

INVESTIGATIVE REPORT

Effects of Pimecrolimus Compared with Triamcinolone Acetonide Cream on Skin Barrier Structure in Atopic Dermatitis: A Randomized, Double-blind, Right–Left Arm Trial

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Patients with atopic dermatitis (AD) have an epidermal barrier dysfunction, which allows invasion of allergens to occur. Stratum corneum skin barrier is formed by corneocytes and extracellular lipids extruded from the epidermal lamellar bodies. In a controlled, randomized, double-blinded, right–left comparison study we investigated the effect of pimecrolimus (PIM) cream compared with triamcinolone acetonide cream (TA) on the skin barrier in 15 patients with symmetrical elbow lesions of AD. In punch biopsies, before and after treatment, skin lipid bilayer and lamellar body structure were examined by transmission electron microscopy (TEM). Partial Eczema Area and Severity Index (pEASI), stratum corneum hydration, and transepidermal water loss (TEWL) were monitored on days 1, 8 and 22. The pEASI was significantly more improved with TA compared with PIM, whereas stratum corneum hydration was slightly more improved after treatment with PIM. The TEM revealed a strong reduction in lamellar bodies in lesional skin of AD; only 32% of the lamellar bodies were normal. A significantly higher number of normal lamellar bodies was found after 3 weeks of treatment with PIM (58%; $p < 0.005$). An increase in lamellar bodies also occurred with TA treatment (46%; $p < 0.05$); however, significantly less than with PIM ($p < 0.05$). Clinical score and TEWL were more improved after treatment with TA, whereas the lamellar bodies were more normal after treatment with PIM. *Key words: calcineurin inhibitor; epidermis; topical corticosteroid; epidermal barrier.*

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Patients with atopic dermatitis (AD) have epidermal barrier dysfunction and inflammation of the skin. The resulting pruritus and burning sensations lead to scratching and self-perpetuation of the disease. The dysfunctional skin barrier allows invasion of allergens and pathogens to occur, which triggers immunological reaction of the

TH-2-mediated pathway (allergy development) and thereafter the TH-1-mediated pathway (inflammation). The pathogenesis of AD is still largely unknown, but an interaction of genetic and environmental factors is likely to play a major role (1–3). Mutations in the filaggrin gene are strong risk factors for ichthyosis vulgaris, AD and asthma (4–8). The "atopic march" describes the development of asthma in patients with AD (9, 10). Recently it has been suggested that allergen penetration into the skin due to a defect in skin barrier function leads to sensitization and causes the development of AD and even hay fever and asthma (11, 12).

The skin barrier is mainly localized in the lower stratum corneum and is maintained by corneocytes and a lipid-enriched intercellular domain. The intercellular lipid bilayers are formed during the extrusion of the epidermal lamellar body into the transition zone between stratum granulosum and stratum corneum (for review see 13). In earlier studies we and others found a disturbed epidermal barrier function in patients with AD not only in lesional skin, but already in non-lesional skin (14). Since 2004 (14) we have consistently seen reduced hydration values in lesional skin in different skin conditions when there are enhanced TEWL values. These findings correlate with the dry skin type or ichthyosis vulgaris frequently seen in atopic patients. It is well known that those patients with dry skin conditions benefit from treatment with emollients as the time between episodes of disease recurrence can be extended.

The most established treatment for AD is topical corticosteroids of different strengths, focussing on immune intervention. The side-effects of corticosteroid treatment, skin atrophy and immune suppression leading to bacterial infection, are well known (2, 15). In recent years calcineurin inhibitors (e.g. pimecrolimus) primarily emerged as topical anti-inflammatory drugs without the risk of skin thinning and without displacement of epidermal Langerhans' cells (16, 17).

In our recent randomized, double-blinded right–left arm study comparing topical treatment with 0.1% betamethasone valerate cream and 1% pimecrolimus cream (PIM) for 3 weeks in AD patients with symmetrical lesions we found a good clinical response to both treatment regimens (15). Skin atrophy in the betamethasone

group was already noticeable after 3 weeks of treatment. Although the transepidermal water loss (TEWL) values as marker of the inside-outside skin barrier function improved significantly more in the betamethasone valerate-treated group, the important skin barrier structures (lamellar bodies and formation of the lipid bilayers in the lower stratum corneum) did not normalize. Unfortunately, the transmission electron microscopy (TEM) investigations in that study could not be statistically evaluated because of the small number of samples. The present study tries to answer the question as to whether there is a significant difference in physiological lamellar bodies and TEWL after moderate strength corticosteroid 0.1% triamcinolone acetonide cream (TA), i.e. a steroid less potent than betamethasone valerate.

