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Skin absorption through atopic dermatitis skin: a systematic review

Running head: Atopic dermatitis and skin absorption

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Key words

Absorption, atopic dermatitis, dermato pharmacokinetics, drugs, penetration, percutaneous

What's known/what's new statements

What's known:

- Atopic dermatitis patients have skin barrier abnormality in both lesional and non-lesional skin.
- Atopic dermatitis patients are frequently exposed to chemicals through anti-inflammatory medicaments and emollients.

What's new:

- Skin absorption is increased about two-fold in atopic dermatitis patients when compared to controls, indicating they are at a higher risk of systemic exposure to chemicals from topical products.

Abstract

Patients with atopic dermatitis have skin barrier impairment in both lesional and non-lesional skin. They are typically exposed to emollients daily and topical anti-inflammatory medicaments intermittently, hereby increasing the risk of developing contact allergy and systemic exposed to chemicals ingredients found in these topical preparations. We systematically searched for studies that investigated skin absorption of various penetrants, including medicaments, in atopic dermatitis patients, but also animals with experimentally induced dermatitis. We identified 40 articles, i.e. 11 human studies examining model penetrants, 26 human studies examining atopic dermatitis drugs and 3 animal studies. We conclude that atopic dermatitis patients have nearly two-fold increased skin absorption when compared to healthy controls. There is a need for well-designed epidemiological and dermato-pharmacokinetic studies that examine to what extent atopic dermatitis patients are systemically exposed to chemicals compared to non-atopics.

INTRODUCTION

Atopic dermatitis (AD) is a common, chronic and relapsing inflammatory skin disease that often begins in infancy, or early childhood.^{1,2} Prevalence of AD is estimated to occur in 10-20% of children in developed countries. AD is characterized by pruritus, xerosis and recurrent episodes of dermatitis in typical anatomical sites. While some patients spontaneously clear, AD does persist in a large proportion of affected individuals.³

The etiopathogenesis of AD is being increasingly understood.¹ While skin inflammation characterized by T helper (Th) 2 cells is predominant in the acute phase, primary or secondary skin barrier impairment characterized by epidermal filaggrin deficiency is observed in both lesional and non-lesional AD skin.⁴ Daily application of emollients provides the basis for

conventional AD therapy, along with topical anti-inflammatory medicaments such as topical corticosteroids (TCS) and calcineurin inhibitors (TCI).⁵

Importantly, AD patients have an elevated exposure to chemicals found in topical products due to frequent use.^{6,7} Moreover absorption of these chemicals appears increased through an impaired skin barrier in both lesional and non-lesional skin.⁸⁻¹⁴ These observations potentially increase the risk in AD patients of developing skin reactions to penetrating chemicals, and possibly also the risk of increased systemic chemical exposure. Accordingly, a higher risk of allergic contact dermatitis to TCS, preservatives, surfactants and metals has been observed in AD patients,¹⁵⁻¹⁸ although other studies have shown opposite associations.^{19,20} Moreover, internal exposure to endocrine disrupting chemicals such as phthalate diesters appears to be increased in AD patients.^{21,22} Finally, TCS use has been linked with diabetes type 2, indicating that skin absorption of chemicals indeed could be a concern in AD patients.^{23,24} This systematic review examined studies that evaluated skin absorption of molecules in AD patients to increase our understanding of this understudied area of dermatology.

MATERIALS AND METHODS

The first author (A-S, H-O) systematically reviewed studies that examined skin absorption in AD patients. Inclusion criteria encompassed human and animal studies where a direct measure of skin absorption was provided and where measurements had been conducted in AD skin or animal skin that had been induced to develop AD like skin disease. Medical databases Pubmed, Embase and Web of Science were searched and studies that fulfilled the inclusion criteria were identified (Figure 1). The following search terms were utilized: (atopic dermatitis OR atopic eczema OR filaggrin) AND (penetration OR percutaneous). An additional search was run on PubMed using the MeSh terms “skin absorption” and “atopic dermatitis”. No limit was set

regarding the range of years. Moreover, we identified studies by the databases function "related articles" and in reference lists of reviews and included articles. The last search was run on 29-05-16 and the process is outlined in Figure 1. Furthermore we identified the molecular weight (MW) and octanol-water partition coefficient ($\log K_{ow}$) of the skin penetrants identified in the articles by searching the chemical databases of PubChem²⁵ and Environmental Protection Agency.²⁶

RESULTS

Forty articles met the inclusion criteria. Relevant definitions for the reviewed articles are provided in Figure 2. A summary of included studies is provided below, and detailed information in Table 1-3.

Human studies examining skin absorption of model penetrants in atopic dermatitis patients

Eleven studies were identified including 473 subjects (range 6-95 subjects) (Figure 3 and Table 1). Subjects were primarily adults (range 15-63 years), and only one study investigated children (AD patients mean age: 10.6 years).¹⁴

Examined chemicals, methods and number of exposures

Studies investigated skin absorption of, respectively, lidocaine and prilocaine (455, Dalton (Da)),⁸ theophylline (180 Da),⁹ lipophilic (423 Da) and hydrophilic dye (524-534 Da),^{10,14} gentian violet dye (408 Da),²⁷ sodium lauryl sulphate (SLS) (288 Da),¹¹ polyethylene glycols (PEG) (150-590 Da),^{12,13} metronidazole (171Da),²⁸ zinc oxide nanoparticles (ZnO-NP) (81 Da)²⁹ and silver (108 Da).³⁰ Methods used to examine skin absorption included blood sampling, skin

microdialysis, dye intensity measurement, and stratum corneum tape stripping. Subjects only underwent single exposure, except for one study where subjects were exposed 1 time daily for 5 consecutive days.³⁰

Comparison between patients with AD and controls

Three studies found increased absorption through lesional AD skin when compared to skin of healthy controls.^{8,9,27} Five studies showed that AD patients had increased skin absorption through non-lesional skin when compared to skin in healthy controls.⁹⁻¹³ One study showed that AD patients had increased skin absorption compared to healthy controls, but gave no information about whether the skin was lesional or non-lesional.¹⁴ One study (n=30) investigating absorption of silver (subjects were exposed to a silver sleeve on both forearms for 8h during 5 consecutive days) found no difference between absorption through non-lesional skin of AD patients and skin of healthy controls.³⁰ One study investigated AD patients by filaggrin gene (FLG) mutation status, but found no difference in skin absorption between genotypes.¹³ No study has yet examined penetration in FLG mutation carriers without AD. Two studies examined differences between controls and, respectively, patients with clinically active AD (defined as pruritic lesions) and patients with clinically inactive AD (defined as patients that had been free of dermatitis for at least 3 months, showing only mild signs at the time – scars, scaling, lichenification or dry skin).^{11,12} In the two studies patients with clinically active AD had increased skin absorption when compared to healthy controls, whereas no difference was observed between patients with clinically inactive AD and healthy controls. Two studies found that skin absorption increased significantly with the severity of AD (evaluated by, respectively, the criteria reported by Rajika and Langeland (1989) and the simple atopic dermatitis symptom score).^{10,14}

Comparison between lesional and non-lesional AD skin

Two studies showed increased skin absorption through lesional skin when compared to non-lesional skin,^{28,29} whereas one study only found an insignificant difference between lesional and non-lesional skin.⁹ Based on the information given in the three studies it was not possible to explain the disparity.

Human studies examining skin absorption of topical corticosteroids and calcineurin inhibitors in atopic dermatitis patients

Twenty-six studies were identified including 1,207 subjects (range 5-631 subjects) (Table 2). Studies investigated skin absorption of TCS (362-467 Da) and TCI (804-810 Da) by measuring their blood concentrations. Sixteen investigated lesional skin,³¹⁻⁴⁶ two investigated the whole body excluding the face and genitalia ^{47,48} and eight gave no information whether the skin was lesional or non-lesional.⁴⁹⁻⁵⁶

Studies investigating topical corticosteroid absorption

Ten studies investigated skin absorption of TCS in AD patients, but none compared to healthy controls.^{31,47,49,51-55,57,58} Three investigated children (1 month-14 years),^{49,51,57} six investigated adults (18-83 years) ^{31,47,53-55,58} and one investigated both children and adults.⁵² Subjects were only exposed to TCS one time throughout the study except for three studies where subjects were exposed respectively, once daily for 7 days, 4 or 5 times with 12h intervals between each application, or 2 times with 4 days between the applications.^{31,53,55} Application of TCS generally resulted in systemic uptake of TCS in AD patients as indicated by plasma cortisol,^{49,51,57} clobetasol propionate,^{47,58} clobetasone butyrate ⁴⁷ and betamethasone benzoate levels.³¹ Higher systemic uptake was found in AD patients younger than 18 months when compared to AD

patients aged 18 months or older,⁵¹ and in patients with clinically severe AD (defined as >40% skin surface involvement and sum of scores for erythema and excoriations 4-6) when compared to patients with clinically moderate and mild AD,⁵¹ and finally in patients with clinically active AD when compared to patients in the remission phase of AD (defined as AD patients in the post acute phase or in the complete remission phase).⁵⁷

Studies investigating topical calcineurin inhibitor absorption

Sixteen studies investigated skin absorption of TCI in patients with active AD, but made no comparison to healthy controls.^{32-46,56} Seven investigated adults (16-79 years),^{34,35,38,39,42,45,56} seven investigated children (4 months-14 years),^{33,36,37,40,41,43,44} and two investigated both children and adults.^{32,46} The subjects were exposed to TCI daily for 1 week to 12 months. Repeated application of TCI caused a low systemic uptake in AD patients as indicated by TCI blood concentration. Seven (44%) of 16 studies showed, in more than 90% of the blood samples, a concentration of TCI below 1 ng/mL.^{37-39,42-45} One study including both children and adults observed no significant difference in systemic uptake.⁴⁶

Skin absorption studies in animal models of atopic dermatitis

Three animal studies were identified (Table 3). Only one gave detailed information on the number of mice included, with a total of 12 mice.⁵⁹ Age of mice in these studies ranged from 7-12 weeks. Two studies compared absorption between skin of flaky tail mice (ft/ft) and control mice (respectively, C57B1&6J+/+ mice and B6 mice),^{59,60} and one compared skin absorption between, respectively, control mice and Balb/c mice that had been sensitized with ovalbumin to induce AD-like skin inflammation.

