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Treatment of Psoriasis Vulgaris with Topical Calcipotriol:

Is the clinical improvement of lesional skin related to a down-regulation of some cell adhesion molecules?

M. L. CAGNONI[1], I. GHERSETICH[2], T. LOTTI[1], M. PIERLEONI[3] and G. LANDI[3]

[1]Department of Dermatology, University of Siena, the [2]Department of Dermatology, University of Florence, and the [3]Department of Dermatology, 'Ospedale Bufalini', Cesena, Italy

Calcipotriol is demonstrably efficacious for the treatment of psoriasis by virtue of its effects on the skin's immune system and on epidermal growth. We performed this study to emphasize the difference in the expression of certain cell adhesion molecules (CAMs) (ICAM-1, ELAM-1, LFA-1, VLA-3, VLA-6) in lesional and perilesional skin of 10 patients with psoriasis, before and after treatment with topical Calcipotriol. We took two biopsies of lesional and perilesional skin from each patient before and after treatment and then performed an immunohistochemical study to observe the expression of these CAMs, utilizing monoclonal antibodies against these adhesion molecules. We noticed reduced levels of infiltrating cells along with the expression of ICAM-1, LFA-1, ELAM-1 and of CAMs VLA-3, VLA-6 in basal and suprabasal keratinocytes. On the basis of these data we hypothesize that, besides epidermal keratinocytes, another target for Calcipotriol may be the skin's own immune system, suggesting that Calcipotriol can modify T lymphocyte activity (IL-1 dependent) through a down-regulation of CAMs. ***Key words: vitamin D analogues; skin immune system; epidermal growth.***

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M. L. Cagnoni, Via Bolognese Vecchia 178, I-50139 Firenze, Italy.

Calcipotriol is the structural analogue of calcitriol, bioactive form of Vitamin D_3. All clinical trials have demonstrated that both have good therapeutic efficacy against hyperproliferative diseases such as psoriasis (1). The efficacy of vitamin D analogues in psoriasis vulgaris was discovered in 1985 by Morimoto et al. They reported a case of significant amelioration of this dermatosis in a patient with psoriasis, treated with oral α-calcidol for a severe osteoporosis (2, 3).

Calcipotriol and calcitriol show similar receptor binding as found in different cells lineages: epidermal keratinocytes, dermal fibroblasts, endothelial cells and activated T lymphocytes (4, 5).

They both have similar effects on the immune system and on the epidermal growth-promoting terminal differentiation and inhibiting proliferation of keratinocytes (6, 7, 8).

The therapeutic index of vitamin D analogues orally administered, is low and a safe and effective dose has not yet been established; in fact, calcitriol implies a high risk of inducing the typical side-effects related to Vitamin D_3: hypercalciuria, hypercalcaemia and bone resorption.

Calcipotriol, on the contrary, as it has been developed for topical treatment of psoriasis, exerts only one-hundredth calcitriol's effect on calcium metabolism (9, 10, 11). Calcipotriol appears not to have a direct anti-inflammatory effect, but seems to modify epidermal growth and T-lymphocyte activity, so modulating the production and release of cytokines (12).

Cell adhesion molecules (CAMs) are cell surface receptors expressed on different cell lineages and involved in cell-cell and cell-matrix interactions in various physiological and pathological conditions. Some of these CAMs are involved in the interactions of lymphocytes with keratinocytes, endothelial cells and inter- and perivascular connective cells. Their expression is often up-regulated by cytokines (13, 14, 15).

These adhesion receptors mediate the trafficking and the homing of lymphocytes from circle to perivascular tissue sites of inflammation through 'transendothelial migration' (16).

MATERIAL AND METHODS

Ten informed outpatients (8 males, 2 females, 25–52 years old, mean 40.5) with symmetrical, plaque-type psoriasis were selected for the study. None of the patients had received antipsoriatic treatment for at least 5 weeks before the study. They all had normal serum levels of calcium. They were treated for 8 weeks with topical calcipotriol (50 μg/g twice daily). No other antipsoriatic treatment was permitted. Blood samples for standard laboratory examinations were repeated after the treatment.

