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Conflict of interest

Ehrhardt Proksch and Regina Fölster-Holst have held lectures for Astellas Pharma GmbH. This study was supported by Astellas Pharma GmbH. Kersten Walter is an employee of Astellas Pharma GmbH.

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Comparison of effects of tacrolimus ointment and mometasone furoate cream on the epidermal barrier of patients with atopic dermatitis

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Summary

Background: The skin barrier plays a crucial role in the pathophysiology of atopic dermatitis. The quality of the skin barrier can be assessed using a new semi-quantitative method to measure intercellular lipid lamellae. This procedure was used to evaluate the influence of the topical application of the calcineurin inhibitor tacrolimus 0.1 % ointment (Protopic®) versus mometasone furoate cream (Ecural®) on the quality of the skin barrier.

Patients and Methods: 20 adult patients with active atopic dermatitis (SCORAD 10–63) were included in an open, non-interventional study. Lesions on their forearms were treated twice daily over 10 days with either tacrolimus 0.1 % ointment or mometasone furoate cream. At the beginning and the end of the treatment period, SCORAD, TEWL and skin hydration were determined and the intercellular lipids were measured using transmission electron microscopy.

Results: The SCORAD improved in both groups nearly to the same extent, whereas TEWL and skin hydration improved significantly only in the tacrolimus group. Using the semi-quantitative analysis of intercellular lipid length per 1,000 nm² intercellular space, a twofold increase for mometasone furoate cream and a fourfold increase for tacrolimus 0.1 % ointment were determined.

Conclusions: In addition to its known antiinflammatory effect, tacrolimus 0.1 % ointment leads also to a measurable increase of the lipids of the skin barrier in patients with atopic dermatitis, exceeding the effect of mometasone furoate cream.

Background

The epidermal permeability barrier protects from excessive water loss from inside to outside (inside-outside barrier) as well as from penetration of noxious agents such as allergens, irritants and microorganisms from outside to inside (outside-inside barrier). It is primarily formed during epidermal differentiation, particularly at the transition from the stratum granulosum to the stratum corneum. During the development of keratinocytes to nucleus-free corneocytes, complex modifications occur depending on the degree of differentiation: altered expression of keratins, formation of the cornified envelope with involucrin and loricrin, production of filaggrin from profilaggrin yielding the natural moisturizing factor

(NMF). Further, lipids synthesized in the keratinocytes are transported in the lamellar bodies and released into the intercellular space at the transition to the stratum corneum. This complex structure consisting of corneocytes and intercellular lipids forms an intact and flexible unit that protects underlying tissue from the penetration of noxious agents and regulates transepidermal water loss [1–7].

Disturbed skin barrier – pathogenetic factor in atopic dermatitis

Atopic dermatitis (AD) is the result of a complex interaction between environmental and genetic factors that influence the epidermal structure and function as well as the immune

system. The result is a hyperreactivity of the skin towards environmental agents [3–4, 8].

A primary cause of AD is a disturbed epidermal skin barrier. This is reflected – even in clinically normal skin – in an increased transepidermal water loss (TEWL) as well as in reduced hydration of the stratum corneum. Further, the penetration of noxious agents and allergens through the skin is facilitated, which can trigger an acute eczema flare via synergistic immunological mechanisms [2–4].

Therapy of atopic dermatitis

Current guidelines [9–11] view the avoidance or reduction of trigger factors as well as the regular application of emollients as the basic therapy of AD. If this does not achieve a satisfactory result, topical corticosteroids are employed. These have an anti-inflammatory action and demonstrate a significant therapeutic effect in clinical studies in comparison to placebo. The use of corticosteroids can be problematic due to the danger of atrophy, especially on the face (periorbital region), scrotum, neck and intertriginous regions [12, 13].

In the event of lack of efficacy of topical corticosteroids, intolerance of corticosteroids or the presence of pre-existing atrophic skin, topical calcineurin inhibitors (tacrolimus, pimecrolimus) are recommended by the guidelines [9]. Through their selective action, they inhibit the synthesis of interleukins (especially IL-2) and other cytokines, suppress T-cell activation and proliferation and interrupt the inflammation cascade [14]. Efficacy and tolerability have been proven for both pimecrolimus and tacrolimus in clinical studies [15]. For example, topical tacrolimus (0.1 %) is as effective as hydrocortisone butyrate, a modern topical corticosteroid of class II (German classification according to Niedner) [12, 16].