Skin absorption of the tracers lanthanum nitrate (325 Da) (in vivo) and calcium green (1200 Da) (in vitro), was assessed in flaky tail mice by, respectively, electron microscopy and dual-photon confocal microscopy.⁵⁹ Another study investigated in vitro skin absorption of fluorescein isothiocyanate (FITC) (389.38 Da) in flaky tail mice by fluorometer.⁶⁰ Both found increased skin absorption in flaky tail mice when compared to control mice. The third investigated, respectively, in vitro skin absorption of tacrolimus (822 Da), 8-methoxypsoralen (216 Da) and methotrexate (454 Da) assessed by a high performance liquid chromatography. Moreover, in vivo skin absorption of FITC (389 Da) and fluorescein isothiocyanate-conjugated dextran (FD4) (454 Da) was assessed by fluorescence and confocal microscopy. The study showed increased absorption through the skin of Balb/c mice with AD-like skin when compared to control mice.⁶¹

DISCUSSION

Main findings

Based on human primarily adult data, skin absorption is increased through AD skin when compared to control skin. Despite data are limited in numbers, it also appears that skin absorption is increased in both lesional and non-lesional AD skin compared to control skin. It is difficult to estimate the magnitude of this difference, but the data suggests that skin absorption in AD skin vs. control skin might be more than doubled (Figure 3). Although data from animal studies should be carefully interpreted, as no good AD model exists, three published studies also found increased skin absorption in AD-like skin when compared to control skin. Finally, studies showed that application of TCS results in systemic uptake in AD patients, whereas application of TCI only results in small amounts of systemic uptake.

Limitations

Several study limitations should be considered. The definition of AD is not always straightforward since various diagnostic criteria exist.² This challenge may potentially lead to misclassification; a risk that is likely highest among controls where a history of AD may sometimes be difficult to establish. Neither AD severity was given in all studies, nor site of test application, though it is highly relevant since there is a regional variation in skin absorption.⁶² For example, the forehead and genital skin has higher skin absorption rate than other skin areas like the forearm.^{62,63} Importantly, severe AD will result in increased barrier impairment when compared to mild disease and hence increase skin absorption. Moreover, skin absorption in non-lesional skin may differ between subtypes of AD and affect the results, e.g. AD patients with *FLG* mutations would be expected to have skin barrier impairment in non-lesional skin as well, whereas other subgroups of AD may tend to only have impairment in lesional skin. While few studies have been performed in children and infants, remember that infants have increased flux due to a high surface area to body weight ratio when compared to adults.⁶⁴ Except from studies investigating application of TCI, only few studies conducted repeated exposures, which is relevant to better mimic real-life.^{30,31,53,55} Several animal models mimic human AD.⁶⁵ Importantly, flaky tail mice (ft/ft) serve as a model for AD characterized by inherent filaggrin deficiency and was used to study skin absorption twice.^{59,60} Balb/c mice develop AD-like skin inflammation following sensitization and challenge with ovalbumin, and this model was used once.⁶¹ Remember that results from these animal models cannot be transferred directly to humans.

Interpretation

Information on skin permeability of subjects with AD is important for dosing purposes in the case of local therapy and risk assessment associated with exposure to topical drugs and chemicals from environment (including occupational exposure). In daily life, skin directly

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contacts various chemicals that might be able to penetrate skin, but also across and result in systemic exposure. Obviously, more permeable skin will allow entrance of a higher amount of a potentially sensitizing or systemically harmful chemical increasing the risk for adverse health effects. Accordingly, internal phthalate exposure was higher in AD patients when compared to controls.^{21,66,67} Although frequency, magnitude and source of epidermal exposure vary between different populations (e.g. children vs. adults, women vs. men), humans of all ages, including atopics, are to some degree exposed to personal care and cosmetic products such as soap, shampoo, hair styling products, make-up, hair dyes, wet wipes and importantly emollients. Notably, AD patients are also repeatedly exposed to anti-inflammatory topical medicaments, in particular during times of increased barrier impairment due to active dermatitis.

TCS are commonly used as AD maintenance treatment,⁵ and may, as shown, result in a systemic uptake.^{49,51,54,57,58} Indeed, patients with severe AD or age under 18 months appear to have increased skin absorption of TCS when compared to patients with mild or moderate AD or older patients, respectively.⁵¹ Notably, TCS use has been associated with adrenal insufficiency, Cushing Syndrome, glaucoma and even diabetes type-2 besides the possible cutaneous side effects.^{23,24,68,69} Most included studies that investigated the systemic absorption of TCS, generally suppressed the endogenous secretion of cortisol with dexamethasone before TCS application. However, three studies did not suppress cortisol before TCS application, but showed that cortisol levels decreased after seven days treatment with TCS (betamethasone butyrate)³¹ and also after just one day of treatment with TCS (clobetasol propionate, clobetasone butyrate).^{47,58} Other studies, not reviewed here, also showed a positive association between TCS and adrenal insufficiency.⁷⁰⁻⁷⁵ Importantly, one study found an association between use of TCS and new-onset diabetes and that the association increased with cumulative duration of TCS use.²⁴ This indicates that AD patients, treated for a long period and perhaps with too potent TCS, potentially may be at risk of developing diabetes type-2. In general, the association between use

of TCS and systemic side effects has so far been weak and few studies investigated patients with AD alone.^{23,58,75} In addition, most studies fail to follow patients long-term for potential side effects and according to a review article, the majority of the moderate to severe side effects are not reported.^{5,23}

Reviewed studies convincingly showed that the systemic uptake of TCI is low even in lesional AD skin. This is likely explained by the large molecular size of TCI (804-810Da) when compared to TCS (362-466 Da). One recent review investigated penetration through damaged skin and showed that a smaller molecular size is associated with increased skin absorption.^{76,77} According to the '500 Da rule', a molecule must be under 500 Da to allow human skin absorption.⁷⁸ However, all TCI are larger than 500 Da and do, to a lesser extent, penetrate the skin of AD patients (Table 2). Notably, one study showed that application of TCI twice daily on the skin in three patients with Netherton Syndrome, resulted in TCI blood levels within or above the established therapeutic range for oral tacrolimus in organ transplant recipients.⁷⁹ Notably, Netherton patients have severe skin barrier deficiency.

Cosmetic products may contain contact sensitizers such as fragrances, surfactants and preservatives that may cause allergic contact dermatitis.⁸⁰ A recent study reported that 89% of 187 hypoallergenic cosmetic pediatric products in the US contained at least one contact allergen, and that 10% contained the potent contact allergen methylisothiazolinone.⁸¹ Interestingly, a higher prevalence of contact sensitization to emollients, fragrances, preservatives and surfactants has been observed in AD patients.^{15-17,82} A French study showed that AD patients frequently reacted on their skin to ingredients found in their emollients and anti-inflammatory medicaments.⁸² However, other studies have found inverse associations, or no association at all.^{2,83} Nonetheless, such data indicate that not only increased topical exposures, but also increased chemical penetration could be a concern in AD patients, and that

use of hypoallergenic products should be advocated. Toys are an inescapable part of children's life and these may contain metal ions such as nickel.⁸⁴ Metal ions can also be released from jewelry, phones, tablets and laptop computers and these have become a common part of children's exposures in industrial countries.⁸⁵ Studies have shown higher prevalence rates of metal sensitization in AD patients, perhaps due to a reduction in epidermal filaggrin levels, hence compromising the metal chelating abilities of the skin.^{17,83,86} Interestingly, patch test reactivity to metals seems to gradually decrease over childhood, perhaps indicating a relatively higher penetration and reactivity in young skin.^{87,88}

Skin absorption of SLS is also increased in patients with AD.¹¹ This is important since some occupations involve daily exposure to irritants, as examples metalwork, healthcare and hairdressing.^{89,90} The increased skin absorption to irritant in AD patients likely makes their already sensitive and irritable skin even more susceptible to irritant contact dermatitis. Indeed, studies have found the risk of occupational irritant contact dermatitis to be significantly increased in AD patients.⁸⁹⁻⁹² Notably, there are indications that adult patients with previous AD in childhood who are continuously exposed on the skin to irritants may first develop irritant contact dermatitis and later AD like skin disease.⁹³

Conclusion

This systemic literature review showed that AD patients have a nearly two-fold increased skin absorption through their skin when compared to healthy controls. Since exposure to chemicals found in topical products is also increased in AD patients as part of their treatment regimen, there is a need for additional epidemiological studies that examine the possible burden of these chemicals. There is also a need for future experimental studies to examine individuals who are repeatedly exposed to topical products to better mimic a real life setting and these studies

should include objective skin measurements. Clinicians should minimize their use of products that contain potent contact allergens and endocrine disrupting chemicals in their AD patients.

