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# Skin Ultrastructure after Calcipotriol Treatment: A Transmission Electron Microscopic and Freeze-fracture Study on Psoriatic Patients

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**Electron microscopic details in psoriatic skin during calcipotriol application are reported. Ten psoriatic patients (PASI score 3–14) were punch-biopsied on typical lesions before treatment and 2, 4 and 8 weeks after it. Two patients dropped out after 1 month because of lack of clinical response. Skin blocks were processed routinely for transmission electron microscopy and freeze-fracture techniques. The first modifications were the disappearance of fine intercellular granular material and the restoration of the granular layer. After 4 weeks desmosomes appeared in greater amounts than at baseline. Horny layer lipid vacuoles and remnants of nuclei as well as multilayering of the basal lamina were only focally observed. Dilatation of dermal capillaries was still seen even after 8 weeks. Freeze-fracture confirmed the data about the desmosomes and revealed a marked decrease of the gap junctions. Calcipotriol basically seems to act similarly to other antipsoriatic agents, though faster. The persistence of microvasculature dilatation could imply the need for long-term therapy.** ***Key words: fine intercellular granular material; multilayering of basal lamina; dilatation of dermal capillaries.***

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During the last two decades the fine structure of psoriatic lesions has been extensively described before therapy (1–3) and after oral retinoids (3, 4), cyclosporine (5) or other specific treatments (6). Although the various antipsoriatic agents exert a different pharmacological influence on the disease, all the ultrastructural data show that the changes observed during regression are basically rather similar and not specific for a given drug (5–7), while only retinoid causes distinctive ultrastructural features (3, 4).

Calcipotriol is a recent and effective topical treatment for mild to moderate psoriasis (8). Reports have been published on its mechanisms of action (9) and on the immunocytochemical features of the affected skin after its application (10). In this work we studied the fine structure of psoriasis, as analyzed by transmission electron microscopy (TEM) and freeze-fracture techniques after 2, 4 and 8 weeks of calcipotriol, focusing our attention on epidermal keratinocytes, basal membrane zone and papillary microcirculation.

## PATIENTS AND METHODS

Ten outpatients with mild to moderate psoriasis (PASI score ranging from 3 to 14) applied calcipotriol ointment 0.005% (Psorcutan, Schering Italia, Milan) twice daily to their skin lesions. Punch biopsies were taken under 1% lidocaine local anesthesia from typical lesions before treatment and from corresponding nearby areas 2, 4, 8 weeks after the administration of the cream. During calcipotriol therapy, gradual regression of the lesions was seen clinically, though with some individual differences (Table I). In two individuals TEM observations were restricted to baseline and 2–4 weeks because of lack of response to the drug, which was stopped after 1 month. Skin blocks, 1–2 $mm^3$ thick, fixed for 3 h in Karnowsky fixative at 4°C and postfixed with 1% osmium tetroxide, were dehydrated in a graded series of alcohol and embedded in epon 812. Ultrathin sections were cut with an LKB ultramicrotome and stained with uranyl acetate and lead citrate.

Freeze-fracturing was performed according to Moor et al. (11). All the specimens were observed with a Philips 501 electron microscope at 80 kv.

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## RESULTS

TEM analysis after topical calcipotriol showed overlapping features in all the patients enrolled in the study, independently of their baseline PASI score.

### *2 weeks of treatment*

An overall improvement toward the features of cured psoriatic skin was detectable.

Multilayering of basal lamina, typically observed in psoriasis, was only focally seen (Fig. 1).

The tonofilaments in spinous cells appeared increased in comparison with baseline and displayed a definite tendency to aggregation.

The cytoplasm of malpighian cells was rich in glycogen and ribosomes; rough endoplasmic reticulum and Golgi apparatus were evident and mitochondria were regularly distributed throughout the cells.

Wider intercellular spaces in association with a slight tendency to desmosomal "stretching", typically seen in the baseline specimens, could be detected only focally, as were rare interdigitating cytoplasmic processes with few desmosomal contacts (Fig. 2 and inset).

