Calmodulin Levels in Psoriasis: The Effect of Treatment

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Epidermal calmodulin levels were measured in lesional and non-lesional skin in 20 psoriatics before and after treatment by a variety of established topical and systemic regimes. Pretreatment, 10 out of 20 patients showed elevated calmodulin levels in lesional epidermis. Clearance of psoriatic plaques was accompanied by a significant overall reduction ($p<0.05$) in epidermal calmodulin, irrespective of the treatment regime used. For the non-lesional epidermis, only 5 out of 17 patients initially showed elevated calmodulin levels and there was no significant reduction in those levels following treatment. (Received October 9, 1985.)

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Calmodulin (CaM) is an intracellular calcium binding protein, molecular weight 16700, which modulates the role of calcium in a variety of intracellular processes. Once calcium has bound to it, CaM activates a number of enzymes, including phospholipase A_2 and ornithine decarboxylase, those levels and those of their products have been shown to be increased in psoriatic skin (1, 2).

Levels of CaM in psoriatic epidermis have been shown to be increased both in the psoriatic plaques and, to a lesser extent, the non-lesional skin, whether measured by a radioimmunoassay of CaM (3, 4, 5), or by assay of biologically active CaM (6, 5).

We set out to determine whether successful treatment of psoriasis by a variety of established topical and systemic methods would lead to a parallel reductionin epidermal CaM levels.

PATIENTS AND METHODS

Patients

Twenty-two patients with psoriasis agreed to take part in this study. None had used methotrexate, etretinate, razoxane or PUVA within the previous year. Two patients were subsequently withdrawn from the study following inadvertant alteration of their treatment regimes. Nineteen of the remaining twenty patients were suffering from active chronic plaque psoriasis unresponsive to outpatient topical treatment and of sufficient severity to necessitate hospital inpatient management. A further patient with less extensive plaque psoriasis agreed to enter the study. Ten patients were male, ten female, mean age 45 years. with active psoriatic plaques covering between 5% and 90% of the skin surface area at the time of entry into the study. Eight patients had stopped all treatment for over one week, but the remainder were unable to abstain completely from treatment due to the severity of their disease. These twelve patients had used varying quantities of either a proprietary diluted topical steroid ointment, or a coal tar and salicylic acid ointment.

Patients were assigned to various treatments by normal clinical criteria (Table I), the patients involvement in the study and the initial epidermal calmodulin levels having no influence over the regime selected, all epidermal calmodulin levels being measured after conclusion of treatment.

Table I. *Epidermal CaM levels before and after treatment*

Treatment	No. of patients	Mean age	Mean area surface involved (%)	No. untreated[a]	Epidermal CaM μg/mg^{-1} protein (mean ± SEM)			
					Lesional		Non-lesional	
					Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Dithranol	8	47	22	4	2.67±1.17	0.94±0.34	0.59±0.2	0.52±0.15
Methotrexate	4	54	18	2	1.05±0.19	0.71±0.24	0.39±0.24	0.35±0.03
PUVA	4	30	43	–	1.16±0.18	0.95±0.33	1.23±0.53	0.85±0.26
Etretinate	2	55	67	–	0.46±0.02	0.56±0.2	0.43±0.13	0.59±0.23
Re-PUVA	1	49	22	1	0.88	0.27	1.02	0.45
Topical steroids	1	40	5	1	3.14	1.57	2.62	2.12

[a] Untreated for over one week.

Topical treatments

Eight patients were treated by a modified Ingram regime, using daily applications of dithranol ointment (rather than paste) combined with daily coal tar baths and UVB phototherapy.

The single outpatient was treated with a potent fluorinated corticosteroid ointment, betamethasone valerate 0.1%, applied twice daily.

Systemic treatments

These were selected where the topical dithranol regime had either proved ineffective on an earlier occasion, had caused irritation or was unsuitable for social or economic reasons.

Four patients were treated with PUVA, this regime being preferred over methotrexate or etretinate for mobile young patients of childbearing age.

Four patients were started on once weekly methotrexate by mouth, following pretreatment normal liver biopsies. Etretinate was selected in two patients with extensive superficial psoriasis, where alcohol abstinence and/or regular weekly attendance were impossible.

One patient was treated with etretinate and PUVA since once again alcohol abstinence was economically and socially impracticable.

Skin sampling

Epidermal shave biopsies approximately 3 mm in diameter were taken freehand by the same operator using intradermal anaesthesia and a No. 22 (Swann-Morton, Sheffield) Scalpel blade. Lesional biopsies (20 patients) were taken from the centre of a representative plaque from which loose scale had been gently removed. Non-lesional biopsies (17 patients) were taken from clinically uninvolved skin at least 2 cm from the edge of a plaque. The forearm was the selected site in all patients, lesional biopsies being taken from the same plaque site before commencing treatment and after successful clearance of psoriasis to eliminate any interlesional variability. Non-lesional biopsies were also taken from adjacent sites before and after treatment.

Methods

Each skin sample was homogenized in 1 ml of 40 mm Tris-HCL, pH 7.0 with 100 μM $CaCl_2$, containing 50 mg/l phenyl methyl sulphonyl fluoride and 50 μl/l pepstatin A using at least 20 strokes of a tight glass Dounce homogenizer at 4°C. Aliquots of this homogenate were immediately taken for protein determination (7). The remainder of the homogenate was heated to 90°C for 6 min and denatured protein removed by centrifugation. We have previously shown recovery of heat stable CaM from such preparations to be approximately 95% (5). Biologically active CaM in these supernatants was measured by the ability of the extracts to activate a CaM-dependent Beef-heart phosphodiesterase (Boehringer, Mannheim, London) which catalyses the hydrolysis of (^{3}H) labelled cyclic AMP (8). CaM activity was determined at three dilutions of each sample and samples were assayed in a minimum of two assays.