As the epidermal barrier plays a central role in the pathophysiology of AD, it appears sensible to examine the available therapy options with respect to their potential for restoring the skin barrier. To date, comparisons between pimecrolimus and betamethasone valerate cream formulations exist, but not for 0.1 % tacrolimus ointment and a topical corticosteroid cream.

Objective

The present study compares the regeneration of intercellular lipid lamellae (ICLL) in patients with AD during therapy with the topical calcineurin inhibitor tacrolimus and the corticosteroid mometasone furoate. Here the epidermal barrier was visualized and evaluated semi-quantitatively using morphometric analysis.

Patients and methods

This open, non-interventional study received a positive ethics vote from the Medical Faculty of the Christian Albrecht University of Kiel. A total of 20 patients with active atopic dermatitis (local SCORAD – SCORing Atopic Dermatitis – between 10 and 63) were included and distributed to two treatment groups: The mometasone furoate group included 6 men (19 to 45 years, SCORAD 10 to 50) as well as 4 women (21 to 31 years, SCORAD 10 to 63). The tacrolimus group consisted of 6 men (19 to 48 years, SCORAD 10 to 45) as well as 4 women (23 to 45 years, SCORAD 10 to 40).

The eczematous lesions on the arms were treated over ten days twice daily either with 0.1 % tacrolimus ointment (Protopic®) or mometasone furoate cream (Ecural®). The local SCORAD, TEWL and hydration were measured at the initiation of therapy (baseline = t_0) and after ten days of treatment (= t_1). Further, samples of the stratum corneum were obtained using Lipbarvis®.

The biophysical measurements as well as the sample removal for transmission electron microscopy (TEM) were performed in a 3 × 6 cm large area on the interior aspects of the forearms. There was an at least 12-hour time period between the last treatment and the sampling at the point of time t_1 .

The measurement of TEWL and hydration was performed after an at least 10-minute acclimatization of the participants at 21 ± 1 °C and 50 ± 5 % relative humidity. Hydration was measured with a Corneometer CM 825 (Courage & Khazaka, Cologne, Germany) using a triple measurement. TEWL was determined using a single measurement (30 s) with a Tewameter TM 300 (Courage & Khazaka, Cologne, Germany).

The removal of the stratum corneum samples using Lipbarvis® and the processing of the samples for the TEM analysis is described by Dähnhardt-Pfeiffer et al. [17]. The TEM analysis shows the intercellular lipids. When in the subsequent morphometric analysis the length of the intercellular lipid lamellae (ICLL) in the intercellular space (ICS) are set into a relationship to the surface area of the intercellular space and calculated with respect to a reference value of $1,000 \text{ nm}^2$ (nICLL), this quotient allows for a semi-quantitative statement on the quality of the skin barrier [17].

Statistical analysis

The examined parameters SCORAD, TEWL, hydration and nICLL were analyzed using the Shapiro-Wilk test for normal distribution at the two measurement time points before treatment (t_0) and after ten days of treatment (t_1). In the event of a significant deviation from a normal distribution, the two treatment groups (mometasone furoate cream and

0.1 % tacrolimus ointment) were compared using the U-test with respect to the values at both measurement time points, otherwise the t-test for independent samples was employed. The comparison of both time points for each medication was performed in the event of significant deviation from a normal distribution using the Wilcoxon two-sample paired signed rank test or with the matched-pairs t-test.

A two-sided analysis was performed and a level of significance of 5 % was presumed. An alpha-adjustment for multiple tests was not performed; the results correspondingly have an explorative and descriptive character. IBM SPSS Statistics 20 (SPSS Inc., an IBM Company, Chicago, IL, USA) was used to perform statistical calculations.

Results

Clinical evaluation (SCORAD) and measurement of biophysical parameters (TEWL, hydration)

During therapy with both mometasone furoate cream and 0.1 % tacrolimus ointment, the clinical picture of AD improved significantly within ten days. The baseline SCORAD for the mometasone group at t_0 was between 10 and 63 and declined at t_1 to 0 to 20 ($p = 0.005$). In the tacrolimus group, the baseline SCORAD (t_0) was between 10 and 45 and improved at t_1 to 0 to 10 ($p = 0.005$) (Figure 1).