All the patients underwent a biopsy on lesional and perilesional skin before and after treatment. Under local anesthesia, samples were taken with a cutaneous biopsy punch, 5 mm. The material was fixed in O.C.T. compound, i.e. an embedding medium that we used for freezing (−80°) our tissue specimens. Cryostat sections (7–8 μm) were studied with an immunohistochemical method (indirect immunoperoxidase). We utilized monoclonal antibodies directed against antigens CD3, CD4, CD8, CD1a, CD36, that respectively identify mature T lymphocytes, T helper/inducer lymphocytes, T cytotoxic/suppressor lymphocytes, antigen-presenting cells, perivascular dendritic cells, and monoclonal antibodies against cell adhesion molecules (CAMs) ICAM-1, ELAM-1, LFA-1 (which may be expressed respectively: ICAM-1 on endothelial cells, keratinocytes, antigen-presenting cells; ELAM-1 on endothelial cells, and LFA-1 on lymphocytes) which represent some of the most important adhesion molecules. We also utilized monoclonal antibodies directed against CAMs VLA-3 and VLA-6, that are expressed on basal and suprabasal keratinocytes.

For quantitative analysis, we used two sections (lesional and perilesional) per patient, before and after treatment, per antibody (CD3, CD4, CD8, CD36, CD1a, ICAM-1, LFA-1, ELAM-1, VLA-3, VLA-6). Expression of ELAM-1, ICAM-1, LFA-1,

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Table I. *Percentage of labelled cells in the dermis of psoriatic skin before and after tratment with topical calcipotriol*

BT = before treatment: AT = after treatment

Pats.	CD3		CD4		CD8		CD1a		CD36	
	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT
1	35	12	40	16	15	13	30	15	18	14
2	20	5	38	18	13	11	35	18	21	15
3	30	0	34	20	10	10	30	23	25	12
4	30	10	36	12	15	14	33	12	16	14
5	22	8	30	15	16	15	27	16	22	20
6	36	5	35	17	18	16	28	10	27	23
7	18	8	32	21	12	12	21	19	19	16
8	28	12	28	10	14	13	34	22	23	15
9	34	10	25	12	12	10	37	20	25	16
10	35	6	35	15	10	9	30	18	20	12

VLA-3, VLA-6 was quantified *ad modum* Messadi et al. for ELAM-1: 0, no staining; 1, weak focal granular staining; 2, moderate staining; 3, strong staining; 4, very strong staining.

RESULTS

Topical application of calcipotriol caused a significant improvement (clinical remission of the lesions) with a decrease of 38 in the PASI score after 8 weeks of treatment ($p < 0.001$).

The urinary and blood parameters of calcium metabolism following treatment were within the normal range.

The immunohistochemical data of our study were as follows (for quantitative data, see Tables I and II):

Lesional skin before treatment

By light microscopy, two main immunohistochemical features were found:

1) Perivascular cellular infiltration.
We have demonstrated with the immunohistochemical method the presence of CD3+, CD4+, CD1a+ and CD36+ cells in the infiltrate.

2) Cell adhesion molecule expression.
We found expression of cell adhesion molecules in the perivascular dermis and in the epidermis of all the samples we studied. In particular we discerned an up-regulation of ICAM-1 (intercellular adhesion molecule-1) (see Fig. 1) by endothelial cells, antigen-presenting cells and keratinocytes and its counter-receptor LFA-1 (Leukocyte function antigen-1) situated on the T lymphocyte surface, whose expression could permit, after a linkage with ICAM-1 expressed on endothelial cells of 'high endothelial venules' (but not only in those cells), the transendothelial migration of T lymphocytes in the perivascular tissue. Finally we have observed a moderate expression of ELAM-1 on endothelial cells and of VLA-3 and VLA-6 on basal and suprabasal keratinocytes. On perilesional skin before treatment we have observed a scanty infiltrate and a slight expression of CAMs.

Table II. *Expression of cell adhesion molecules (ICAM-1, ELAM-1, LFA-1, VLA-3, VLA-6) in the epidermis and dermis of psoriatic skin, before and after an 8-week treatment with topical calcipotrol*

BT = before treatment: AT = after treatment, P = patients

CAMs	ICAM-1		ELAM-1		LFA-1		VLA-3		VLA-6	
Pats.	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT
1	++++	+	+++	+	+	+	+	+	+++	+
2	+++	+	+	+	+	−	+++	+	+	+
3	+++	+	+	+	+	−	+	+	+	+
4	+++	+	+++	+	++++	+	+	+	+	+
5	+++	+	+	+	+++	+	+	+	+	+
6	+++	+	+++	+	+	−	+	+	+	+
7	+++	+	+	+	+++	+	+	+	+++	+
8	++++	+	+++	+	+++	+	+	+	+	+
9	+++	+	+	+	+++	+	+	+	+	+
10	++++	+	+++	+	++++	+	+	+	+	+