METHODS

Patients, treatment regimens, and analysis methods

In a controlled, randomized, double-blinded right-left arm comparison study we investigated the effect of 1% PIM cream¹ on skin barrier structure and lamellar body formation in AD. TA 0.1% cream treatment served as verum control. We included 15 patients with mild-to-moderate AD according to our previous trial protocol (15). All data evaluation including TEM was performed in a blinded manner. Clinical assessment (partial Eczema Area and Severity Index (pEASI)), biophysical measurements: TEWL, stratum corneum hydration, histological, immunochemical, and proliferation assay, and TEM, and statistical methods were performed analogously to ref. 15. Stratum corneum integrity was measured by counting tape strips until TEWL reached values above 30 g/m²/h. The study was approved by the local ethics commission and the German Federal Institute for Pharmaceuticals and Medical Products (EudraCT-No. 2007-003106-99). Mean and standard error of the mean are used throughout.

RESULTS

Clinical assessment

All 15 patients (7 women and 8 men, age 21–45 years) completed the study according to the protocol. Both groups started with almost identical scores, and both treatment regimens led to an improvement in clinical symptoms. Clinical scoring revealed a faster improvement in the TA-treated group compared with the PIM-treated group. Already at day 8 there was a significant difference between the treatment groups ($p=0.022$). The difference was even more pronounced at day 22 ($p=0.0008$, Fig. 1A). The lesion size at the start of the study was similar in both groups and consistent with the study's entry criteria. The lesion size reduced in both groups, but did

so more quickly in the TA group: at the day 8 time-point the difference reached a p -value 0.2072, and at the day 22 time-point $p=0.0135$ (Fig. 1B). The visual analogue scale from the patient's logbook revealed a reduction in pruritus in both treatment groups. However, in the PIM-treated group there was a transitory increase in pruritus followed by significant decrease in pruritus ($p=0.0010$, Fig. 1C). The TA-treated arm improved continuously ($p<0.0001$). The difference between the treatment groups was statistically significant at most of the time-points ($p<0.05$). Although all patients were introduced to the fingertip-unit method for applying cream, overall cream usage varied widely over the course of the treatment in the range 4.5–43 g. The difference in cream usage was up to 8 g, but the side differences distributed equally in both groups (Fig. 1D).

Biophysical assessment (transepidermal water loss, stratum corneum hydration and integrity)

An almost 3-fold enhanced TEWL was seen in lesional skin compared with non-lesional skin in the defined area of the atopic patients (Fig. 2A and C). But even non-lesional skin showed absolute TEWL values of around 10 g/m²/h, representing higher values compared with healthy volunteers (14). There was no significant change in non-lesional skin during the treatment period in either regimen (Fig. 2A). Similar results were found for stratum corneum hydration in non-lesional skin (data not shown).

Lesional skin TEWL improved in both groups during treatment. The TEWL values in the TA-treated group were more reduced compared with the PIM-treated group ($p=0.1036$ at day 8 and $p=0.0003$ at day 22) (Fig. 2C). The stratum corneum hydration was slightly reduced at day 8 after TA treatment, but showed a tendency toward improvement in both groups at day 22 (n.s.).

The stratum corneum integrity (assessed as the mean number of tape-strips required for TEWL to exceed 30 g/m²/h) improved by approximately 16% in both groups (PIM: from 64.0 to 54.3, $p=0.2585$; TA: from 64.0 to 52.7, $p=0.1929$).

Proliferation assay and histological analysis

Using Ki-67 antibodies the proliferation rate of keratinocytes was reduced by 33% in the PIM-treated group ($p=0.1235$) and by 70% in the TA-treated group ($p=0.0002$) compared with untreated lesional skin. The difference between the treatment groups was highly significant ($p=0.0088$, Fig. 3A). The proliferation-associated K16 correlated with the proliferation analysis (Fig. 3B). The untreated lesional epidermis was twice as thick as healthy skin. Epidermal thickness was reduced ($p=0.275$) after PIM treatment (Fig. 3C).

A broadening of the staining band for involucrin is seen in lesional compared with non-lesional skin and the skin of healthy controls (14). The thickness of the stai-

¹ According to German law (AMG=Arzneimittelgesetz) the manufacturer of the cream preparations is the hospital pharmacy of the Universitätsklinikum Schleswig-Holstein, Campus Kiel, Kiel Germany because the hospital pharmacy refilled the commercially available creams Elidel Cream® (Novartis Pharma GmbH, Nuremberg, Germany) and Triamgalen® (GALENpharma GmbH, Kiel, Germany) into blinded cream tubes.

