

Percutaneous Absorption of Pimecrolimus Is Not Increased in Patients with Moderate to Severe Atopic Dermatitis when Pimecrolimus Cream 1% Is Applied under Occlusion

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Key Words

Atopic dermatitis • Calcineurin inhibitor • Occlusion • Pharmacokinetics • Pimecrolimus • Safety, skin permeation • Systemic exposure • Topical administration

Abstract

Aim: To evaluate the systemic exposure of pimecrolimus cream 1% applied under occlusion in atopic dermatitis (AD) patients. **Methods:** A noncomparative, open-label study conducted in 3 groups of moderate to severe AD patients: A (adults, n = 9), B (adolescents, n = 4) and C (children, n = 6). Pimecrolimus cream 1% was applied twice daily for 8.5 days with overnight occlusion in patients with investigator's global assessment scores of ≥ 3 and AD involving at least 30% of their body surface area. Pimecrolimus blood concentrations were analyzed. **Results:** The highest pimecrolimus blood concentrations observed in adults, adolescents and children were 1.84, 0.55 and 1.29 ng/ml, respectively. Pimecrolimus blood concentrations and affected body surface area showed no apparent correlation. **Conclusion:** No measurable differences were found in pimecrolimus blood concentrations, efficacy and safety profile when pimecrolimus

cream 1% was applied under occlusion versus application without occlusion. These findings reflect the high lipophilic properties of pimecrolimus.

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease that affects all age groups and is the most prevalent in children. It is characterized by intense pruritus, and its course is marked by exacerbations and remissions [1]. Although the traditional mainstay of active treatment is topical corticosteroids, some patients do not adequately respond to them. Furthermore, the use of corticosteroids in AD is also limited due to their potential for adverse events (AEs) such as skin atrophy or striae [2].

Pimecrolimus cream 1% has been shown to be effective in adolescents and adults with AD who are intolerant to or dependent on topical corticosteroids. It can also be used for treatment of sensitive skin (e.g. face and other sensitive skin areas, skin of infants), as it has no atrophogenic potential [3–5]. In addition, pimecrolimus cream

reduces the requirement of topical corticosteroids in the management of severe pediatric AD [6]. Pimecrolimus has a favorable tolerability and safety profile in adults, adolescents and children and is absorbed at very low levels even in children [7, 8].

Pimecrolimus, applied as 1% cream on the skin, penetrates the skin. However, permeation through the skin and systemic absorption are low so that blood levels of pimecrolimus are generally below 2 ng/ml, and largely even less than 0.5 ng/ml, in both pediatric and adult patients [8–16]. Low skin permeation has also been observed with human skin in vitro. When treated with pimecrolimus 1% cream for 24 h, only about 3% each was absorbed into the epidermis (including the stratum corneum) and the dermis [17]. Human skin penetration of pimecrolimus in vitro was similar to that of tacrolimus, another calcineurin inhibitor and corticosteroids; however, permeation through the skin and systemic exposure to pimecrolimus were lower than with tacrolimus and much lower than with corticosteroids. The lower skin permeation of pimecrolimus was explained by its higher lipophilicity (compared with tacrolimus and corticosteroids) and larger molecular weight (compared with corticosteroids) [18].

Occlusion is often used to enhance percutaneous absorption of topically applied drugs [19, 20]. An occlusive therapy with pimecrolimus cream 1% leads to marked improvements and long-term clearance of relapsing dys-hydrosiform eczema of the hand and foot [21]. In chronic hand dermatitis, treatment with pimecrolimus cream under occlusion was shown to be associated with low systemic exposure and a good efficacy and safety profile [22]. Thus, the present study was designed to evaluate primarily the systemic exposure of AD patients after pimecrolimus cream 1% had been applied under occlusion.

Methods

Study Design

This was a noncomparative, open-label study, conducted at 2 centers in Germany. The study comprised a screening period of 1 day to 1 week, followed by a treatment period of 8.5 days, and a final evaluation period of 1 week after the last application of pimecrolimus cream 1%. The study was conducted according to the ethical principles of the Declaration of Helsinki and was approved by the respective independent ethics committee or institutional review board. Informed consent was obtained from each patient before entering the study.