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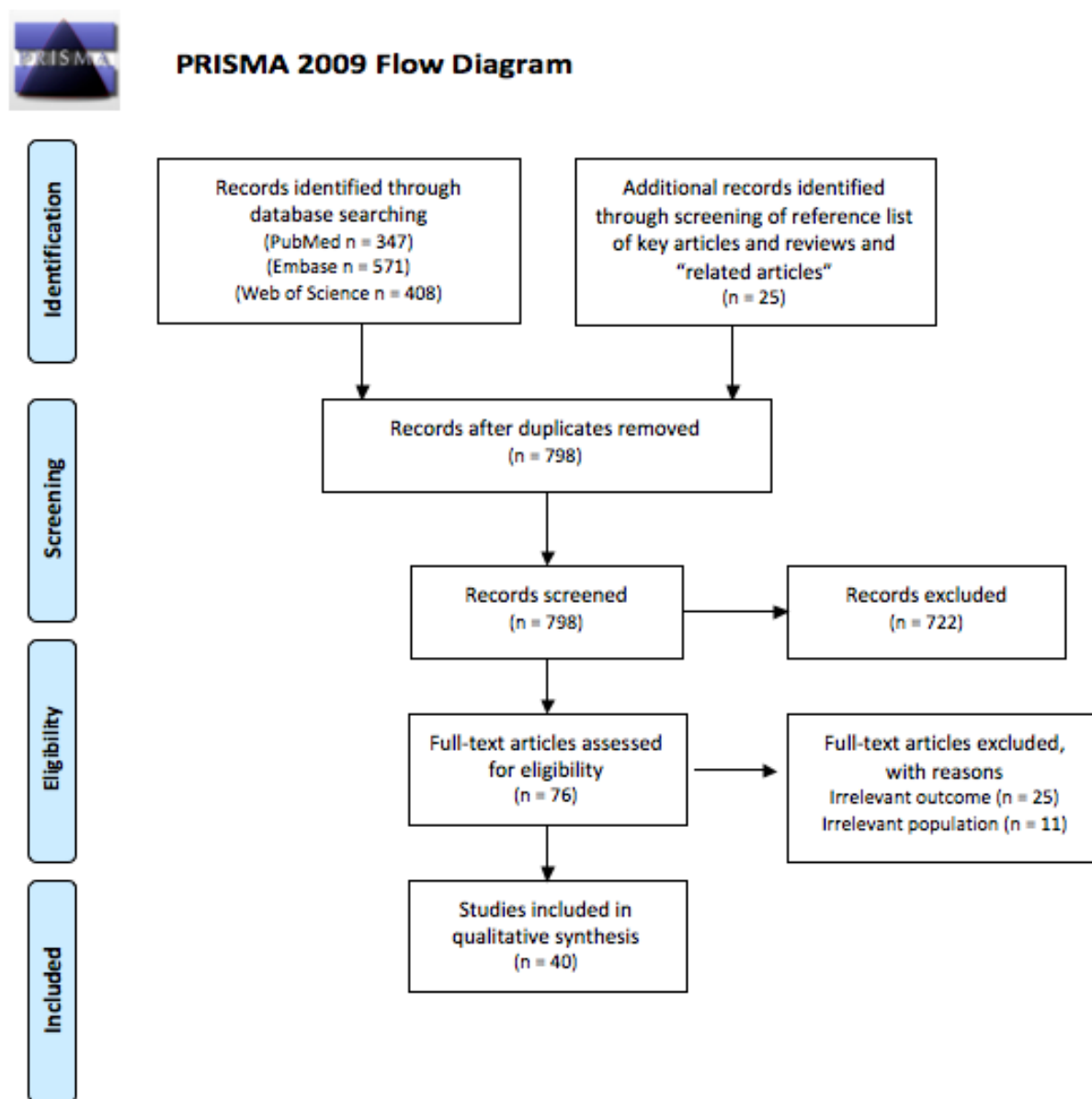
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FIGURES

Figure 1. Flow diagram



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med* 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit www.prisma-statement.org.

Figure 2. Selected definitions used in the reviewed articles.

Skin absorption is a general term that describes the transport of a substance/penetrant from the environment through the skin into systemic circulation and can be divided into several steps*:

- penetration which represents the entry of a substance into particular layer of the organ (e.g. entrance of a substance into the uppermost layer of the skin, the stratum corneum).
- permeation is the penetration of a substance through one layer into another layer, which is both functionally and structurally different from the first layer.
- resorption is the uptake of a substance into the skin lymph and vascular system (blood vessels) which can lead to entry into the systemic circulation.

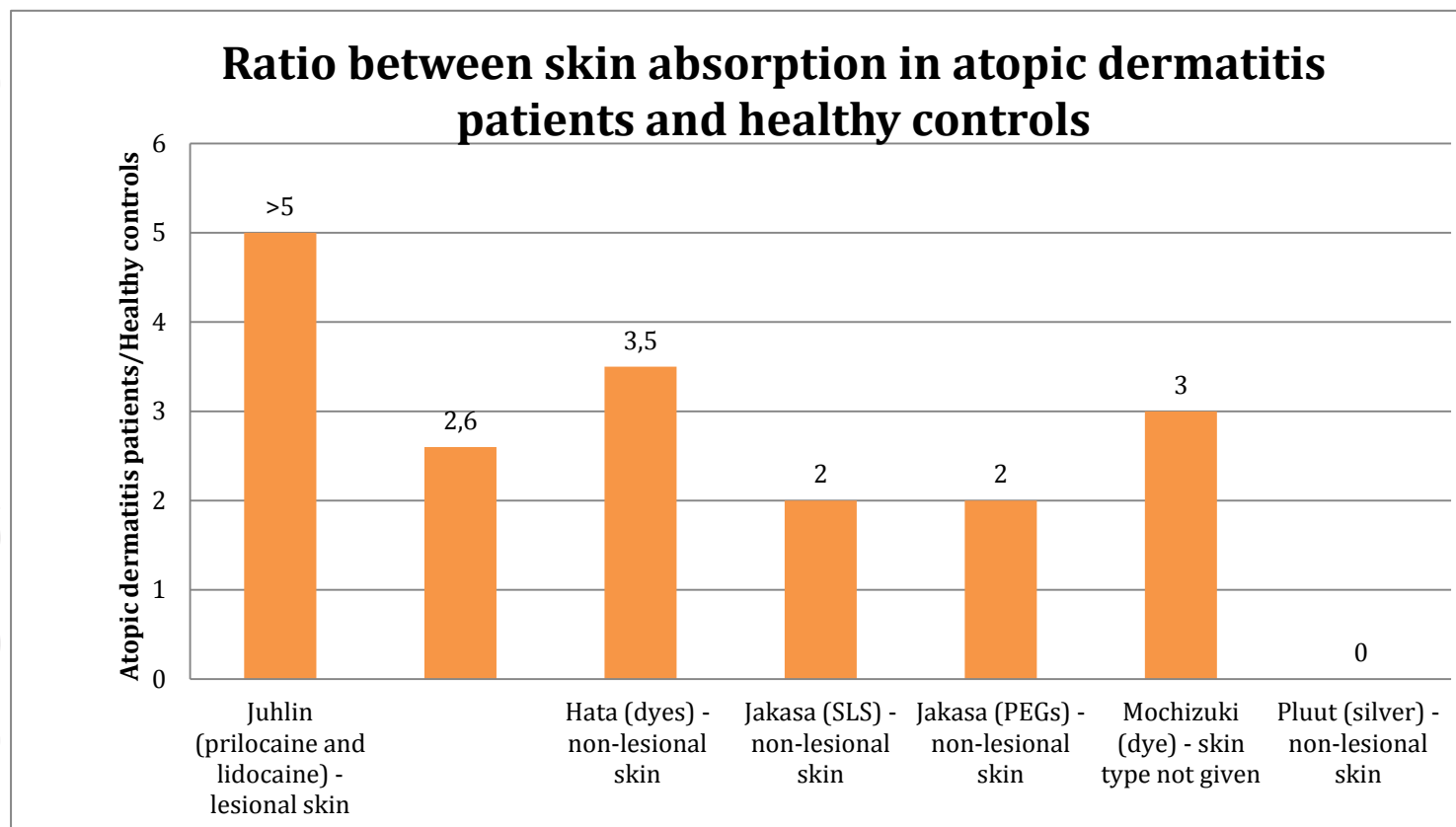
Diffusion is a velocity of a transport of a substance through the skin layer.