The fine intercellular granular material usually found in fully developed psoriasis or after the use of retinoids was not observed, neither in the intercellular spaces nor below the basal lamina. The granular layer quite regularly displayed a fair amount of keratohyalin (Fig. 3), and granular cells on average appeared morphologically normal. Desmosomes and keratinosomes were detectable in great amounts and well structured; gap junctions were frequently found with freeze-fracture (Fig. 4). The horny layer was 15–26 cell thick, with occasional remnants of nuclei. Lipid vacuoles (=electron microscopic parakeratosis) within horny cells were rare (Fig. 3). Langerhans' cells, polymorphonuclear leukocytes and



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Table I. *Clinical evolution of patients*
E = erythema, S = scaling, I = infiltration.

| Patient no. | Age | PASI | | | | Note |
|---|---|---|---|---|---|---|
| | | 0 | 2wks | 4wks | 8wks | |
| 1 | 33 | 13.2 | 10.1 | 6.1 | 3.8 | E I S |
| 2 | 70 | 7.0 | 7.0 | 5.0 | – | * |
| 3 | 60 | 14.0 | 12.0 | 12.0 | – | * |
| 4 | 39 | 8.3 | 4.9 | 2.7 | 2.9 | E I S |
| 5 | 61 | 6.4 | 4.7 | 2.1 | 1.8 | E I S |
| 6 | 70 | 8.1 | 5.8 | 4.9 | 3.3 | E I S |
| 7 | 35 | 3.0 | 2.6 | 1.4 | 2.4 | E |
| 8 | 61 | 13.8 | 10.0 | 8.0 | 8.6 | E I S |
| 9 | 62 | 14.0 | 10.8 | 8.3 | 3.4 | E I |
| 10 | 61 | 13.5 | 6.3 | 6.3 | 3.4 | E S |

* = therapy stopped due to lack of response.

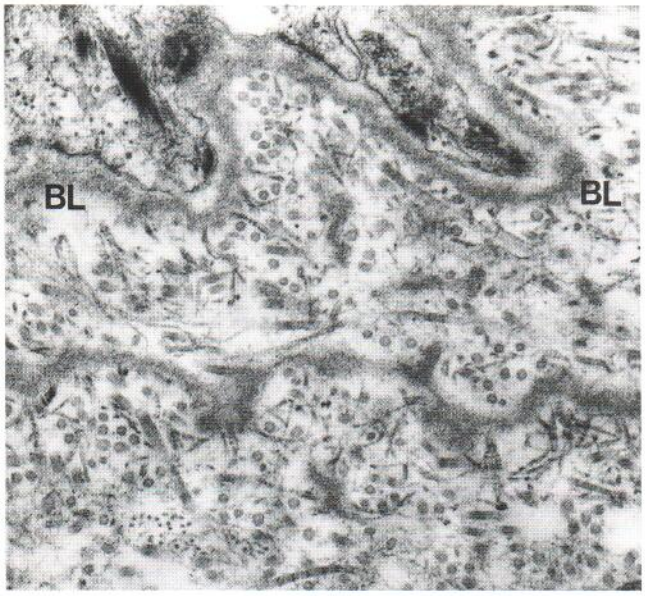


*Fig. 1.* Dermo-epidermal junction: multilayering of the basal lamina (*BL*) (×47250).

lymphocytes appeared morphologically normal and were detected only occasionally.

Most of the dermal capillaries appeared to be normally structured, even though the lumen was often enlarged. An overall tendency to compacting of the collagen fibres was also seen. The dermal infiltrate was mainly perivascular and consisted mostly of normal lymphocytes and macrophages.

*4 weeks of treatment*

The ultrastructural picture observed at this time showed features similar to those described above. The only distinctive aspects detected are resumed in the following points.

The basal lamina showed a more regular pattern without multilayering.

The widening of the intercellular spaces in the malpighian layers was greatly reduced, and desmosomes appeared normal in number and structure. This last finding was confirmed by freeze-fracture, which also showed a persistent increase of gap