Patients with moderate to severe AD involving at least 30% of their body surface area (as determined by the rule of nine or the

Lund and Browder chart) who fulfilled the Hanifin and Rajka diagnostic criteria for AD with a score of ≥ 3 at the investigator's global assessment (IGA) were enrolled. Patients treated with phototherapy or systemic corticosteroids within 4 weeks prior to baseline, topical therapy (tar and corticosteroids) within 3 days prior to baseline, nephrotoxic drugs or any CYP3A4 inhibitors or inducers within 2 weeks prior to baseline were excluded. Also excluded were pregnant or lactating female patients as well as patients with a history of any malignancy, lymphoproliferative disorders, immunosuppression, erythroderma, Netherton's syndrome and hypersensitivity. The use of bland emollients (urea- or salicylate-free) was permitted if applied at least 1 h after the trial medication. Patients were stratified by age: group A comprised adults aged >18 years, group B adolescents aged 12–17 years and group C children aged 2–11 years. Groups B and C were treated in parallel only after confirmation of the safety of pimecrolimus cream 1% under occlusion in group A.

The primary objectives of this study were to measure the concentration of pimecrolimus in blood after twice daily applications of pimecrolimus cream 1% over 8.5 days, with overnight occlusion, in patients with moderate to severe AD, and to evaluate the topical and systemic tolerability of pimecrolimus cream 1% in this treatment regimen. The preliminary data collected on the efficacy of pimecrolimus cream 1% in this treatment regimen were exploratory.

Treatment

Pimecrolimus cream 1% was applied twice daily, approximately every 12 h, for 8.5 days (the first and last applications were in the morning on days 1 and 9, respectively). In the morning, pimecrolimus cream 1% was applied on all lesional skin areas, including those on the face. The total body surface area (TBSA) affected at baseline and treated with the morning dose administration is listed in table 1. In the evening, all lesions were treated again. In addition, each evening study medication was applied to the entire skin surface of the 4 limbs (lesional and nonlesional skin areas, except predefined venipuncture sites), and these areas were wrapped with plastic film. Wrapping was carried out only in the evening (except for the morning application on day 9, which was also wrapped). The occlusive wrapping was left in place overnight for at least 8 h. Patients were permitted to apply the required amount of pimecrolimus 1% on each occasion, and the amount of cream used during the treatment period was estimated on day 9 by weighing all tubes that were dispensed on day 1. For adults and children aged ≥ 8 years, the head and neck, the upper extremities, the trunk and the lower extremities were assigned proportionate body surface areas of 10, 20, 30 and 40%, roughly consistent with the rule of nine for adults. For children aged below 8 years, a modification assigned 20% (head and neck), 20% (upper extremities), 30% (trunk) and 30% (lower extremities) to the 4 regions, respectively. The area treated overnight under occlusion on the upper limbs represented about 20% of the TBSA. The treated area of the lower limbs represented another 40% of the TBSA for adults and 30% of the TBSA for children. Therefore, the TBSA treated under occlusion was about 50% in children and about 60% in adults. Thus, the occlusive overnight treatment was similar within patient groups, whereas the nonocclusive morning treatment was applied to the affected TBSA, which represented an individual and a variable component to the whole daily exposure to pimecrolimus.

Pharmacokinetic Assessments

Blood samples were collected on day 1 at 0 h, on day 9 at 0 h (within 10 min prior to the morning application and wrapping), and at 2, 4 and 8 h after the application and wrapping, and finally after study completion on day 16. Blood samples were analyzed by a validated liquid chromatography-mass spectrometry method. Pharmacokinetic variables were assessed for day 9 if at least 3 out of 4 measured concentration values were above the limit of quantification. The limit of quantification was 0.3 ng/ml for samples from adults. During the study, for the analysis of samples from adolescents and children, the limit of quantification was improved to 0.05 ng/ml. The following pharmacokinetic variables were determined using noncompartmental methods: $AUC_{0-8\text{ h}}$ (area under the whole-blood concentration/time curve from time 0 to 8 h), $AUC_{0-12\text{ h}}$, $AUC_{0-24\text{ h}}$, maximum observed whole-blood concentration and time of maximum observed whole-blood concentration. AUC variables were calculated by the linear trapezoidal method. The $AUC_{0-8\text{ h}}$ was calculated using the concentrations measured at 0, 2, 4 and 8 h. The $AUC_{0-12\text{ h}}$ was extrapolated from $AUC_{0-8\text{ h}}$ assuming steady-state conditions, that is the concentration $C_{12\text{ h}}$ was assumed to be equal to $C_{0\text{ h}}$. Finally, the $AUC_{0-24\text{ h}}$ was estimated as $2 \times AUC_{0-12\text{ h}}$. The potential association between blood concentrations and TBSA affected at baseline and TBSA treated was evaluated graphically.

