Dermal absorption of chemicals through normal and compromised skin

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Percutaneous penetration of sodium lauryl sulphate is increased in uninvolved skin of atopic dermatitis patients compared to control subjects
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Abstract

**Background:** Involved regions of the skin in atop dermatitis (AD) patients have been shown to have higher trans-epidermal water loss (TEWL) indicating compromised skin barrier. Whether also uninvolved skin has diminished barrier is controversial.

**Objectives:** To study the penetration of sodium lauryl sulphate (SLS) into uninvolved skin of AD patients compared to the skin of control subjects.

**Methods:** The percutaneous penetration was assessed using the tape stripping technique of the stratum corneum (SC). Twenty AD patients and 20 healthy subjects were exposed to 1% SLS for four hours on mid volar forearm. After the end of exposure the SC was removed by adhesive tape. In each consecutive strip, the amount of SLS was determined. Using Fick’s second law of diffusion, diffusivity and partition coefficient of SLS between water and SC were deduced.

**Results:** The SC thickness was similar in both groups; however the TEWL was higher in AD patients compared to that of the control group (8.4 ± 4.3 and 6.3 ± 2.1 g m\(^{-2}\) h\(^{-1}\), respectively). There was a correlation between SC thickness and TEWL in control subjects but no correlation was found in AD patients. The diffusivity of SLS through uninvolved AD skin was higher compared to normal skin (12.7 ± 5.8 x 10\(^{-9}\) and 6.1 ± 3.1 x 10\(^{-9}\) cm\(^2\) h\(^{-1}\), respectively) while the partition coefficient between SC and water was lower (137 ± 64 and 193 ± 101, respectively).

**Conclusion:** The results show different penetration profile of SLS into the SC of AD patients compared to control subjects. This indicates that even non-involved skin in AD patients has altered barrier emphasizing importance of skin protection and prevention of skin contact with chemicals.
Chapter 4: Section 4.1

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease associated with cutaneous hyperactivity to environmental agents and is characterized by pruritic lesions with dryness and typical distribution and morphology. The affected regions of the skin in AD patients show higher trans-epidermal water loss (TEWL) in comparison to the normal skin, in other words, lower capacity to hold water\(^1\). Higher permeability of affected skin was shown also for theophylline\(^2\) and polyethylene glycols (Jakasa et al., manuscript in preparation). The literature data concerning permeability of uninvolved AD skin is contradictory. Some authors reported higher TEWL in patients with AD history\(^1,3-8\) while others found no difference in comparison to normal skin\(^5,9-12\). In several studies it was suggested that the higher susceptibility to irritation in AD patients\(^6,10-11,13\) might partly be explained by higher skin permeability\(^14\). Increased susceptibility of AD patients to irritation induced by sodium lauryl sulphate (SLS) was shown both in involved and uninvolved AD skin\(^6\). Impaired skin barrier in AD has often connected to the different lipid composition and structure of atopic skin. Previous studies have demonstrated that barrier impairment coincides with alterations in the amount and composition of stratum corneum (SC) ceramides in AD skin\(^5,15-16\). In involved as well as in uninvolved skin of AD patients reduced ceramide content and decreased percentages of Cer1 and Cer3 were reported.\(^5,15-17\)

In contrast to this, Matsumoto et al.\(^18\) found that the reduction of Cer 1 is restricted to the involved AD skin and is not extended to uninvolved area, which is consistent with data of Farwanah et al.\(^19\)

The question whether the barrier function in uninvolved skin in AD patients is compromised as compared to control skin is still open. In the present study we investigated the penetration of SLS into the SC of uninvolved skin of AD patients and into the SC of the skin of control subjects. SLS is a common ingredient of soaps and cosmetic products and atopic persons are known to be sensitive to these. In addition, in a previous study we have shown that atopic persons, although with no history of AD, appeared to have higher skin diffusibility and to be more susceptible to SLS irritation than non-atopic subjects.\(^14\)

Subjects and methods

Study population

Twenty AD patients, 12 male and 8 female, mean age 29 years (range 18-54 years) and 20 healthy subjects, 11 male and 9 female, mean age 32 years (range 18-55
years), all Caucasians participated in the study. The study was carried out in June and July 2004.