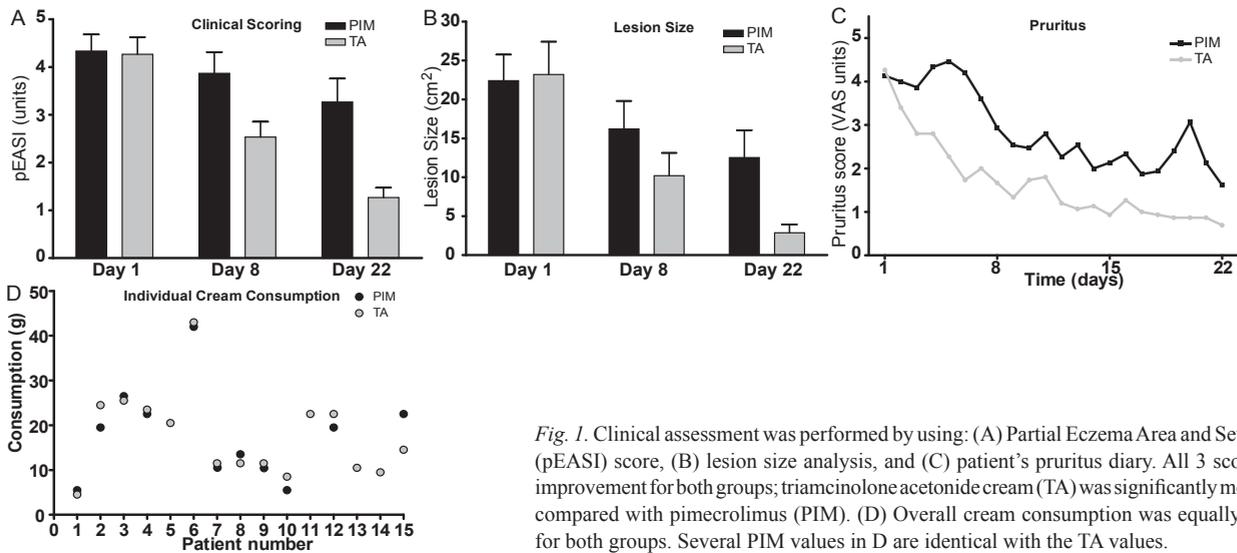


Fig. 1. Clinical assessment was performed by using: (A) Partial Eczema Area and Severity Index (pEASI) score, (B) lesion size analysis, and (C) patient’s pruritus diary. All 3 scores showed improvement for both groups; triamcinolone acetonide cream (TA) was significantly more effective compared with pimecrolimus (PIM). (D) Overall cream consumption was equally distributed for both groups. Several PIM values in D are identical with the TA values.

ned band was reduced by PIM treatment ($p=0.1517$), and more pronounced by TA treatment ($p=0.0041$) compared with untreated skin (data not shown). However, differences between both treatment regimens did not reach significance ($p=0.1457$). The score for loricrin immunostaining did not change significantly during the treatment period in either the PIM or the TA group (data not shown). Filaggrin expression showed a tendency toward enhancement after PIM treatment (n.s.), whereas no change occurred after TA treatment.

Ultrastructural analysis

Lamellar bodies (49–99 for each sample) were analysed in the stratum granulosum/stratum corneum interface for quantification if they contained at least 50% lamellar structures. The TEM investigations revealed a lack of physiological lamellar bodies in lesional skin of AD (only 34% compared with healthy control, $p < 0.0001$). A significantly higher number of physiological lamellar

bodies was found after 3 weeks of treatment with PIM (+86%, $p < 0.0001$ compared with untreated AD) and after 3 weeks of TA treatment (+46%, $p = 0.0035$ compared with untreated AD). The 2 treatment regimens differed significantly in favour of the PIM-treated group ($p = 0.0457$; Fig. 3D). The lack of physiological lamellar body extrusion probably leads to an inchoate skin barrier after TA treatment, whereas PIM treatment led to a more physiological formation of the lipid bilayers.