Efficacy and Safety Assessments

Efficacy assessments included the measurement of the extent of lesions and IGA and eczema area and severity index (EASI) scores [23]. The percentage of the TBSA affected by lesions was evaluated using the rule of nine in adults or the Lund and Browder chart for children. IGA is a 6-point static score that assesses disease severity on the basis of morphology (ranging from 0 = clear to 5 = very severe). The IGA and EASI scores were assessed at screening, baseline, day 9 and after study completion (day 16). The safety assessments included monitoring and recording of all AEs and serious AEs.

Statistical Methods

Descriptive statistics of pharmacokinetic parameters included mean, standard deviation (SD) and median. The range of values was presented for selected variables. Since time of maximum observed whole-blood concentration is generally evaluated by a non-parametric method, median and range values were given for this parameter.

All patients who completed the trial with evaluable efficacy measurements were included in the exploratory efficacy data analysis. All patients who received at least 1 treatment were included in the safety and tolerability evaluation. As with the pharmacokinetic variables, descriptive statistics were used for analysis of the efficacy and safety variables.

Results

Out of the 20 patients screened, 19 were given the study medication. Sixteen patients completed the study (7, 4 and 5 patients in groups A, B and C, respectively), while 3 patients discontinued the study. The reasons for

Table 1. Patient demographics and baseline disease characteristics

	Group A (adults, n = 9)	Group B (adolescents, n = 4)	Group C (children, n = 6)
Age, years	39.6 ± 16.7	14.0 ± 1.4	5.8 ± 2.8
Gender			
Male	6 (66.7)	3 (75)	3 (50)
Female	3 (33.3)	1 (25)	3 (50)
Race			
Caucasian	8 (88.9)	3 (75)	3 (50)
Black	1 (11.1)	–	2 (33.3)
Asian	–	1 (25)	1 (16.7)
Weight, kg	74.0 ± 17.1	69.9 ± 14.4	24.8 ± 10.8
Height, cm	173.1 ± 9.8	167.0 ± 7.7	115.3 ± 17.3
BMI, kg/m ²	24.5 ± 3.5	25.1 ± 5.1	17.9 ± 2.2
TBSA affected, %	43.4 ± 16.1	35.8 ± 9.6	44.2 ± 15.3
IGA score	3.7 ± 0.5	3.8 ± 0.5	3.3 ± 0.5
EASI score	29.7 ± 12.2	26.4 ± 7.3	26.5 ± 6.3

Results are means ± SD or numbers with percentages in parentheses. BMI = Body mass index; EASI = eczema area and severity index.

discontinuation included an AE (n = 1) and withdrawal of consent (n = 2). The patient disposition is shown in figure 1. The mean ages of patients in groups A, B and C were 39.6, 14.0 and 5.8 years, respectively. The average severity of the disease was comparable across the groups. Patient demographics and baseline disease characteristics are summarized in table 1. The dose variation observed during the study treatment was based on the nature of treatment, amount of cream applied, and area of body covered. The total amount of cream applied (mean ± SD) during the study was 348 ± 230, 220 ± 73.1 and 141 ± 78.4 g in groups A, B and C, respectively.

Pharmacokinetics

Seven patients in group A, 4 in group B and 2 in group C provided complete blood samples as per protocol. The mean concentrations of pimecrolimus in blood on day 9 exhibited a plateau over a time interval of 0–8 h (fig. 2). There was no obvious difference between the study groups. All mean pimecrolimus blood concentrations were below 0.8 ng/ml. The determined pharmacokinetic variables (with mean, SD and range) are summarized in table 2. The $AUC_{0-24\text{ h}}$ was estimated in 10 patients across the groups (groups A and B: n = 4; group C: n = 2) and was in the range of 2.4–41.4 ng·h/ml. The highest individual blood concentrations observed during the study