The TEWL also declined with both therapy options after ten days of therapy (mometasone furoate group: $t_0 = 17 \pm 8$, $t_1 = 13 \pm 6$ and, respectively, tacrolimus group: $t_0 = 17 \pm 8$, $t_1 = 10 \pm 5$). The difference to the baseline was significant in the tacrolimus group ($p = 0.021$) (Figure 2).

While the hydration of the skin remained nearly constant in the mometasone furoate group ($t_0 = 22 \pm 17$, $t_1 = 22 \pm 12$), in the tacrolimus group a doubling of skin moisture occurred ($t_0 = 14 \pm 5$, $t_1 = 30 \pm 11$; $p = 0.017$) (Figure 3).

TEM and morphometric analysis of the intercellular lipids

The most distinct differences between treatment with 0.1 % tacrolimus ointment and mometasone furoate cream were seen in electron microscopy and the semi-quantitative analysis of the intercellular lipid lamellae of the stratum corneum. At baseline (t_0) the organization of the ICLL was clearly disturbed in both patient groups. Few lipid lamellae were found in the intercellular space between the corneocytes in the stratum corneum. These lipid lamellae are found – as often in AD – typically more along the outer edges of the intercellular space (Figure 4a). After 10 days of therapy with mometasone furoate, more lipid lamellae distributed diffusely throughout the intercellular space can be recognized (Figure 4b).

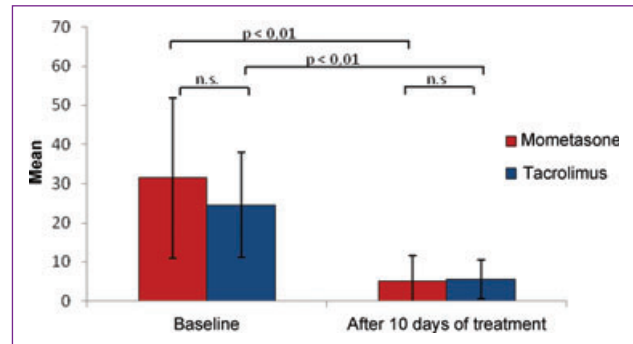


Figure 1 SCORAD: Significant decrease of clinical symptoms of AD after 10 days of treatment with either mometasone furoate cream or tacrolimus ointment.

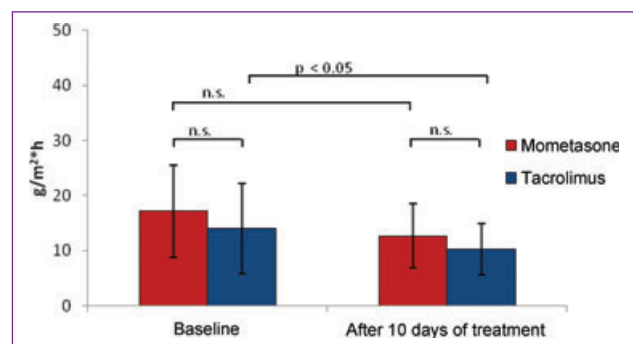


Figure 2 TEWL: decrease of TEWL after 10 days of treatment with either mometasone furoate cream or tacrolimus ointment. Only for tacrolimus ointment is the difference significant.

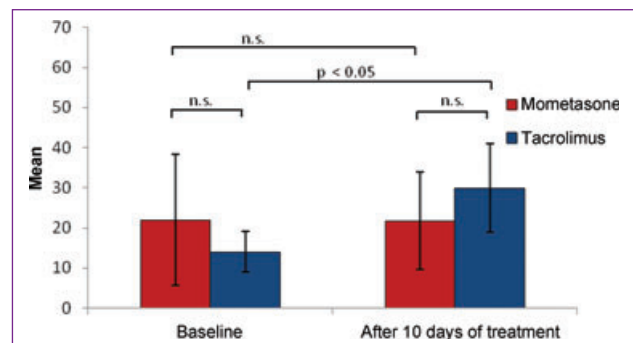


Figure 3 Skin hydration: significant increase of skin hydration following a 10-day treatment with topical tacrolimus ointment.

In the tacrolimus group at baseline t_0 there was a reduced number of lipid lamellae in the intercellular spaces of the stratum corneum as is typical for AD (Figure 5a). At t_1 a distinct increase in the number of lipid lamellae in the intercellular space was seen (Figure 5b).