AD patients were recruited from the outpatient clinic of the Academic Medical Center and diagnosed according to Hanifin & Rajka criteria. We excluded patients that had received systemic therapy, such as corticosteroids and immunosuppressants, or photo-therapy in the past two years. Subjects with concomitant ichthyosis vulgaris were excluded. The test sites, both mid volar arms were free of dermatitis for at least 3 months prior to the experiment. The total eczema area and severity index (EASI, the maximum is 72 points) was assessed in AD patients. The severity of the disease was mild in all patients and the median EASI score was 1.7 (ranging from 0.2 to 22.8). Twelve patients had active AD (pruritic lesions) and eight had inactive AD (free of dermatitis for at least 3 months, at the time showing only mild signs: scars, scaling, lichenification or dry skin) on body parts other than test sites.

Control subjects had no visible skin damage and no history of past or present AD and other dermatological diseases.

All subjects completed the Erlangen questionnaire from which an Atopy Score (the maximum is 34 points and having a score ≥ 10 is considered as atopy) was derived. AD patients and control subjects had a score of 17.4 ± 6.6 and 3.0 ± 2.4 (mean ± SD), respectively.

Participants were not allowed to use soap, moisturizers or any other cosmetics and creams on the lower mid volar arms 48 hours prior to and during the experiments. Written informed consent was obtained from all subjects prior to the experiment. The Medical Ethical Committee of the Academic Medical Center, University of Amsterdam approved the experimental protocol.

**Penetration experiment**

The subjects were exposed for four hours on both volar arms to 1% SLS in water (200 µL, ≥ 99% purity, Fluka, Buchs, Switzerland) using patch test chambers (Finn chambers®, 18 mm in diameter, Epitest Ltd., Finland). Before application and after patch removal, TEWL was measured on application sites. TEWL was measured with an Evaporimeter (VapoMeter SWL2g, Delfin Technologies, Ltd., Kuopio, Finland). The measurement was described in detail elsewhere. Twenty minutes prior to the measurements, the subjects rested with their sleeves rolled-up in the examination room, where temperature was 20-22 °C and relative humidity ranged between 50 and 60%. Fifteen minutes after the end of exposure the SC layers were sequentially
removed with pre-cut Diamond tape pieces, 19 x 25 mm (Diamond Ultra Clear tape, The Sellotape® Company, the Netherlands). Templates of Scanpor® tape were fixed on the skin around application spot to limit the tape stripping area (18 mm in diameter). The tape pieces were consecutively applied to the test site and uniformly pressed with 1 kg stainless steel roller that was moved 20 times in two directions. The sites were stripped multidirectionally with one quick movement until the SC was totally removed as observed by shiny and reddish appearance of the skin, feeling of burning sensation by subjects when last tape strips were taken off and by measuring TEWL > 100 g m⁻² h⁻¹. Each subsequent strip was placed into a glass vial and stored at -20 °C until analysis. The stripping of each site was completed within 40 minutes. The SC from non-exposed site was stripped off and served as negative control.

Analytical procedure

The concentration of SLS on each strip was determined spectrophotometrically using the adjusted method of Rusconi et al. In brief, 2 ml of methanol (J. T. Baker, Deventer, The Netherlands) was added to the vials and shaken for one hour (TPM-2 shaker-Sarstedt, Numbrecht, Germany) to extract the SLS from the tapes. Standards of SLS for the calibration curve were prepared in methanol (2.3 – 50 μg cm⁻³) and 20 μl from each standard and sample was pipetted into a 96-wells plate. After evaporation of the methanol, 200 μl Stains-All® working solution was added to each well and absorbance was read at 450 nm (Model 680 Microplate reader, Bio-Rad Laboratories, Hercules, CA, USA).