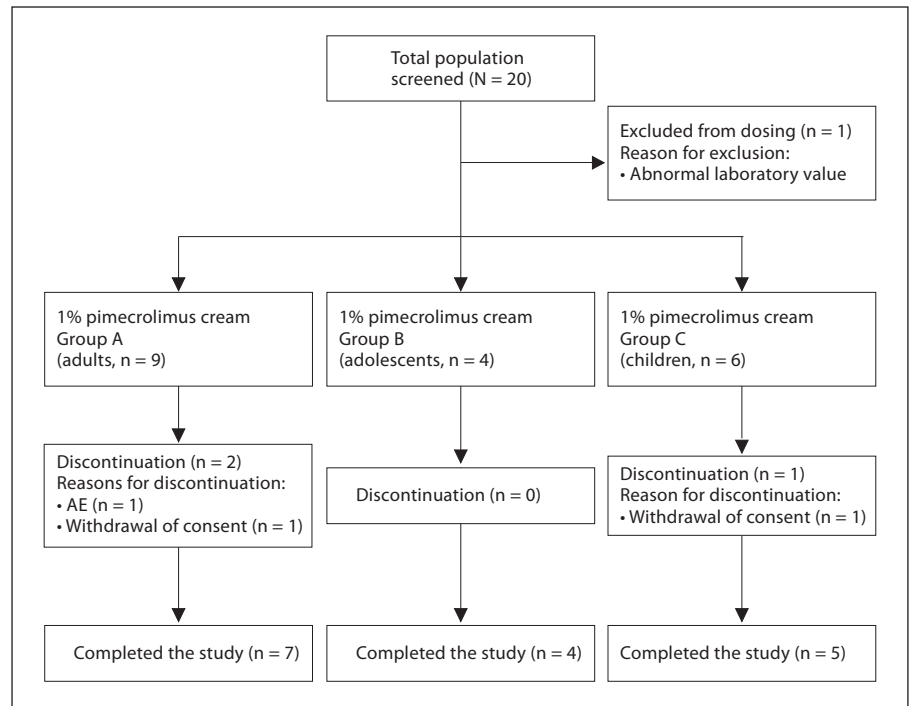


Fig. 1. Patient disposition.

with 1% pimecrolimus cream under occlusion were 1.84, 0.55 and 1.29 ng/ml in adults, adolescents and children, respectively. Given the large variability, there was no obvious difference in the pharmacokinetic variables between the study groups.

A total of 71 blood samples were collected and analyzed. The frequency distribution of all measured blood concentrations of pimecrolimus under occlusion is shown in figure 3a. Eighty-four percent of all samples measured were below 0.5 ng/ml. There was no obvious difference between the study groups. For comparison, data from previously published studies of pimecrolimus without occlusion in AD patients are shown in figure 3b. The similarity between the data indicates that occlusion did not cause any increase in skin penetration, that is in systemic exposure to pimecrolimus.

To check for a potential relation between pimecrolimus blood levels and the TBSA affected, all measured blood concentrations were plotted versus affected TBSA (fig. 4). For the whole patient group with affected TBSA in the range of 30–72% comprising adults, adolescents and children, blood levels showed strong variability but no correlation with affected TBSA. As an apparent exception, the highest blood concentration was observed in an adult patient with an affected TBSA of 80%; however, these concentrations were still within the concen-

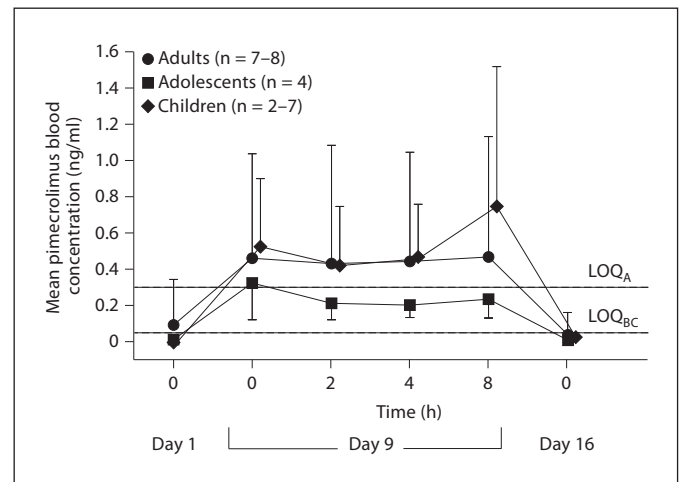


Fig. 2. Mean ($\pm$ SD) pimecrolimus blood concentrations with pimecrolimus cream 1% twice daily under overnight occlusion. LOQA = Limit of quantification in adults; LOQBC = limit of quantification in adolescents and children; concentrations below the limit of quantification are shown as 0 values.

tration range observed in nonocclusive studies, and the results are consistent with published data showing no correlation with the percentage of affected TBSA [10, 12, 14, 15].