Flux is mass of a substance passing through a unit area of skin per unit of time

Octanol-water partition coefficient is the ratio of concentrations of a substance distributed between two immiscible phases, octanol and water

*Reference: Environmental Health Criteria 235, DERMAL ABSORPTION, World Health Organization, 2006 and

Figure 3. Ratio between skin absorption in atopic dermatitis patients and healthy controls.



SLS: sodium lauryl sulphate, PEGs: polyethylene glycols.

Figure 3 shows the ratio between skin absorption in skin of atopic dermatitis patients and skin of healthy controls. The results are rough estimates based on information provided in the studies.

TABLES

Table 1: Human studies examining skin absorption in atopic dermatitis patients

Reference	Study participants	AD diagnosis	Type of skin	Test material/exposure conditions/technique	Endpoints	Main findings
Juhlin et al. (1989)	AD patients: n=2, F/M=2/0, age: 18-24 years Healthy controls: n=3, F/M not given, age: 20-60 years Contact dermatitis patients: n=1, F/M=1/0, age: 18-24 years Psoriasis patients: n=8, F/M not given, age: 20-63 years	Definition of AD diagnosis not given. AD patients had active disease. Severity of disease not given.	Lesional	Test compound: mixture of 4-6 g of 5% lidocaine and prilocaine (MW=455 Da) as EMLA cream. $\log K_{ow}$ (lidocaine)=2,26 and $\log K_{ow}$ (prilocaine)=2,11. Exposure condition: subjects exposed for 1 hour (h) on the forearm under occlusion. Technique: absorption assessed by a vein puncture from the antecubital vein.	Lidocaine and prilocaine concentrations in the general circulation and draining vein.	Skin of AD patients showed increased skin resorption of lidocaine and prilocaine when compared with healthy controls. 1h after application, the plasma concentrations in the general circulation were 50-450 ng/mL in AD patients, <10-162 ng/mL in psoriasis patients, <10-20 ng/mL in contact dermatitis patients and non-detectable in healthy controls (<10 ng/mL). In the draining vein, concentrations were higher than in the general circulation. Highest concentrations were found in AD patients (9.560-13.070 ng/mL), 10.000 times higher than in healthy controls (<10 – 12 ng/mL).
Yoshiike et al. (1993)	AD patients: n=20, F/M=9/11, age: 15-32 years Healthy controls: n=15, sex and age matched with AD patients	AD patients diagnosed according to the Hanifin and Rajka criteria. Activity and the severity of AD not given.	Lesional and non-lesional	Test compound: theophylline (MW=180 Da) in n-octanol. $\log K_{ow}$ (theophylline) =-0,02. Exposure condition: stratum corneum sheets exposed for 2h using round diffusion chambers (Boyden chamber, 4 mm in diameter). Technique: absorption assessed by using stratum corneum sheets obtained from overarm skin by using the cantharidin blister technique and analyzed by a spectrophotometer.	Theophylline penetration rates through stratum corneum.	Skin of AD patients showed increased penetration of theophylline when compared with healthy controls. Determined theophylline penetration rates were significantly higher in lesional ($12,5 \pm 2,5 \mu\text{g/mL/h}$) and non-lesional skin ($9,8 \pm 2,7 \mu\text{g/mL/h}$) of AD patients when compared to skin of healthy controls ($4,9 \pm 1 \mu\text{g/mL/h}$). However, there was no significant difference between lesional and non-lesional AD skin.
Hata et al. (2002)	AD patients: n=27, F/M=17/10, age: 19-44 years Healthy controls: n=18, F/M=11/7, age: 20-56 years	AD patients diagnosed according to the Hanifin and Rajka criteria. Activity of AD not given. Severity of the disease was grouped into mild, moderate and severe by the criteria reported by Rajika and Langeland (1989).	Non-lesional	Test compound: 649 μL of a lipophilic dye (MW=423 Da) and a hydrophilic dye (MW=524 Da) in 95% ethanol and 5% water. $\log K_{ow}$ (lipophilic dye) = no data available and $\log K_{ow}$ (hydrophilic dye)= no data available. Exposure conditions: subjects exposed for 2 and 5 minutes on the forearm using patch test chambers. Technique: absorption assessed by using noninvasive tape stripping and measurements of	Photoacoustic signals of both dyes	Skin of AD patients showed increased penetration of the dyes when compared with healthy controls. Determined photoacoustic signal intensity was higher in non-lesional skin of AD patients than in skin of healthy controls ($p < 0.001$). Signal intensity obtained with the hydrophilic dye increased significantly with the severity of AD.

				photoacoustic signals.		
Jakasa et al. (2006)	Subjects of Caucasian origin AD patients: n=20, F/M=8/12, mean age: 29 years (range 18-54 years) Healthy controls: n=20, F/M=9/11, mean age: 32 years (range 18-55 years)	AD patients diagnosed according to the Hanifin and Rajka criteria. 12 had active AD (pruritic lesions) and 8 had inactive AD (they had been free of dermatitis for at least 3 months, showing only mild signs at the time – scars, scaling, lichenification, or dry skin) on body parts other than the test sites. Severity of the disease was mild in all the patients and median EASI score (max. 72 points) was 1.7 (ranging from 0.2 to 22.8). Erlangen atopy score (max. 34 points) was 17.4 ± 6.6 in AD patients and 3.0 ± 2.4 in healthy controls.	Non-lesional	Test compound: 1% SLS (MW=288 Da) in water. logK _{ow} (SLS)=1.60. Exposure condition: subjects exposed for 4h on both forearms using round patch test chambers (Finn chambers, 18 mm in diameter). Technique: absorption assessed by using noninvasive tape stripping of stratum corneum and approach based on Fick's second law of diffusion for non-steady state condition.	Diffusion coefficient of SLS.	Skin of AD patients showed increased penetration of SLS when compared with healthy controls. Determined apparent diffusion coefficient was approximately twice as high in AD patients (12,7 ± 5,8 · 10 ⁻⁹ cm ² h ⁻¹) as in healthy controls (6,2 ± 3,0 · 10 ⁻⁹ cm ² h ⁻¹). Diffusion coefficient was significantly higher in patients with active AD compared with patients with inactive AD (p=0.016), but no significant difference was found between patients with inactive AD and healthy controls (p>0.05).
Jakasa et al. (2007)	Subjects of Caucasian origin AD patients: n=20, F/M=8/12, mean age of 29 years (range 18-54 years) Healthy controls: n=20, F/M=9/11, mean age: 32 years (range 18-55 years)	AD patients diagnosed according to the Hanifin and Rajka criteria. Twelve had active AD (pruritic lesions) and eight had inactive AD (they had been free of dermatitis for at least 3 months, showing only mild signs at the time – scars, scaling, lichenification, or dry skin) on body parts other than the test sites. Severity of the disease was mild in all and median EASI score (max. 72 points) was 1.7 (ranging from 0.2 to 22.8). Erlangen atopy score (max. 34 points) was 17.4 ± 6.6 in AD patients and 3.0 ± 2.4 in healthy controls.	Non-lesional	Test compound: mixture of PEGs of different MWs (160 – 590 Da) in water. logK _{ow} (PEGs)~1.6 which does not change greatly with molecular size. Exposure condition: subjects exposed for 6h on both mid-volar arms using round patch test chambers (Finn chambers, 18 mm in diameter). Technique: absorption assessed by using noninvasive tape stripping of stratum corneum and approach based on Fick's second law of diffusion for non-steady state condition.	Diffusion coefficient of PEGs	Skin of AD patients showed increased penetration of all PEGs when compared with healthy controls. Determined apparent diffusion coefficient was approximately twice as high for PEGs with MW range 282-590 Da in AD patients as in healthy controls, and for PEG with MW of 150 Da, diffusion coefficient was only 60% higher. As an example, the diffusion coefficient of PEG414 was 6,7 ± 3,6 · 10 ⁻⁹ cm ² h ⁻¹ for non-lesional skin of AD patients and 3,5 ± 2,2 · 10 ⁻⁹ cm ² h ⁻¹ for healthy controls. Diffusion coefficient also decreased with increasing MW of PEGs in both groups. No significant difference in diffusion coefficient between patients with inactive AD and healthy controls, or between AD patients with active and inactive AD, was found.
Jensen et al. (2009)	AD patients: n=4 Healthy controls: n=15 Gender or age not given	AD patients diagnosed according to the Hanifin and Rajka criteria. All had active AD with target lesion score 3-8 (on a scale 0-12) affecting the upper limbs by at least 10%. Some patients were treated twice daily for 3 weeks on 1 upper limb with 1% pimecrolimus and on the other limb with 0,1% betamethasone	Lesional skin	Test compound: 30 µL of 0,1% dye gentian violet (MW=408 Da) logK _{ow} (dye)=0,51. Exposure condition: subjects exposed for 69 seconds using a paper carrier to apply dye. Technique: absorption assessed by using the Minolta Chroma Meter to determine color intensity.	Dye penetration	Skin of AD patients showed increased penetration of dye when compared with healthy controls. Determined dye penetration was significantly higher in untreated AD patients compared with treated AD patients and in untreated AD patients compared with healthy controls. There was no significant difference between the pimecrolimus and betamethasone treated groups. In the skin of healthy controls no dye penetration occurred.