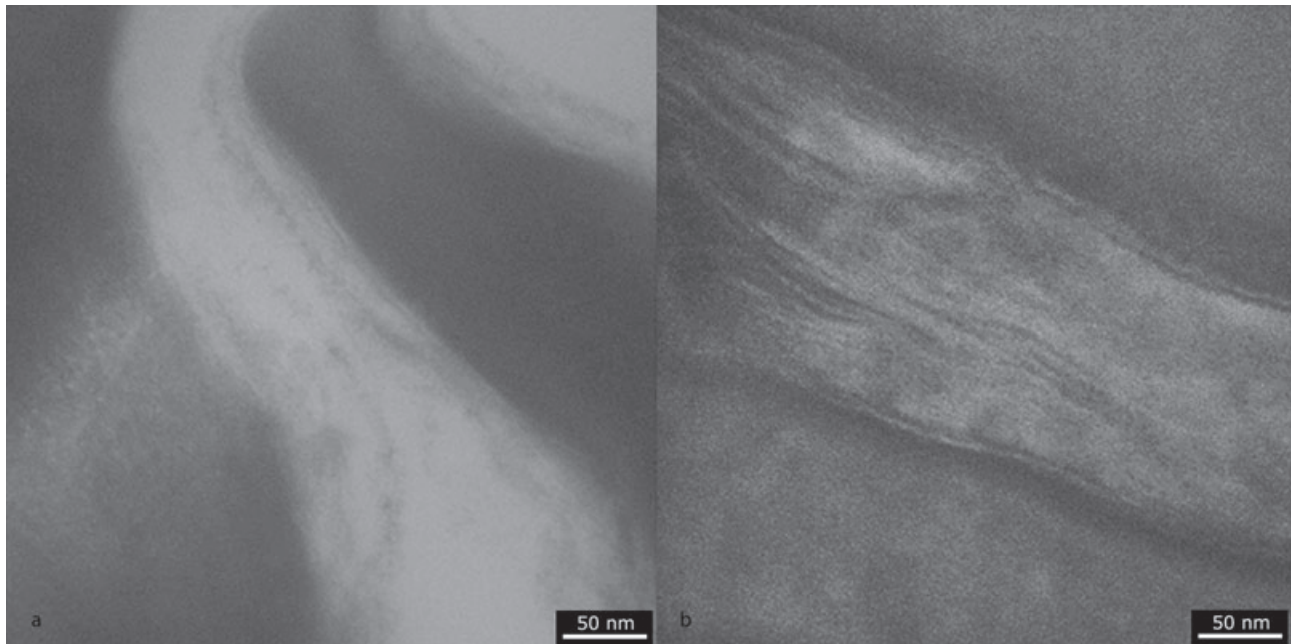


Figure 4 TEM: Depiction of lipid lamellae in the intercellular space. Before treatment (t_0) (a). After 10 days of treatment with mometasone furoate cream (t_1), more lipid lamellae are visible and diffusely spread throughout the intercellular space (b).