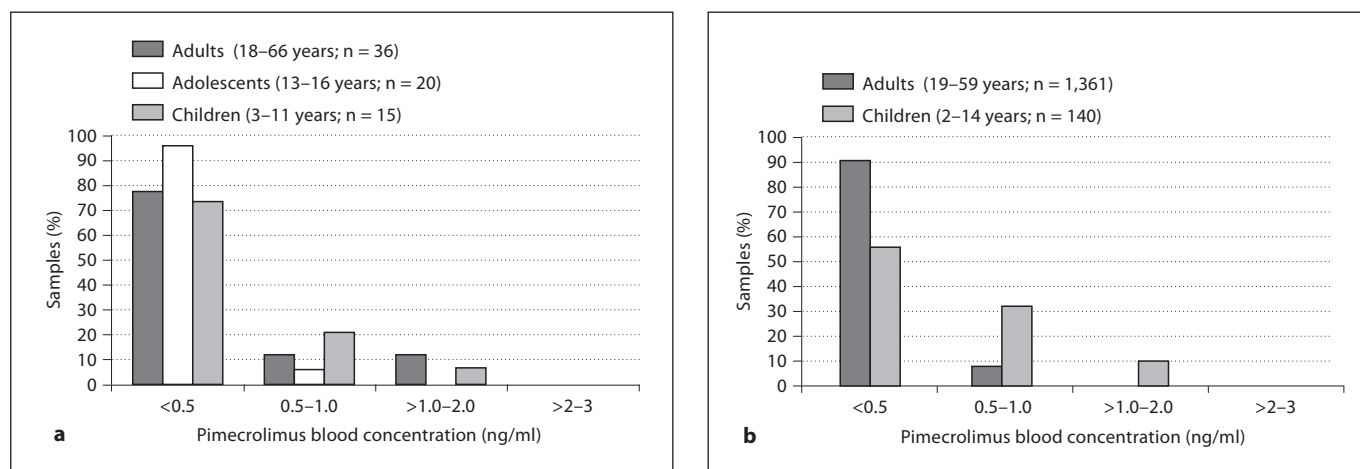


Fig. 3. Frequency distribution of pimecrolimus blood concentrations; n = number of blood samples analyzed in each group. Comparison of treatment under occlusion (a) and with nonocclusive treatment (b); pooled data from Harper et al. [10], Van Leent et al. [11, 14] and Allen et al. [13].

Table 2. Pharmacokinetic variables for pimecrolimus blood concentrations on day 9 following treatment with pimecrolimus cream 1% twice daily under occlusion

	Group A (adults, n = 4)		Group B (adolescents, n = 4)		Group C (children, n = 2)	
	mean $\pm$ SD	range	mean $\pm$ SD	range	mean $\pm$ SD	values ^a
$t_{\max}$, h	4 ^b	0-8	2 ^b	0-8.2	6 ^b	4; 8
$C_{\max}$, ng/ml	0.89 $\pm$ 0.66	0.36-1.84	0.36 $\pm$ 0.18	0.11-0.55	0.76	0.23; 1.29
t_{last} , h	8.00 $\pm$ 0.05	7.98-8.08	8.06 $\pm$ 0.13	7.97-8.25	8.00	8.0; 8.0
AUC _{0-8 h} , ng·h/ml	6.21 $\pm$ 5.21	2.47-13.91	1.84 $\pm$ 0.68	0.85-2.30	4.90	1.76; 8.05
AUC _{0-12 h} , ng·h/ml	9.29 $\pm$ 7.82	3.18-20.70	2.94 $\pm$ 1.14	1.24-3.64	7.57	2.61; 12.52
AUC _{0-24 h} , ng·h/ml	18.57 $\pm$ 15.63	6.35-41.40	5.87 $\pm$ 2.28	2.47-7.29	15.13	5.21; 25.05

$t_{\max}$ = Time of maximum observed whole-blood concentration; t_{last} = time of last quantifiable whole-blood concentration; $C_{\max}$ = maximum observed whole-blood concentration. For calculation of pharmacokinetic parameters, see text.

^a Data reported across 2 patients. ^b Values reported as median.

Efficacy

Treatment with pimecrolimus cream 1% was effective in all 3 groups. The efficacy results are summarized in table 3. The average reduction in EASI scores from baseline at the end of the study were -6.2, -9.5 and -14.1 in adults, adolescents and children. With pimecrolimus cream 1% under occlusion, children showed a greater improvement in disease from baseline compared with adolescents and adults, which is evident from the mean average changes of EASI at the end of the study. None of these moderate to severe AD patients achieved an IGA score of 0 or 1 (no or almost no disease) on day 9, but the ratios