		and some patients worked as placebo controls.				
Mochizuki et al. (2009)	AD patients: n = 42, F/M=23/19, mean age: 10,6 years Healthy controls: n = 26, F/M=14/12, mean age: 22,3 years Patients with dry skin: n = 27, F/M=17/10, mean age: 18,4 years	AD patients diagnosed according to the Japanese Dermatological Association criteria for the diagnosis of atopic dermatitis. Simple 'atopic dermatitis symptom score' classified severity of the disease.	Not given	Test compound: hydrophilic yellow dye, tartrazine (MW=556 Da) dissolved in saline. $\log K_{ow}(\text{dye}) = -10,17$. Exposure condition: subjects exposed on both forearms, further information not given. Technique: absorption assessed by a tristimulus photocolormeter (model CR-400) measuring the skin color objectively.	Skin transparency index (out-in skin transparency)	Skin of AD patients showed increased penetration of tartrazine when compared with healthy controls. Determined skin transparency index, using tartrazine, was significantly higher in AD patients than in healthy controls ($p < 0.001$) and in patients with dry skin ($p = 0.022$). Skin transparency index using tartrazine, increased significantly with AD severity.
Ortiz et al. (2009)	Lesional and non-lesional skin of AD patients: n = 6, F/M = 1/5, age: 19-45 years	Definition of AD diagnose not given. All had active AD. Severity of the disease not given.	Lesional and non-lesional	Test compound: MTZ in a cream formulation (flagyl 1%) (MW=171 Da). $\log K_{ow}(\text{MTZ}) = -0,02$. Exposure condition: subjects exposed on the forearm. Technique: penetration assessed by using invasive microdialysis probes and by using noninvasive tape stripping.	Skin concentration of MTZ Content of MTZ in stratum corneum	Lesional skin of AD patients showed increased penetration of MTZ when compared with non-lesional skin of AD patients. Determined skin concentration of MTZ was 2.4 fold higher ($p = 0,004$) in lesional skin of AD patients ($671,7 \pm 127,9 \text{ ng}/\mu\text{L}\cdot\text{min}$) than in non-lesional skin of AD patients ($285,5 \pm 66,3 \text{ ng}/\mu\text{L}\cdot\text{min}$). Content of MTZ in the stratum corneum after 30 minutes of exposure was 15% lower ($p = 0,334$) in lesional skin of AD patients ($34,3 \pm 13,0 \mu\text{g}/\text{cm}^2$) than in non-lesional skin of AD patients ($39,3 \pm 11,0 \mu\text{g}/\text{cm}^2$).
Jakasa et al. (2010)	Subjects of Caucasian origin AD patients without FLG mutations: n=15, F/M=11/4, mean age: 24 years (range 18-52 years) AD patients with mutations in one allele of FLG: n=17, F/M=10/7, mean age: 26 years (range 19-60 years) Healthy controls without FLG mutations: n=20, F/M=11/9, mean age: 27 years (range 21-56 years)	AD patients diagnosed according to the Hanifin and Rajka criteria. Activity of AD not given. Severity of the disease was mild in all patients and the median EASI score (max. 72 points) was 1.2 (ranging from 0.4 to 22.8) in AD patients without FLG mutations and 0.9 (ranging from 0.0-0.3) in AD patients with FLG mutations.	Non-lesional	Test compound: PEG (MW=370 Da) in water. $\log K_{ow}(\text{PEG}) \approx -1.6$. Exposure condition: subjects exposed for 6h on the forearm using round patch test chambers (Finn chambers, 18 mm in diameter). Technique: absorption assessed by using noninvasive tape stripping of stratum corneum and approach based on Fick's second law of diffusion for non-steady state condition.	Diffusion coefficient of PEG	Skin of AD patients showed increased penetration of PEG when compared with healthy controls. Determined diffusion coefficient was higher for PEG in non-lesional skin of AD patients, irrespective of their FLG genotype, than in healthy controls without FLG mutations. No difference between AD patients according to FLG status was identified.

Lin et al. (2011)	Lesional and non-lesional skin of AD patients: n=3 Psoriasis patients: n=5 Gender or age not given.	Definition of AD diagnose not given. All had active AD. Severity of the disease not given.	Lesional and non-lesional	Test compound: 60% of ZnO-NP (MW=81 Da) solution which consist of silicate-coated ZnO-NP dispersed in caprylic/capric triglycerides. $\log K_{ow}(\text{ZnO-NP})$ = no data available. Exposure condition: subjects exposed for 2h on the forearm, directly. Technique: absorption assessed by in vivo multiphoton images.	ZnO-NP signals in the skin	Lesional skin of AD patients showed increased penetration of ZnO-NP when compared with non-lesional skin of AD patients. Determined ZnO-NP signals were significantly higher ($p<0.01$) in the stratum corneum of lesional skin of AD and psoriasis patients than in non-lesional skin of AD- and psoriasis patients. No distinction between AD- and psoriasis patients was made.
Pluut et al. (2015)	Subjects of Caucasian origins AD patients: n=15, mean age: 27 years Healthy controls: n=15, mean age: 42 years F/M=15/15	AD patients diagnosed according to the UK Working party criteria. Activity and severity of the disease not given.	Non-lesional	Test compound: 3,6% silver (MW=108 Da) in a sleeve consistent of 79% Micromodal fibre, 13% Polyamide Ag and 7% Lycra.. $\log K_{ow}(\text{silver})$ = no data available. Exposure condition: subjects exposed for 8h during 5 days on both forearms. Technique: absorption assessed by using noninvasive tape stripping of stratum corneum and the approach based on Fick's second law of diffusion for non-steady state condition.	Dermal flux of silver, stratum corneum concentration of silver and urine silver concentrations	Skin of AD patients showed no significant increase in penetration and resorption of silver when compared with healthy controls. No significant difference in dermal flux of silver was observed between AD patients (0,23 ng/cm ² /h) and healthy controls (0,20 ng/cm ² /h). Silver in stratum corneum increased from day 2 to day 5, but there was no significant difference between AD patients and healthy controls. No significant increase of silver in urine either in AD patients or healthy controls.

AD: atopic dermatitis, n: number of participants, F/M: female/males, MW: molecular weight, $\log K_{ow}$: octanol-water partition coefficient, SLS: sodium lauryl sulphate, PEGs: polyethylene glycols, MTZ: metronidazole, ZnO-NP: zinc oxide nanoparticles.

Table 2: Human studies examining skin absorption of glucocorticoids and calcineurin inhibitors in atopic dermatitis patients

Reference	Study participants	AD diagnosis	Type of skin	Test material/exposure conditions/technique	Endpoints	Main findings
Mizuchi et al. (1976)	AD patients, nummular eczema, psoriasis and prurigo nodularis: n=16, F/M=4/12, age: 17-83 years	No information given.	Lesional	Test compound: 5, 10 or 20 g of 0,025% betamethasone 17-benzoate cream (MW=497 Da, $\log K_{ow}$ =3,92). Exposure conditions: subjects exposed for 14h, massaged into skin and covered with plastic film. After 4 days with steroid free cream, subjects were exposed for 7 days under occlusion. Technique: absorption assessed by blood samples and betamethasone 17-benzoate radioimmunoassay.	Plasma betamethasone 17-benzoate levels	Plasma betamethasone 17-benzoate was detectable on days 7 (0,41-3,97 ng/mL) and 11 (0,34-4,89 ng/mL) in the patients treated with betamethasone 17-benzoates. No distinction between AD- and psoriasis patients was made.