DISCUSSION

The skin seems to be the central organ for the "atopic march". A dysfunctional skin barrier may lead to allergen penetration and sensitization to environmental allergens, which may contribute to the development of allergic asthma (18). Therefore, the repair of the skin’s barrier function becomes an important objective in the treatment of AD. The present study compared the influence of a moderate strength corticosteroid cream (0.1%

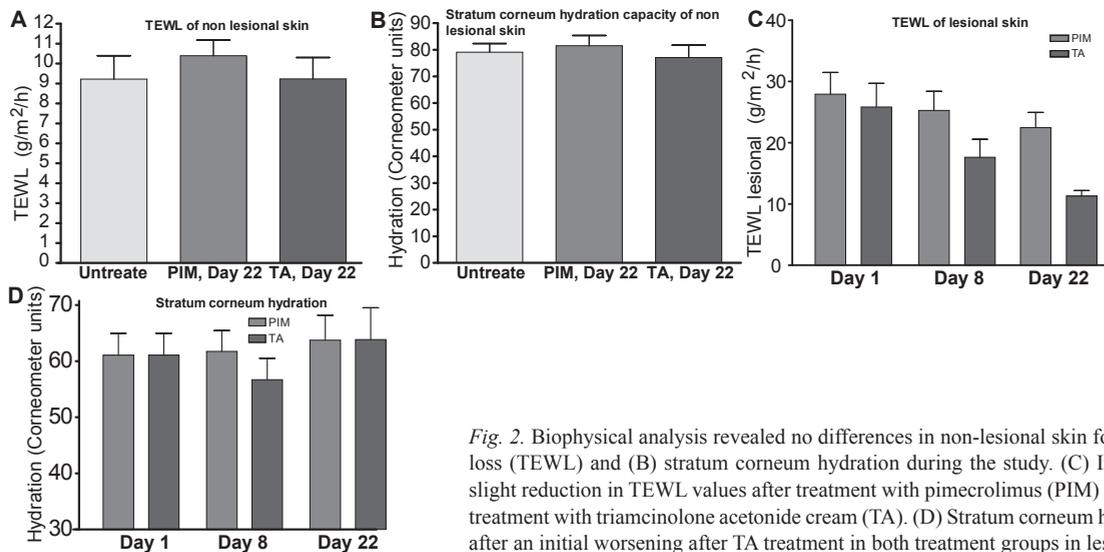


Fig. 2. Biophysical analysis revealed no differences in non-lesional skin for (A) transepidermal water loss (TEWL) and (B) stratum corneum hydration during the study. (C) In lesional skin there was a slight reduction in TEWL values after treatment with pimecrolimus (PIM) and a strong reduction after treatment with triamcinolone acetonide cream (TA). (D) Stratum corneum hydration improved slightly, after an initial worsening after TA treatment in both treatment groups in lesional skin.

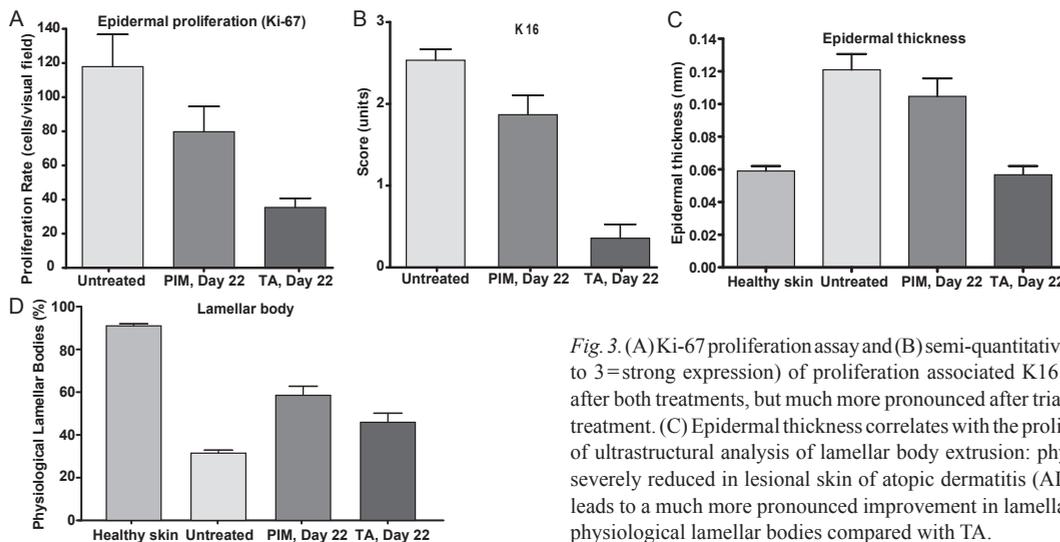


Fig. 3. (A) Ki-67 proliferation assay and (B) semi-quantitative evaluation (score from 0 = absent to 3 = strong expression) of proliferation associated K16 revealed a significant reduction after both treatments, but much more pronounced after triamcinolone acetonide cream (TA) treatment. (C) Epidermal thickness correlates with the proliferation rates. (D) Quantification of ultrastructural analysis of lamellar body extrusion: physiological lamellar bodies were severely reduced in lesional skin of atopic dermatitis (AD) patients. Pimecrolimus (PIM) leads to a much more pronounced improvement in lamellar body extrusion and increase in physiological lamellar bodies compared with TA.