A protein analysis was used to measure the amount of SC removed by each tape strip and to assess the depth of the consecutive SC strip. The methanol residue, containing tape strip with precipitated proteins on it after SLS analysis, was evaporated. 1 ml of 1 M NaOH was added to the strip and the vials were shaken for two hours. The samples were left at room temperature overnight and the next day they were once more shaken for two hours. 1 ml of 1M HCl was added to the vials to neutralize the basic solution. The protein assay was based on the modified method of Dreher et al. and performed according to Bio-Rad DC protein microassay using commercially available bovine serum albumin (BSA) for standardization. Absorbance at 655 nm was measured using the Bio Rad 680 microplate reader.

The concentration of SLS on each strip was normalized for the amount of proteins and expressed as μg SLS/μg protein. Assuming the SC density of 1 g cm⁻³ and a uniform distribution of SC on the tapes the protein mass removed was converted to a volume enabling estimation of the depth of each strip in the SC and total thickness of the removed SC.
Data analysis
The concentration of SLS on each strip was plotted as a function of the relative SC depth. For the estimation of the penetration parameters we used the approach based on Fick’s second law of diffusion described in detail elsewhere\textsuperscript{28-29} (Fig 1) where $C_{veh}$ is the applied SLS concentration (μg cm\(^{-3}\)), $C(x)$ is the SLS concentration (μg cm\(^{-3}\)) at depth $x$, $K$ is the SC/water partition coefficient, $L$ is the total thickness of the SC (μm), $D$ is the diffusivity of SLS through the skin (cm\(^2\) h\(^{-1}\)) and $t$ is the exposure duration (h). The non-steady state diffusion equation (Fig 1) was fitted to the data where the rate constant for diffusion across SC ($D/L^2$, h\(^{-1}\)) was obtained from the decay of $C(x)$ as a function of $x$ and $K$ was obtained from the intercept at $x = 0$. The penetration parameters were derived from individual experiments and were averaged. The first strip was not included in the regression analysis, as it contained some residue of SLS on the surface of the skin after the end of exposure. For curve fitting and statistical calculations, Prism 4 software was used (Graph Pad, San Diego, CA, USA).

For statistical calculations Student’s t-test and one-way ANOVA with Bonferroni post test were used. $P$ value < 0.05 was considered significant.

$$C(x) = KC_{veh} \left\{ 1 - \frac{x}{L} \right\} - \sum_{n=1}^{\infty} \frac{2}{n\pi}KC_{veh} \sin\left( \frac{n\pi x}{L} \right) \exp\left( -\frac{Dn^2\pi^2 t}{L^2} \right)$$

*Fig 1: Fick’s second law of diffusion.*

Using the results of duplicate dermal exposures on two volar forearms we calculated the intra-subject variability as well as the inter-subject variability in a restricted sense, i.e. after eliminating the intra-subject variability. For the latter we used the coefficient of variation = {[(between subject variance−within subject variance)/2]\(^{1/2}\)/mean. We assume that the intra-individual variation predominantly consists of the measurement variation and in a small part of the difference in permeability between both measured sites.
Results

To remove the SC completely, on average 22 ± 9 strips for control subjects and 28 ± 9 for the AD patients were needed. The average amount of proteins removed from the exposure sites of the control subjects was 2206 ± 644 µg corresponding to SC thickness of 8.7 ± 2.5 µm that of AD patients was 2394 ± 491 µg corresponding to SC thickness of 9.4 ± 1.9 µm. Statistically acceptable ($r^2 \geq 0.95$) curve fitting was obtained for all control subjects and AD patients. For two control subjects and two AD patients fitting was obtained only for one of the duplicate measurement and for four control subjects curve fitting could only be performed using the pooled duplicate data. Figure 2 shows the SLS concentration profile across SC for one typical control subject and one AD patient as well as fitted curve obtained by non-linear regression analysis (dashed lines).