Hehir et al. (1983)	AD patients: n=9, F/M=3/6, age: 16-80 years Psoriasis patients: n=11, F/M=6/5, age: 16-80 years	Definition of AD diagnose not given. All had active AD with 40-95% of body surface area affected.	Whole body excluding the face, genitalia and the arm covered by tubegaze	Test compound: 25 g of 0,05% clobetasol 17-propionate cream (MW=483 Da, logK _{ow} =3,28) or 30 g of 0,05% clobetasone 17-butyrate cream (MW=479 Da, logK _{ow} =3,76) Exposure conditions: subjects exposed one time on the whole body excluding face, genitalia and the arm and covered by tubegaze. Technique: absorption assessed by blood samples and clobetasol 17-propionate or clobetasone 17-butyrate radioimmunoassay.	Plasma clobetasol propionate levels Plasma clobetasone butyrate levels	Plasma clobetasol propionate levels varied between 0-15,8 ng/mL and 0-4,5 ng/mL after one application in respectively, AD patients and psoriasis patients. Plasma clobetasone butyrate levels varied between 0-1 ng/mL and 0,1-0,58 ng/mL after one application in respectively, AD patients and psoriasis patients.
Turpeinen et al. (1986)	AD patients: n=14, F/M=8/6, age: 3 months – 14 years Seborrheic dermatitis patients: n=4, F/M=2/2, age: 1,5–14 months	Definition of AD diagnose not given. All patients had active dermatitis with 30-90% of their body affected.	Not given	Test compound: 4,5-106 g of 1% hydrocortisone cream (MW=362 Da, logK _{ow} =1,61). Exposure conditions: subjects exposed for 4h or 24h. Before application endogenous secretion of cortisol was suppressed with dexamethasone. Technique: absorption assessed by blood samples and cortisol (¹²⁵ I) radioimmunoassay.	Plasma cortisol levels	4h absorption test: Plasma cortisol levels increased in 8 of 9 patients ranging from 212-2669 nmol/L. 24h absorption test: Plasma cortisol levels increased in 6 of 9 patients ranging from 98-1405 nmol/L.
Turpeinen et al. (1988)	AD patients and seborrheic dermatitis patients: n = 55, F/M=23/32, age: 1 month – 14 years	Definition of AD diagnose not given. Severity of the disease was assessed by scoring erythema and excoriations on a scale from 0-3 (0=none, 1=mild, 2=moderate and 3=severe). 20 had severe dermatitis, 17 had moderate dermatitis and 18 had mild dermatitis.	Not given	Test compound: 1% hydrocortisone cream (MW=362 Da, logK _{ow} =1,61). Exposure conditions: subjects exposed for 4h. Before application endogenous secretion of cortisol suppressed with dexamethasone. Technique: absorption assessed by blood samples and cortisol (¹²⁵ I) radioimmunoassay.	Plasma cortisol levels	Plasma cortisol levels increased on average with 537 ± 589 nmol/L in severe dermatitis patients, 193 ± 175 nmol/L in moderate dermatitis patients and 107 ± 61 nmol/L in mild dermatitis patients. Increase was significantly higher in severe dermatitis patients compared with moderate dermatitis patients and in moderate dermatitis patients compared with mild dermatitis patients. In severe and mild dermatitis patients the increase significantly higher in patients under 18 months than in patients aged 18 months or over.
Turpeinen et al. (1988)	AD patients: n=16, F/M=8/8, age: 5 months – 12 years Seborrheic dermatitis patients: n=4, F/M=2/2, age: 1-12 months	Definition of AD diagnose not given. Some were in acute phase of dermatitis (erythema and excoriations) and some were in remission phase of dermatitis (no erythema or excoriations).	Not given	Test compound: 11-91 g of 1% hydrocortisone cream (MW=362 Da, logK _{ow} =1,61). Exposure conditions: subjects exposed for 4h. Before application endogenous secretion of cortisol suppressed with dexamethasone. Technique: absorption assessed by blood samples and cortisol (¹²⁵ I) radioimmunoassay.	Plasma cortisol levels	Plasma cortisol levels increased with 47-961 nmol/L in patients in the acute phase of dermatitis and 18-241 nmol/L in patients in the remission phase of dermatitis. Increase was significantly higher in patients in the acute phase of dermatitis when compared to patients in the remission of dermatitis.
Turpeinen et al. (1991)	AD patients: n=7, adults=3 and children=4, F/M=3/4, age: 4 months – 39 years	Definition of AD diagnose not given. All had active AD with 60-90% of body affected. Their disease scored by grading erythema and excoriations, sum of the score had a median=4 (range 3-5), with a maximum score of 6.	Not given	Test compound: 33-75 g of 1% hydrocortisone cream (MW=362 Da, logK _{ow} =1,61). Exposure conditions: subjects exposed for 24h or 28h. Before application endogenous secretion of cortisol suppressed with dexamethasone. Technique: absorption assessed by blood samples and cortisol (¹²⁵ I) radioimmunoassay.	Plasma cortisol levels	Plasma cortisol levels increased after the first 4h. At 12h and 24h plasma cortisol levels tended to decrease but levels were still elevated compared to before the application of hydrocortisone.
Aalto-Korte et al. (1995)	AD patients: n=6, F/M=3/3, age: 21-63 years	Definition of AD diagnose not given. All had active AD with 90-100% of body affected.	Not given	Test compound: 50 g of 1% hydrocortisone cream (MW=362 Da, logK _{ow} =1,61). Exposure conditions: subjects exposed for 24h. Before application endogenous secretion of	Plasma cortisol levels	Plasma cortisol levels increased with 273-908 nmol/L. Between 19-93 mg of the 50 g of 1% hydrocortisone was absorbed systemically.

				cortisol suppressed with dexamethasone. Technique: absorption assessed by blood samples and cortisol (¹²⁵ I) radioimmunoassay.		
Aalto-Korte et al. (1995)	AD patients: n=9, F/M=3/6, age: 23-70 years	Definition of AD diagnose not given. All had active AD with at least 50% of body affected at entry day. Severity of disease graded by scoring system of Rajka and Langeland (1989) with a median score of 8 at entry day (ranged from 5,5-8). Clinical activity scores were 26,5-71 at entry day and 2-9,5 at day 5.	Not given	Test compound: 1% hydrocortisone cream (MW=362 Da, logK _{ow} =1,61). Exposure conditions: subjects exposed for 4h at entry day and day 5 and in between treated for dermatitis. Before application endogenous secretion of cortisol was suppressed with dexamethasone. Technique: absorption assessed by blood samples and cortisol (¹²⁵ I) radioimmunoassay.	Plasma cortisol levels	Plasma cortisol levels increased with 49-614 nmol/L (median, 102nmol/L) after application at entry day. At day 5 increase was 0-26 nmol/L (median, 0 nmol/L). Difference between entry day and day 5 was statistically significant (p<0,005).
Aalto-Korte et al. (1995)	AD patients: Group 1: n=6, F/M=5/1, median age: 23 years (range 20-52 years) Group 2: n=6, F/M=3/3, median age: 18 years (range 17-28 years) Group 3: n=7, F/M=6/1, median age: 26 years (range 18-56 years)	Definition of AD diagnose not given. All had active AD with 50-100% of body affected.	Not given	Test compound: 50 g of 1% hydrocortisone cream (MW=362 Da, logK _{ow} =1,61). Exposure conditions: before exposure endogenous secretion of cortisol was suppressed with dexamethasone. Group 1: subjects exposed 4 times with 12h intervals. Group 2: subjects exposed 5 times with 12h intervals. Group 3: subjects exposed once. Technique: absorption assessed by blood samples and cortisol (¹²⁵ I) radioimmunoassay.	Plasma cortisol levels	Systemic absorption of hydrocortisone is seen in AD patients after first two applications but systemic absorption decrease after the third and fourth application of hydrocortisone. Group 1: Plasma cortisol levels increased significantly after the first two exposures. After third exposure plasma cortisol levels began to decrease. Group 2: Plasma cortisol levels decreased significantly after the third and fourth applications. Group 3: Plasma cortisol levels increased after application of hydrocortisone.
Alaiti et al. (1998)	AD patients: n=29, F/M=21/18, age: 5-75 years	AD patients diagnosed according to the Hanifin and Rajka criteria. All had active AD with more than 30% of body surface area affected. Severity of the disease was graded by scoring system of Rajka and Langeland (1989), 32 had moderate AD and 7 had severe AD.	Lesional skin	Test compound: 0,3% tacrolimus cream (MW=804 Da, logK _{ow} =3,03). Exposure conditions: subjects exposed twice daily for one week. Technique: absorption assessed by blood samples and enzyme immunosorbent assay.	Tacrolimus blood concentration	On day 1 tacrolimus maximum mean blood concentration varied between 0,1-0,35 ng/mL. Tacrolimus blood concentration varied between >0,05-5,65 ng/mL through the week.
Harper et al. (2001)	AD patients: n=10, age: 1-4 years Gender not given.	AD patients diagnosed according to the Hanifin and Rajka criteria. All had active AD with 23-69% of body surface area affected. EASI score ranged from 10,5-43,6.	Lesional skin	Test compound: 1% pimecrolimus cream (MW=810 Da, logK _{ow} =4,4). Exposure conditions: subjects exposed twice daily for 3 weeks. Technique: absorption assessed by blood samples and pimecrolimus radioimmunoassay.	Pimecrolimus blood concentration	Pimecrolimus blood concentration varied between <0,5-1,8 ng/mL after 4 days and <0,5-1,6 ng/mL after 22 days. 63% of blood samples had a pimecrolimus concentration below 0,5 ng/mL.