TA) with the calcineurin inhibitor PIM as 1% cream formulation on the epidermal skin barrier structure and clinical outcome in AD patients.

We found that according to pEASI and lesion size, TA improved clinical symptoms more effectively than PIM. When comparing the previous (15) and the present study the improvement after treatment with PIM was almost the same in both studies (though pEASI was approximately 10% higher in the present study). The result seems surprising, because betamethasone valerate is regarded to be more potent than TA, according to US classification (19). However, in some cases greater clinical improvement using a less potent corticosteroid has been found (20), and this effect may be already related to the fact that the disrupted barrier in AD recovers more after the use of a less potent steroid, as we describe here.

Pruritus is a patient's subjective parameter and therefore it was evaluated using a visual analogue scale in the patient's diary. Both treatment groups improved significantly, but TA treatment was slightly more effective than PIM and the pruritus reduction was more continuous.

Our biophysical evaluation confirmed previous studies showing that corticosteroid treatment influences TEWL. It has been shown previously that application of clobetasol propionate cream to healthy skin for 3 weeks led to the well-known thinning of the skin and an increase in TEWL (21). We previously found that betamethasone valerate cream in AD leads to a reduction in TEWL (15). In the present study, TA reduced TEWL to a similar extent, but the effects were less pronounced than those found for betamethasone valerate in our previous study (15). Thus TEWL measurements do not seem to be reliable markers to investigate effects of corticosteroids on the skin barrier in AD, because they do not correlate with skin barrier structure under these circumstances. Most of the reduction in TEWL may be related to the well-known vasoconstrictive effect of corticosteroids.

The potency of corticosteroids is determined by the vasoconstriction test (21). Vasoconstriction causes reduced fluid flow into the dermis and the epidermis, and conceivably reduces TEWL. TEWL depends on the barrier component plus a driving force component. The essential part of this component is the blood flow (22).

A second biophysical marker, the hydration of the stratum corneum improved very little in either group. Dry skin is well known in AD and increase in stratum corneum hydration may be a sign of the improved epidermal differentiation including an increase in filaggrin. Filaggrin break-down products are important for water binding of the skin (23).

The severe hyperproliferation in lesional skin of AD patients, as shown by the Ki-67 antibody and proliferation-associated K16, was reduced in both treatment groups. The reduction was much more pronounced with TA compared with PIM. The reduction after PIM treatment was similar, as in our previous study (15). After TA treatment the proliferation values were lower than in non-lesional skin of AD patients and showed a tendency to be lower compared with healthy controls, as shown in our previous study (14). Epidermal thickness was slightly lower compared with healthy skin, meaning that a slight epidermal atrophy is already present at 3 weeks of treatment, though not as pronounced as with betamethasone valerate (15).

Semi-quantitative evaluation of cornified envelope protein expression revealed no significant differences between both treatment groups with the exception of involucrin expression, which was reduced after 3 weeks of treatment with TA compared with untreated lesional skin of AD patients. Involucrin binds covalently ceramides of the lipid bilayer structure and anchors in the cornified envelopes of the corneocytes. Therefore, a reduction in involucrin expression is not desirable. There was a tendency toward an increase in filaggrin expression after PIM treatment which is in agreement with the slight

increase skin hydration. Filaggrin breakdown products are important for stratum corneum water binding (23).

A major focus of the present study was to evaluate the effect of TA and PIM on ultrastructural skin barrier structures. Three weeks of PIM treatment led to a highly significant increase in the number of physiological lamellar bodies. In contrast to our previous study with betamethasone valerate cream (15), TA also showed an increase in the number of physiological lamellar bodies, although the effect was much less than that seen with PIM. Unfortunately, there is no established system for assessing the quality of lipid bilayer architecture (24), but the lipid bilayer architecture appears normalized only after PIM treatment, confirming the preliminary results from the previous study (15) using the more potent betamethasone.

In summary, the calcineurin inhibitor PIM and the corticosteroid TA differ not only in their effect on inflammation but also in their influence on the skin barrier structure. We confirmed the reconstructive effect of PIM on lamellar body extrusion and lipid bilayer architecture, whereas TA and the previously examined betamethasone valerate show similar clinical, histochemical, and ultrastructural effects. Speculatively, PIM seems superior in repairing skin barrier architecture compared with corticosteroids of medium or strong potency, which may prevent allergen penetration and relapse of AD.

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