The penetration parameters are summarized in Table 1. We have found no substantial difference between the two groups for SC thickness. TEWL was higher in AD patients when compared to control subjects. A significant correlation between SC thickness and TEWL was found in control subjects ($r = -0.59$, $p = 0.003$): the thinner the SC, the higher the TEWL but no significant correlation was found in AD patients ($r = -0.14$, $p = 0.55$) (Figure 3).

![Graph](image.png)

**Fig 2:** SLS concentration decay as a function of SC depth ($x/L$) in one control subject and one AD patients after 4 hour exposure to 1% SLS. Non-linear regression analysis was used to fit the equation given in Fig 1 to the data (dashed lines).
Table 1. SLS penetration parameters (*mean ± SD*) and corresponding inter- and intra-individual variations for AD patients and control subjects.

<table>
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<th>Unit</th>
<th>Control subjects</th>
<th>AD patients</th>
<th>p-value*</th>
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<tr>
<td>Number of subjects</td>
<td></td>
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<tr>
<td>SC thickness</td>
<td>µm</td>
<td>8.7 ± 2.5</td>
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<td>Baseline TEWL</td>
<td>g m⁻² h⁻¹</td>
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<td>Diffusivity</td>
<td>10⁻⁹ cm² h⁻¹</td>
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<td>12.7 ± 5.8</td>
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<td>SC/water partition</td>
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<td>196 ± 107</td>
<td>137 ± 64</td>
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<td>coefficient</td>
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<tr>
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*Independent sample T-test: ¹(one-sided), ²(two-sided), ns = not significant

Fig 3: Correlation between basal TEWL and SC thickness determined in control subjects and AD patients.
The diffusivity was twice as high in AD patients (12.7 ± 5.8 x 10⁻⁹ cm² h⁻¹) when compared to control subjects (6.1 ± 3.1 x 10⁻⁹ cm² h⁻¹, p < 0.001). We also compared, using one-way ANOVA test, control subjects and AD patients according to state of disease; patients with active and inactive AD, and the mean values of diffusivity in three groups were significantly different (p < 0.001) (Figure 4). The mean value of diffusivity in patients with active AD was significantly higher compared to AD patients with inactive AD (p = 0.017) and control subjects (p < 0.001), but no significant difference was found between patients with inactive AD and control subjects (p > 0.05).

The partition coefficient was somewhat lower in AD patients (p < 0.05) than in control subjects. The mean values for the partition coefficient were not significantly different when we compared all three groups (P > 0.05) using ANOVA. Nevertheless, there was a trend of decreasing partition coefficient with state of disease (Figure 4).

![Graph showing diffusivity (D) and SC/water partition coefficient (K) of SLS in patients with active AD (AD_A, n=12), inactive AD (AD_i, n=8), and control subjects (n=20).](image)

**Fig 4:** Diffusivity (D) and SC/water partition coefficient (K) of SLS in patients with active AD (AD_A, n=12), inactive AD (AD_i, n=8), and control subjects (n=20).

To obtain insight into intra-individual and inter-individual variation in diffusivity and partition coefficient we have used available duplicate data for 18 AD patients and 14 control subjects as shown in Table 1. The inter-individual variation, expressed as the coefficient of variation (CV), in diffusivity amounted to 48% and 55% in control subjects and AD patients, respectively, while CV for partition coefficient amounted to 46% in both groups. Overall, the inter-individual variation was higher than intra-individual variation in both parameters in both groups. The intra-individual variation in diffusivity was higher in AD patients, while for partition coefficient it was lower then in control subjects.
Chapter 4: Section 4.1

Discussion

In the present study we have assessed the penetration of SLS into the SC of AD patients and control subjects using the non-invasive tape stripping technique. We have shown an increased diffusion in uninvolved AD skin when compared to normal skin.