Soter et al. (2001)	Subjects of Caucasian (n=422), African-American (n=167) and other (n=42) origin AD patients: n=631, F/M=357/274, age: 16-79 years	AD patients diagnosed according to the Hanifin and Rajka criteria. All had active AD with 10-100% of body surface area affected. Severity of the disease was graded by scoring system of Rajka and Langeland (1989), all had moderate or severe AD.	Lesional skin	Test compound: 0,03% or 0,1% tacrolimus cream (MW=804 Da, $\log K_{ow}=3,03$.) Exposure conditions: subjects exposed twice daily until 1 week after complete clearance or up to 12 weeks. Technique: absorption assessed by blood samples and a validated enzyme-linked immunosorbent assay method.	Tacrolimus blood concentration	80% of blood samples had a tacrolimus concentration below 0,5 ng/mL.
Van Leent et al. (2002)	Subjects of Caucasian origin AD patients: n=12, F/M=4/8, mean age: 23,4 years (range 19-54 years)	AD patients diagnosed according to the Hanifin and Rajka criteria. All had active AD with 15-59% of body surface area affected. Severity of the disease was moderate to severe and the clinical scores for atopic dermatitis were 46,1 (range 21-63) for the SCORAD index and 9,4 (range 3-14) for the Hanifin score.	Lesional skin	Test compound: 1% pimecrolimus cream (MW=810 Da, $\log K_{ow}=4,4$.) Exposure conditions: subjects exposed twice daily for 3 weeks. Technique: absorption assessed by blood samples and pimecrolimus radioimmunoassay.	Pimecrolimus blood concentration	Pimecrolimus blood concentration varied between <0,5-1,4 ng/mL in the 3 weeks. 78% of blood samples had a pimecrolimus concentration below 0,5 ng/mL.
Allen et al. (2003)	AD patients: n=26, F/M=14/12, age: 4 months - 14 years	AD patients diagnosed according to the Hanifin and Rajka criteria or the criteria of Sampson. All had active dermatitis with 21-80% of body surface area affected. EASI score ranged from 10,8-35,6.	Lesional skin	Test compound: 0,7-15 g of 1% pimecrolimus cream (MW=810 Da, $\log K_{ow}=4,4$.) Exposure conditions: subjects exposed twice daily for 3 weeks. Technique: absorption assessed by blood samples and pimecrolimus radioimmunoassay.	Pimecrolimus blood concentration	Pimecrolimus blood concentration varied between <0,5-2,6 ng/mL in the 3 weeks. 44,7% of blood samples had a pimecrolimus concentration below 0,5 ng/mL.
Draeos et al. (2005)	Subjects of Caucasian (n=28), African-American (n=5), Oriental (n=3) or other origin (n=1) AD patients treated with pimecrolimus: n=18, F/M=12/6, mean age: 40,9 ± 14,3 years AD patients treated with tacrolimus: n=19, F/M=17/2, mean age: 42,5 ± 15,1 years	Definition of AD diagnose not given. All had active AD. Severity of the disease was diagnosed according to Investigators' Global Assessment, 18 had moderate AD, 17 had severe AD and 2 had very severe AD.	Not given	Test compound: 0,7-15 g of 1% pimecrolimus cream (MW=810 Da, $\log K_{ow}=4,4$) or tacrolimus cream (MW=804 Da, $\log K_{ow}=3,03$.) Exposure conditions: subjects exposed twice daily for 13 days. Technique: absorption assessed by blood samples and high-performance liquid chromatography-mass spectrometry detection.	Tacrolimus and pimecrolimus blood concentration	36% and 12% of blood samples had detectable levels of tacrolimus and pimecrolimus, respectively. Highest tacrolimus and pimecrolimus blood concentration observed was 2,39 ng/mL and 1,51 ng/mL respectively.
Harper et al. (2005)	Subjects of Caucasian (n=31), African-American (n=2) and other (n=6) origin	AD patients diagnosed according to the Hanifin and Rajka criteria. All had active AD with 11-94% of body surface area affected. 16 had moderate AD and 23	Lesional skin at day 1 but the treatment area	Test compound: 1% tacrolimus cream (MW=804 Da, $\log K_{ow}=3,03$.) Exposure conditions: subjects exposed twice daily for 14 days.	Tacrolimus blood concentration	92% of blood samples had a tacrolimus concentration below 1 ng/mL. Tacrolimus concentration increased with increasing size of treated body surface area.

	AD patients: n=39, F/M=17/22, age: 6-12 years	had severe AD.	remained the same throughout the study irrespective of improvement in the skin condition	Technique: absorption assessed by blood samples and high-pressure liquid chromatography with tandem mass spectrometry detection.		
Ling et al. (2005)	Subjects of Caucasian (n=29), African-American (n=16) and Oriental (n=1) origin AD patients: Group 1: n=24, F/M=13/11, mean age: 40,5 years Group 2: n=25, F/M=14/11, mean age: 36,1 years	AD patients diagnosed according to the Hanifin and Rajka criteria. All had active AD with at least 30% of body surface area affected. 57% had moderate AD with a pruritus severity score of 2 and 39% had severe AD with a pruritus severity score of 3.	Lesional skin	Test compound: 1% pimecrolimus cream (MW=810 Da, logK _{ow} =4,4). Exposure conditions: Group 1: subjects exposed twice daily for 1 week. Group 2: subjects exposed 4 times daily for 1 week. Technique: absorption assessed by blood samples and high-pressure liquid chromatography with mass spectrometry detection.	Pimecrolimus blood concentration	95% of blood samples had a pimecrolimus concentration below 0,5 ng/mL. 3 patients had a pimecrolimus concentration above 0,5 ng/mL, 2 from group 1 and 1 from group 2. Their pimecrolimus concentration varied between 0,53-1,37 ng/mL.
Rubins et al. (2005)	AD patients: n=32, age: > 18 years Gender not given	AD patients diagnosed according to the Hanifin and Rajka criteria. Severity of disease was graded by scoring system of Rajka and Langeland (1989), all had moderate to severe AD (score >4,5).	Lesional skin	Test compound: 1% tacrolimus cream (MW=804 Da, logK _{ow} =3,03.) Exposure conditions: subjects exposed twice daily for 14 days. Technique: absorption assessed by blood samples and high-pressure liquid chromatography with mass spectrometry detection.	Tacrolimus blood concentration	96% of blood samples had a tacrolimus concentration below 1 ng/mL. Tacrolimus concentration increased with increasing size of treated body surface area.
Staab et al. (2005)	Subjects of Caucasian (n=11), African-American (n=7) and Oriental (n=3) origin AD patients: n=21, F/M=6/15, age: 3-23 months	AD patients diagnosed according to the Sampson diagnostic criteria. All had active AD with 10-92% of body surface area affected. EASI score ranged from 1,4- 56,4.	Lesional skin	Test compound: 1% pimecrolimus cream (MW=810 Da, logK _{ow} =4,4). Exposure conditions: subjects exposed twice daily for 3 weeks. Technique: absorption assessed by blood samples and high-pressure liquid chromatography with mass spectrometry detection.	Pimecrolimus blood concentration	Pimecrolimus blood concentration varied between <0,1-2,26 ng/mL in the 3 weeks. 71% of blood samples had a pimecrolimus concentration below 0,5 ng/mL.
Lakhanpaul et al. (2006)	AD patients: n=5, F/M=3/2, age: 5,7-11,9 months Gender not given.	AD patients diagnosed according to the Sampson diagnostic criteria. All had active AD with 39-52% of body surface area affected. Severity of the disease was moderate to severe.	Lesional skin	Test compound: 1% pimecrolimus cream (MW=810 Da, logK _{ow} =4,4). Exposure conditions: subjects exposed when needed for a year. The mean number of days subjects were exposed was 332 days. Technique: absorption assessed by blood samples and high-pressure liquid chromatography with tandem mass spectrometry detection.	Pimecrolimus blood concentration	Pimecrolimus blood concentration was on average 0,32 ± 0,35 ng/mL after 27 weeks and 0,68 ± 0,76 ng/mL after 53 weeks. Concentration varied between <0,5-1,94 ng/mL.