Statistics

Values are expressed as the means ±SEM. Differences between means were compared by the paired *t*-test. Values of $p<0.05$ were taken as statistically significant.

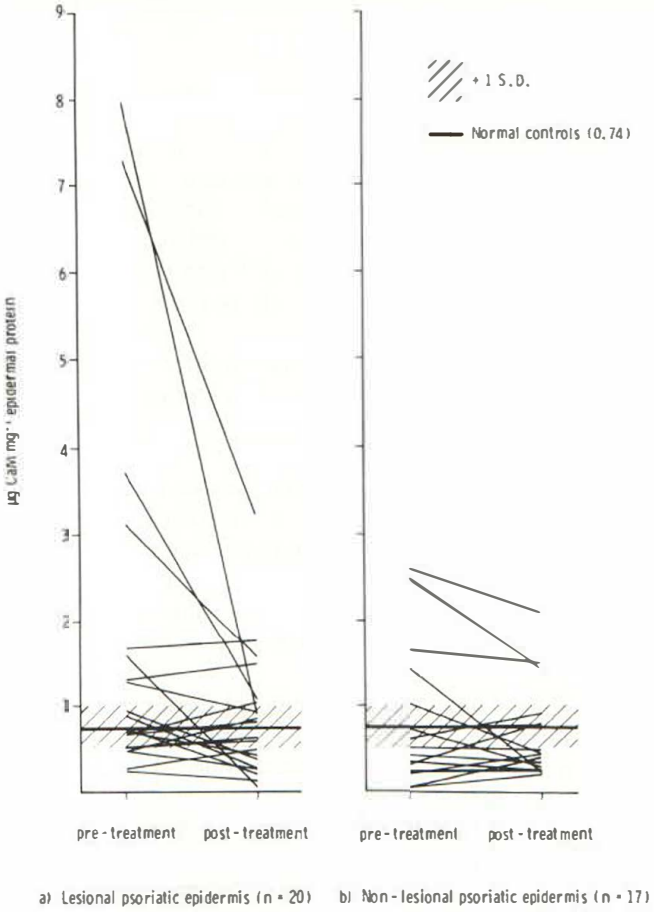

Fig. 1. The effect of treatment on the CaM levels of psoriatic epidermis.

RESULTS

Lesional epidermis

For the 20 patients there was an overall significant decrease in mean epidermal CaM levels (±SEM) from 1.76±0.49 μg CaM mg^{-1} epidermal protein to 0.87±16 μg CaM mg^{-1} ($p<0.05$).

Closer analysis of the results (Fig. 1 *a*, Table I) shows that irrespective of the treatment used those patients who had elevated CaM levels pre-treatment ($n=10$) showed a significant decrease following treatment ($p<0.05$). Those patients ($n=10$) with levels equal to or less than 0.72 μg CaM mg^{-1} epidermal protein (the mean value obtained from 16 healthy controls) showed no significant alteration in levels.

Non-lesional epidermis

There was no significant overall reduction in CaM levels in the non-lesional psoriatic epidermis following treatment (Fig. 1 *b*). However, the majority of the patients had epidermal CaM values pre-treatment which were within the normal range, in contrast to our earlier findings (6) and to those of a later study (4).

DISCUSSION

Psoriatic plaques have been reported to contain from 2 (4, 5) to 30 (3) times more CaM than the skin of normal volunteers. We (5, 6) and others (4) have been unable to confirm the original report of a 30-fold elevation in plaque CaM but find instead a more modest 2–3-fold elevation, irrespective of whether CaM is measured by radioimmunoassay (4, 5) or by assay for biological activity (5). Levels of CaM in the non-lesional skin are elevated to a lesser degree (4, 5). In the present study, we have found little correlation between the severity and extent of psoriasis and plaque CaM levels (Table I), and it is also apparent (Fig. 1) that some patients had plaque CaM levels within the normal range. Although this implies that CaM activity may not be a primary defect of psoriatic skin, CaM activation represents an amplification step in many enzymatic processes, and even a 2–3-fold increase in epidermal CaM levels might be expected to produce wide-ranging effects. Accordingly, whether or not increased CaM activity in psoriatic epidermis is one of the initiating abnormalities in psoriasis, such an abnormality may offer an opportunity to explore a new therapeutic approach in psoriasis.

We have shown that following successful treatment by a variety of regimes, epidermal CaM levels in lesional psoriatic skin fall significantly overall. Only a small reduction in CaM levels was observed in the non-lesional psoriatic epidermis, in which for the majority of patients, CaM levels were not elevated pre-treatment.

It seems apparent from both groups that epidermal CaM levels which are initially elevated tend to fall toward the "normal range" after treatment, while there is little change in those skins where pre-treatment CaM levels are already low.

The individual treatment groups were small, and none produced any significant change. Although the dithranol treated group appears to show a trend towards a significant reduction in the lesional skin, this was the largest group (8 patients) and also had the highest initial CaM levels. (All CaM levels were determined at the conclusion of the study and did not influence the choice of treatment.) Thus we cannot from this study compare the efficacy of the different treatment regimes in affecting epidermal CaM levels.

Nevertheless, our findings of an overall decrease in CaM levels in the lesional psoriatic epidermis following treatment suggests that there is potential for the investigation of drugs possessing known Calmodulin antagonist activity, as a possible new class of treatment for psoriasis.

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