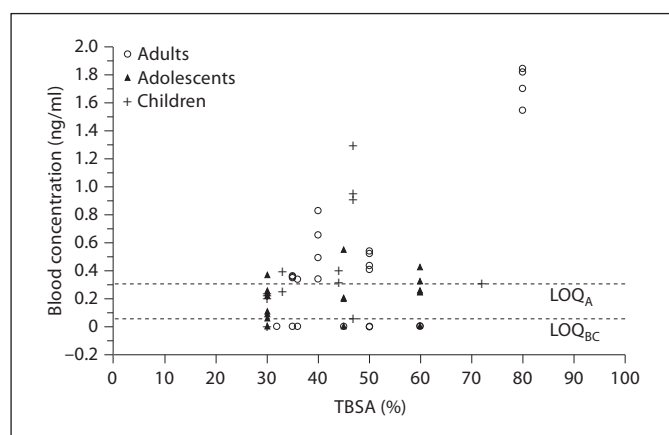
(in percentage) of patients with severe disease at baseline to day 9 were 66.7/42.9 for group A, 75.0/25.0 for group B and 42.9/0 for group C. Conversely, the ratios of patients (in percentage) with mild disease at baseline to day 9 were 0/0 for group A, 0/25 for group B and 0/66.7 for group C.

Safety

Pimecrolimus cream was well tolerated within various study populations. Fifty nonserious AEs were reported, of which the majority (40) were moderate and 5 were severe AEs (table 4). Most of the AEs were assessed by the investigator as related to skin wrapping (45/50 AEs), and

Table 3. Efficacy analysis in the 3 groups treated with pimecrolimus cream 1% under occlusion

Groups	TBSA affected, %		EASI score	
	baseline	day 9	baseline	day 9
<i>Group A (adults)</i>	(n = 9)	(n = 7)	(n = 9)	(n = 7)
Median	36.0	40.0	25.1	17.6
Mean $\pm$ SD	43.4 $\pm$ 16.1	38.6 $\pm$ 16.0	29.7 $\pm$ 12.2	22.7 $\pm$ 11.1
Range	30–80	20–70	18–56	14–40
<i>Group B (adolescents)</i>	(n = 4)	(n = 4)	(n = 4)	(n = 4)
Median	31.5	29.0	26.1	18.7
Mean $\pm$ SD	35.8 $\pm$ 9.6	36.5 $\pm$ 30.4	26.4 $\pm$ 7.3	21.5 $\pm$ 16.3
Range	30–50	10–78	18–35	6–43
<i>Group C (children)</i>	(n = 6)	(n = 6)	(n = 6)	(n = 6)
Median	40.5	19.0	25.1	12.3
Mean $\pm$ SD	44.2 $\pm$ 15.3	24.8 $\pm$ 13.8	26.5 $\pm$ 6.3	11.9 $\pm$ 5.6
Range	30–72	11–44	18–36	6–19

**Fig. 4.** Scatter plot of all measured pimecrolimus blood concentrations versus TBSA treated. LOQ_A = Limit of quantification in adults (0.3 ng/ml); LOQ_{BC} = limit of quantification in adolescents and children (0.05 ng/ml); concentrations below the limit of quantification are shown as 0 values.

among these, sweating ($n = 10$) and itching ($n = 13$) were the most common. There was a relatively higher number of AEs in group B (25 AEs in 4 patients). The majority of these events in group B were not considered by the investigator to be related to the study medication (22/25 events). There were no deaths or serious AEs reported during the study. One patient (group A) discontinued the study because of an AE caused by severe itching under the occlusive wrap.

Discussion

Pimecrolimus, a macrolide immunomodulator belonging to the family of calcineurin inhibitors, inhibits T cell proliferation, mast cell degranulation, and production and release of interleukins 2 and 4, γ -interferon and tumor necrosis factor α . In addition, calcineurin has been shown to negatively regulate Toll-like receptor-mediated signaling [24, 25]. Consistent with this finding, pimecrolimus has been shown to enhance antimicrobial peptide expression in human keratinocytes in vitro, suggesting a possible role of topical pimecrolimus directly affecting keratinocytes in vivo [26]. However, unlike corticosteroids, pimecrolimus does not affect endothelial cells and fibroblasts and hence does not induce skin atrophy. Moreover, the safety profile of pimecrolimus has been well described in both adults and children with AD [25].