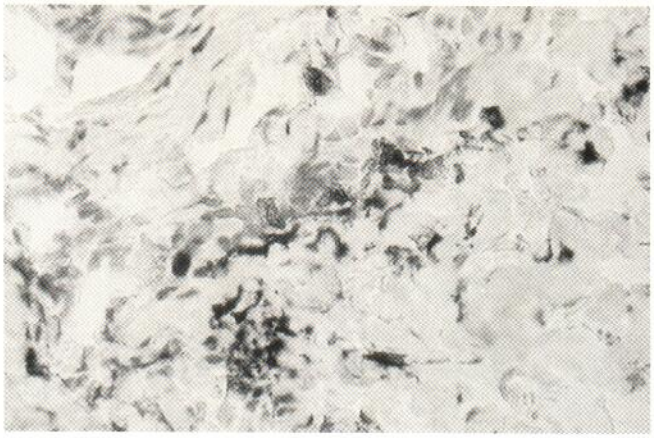

Fig. 1. Significant expression of ICAM-1 in the dermis of psoriatic skin before treatment. 40×.

Lesional skin after treatment

By light microscopy we have observed a significant reduction of CD3, CD4, CD1a and CD36 perivascular cellular infiltrate, a strong down-regulation of ICAM-1 (see Fig. 2), LFA-1, ELAM-1 and a moderate reduction of the expression of VLA-3

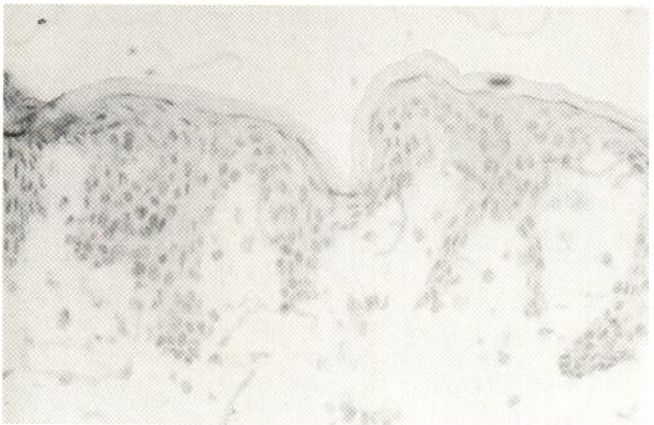

Fig. 2. Significant down-regulation of ICAM-1 expression after treatment. 25×.

and VLA-6 (see Table II). In particular we have discerned a very strong reduction of LFA-1 expression.

On perilesional skin, the infiltrate, present in moderate quantities before the treatment, was now completely absent. Some placebo-treated plaques in a patient with psoriasis have not improved.

DISCUSSION

All clinical trials have shown that topical calcipotriol is of significant therapeutic benefit in psoriasis vulgaris (17). Its mode of action has not been completely elucidated but it does not seem to have a direct anti-inflammatory action (12). Besides modulating epidermal growth-promoting terminal differentiation and inhibiting proliferation of keratinocytes, calcipotriol seems to have an immunoregulatory role that involves the skin immune system. In our data we have shown that VLA-3 and VLA-6 expression are not significantly modified by calcipotriol, while we have found a marked decrease in infiltrating cells and a down-regulation of ICAM-1, ELAM-1 and LFA-1 (18).

What is commonly accepted is the correlation between several weeks of treatment and a significant reduction of the perivascular cellular infiltrate on lesional and perilesional skin, represented mainly by activated T-lymphocytes (CD4+) and antigen-presenting cells (CD1a+) (19).

This reduction could be partly linked to the effect of this drug's inhibition of the production and release of cytokines. In fact calcipotriol reduces the release of various soluble factors (IL-2, IL-6, TNF) that normally are released by epidermal keratinocytes, monocytes and activated T-lymphocytes (20). Subsequently, this reduction could also be correlated to the effect of calcipotriol inhibiting the response of lymphocytes to IL-1 (21).

In this context, the role played by cell adhesion molecules is noteworthy. These adhesion receptors are known to mediate the trafficking and the homing of lymphocytes from the blood to the area site of inflammation, so representing the first step in the pathogenesis of many systemic and also cutaneous diseases.

On the basis of the results of our study, the reduction of cytokine release due to the therapy with topical calcipotriol might indicate/govern/regulate the down-regulation of the adhesion molecules ICAM-1, ELAM-1, LFA-1. In conclusion, calcipotriol may reduce the release of cytokines from different cell lineages, indicate/govern/regulate a down-regulation of CAMs that are known to mediate the passage of activated T-lymphocytes in the dermis and in the epidermis, thus producing a reduction of cellular infiltrate in psoriasis.

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