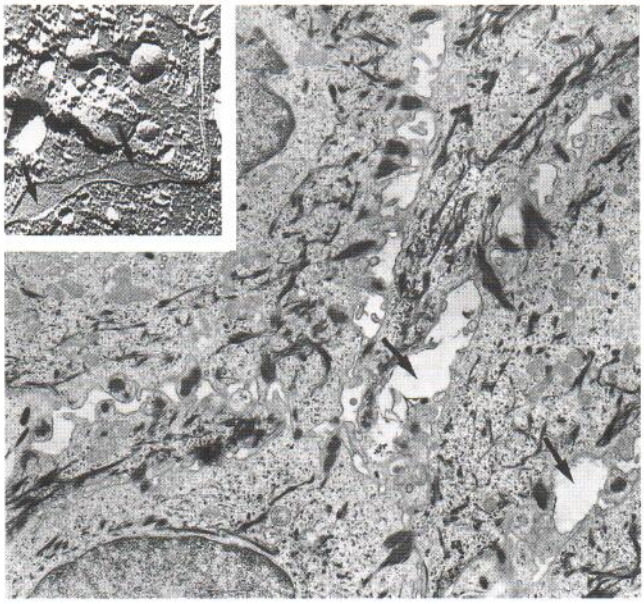

*Fig. 2.* Suprabasal layer after 2 weeks' treatment: a definite widening of the intercellular spaces (*arrows*) is detectable only focally (×31000).

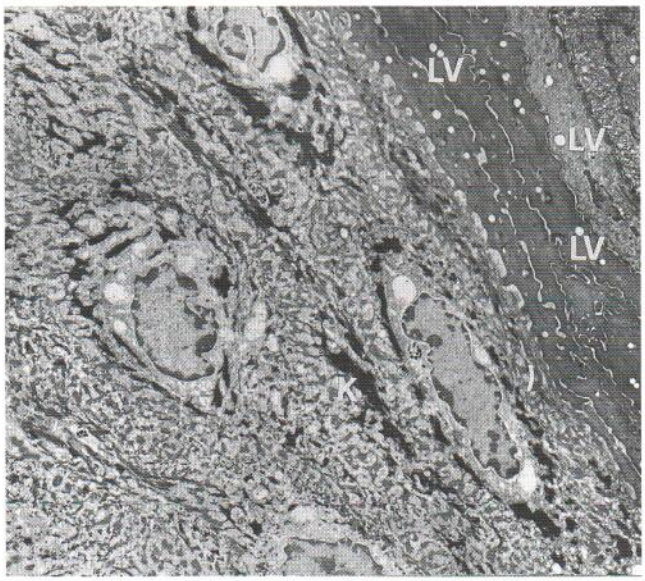


*Fig. 3.* Granular layer after 2 weeks' treatment: keratohyalin granules (*K*) are regularly distributed. Scanty lipid vacuoles (*LV*) are found in stratum corneum (×4180).

junctions and several micropinocytotic vesicles (Fig. 5). The granular layers showed a compact net of keratohyalin granules, well fused with tonofilaments. The horny layer was generally orthokeratotic, with very rare lipid vacuoles.

These features correlated strictly with the clinical behaviour of the disease. In fact, the specimens from patients with resistant psoriasis still showed defined intercellular widening and defective desmosomes (Fig. 6), together with evident capillary enlargement and a less structured keratohyalin tonofilament pattern. Nevertheless, no deposition of the fine intercellular granular material was detectable even in these cases.


*Fig. 4.* The plasma membrane of keratinocytes frequently displays well developed gap junctions (*GJ*) after 2 weeks' treatment (×119600).

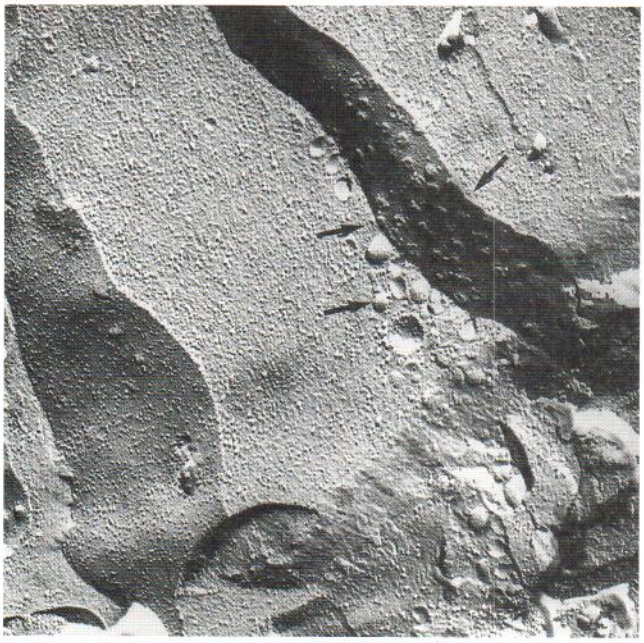

*Fig. 5.* Plasma membrane of keratinocytes after 1 month of treatment: micropinocytotic vesicles (*arrows*) are frequently observed (×42600).