Skin permeation and systemic absorption of pimecrolimus in AD patients have been shown to be generally low. This was observed even when pimecrolimus cream 1% was applied to large body surfaces up to 92% [11, 13]. In an in vitro investigation comparing the penetration and permeation properties of pimecrolimus and 3 corticosteroids (betamethasone-17-valerate, clobetasol-17-propionate and diflucortolone-21-valerate), concentrations of pimecrolimus and corticosteroids in human skin were of the same order of magnitude; permeation of pimecrolimus through human skin was, however, lower by factors of 70–110 compared with the corticosteroids. Compared with tacrolimus in human or pig skin, similar drug con-

Table 4. Adverse events overall and frequently affected system organ classes

	Group A (adults, n = 9)	Group B (adolescents, n = 4)	Group C (children, n = 6)	Total (N = 19)
Any body system				
Total	9 (100)	4 (100)	5 (83.3)	18 (94.7)
General disorders and administrative site conditions				
Application site discomfort	2 (22.2)	0 (0)	0 (0)	2 (10.5)
Application site irritation	0 (0)	1 (25)	0 (0)	1 (5.3)
Application site pain	1 (11.1)	0 (0)	0 (0)	1 (5.3)
Application site perspiration	0 (0)	3 (75)	0 (0)	3 (15.8)
Application site pruritus	5 (55.6)	3 (75)	1 (16.7)	9 (47.4)
Heat sensation in the face	0 (0)	1 (25)	0 (0)	1 (5.3)
Peripheral edema	1 (11.1)	0 (0)	0 (0)	1 (5.3)
Infections and infestations				
Infected dermatitis	3 (33.3)	0 (0)	0 (0)	3 (15.8)
Folliculitis	1 (11.1)	1 (25)	0 (0)	2 (10.5)
Nasopharyngitis	1 (11.1)	2 (50)	0 (0)	3 (15.8)
Perichondritis	0 (0)	1 (25)	0 (0)	1 (5.3)
Psychiatric disorders				
Sleep disorder	1 (11.1)	0 (0)	0 (0)	1 (5.3)
Respiratory, thoracic and mediastinal disorders				
Cough	0 (0)	0 (0)	2 (33.3)	2 (10.5)
Skin and subcutaneous tissue disorders				
Dyshidrosis	1 (11.1)	0 (0)	0 (0)	1 (5.3)
Pain of the skin	0 (0)	0 (0)	1 (16.7)	1 (5.3)
Pruritus	0 (0)	0 (0)	3 (50)	3 (15.8)
Skin disorder	1 (11.1)	0 (0)	0 (0)	1 (5.3)
Swollen face	1 (11.1)	0 (0)	0 (0)	1 (5.3)

Figures are numbers with percentages in parentheses.

centrations were measured in the skin, whereas permeation of pimecrolimus through the skin was consistently lower by a factor of 9–10. It is noteworthy that lipophilicity was the highest for pimecrolimus, based on the octanol/water distribution coefficient value (6.99) being higher by factors of 8 and 25–450 than that of tacrolimus (6.09) and the 3 corticosteroids, respectively (range 4.34–5.60). The low permeation of pimecrolimus may thus be explained by its higher lipophilicity (compared with tacrolimus and corticosteroids), and further by its higher molecular weight (compared with corticosteroids) [18, 27]. Additionally, in an in vitro study, the binding of pimecrolimus to human skin proteins was approximately 3-fold higher than that of tacrolimus, and the free fraction of pimecrolimus in human plasma was approximately 9-fold lower than that of tacrolimus [28]. All these findings, consistent with the clinical pharmacokinetic data, may explain the low level of systemic absorption following topical application of pimecrolimus.

Applying drugs under occlusion inhibits physiological water loss and increases the skin temperature. This may help in forming a drug reservoir within the stratum corneum [19, 20]. As topical formulations are often applied under occlusion, the present study was designed to evaluate the efficacy, safety and systemic exposure of pimecrolimus cream 1% under occlusion in adult and pediatric patients with moderate to severe AD.

In this study, pimecrolimus cream 1% under occlusion was effective in all 3 age groups (i.e. adults, adolescents and children) as shown by the average reduction in the EASI scores compared with the baseline. Consistent with previous studies, efficacy was the most pronounced in younger patients, possibly due to a more sensitive skin in this population.

The majority of the AEs were moderate and were related to the occlusive procedure rather than to the treatment. No serious AEs were reported in the study. Only 1 patient withdrew from the study because of an AE,

which was caused by increased itching due to occlusive wrapping.

All mean pimecrolimus blood concentrations, in all age groups, were below 0.8 ng/ml. The highest individual maximum observed whole-blood concentration value, measured in an adult, was 1.84 ng/ml. This is in concurrence with the findings from previous studies observed without occlusion in infants and children [10, 12, 29]. The highest ever reported individual blood concentration of pimecrolimus, 2.6 ng/ml, was measured in a nonocclusive study in infants [13]. The frequency distribution of blood concentrations of pimecrolimus cream 1% under occlusion, shown in figure 3a, showed that there was no relevant difference between the study groups. Further, the results were consistent with the findings from numerous published studies without occlusion [8–15]. For comparison, the data from 4 of these published studies were combined and are shown in figure 3b. Figure 3 shows that there was no relevant difference between occlusive and nonocclusive treatments. Thus, for pimecrolimus 1% cream, occlusion does not cause any increase in skin penetration and systemic exposure.