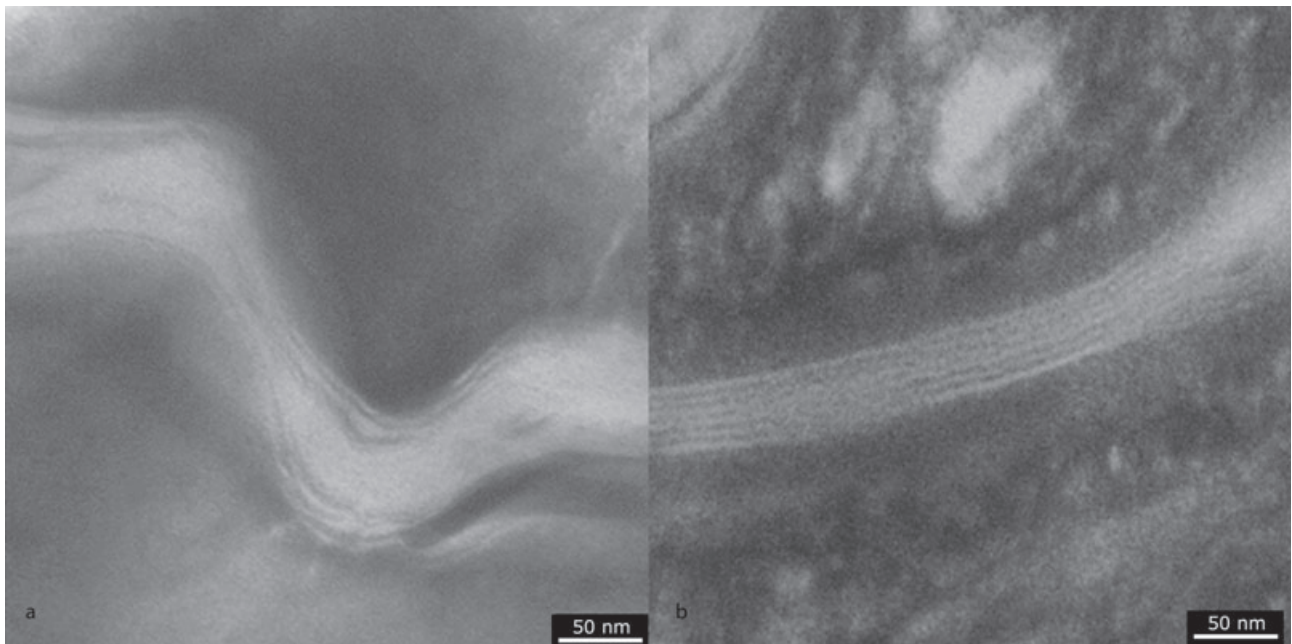


Figure 5 TEM: Depiction of lipid lamellae in the intercellular space. Before treatment (t_0) (a). After 10 days of treatment with tacrolimus ointment (t_1), clearly more lipid lamellae are visible and the intercellular space is widely filled with lipid lamellae (b).

The semi-quantitative analysis for both study medications in comparison to baseline revealed a significant increase in the number of lipid lamellae in the intercellular space ($p < 0.001$). In the mometasone furoate group, the baseline

value for the length of the intercellular lipids per 1,000 nm² intercellular space increased twofold from 37 ± 7 at t_0 to 75 ± 14 ($p < 0.001$) at t_1 . In the tacrolimus group, the baseline value (t_0) was 40 ± 5 and increased at t_1 fourfold to 155 ± 23

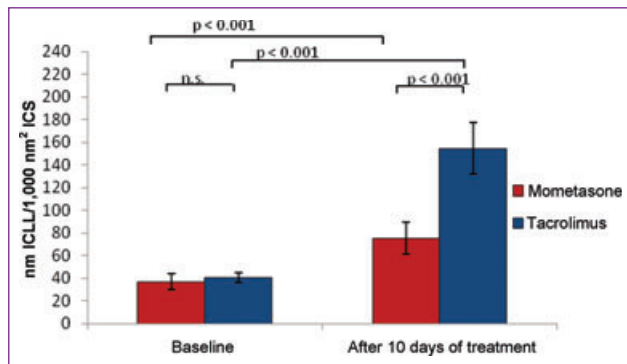


Figure 6 Reorganization of the skin barrier: The semi-quantitative analysis of intercellular lipids shows a significant increase in the number of lipid lamellae in the intercellular spaces ($p < 0.001$ for tacrolimus ointment and mometasone furoate cream). In comparison to baseline, the length of intercellular lipids per 1,000 nm² intercellular space increased twofold in the mometasone furoate cream group from 37 ± 7 to 75 ± 14 . In the treatment group with tacrolimus ointment, the value increased almost fourfold in comparison to baseline (t_0 : 40 ± 5 , t_1 : 155 ± 23).

($p < 0.001$). When one compares both treatment groups with respect to the reorganization of the lipid lamellae in the intercellular space with each other, treatment with tacrolimus was significantly superior to that with mometasone furoate (Figure 6).

Discussion

The most important constituent of the permeability barrier are the intercellular lipid lamellae in the stratum corneum. During epidermal differentiation these lipids are synthesized in the keratinocytes, stored in the lamellar bodies and released into the intercellular spaces of the stratum corneum. The synthesis and storage of the lipids in the lamellar bodies and their release is essential for an effective permeability barrier that protects the organism from penetration by noxious agents and regulates transepidermal water loss.

In atopic dermatitis the barrier function of the skin is distinctly impaired; TEWL is already elevated in clinically normal skin as opposed to healthy persons [2]. Therefore, in the current S2-guideline, basic therapy with emollients is recommended as the foundation of every further therapeutic intervention [9]. In a disease characterized by reduced function of the permeability barrier, therapeutic measures should also be studied with respect to their effect on this skin barrier. In an

acute flare of atopic dermatitis, TEWL rises significantly. The primary cause is the disorganization of the intercellular lipid lamellae during the inflammatory reaction so that the complex system of the epidermal permeability barrier has only limited functional capabilities.