Van Leent et al. (2007)	Subjects of Caucasian (n=37) and Oriental (n=3) origin AD patients: n=40, F/M=19/21, age: 19-59 years	AD patients diagnosed according to the Hanifin and Rajka criteria. All had active AD with 20-62% of body surface area affected. Severity of the disease was moderate to severe.	Lesional skin	Test compound: 1% pimecrolimus cream (MW=810 Da, logK _{ow} =4,4). Exposure conditions: subjects exposed twice daily for up to 12 months. Technique: absorption assessed by blood samples and pimecrolimus radioimmunoassay.	Pimecrolimus blood concentration	Pimecrolimus blood concentration varied between <0,5-0,8 ng/mL in the 12 months. 98% of blood samples had a pimecrolimus concentration below 0,5 ng/mL.
F. Eichenfield et al. (2007)	Subjects of Oriental origin AD patients: n=17, F/M=13/4, mean age: 3,7 years (range 3,6 months – 11,6 years)	Definition of AD diagnose not given. All had active AD with 10-48% of body surface area affected. 64,7% had moderate AD as indicated by Investigators' Global Assessment score of 3.	Lesional skin	Test compound: 1% pimecrolimus cream (MW=810 Da, logK _{ow} =4,4). Exposure conditions: subjects exposed twice daily for 3 weeks. Technique: absorption assessed by blood samples and high-pressure liquid chromatography with mass spectrometry detection.	Pimecrolimus blood concentration	On day 1, 94% of blood samples had a pimecrolimus concentration below 0,5 ng/mL. On days 10 and 22, 93% and 100% of blood samples, respectively, had a pimecrolimus concentration below 0,5 ng/mL. No correlation between concentration of pimecrolimus and size of treated body surface area.
Reitamo et al. (2009)	Subjects of Caucasian (n=42), Oriental (n=2) and other (n=2) origin AD patients: n=46, F/M=20/26, mean age: 14,5 months (range 5-23 months)	AD patients diagnosed according to the Hanifin and Rajka criteria. All had active AD with at least 5% of body surface area affected.	Lesional skin at day 1 but the treatment area remained the same throughout the study irrespective of improvement in the skin condition	Test compound: 0,03% tacrolimus cream (MW=804 Da, logK _{ow} =3,03.) Exposure conditions: subjects exposed 1 or 2 times daily for 14 days. Technique: absorption assessed by blood samples and high-pressure liquid chromatography with tandem mass spectrometry detection.	Tacrolimus blood concentration	97% of blood samples had a tacrolimus concentration below 1 ng/mL. Tacrolimus concentration increased with increasing size of treated body surface area.
Undre et al. (2009)	Subjects of Caucasian origin AD patients: n=11, F/M=5/6, age: 19-70 years	AD patients diagnosed according to the Hanifin and Rajka criteria. All had active AD with at least 0,3 m ² of body surface area affected. Severity of the disease graded by the scoring system of Rajka and Langeland (1989), all had moderate to severe AD (score >4,5).	Lesional skin	Test compound: 0,1% tacrolimus ointment (MW=804 Da, logK _{ow} =3,03.) Exposure conditions: subjects exposed twice daily for 2 weeks. Technique: absorption assessed by blood samples and skin biopsies and high performance liquid chromatography with tandem mass spectrometry detection.	Tacrolimus skin concentration Tacrolimus blood concentration	On day 1 tacrolimus skin concentration was 94 ± 20 ng cm ⁻³ . On day 14 tacrolimus skin concentration was 595 ± 98 ng cm ⁻³ . Concentration decreased with increasing depth in the skin. On day 1 and 14, tacrolimus blood concentration was 0,13 ± 0,16 ng/mL and 0,33 ± 0,34 ng/mL, respectively. 94% of blood samples had a tacrolimus concentration below 1 ng/mL.
Thaci et al. (2010)	Subjects of Caucasian (n=14), African-American (n=3) and Oriental (n=2) origin Adult AD patients: n=9, F/M=3/6, mean age: 39,6 years	AD patients diagnosed according to the Hanifin and Rajka criteria. All had active AD with at least 30% of body surface area affected. Severity of disease was moderate to severe and EASI score was 29,7±12,2, 26,4±7,3 and 26,5±6,3 for adults, adolescents and children, respectively.	Lesional and non-lesional skin	Test compound: 1% pimecrolimus cream (MW=810 Da, logK _{ow} =4,4). Exposure conditions: subjects exposed twice daily for 8.5 days. Open exposure at day and closed exposure overnight. Technique: absorption assessed by blood samples and high-pressure liquid chromatography with mass spectrometry detection.	Pimecrolimus blood concentration	Maximum pimecrolimus blood concentration varied between 0,36-1,84 ng/mL in adults, 0,11-0,55 ng/mL in adolescents and 0,23-1,29 ng/mL in children. No significant difference between the three groups. No correlation between tacrolimus concentration and size of treated body surface area.

	Adolescents AD patients: n=4, F/M=1/3, mean age: 14 years Children AD patients: n=6, F/M=3/3, mean age: 5,8 years					
Van Velsen et al. (2012)	AD patients: n=25, F/M=15/10, age: 18-71 years	AD patients diagnosed according to the Hanifin and Rajka criteria. All had severe AD.	Whole body excluding the face and genitalia	Test compound: 20-30 g of 0,05% clobetasol propionate (CLO) (MW=483 Da, logK _{ow} =3,28) Exposure conditions: subjects exposed for 1 day to 1 or 2 applications. Technique: absorption assessed by blood samples and validated LC/MS/MS method determining serum CLO levels.	Serum CLO levels	Serum CLO levels varied between 0,173-4,504 ng/mL after 1 application and 0,112-4207 ng/mL after 2 applications. No significant difference between 2 groups.

AD: atopic dermatitis, n: number of participants, F/M: female/males, MW: molecular weight, logK_{ow}: octanol-water partition coefficient, CLO: clobetasol propionate

Table 3: Animal studies

Reference	Animal	Test material/exposure conditions/technique	Endpoints	Main findings
Scharschmidt et al. (2009)	In vivo: Control mice(C57B1&6j+/+ mice) and flaky tail mice (ft/ft mice) n=6 age 12 weeks. In vitro: Freshly obtained explants from control mice (C57B1&6j+/+ mice) and flaky tail mice (ft/ft mice). n=6 age: 12 weeks Gender not given.	In vivo: Test compound: 4% lanthanum nitrate solution (MW=325 Da, logK _{ow} = no data available) in 4% sucrose in 0,05 M Tris buffer, pH 7,5. Exposure conditions: one flank immersed for 30 minutes – 2h in 4% lanthanum nitrate solution, while under general anesthesia. Technique: absorption assessed by electron microscopy. In vitro: Test compound: water soluble fluorophore, calcium green (MW=1200 Da, logK _{ow} = no data available) in nanopure water. Exposure conditions: explants exposed to soluble fluorophore, calcium green. Technique: absorption assessed by dual-photon confocal microscopy.	In vivo: Penetration of tracer (lanthanum nitrate) in stratum corneum In vitro: Penetration of tracer (calcium green) in stratum corneum	Skin of flaky tail mice showed increased penetration of the tracers when compared with control mice. In vivo: After 3h, tracer penetrated 4 layers of the stratum corneum of flaky tail mice while no tracer penetrated the stratum corneum of control mice. In vitro: The penetration was higher in the skin of flaky tail mice than in the skin of control mice.
Moniaga et al. (2010)	Control mice (B6 mice) and flaky tail mice (ft/ft mice). F/M: only females age: 8 weeks	Test compound: 100 µL of 1% FITC diluted in acetone and dibutyl phthalate (1:4) (MW=389 Da, logK _{ow} = no data available). Exposure conditions: shaved dorsal skin of mice	Amount of FITC in epidermis and dermis	Skin of flaky tail mice showed increased penetration of the tracers when compared with control mice Epidermis of flaky tail mice contained higher amounts of FITC than epidermis of control mice. Neither contained FITC in dermis.

	Number of mice not given.	exposed to 1% FITC. Technique: absorption assessed by a fluorometer.		
Fang et al. (2016)	Balb/c mice with intact skin and balb/c mice exposed to ovalbumin to induce AD-like skin: age: 7 weeks Number of mice or gender not given.	In vivo: Test compound: 0,2 mL FITC (MW=389 Da, $\log K_{ow}$ =no data available) or 0,56% FD4 (MW=4000 Da, $\log K_{ow}$ = no data available) in pH 7,4 buffer. Exposure conditions: exposed for 6h to a glass cylinder filled with 0,2 mL FITC or FD4 attached to the mouse back. Technique: absorption assessed by fluorescence and confocal microscopy. In vitro: Test compound: 4% tacrolimus (MW=822 Da, $\log K_{ow}$ = 3, in 30% PEG400/water, 0,8% 8-methoxypsoralen (MW=216 Da, $\log K_{ow}$ = 1,93) in 40% PEG400/pH 4,7 buffer, 1% methotrexate (MW=454 Da, $\log K_{ow}$ = -1,85) in water or 0,56% FD4 in pH 7,4 buffer. Exposure conditions: Mouse skin exposed to tacrolimus, 8-methoxypsoralen, methotrexate or FD4 in a Franz cell diffusion chamber. Technique: absorption assessed by a high performance liquid chromatography.	In vivo: Amount of FITC and FD4 signals in the skin- In vitro: Dermal flux of drugs and accumulation of drugs in the skin.	Skin of AD-like mice showed increased penetration of the penetrants when compared with control mice. In vivo: FITC and FD4 signals were stronger and had penetrated to deeper layers in atopic dermatitis-like skin than in healthy intact skin. In vitro: Tacrolimus: The dermal flux was below detection limit, but tacrolimus accumulation was 2.65-fold higher ($p<0.05$) in atopic dermatitis-like skin (0,66 $\mu\text{g}/\text{mg}$) than in healthy intact skin (0,25 $\mu\text{g}/\text{mg}$). 8-methoxypsoralen: Dermal flux was 2.4-fold higher ($p<0.05$) in atopic dermatitis-like skin than in healthy intact skin, but accumulation of 8-methoxypsoralen was 0.6 fold lower in atopic dermatitis like-skin than in healthy intact skin. Methotrexate: Dermal flux was 17,5-fold higher in atopic dermatitis-like skin than in healthy intact skin and accumulation of methotrexate was 2-fold higher ($p<0.05$) in atopic dermatitis-like skin than in healthy intact skin. FD4: Dermal flux was 17.5-fold higher in atopic dermatitis-like skin than in healthy intact skin and there was only accumulation of FD4 in atopic dermatitis-like skin (0,46 $\mu\text{g}/\text{mg}$) and no accumulation in healthy intact skin.

AD: atopic dermatitis, n: number of participants, MW: molecular weight, $\log K_{ow}$: octanol-water partition coefficient