The conclusion is further supported by the following data comparison: after occlusive treatment, pimecrolimus blood concentrations were below 0.5 ng/ml in 84% of all samples (fig. 3a), and 27% of all samples (not shown) had levels below the limit of quantification (0.05 or 0.3 ng/ml). In a review publication, it was shown that in about 60% of all blood samples from pediatric patients (nonocclusive treatment), pimecrolimus blood concentrations were below 0.5 ng/ml [9]. Further, 27% were below the limit of quantification (0.1 ng/ml) [29].

Because of the low blood levels measured, calculation of AUC variables was feasible for only a few individuals per group. The highest individual value for AUC_{0-24h} was 41.4 ng·h/ml, which was found in an adult who was an outlier compared with the remaining study population. This AUC_{0-24h} value was slightly above the hitherto published highest value of 37.6 ng·h/ml (calculated as twice the reported AUC_{0-12h} of 18.8 ng·h/ml) [10, 16]. Nevertheless, the systemic exposure of this patient as well as that of all other patients in this study was approximately in the same range as in the nonocclusive studies published previously [8–15].

Figure 4 shows a scatter plot of all measured blood concentration values versus affected TBSA. Since the number of patients and observations was limited, no final conclusion can be drawn; however, for the patient group in the affected TBSA range of 30–72%, there was clearly no correlation between affected TBSA and systemic ex-

posure. The highest individual blood levels were measured in 1 adult patient who exhibited an affected TBSA of 80% (fig. 4). However, from this single observation no tendency can be inferred to a higher systemic exposure with more highly affected (and treated) TBSA, that is to a dose-related exposure.

It is noteworthy that a minimal affected-TBSA-dependent increase in blood concentration has been determined by Allen et al. [13], although, given the strongly varying blood concentrations, the very small tendency was judged to be of no relevance. Further, in numerous published studies where affected TBSA ranged from 0 to 90%, the blood levels of pimecrolimus were generally low and variable, with no correlation with affected TBSA [9, 12, 14, 15].

It should be noted that the TBSA treated overnight under occlusion (4 wrapped limbs) was similar across the study population, while the nonocclusive morning treatment on the affected TBSA represented an individual and variable contribution to the drug exposure of each patient. The variable treatment component might have been expected to lead to a TBSA-dependent systemic exposure; however, the study results do not support that hypothesis.

In summary, we did not find any apparent difference in pimecrolimus blood concentrations when pimecrolimus cream 1% was applied either with or without occlusion. Similarly, in a previous study with occlusive treatment of hand dermatitis with pimecrolimus cream 1%, occlusion did not cause increased absorption and blood levels [22]. In contrast, absorption of topical corticosteroid drugs was reported to increase when administered under occlusion [30, 31]. The effect of occlusion on percutaneous absorption of a drug may be influenced by its physicochemical properties, site of application and nature of the vehicle [19]. Under occlusion, the absorption of different steroid hormones increased with increasing lipophilicity of the molecule [32]. However, even if occlusion of a topically applied formulation may often increase penetration into the skin, this effect is not always observed.

The present investigation showed that occlusion had no impact on the percutaneous absorption of pimecrolimus. The most likely explanation for this observation is the high lipophilicity of pimecrolimus. As discussed above, based on the octanol/water distribution coefficient, pimecrolimus is more lipophilic than tacrolimus, and far more so than corticosteroids [18, 27]. This is in line with the protein-binding properties of pimecrolimus. The higher nonsaturated binding of proteins for pimecrolimus may increase the local drug concentration

after topical administration and hence reduce permeation through the skin [28]. Occlusion is likely to maintain, if not increase, the water content in the epidermis and stratum corneum. Thus, under occlusion, the upper skin layers become a more hydrophilic environment. It is plausible that in this environment, penetration and permeation of the highly lipophilic drug pimecrolimus are not favored. Therefore, the results of this study appear to reflect the physicochemical properties of pimecrolimus.

Conclusion

No formal inferential analysis was done as the study was not adequately powered for significance testing of a statistical hypothesis. However, the results indicate that occlusive treatment with pimecrolimus cream 1% over 8.5 days does not cause an increase in the pimecrolimus blood concentrations, compared with nonocclusive treat-

ment. With the exception of AEs reported by the investigator as related to occlusion, the safety profile was comparable to the studies conducted without occlusion.

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