Good clinical efficacy of both therapies

The aim of the present pilot study was to compare the effects of the modern topical corticosteroid mometasone furoate (therapeutic index [TIX] 2.0) with the topical calcineurin inhibitor tacrolimus with respect to the regeneration capability of the intercellular lipids during short-term use. Thus, a new semi-quantitative method was employed [17]. After ten days of topical application of mometasone furoate cream or 0.1 % tacrolimus ointment, a significant improvement of the skin status was seen in both patient groups: SCORAD decreased by 83 % in the mometasone furoate group and by 78 % in the tacrolimus group, respectively. This rapid improvement of clinical features corresponds to the clinical experience with both of these topical agents in AD. For example, Schnopp et al. demonstrated that both active agents are similarly effective in dyshidrotic hand dermatitis [18].

The good efficacy of both therapeutic agents is reflected not only by the decrease in clinical symptoms but also by changes biophysical measurements such as TEWL and hydration. Using the TEWL values as a marker of the barrier, a decline occurred in both groups, but was significant only in the tacrolimus group.

The low TEWL in the mometasone furoate group could in part be explained by the well-known vasoconstriction of the corticosteroids. Further, TEWL is also dependent on perfusion [19].

Stratum corneum hydration improved distinctly in the tacrolimus group, but not in the mometasone furoate group. This phenomenon is probably due to the improved barrier during tacrolimus treatment, while the effects of corticosteroids on the barrier are less distinct. Further, corticosteroids probably reduce the synthesis of water-binding substances through their strong anti-proliferative action.

Studies on the morphology of the stratum corneum demonstrate that the intercellular lipid lamellae (ICLL) are well-suited as markers for the function of the inside-outside and outside-inside barrier [3]. According to semi-quantitative analyses, healthy skin has an nICLL of about 190 nm/1,000 nm², compared to lesional atopic skin with about 40 nm/1,000 nm² [17]. During the 10-day therapy the nICLL in the mometasone furoate group rose about twofold to 80 nm/1,000 nm², reaching about 40 % of the level of intercellular lipids in healthy skin. In the tacrolimus group, nICLL rose almost fourfold to about 160 nm/

1,000 nm² – a value corresponding to 83 % of the normal value of healthy skin.

In this pilot study, no comparison with the vehicles without active agents was performed, so that the effect of the respective vehicles on these results can not be evaluated. In contrast to the cream containing an emulsifier, 0.1 % tacrolimus ointment contains no emulsifiers. Vehicles containing emulsifiers could impact the reorganization of the lipid lamellae [20], so that an overlapping of different effects can not be excluded for the cream.

Jensen et al. cast some light on this question [19]. They examined the effects of two cream formulations containing emulsifiers as well as either pimecrolimus or betamethasone valerate. The pimecrolimus cream proved to be distinctly more effective in barrier repair than the betamethasone valerate cream. We suspect that during therapy with topical calcineurin inhibitors, the endogenous lipid synthesis of the keratinocytes regenerates more rapidly than during topical corticosteroid therapy. Further studies that also examine the vehicles are needed in order to confirm this assumption.

Our results stand in contrast to other studies that report a negative effect on the permeability barrier during the use of tacrolimus [21]. Kim et al. explain the reduction of the epidermal lipid synthesis during the use of tacrolimus on healthy human and mouse skin via inhibition of production and release of IL-1 α [21]. However, they employed TEWL to study only the recovery of the barrier after artificial disturbance but not the barrier itself. In contrast, our study on patients with an active flare of AD demonstrated another result.

We found that TEWL, measurement of skin hydration and Lipbarvis® technology demonstrated a positive effect on the barrier within ten days of treatment with both agents, with 0.1 % tacrolimus ointment being distinctly more effective with respect to the reorganization of the intercellular lipids in the stratum corneum.

This study has the characteristics of a pilot study. It is desirable that the observed results be confirmed in a larger, controlled, randomized and double-blind study and the effect of different active agent-free vehicles be examined.

Conclusions

We conclude that both medications lead to improved measurable reorganization of the intercellular lipids in the intercellular space of the stratum corneum; the change is significantly more distinct for 0.1 % tacrolimus ointment than for mometasone furoate cream.

As expected, both mometasone furoate cream and 0.1 % tacrolimus ointment demonstrate a distinct anti-inflammatory effect.

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