \leq 0.005$ ; synergism: + =  $p \leq 0.05$ ; ++ =  $p \leq 0.005$ .)

T-cells. In contrast, no substantial effects on epidermal growth and differentiation parameters were observed. These data are at variance with previous studies on dithranol incorporated in cream or petrolatum, which showed a relative persistence of the inflammatory infiltrate with more pronounced effects on epidermal growth and differentiation (5, 6). During treatment with dithranol in emulsifying ointment the accumulation of T-lymphocytes and CD14 cells persisted up to 8 weeks whereas the suprabasal expression of keratin 16 as well as the number

of Ki-67-positive nuclei has diminished considerably during treatment (5). The immunohistochemical results in this study were analysed using the same methodology as in the present study. It is of interest that the relative reduction of the PASI-score in the previous study (5) was 77%; in the present study the reduction of PASI was 69%. In a histochemical study on the effect of dithranol in petrolatum on psoriasis by Braun-Falco et al. (6), besides changes in parameters for differentiation, the persisting inflammatory reaction was a remarkable finding. Although comparisons between different studies have their limitations, the statistical validation of the immunohistochemical studies demonstrates that Micanol base has a profile of immunohistochemical changes that is different from more traditional vehicles.

Phototherapy with UVB had substantial effects on expression of transglutaminase, involucrin, the recruitment of cycling cells and the accumulation of T-cells. Only a borderline significant effect was seen with respect to filaggrin expression. UVB phototherapy and dithranol treatment have markedly different profiles of effects on the skin compared to other local anti-psoriatic therapies, for instance vitamin D<sub>3</sub> analogues (calcipotriol, calcitriol and tacalcitol) (2, 7–9). These different histological changes are reflected in the different sequential clinical changes of the psoriatic plaque as a result of each therapy. UVB ( $\lambda=290-320$  nm) has major effects on the epidermis, resulting in DNA damage by formation of pyrimidine dimers and hence interfering with macromolecule synthesis and cell division (10). The *in vivo* effect of vitamin D<sub>3</sub> analogues is primarily on epidermal proliferation and differentiation, partly via binding with the vitamin D<sub>3</sub> receptor, resulting in a cascade of nuclear mechanisms, and partly via non-genomic mechanisms (11). Dithranol is thought to have its effects predominantly via auto-oxidation and the formation of free radicals, thereby causing a cascade of effects (12). The so-called "minimum structure for anti-psoriatic activity" is responsible for the anti-psoriatic effects, also causing irritation and staining of the skin (13). Short-contact application schedules constitute one method of decreasing irritation and discomfort for the patient. Dithranol in the formulation of the present study is another method of decreasing the adverse events. One could speculate that dithranol in Micanol delivers the active substance more directly in the dermal infiltrate, where auto-oxidation starts, leaving the epidermis relatively unaffected. Such might explain the low irritancy of Micanol treatment.

In the combination therapy, a statistically significant synergistic clinical effect was demonstrated. The combination therapy also revealed evident immunohistochemical synergistic effects on the recruitment of cycling cells, involucrin above the dermal papilla, filaggrin in the stratum corneum, PMN and T-cells. It is intriguing that the immunohistochemical effects in the combination therapy cannot always directly be predicted from adding up the effects in either monotherapy. The present study suggests that the immunohistochemical effects of both therapies are mingled and amplified, resulting in a new profile of immunohistochemical changes.

In literature, so far, no consensus has been reached as to the synergism of the combination of dithranol and phototherapy (14–18). Biochemical studies revealed that UVB increases dithranol activity (19). But it is well established that during optimised dithranol therapy with the gold standard of 24-h applications in an in-patient setting, UVB does not improve the anti-psoriatic efficacy (20, 21). On the other hand,

less optimised dithranol treatment (short contact treatment with dithranol cream at home) is enhanced by phototherapy, as demonstrated in the present clinical study. It is a well-established fact that remissions last longer when UVB is added (22). It is attractive to hypothesise that during less optimised dithranol treatment, in which less irritation is encountered, the effects of the dithranol therapy are enhanced by the effects of UVB on epidermal processes.

In conclusion, the present study suggests that Micanol predominantly has anti-inflammatory immunohistochemical effects. However, comparative studies with different dithranol formulations have to be carried out to prove this statement definitively. UVB had a broad spectrum of reductions in immunohistochemical parameters. The combination appeared to result in clinical and multiple immunohistochemical synergistic effects.

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