*8 weeks of treatment*

Lesional skin showed almost complete regression to the characteristics visible in normal individuals (Fig. 7). Papillary oedema disappeared and an inflammatory infiltrate (mainly composed of perivascular macrophages) was only focally seen.

The only residual feature suggesting "psoriatic" markers was the frequent papillary microvessel enlargement, which, however, was not associated with endothelial gaps, bridged fenestrations of endothelial cell layer and/or abnormal basement membrane material. The most impressive freeze-fracture finding was the marked reduction of gap junctions.

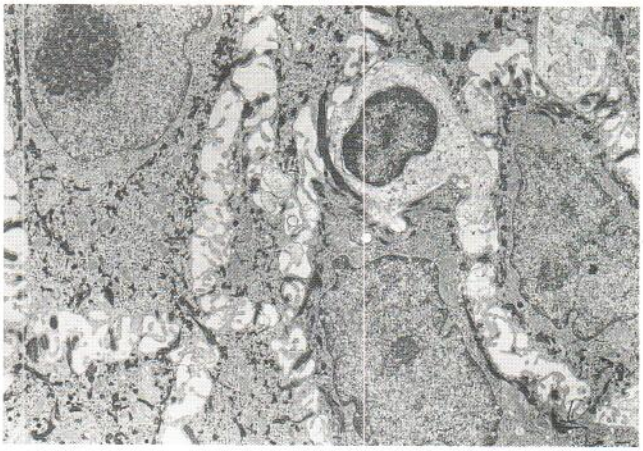

*Fig. 6.* Psoriasis resistant to treatment: large intercellular spaces with reduced desmosomal contact (×8580).

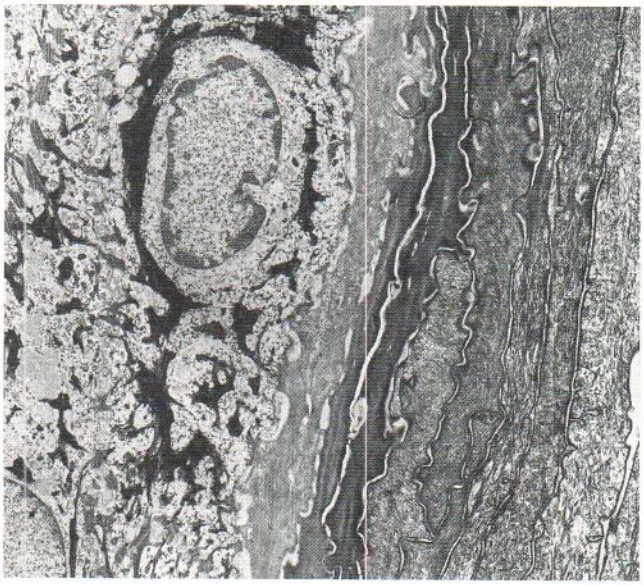

*Fig. 7.* After 2 months of treatment the granular and horny layers display the features of healthy skin (×6750).

## DISCUSSION

Skin TEM observations after calcipotriol application confirmed the efficacy of the drug in treating mild psoriasis. The normalization of the epidermal and dermal defects observed in the disease was progressive and not strictly time-related, even though evident changes were detectable after only 2 weeks. The intercellular fine granular material, considered in the past as a possible product of psoriatic keratinocytes and probably also stimulated by retinoids (12), was the first TEM psoriatic marker which completely regressed, even if the disease was resistant to the drug.

Secondly, calcipotriol appeared to improve psoriatic skin faster than etretinate and Goeckerman' regimen, even if it basically restored the granular layer and normalized the keratinization processes in a manner similar to that observed with the other treatments (7).

In addition, calcipotriol restored endothelial structure to normal, even if a persistent dilation could be still detected

with TEM at 8 weeks. This ultrastructural observation correlated with the clinical evidence of residual erythema after the disappearance of the other psoriatic signs. According to Braverman & Sibley's theories (6), this lack of effect on the vessels could explain the fast relapse of psoriasis after calcipotriol interruption and the need for long-term maintenance therapy. In our opinion, the tendency to compacting of the collagen fibres, already described by Kamino-Kamino et al. (3), could be linked to a reduction of dermal oedema.

The action on the dermal cellular infiltrate might also indicate a slight anti-inflammatory effect of calcipotriol, already described by others (10, 13).

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