The thickness of SC as calculated from the amount of proteins removed by tape strips was nearly the same in both groups. The skin of AD patients, however, showed increased TEWL when compared to the skin of normal subjects (Table 1) indicating less effective skin barrier for water. This is in agreement with the results of Laudanska et al.\textsuperscript{30}, who also found higher TEWL in AD patients in the state of remission of the skin lesions and of Seidenari et al.\textsuperscript{1} reporting higher TEWL in involved and uninvolved skin of children affected by AD. The TEWL in control subjects showed to be inversely dependent on the SC thickness, which is in agreement with other studies.\textsuperscript{31,32} However this relationship was not found in AD patients suggesting that in atopic skin other factors besides skin thickness play a role in skin permeability for water. Altered composition and structure of the SC in atopic skin might at least partly be responsible for this. A reduction of Cer 3 was previously found to correlate with an increased TEWL in both involved as well as uninvolved skin.\textsuperscript{5} Since epidermal lipids are essential for the proper barrier function and prevention of excessive water loss, the decreased amount of lipids would be responsible for the loss of barrier function and likely also for higher permeability of foreign substances.

The applied method of skin stripping enabled us to estimate two parameters which determined the permeability, diffusivity and partition coefficient of SLS. According to the Fick's law of diffusion, these two parameters determine the skin flux of a penetrant and its concentration in the SC. Both parameters, the diffusivity, which reflects the resistance of SC toward movement of SLS, and the partition coefficient, are dependent on the composition and structure of the SC. We found the average diffusivity across SC to be two times higher in the skin of AD patients when compared to control subjects. We have also looked into the differences in diffusivity of AD patients according to state of disease (Figure 4). The diffusivity was higher in patients with active AD when compared to those with inactive AD and control subjects but there was no significant difference between patients with inactive AD and control subjects. However, there is a clear trend of increasing diffusion of SLS with state of disease. This indicates that state of disease influences the permeability of the skin visibly not affected by AD.
These findings are in line with a study of de Jongh et al.\textsuperscript{14}, where atopic persons (although with no history of AD) showed approximately 1.5 times higher diffusivity for SLS than non-atopics. In our accompanying study consisting of the same subjects we found higher diffusivity also for polyethylene glycols of different molecular sizes in the skin of AD patients compared to that of control subjects (Jakasa et al., manuscript in preparation). Yosiike et al.\textsuperscript{2} reported increased penetration of theophylline not only in involved but also in uninvolved AD skin compared to control subjects. All these findings indicate that uninvolved atopic skin is more permeable for different compounds concerning their hydrophilicity and molecular size.

The skin of AD patients showed a 30% lower solubility of SLS compared to normal skin, although the difference was not as high as by diffusion. The partition coefficient was not significantly different when two groups of AD patients and control subjects were compared; but there was a trend of decreasing partition of SLS into the SC with state of disease. Estimation of the partition coefficient, using the method applied in the present study, is associated with higher uncertainty compared to diffusivity as the quality of the first data points largely influences the estimation outcome. To overcome this problem, a second prolonged experiment was recommended\textsuperscript{33} where the curve becomes a straight line at steady-state and estimation of partition coefficient is less dependent on the error from the superficial strips. However, in a present study we chose a relatively short exposure duration since longer exposure to SLS would more likely lead to the alteration of the skin barrier which might change the SC permeability.

In the present study we observed substantial inter- and intra-individual variation in both penetration parameters. The intra-individual variation in diffusivity was higher in AD patients compared to control subjects, which may be attributed to the more pronounced difference in composition and structure of the skin in different skin areas. At the same time the intra-individual variability in the partition coefficient was lower in AD patients. As mentioned earlier, the determination of partition coefficient in a non-steady state is largely influenced by the quality of the first data points. As the SLS concentration/SC depth curve (Fig 2) approaches linearity, as is the case with AD patients, the intercept value from which partition coefficient is derived is less dependent on first data points and, therefore, the intra-individual variation decreases.

To summarize, the skin of AD patients showed increased percutaneous penetration of SLS when compared to control subjects supporting the hypothesis of impaired skin barrier even in the non-involved skin. As a consequence, we expect that the defect skin barrier of AD patients will facilitate absorption of other chemicals, which could
lead to the higher susceptibility for local skin effects. This emphasizes the importance of continuous skin protection and maintenance of the skin barrier.

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