Individual susceptibility to chronic irritant contact dermatitis

de Jongh, C.M.

Citation for published version (APA):
de Jongh, C. M. (2008). Individual susceptibility to chronic irritant contact dermatitis

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 2.2

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

I. Jakaša¹, C.M. de Jongh¹, M.M. Verberk¹, J.D. Bos², S. Kežić¹

British Journal of Dermatology 2006; 155: 104-109

¹Coronel Institute of Occupational Health, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands; ²Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands
Chapter 2.2

Abstract

**Background** Involved regions of the skin in patients with atopic dermatitis (AD) have been shown to have higher transepidermal water loss (TEWL), indicating a compromised skin barrier. Whether uninvolved skin also has diminished barrier characteristics is controversial.

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

**Methods** Percutaneous penetration was assessed using the tape stripping technique on the stratum corneum (SC). Twenty patients with AD and 20 healthy subjects were exposed to 1% SLS for 4 h on mid-volar forearm. After the end of exposure the SC was removed by adhesive tape. The amount of SLS was determined in each consecutive strip. Fick’s second law of diffusion was used to deduce the diffusivity and the partition coefficient of SLS between water and the SC.

**Results** The SC thickness was similar in both groups; however, the TEWL was higher in patients with AD compared with that of the control group (mean ± SD 8.4 ± 4.3 and 6.3 ± 2.0 g m$^{-2}$ h$^{-1}$, respectively). There was a correlation between SC thickness and TEWL in control subjects but no correlation was found in patients with AD. The diffusivity of SLS through uninvolved AD skin was higher compared with normal skin (mean ± SD 12.7 ± 5.8 x 10$^{-9}$ and 6.2 ± 3.0 x 10$^{-9}$ cm$^{2}$ h$^{-1}$, respectively), while the partition coefficient between SC and water was lower (mean ± SD 137 ± 64 and 196 ± 107, respectively).

**Conclusions** The results show a different penetration profile of SLS into the SC of patients with AD compared with control subjects. This indicates that even noninvolved skin in patients with AD has altered barrier characteristics, emphasizing the importance of skin protection and prevention of skin contact with chemicals.
Percutaneous penetration of SLS is increased in patients with AD

**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 patients with AD show higher transepidermal water loss (TEWL) in comparison with 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.\(^3\) The literature data concerning permeability of uninvolved AD skin are contradictory. Some authors reported higher TEWL in patients with a history of AD,\(^1,4-8\) while others found no difference in comparison with normal skin.\(^9-13\) In several studies it was suggested that the higher susceptibility to irritation in patients with AD\(^6,11,12,14\) might partly be explained by higher skin permeability.\(^15\) Increased susceptibility of patients with AD to irritation induced by sodium lauryl sulphate (SLS) was shown both in involved and in uninvolved AD skin.\(^6\) The impaired skin barrier in AD has often been 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.\(^10,16,17\) Reduced ceramide content and decreased percentages of Cer 1 and Cer 3 were reported in involved as well as in uninvolved skin of patients with AD.\(^10,16-18\) In contrast to this, Matsumoto et al.\(^19\) found that the reduction of Cer 1 is restricted to involved AD skin and is not extended to uninvolved area, which is consistent with data of Farwanah et al.\(^20\)

The question whether the barrier function in uninvolved skin in patients with AD is compromised as compared with control skin is still open. In the present study we investigated the penetration of SLS into the SC of uninvolved skin of patients with AD 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 nonatopic subjects.\(^15\)
Subjects and methods

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

Patients with AD were recruited from the outpatient clinic of the Academic Medical Center and diagnosed according to Hanifin and Rajka criteria. We excluded patients who had received systemic therapy, such as corticosteroids and immunosuppressants, or phototherapy 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 patients with AD. The severity of the disease was mild in all patients and the median EASI score was 1.7 (range 0.2-22.8). 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. Patients with AD 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 h 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 4 h 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, Tuusula, Finland). Before application and after patch removal, TEWL was measured on application sites. TEWL was measured with an Evaporimeter (VapoMeter
Percutaneous penetration of SLS is increased in patients with AD.

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 was 50-60%. Fifteen minutes after the end of exposure the SC layers were sequentially removed with precut Diamond tape pieces, 19 x 25 mm (Diamond Ultra Clear tape, The Sellotape® Company, Eindhoven, the Netherlands). Templates of Scanpor® tape (Epitext) were fixed on the skin around the application site to limit the tape stripping area (18 mm in diameter). The tape pieces were consecutively applied to the test site and uniformly pressed with a 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, a burning sensation in subjects when the 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 min. The SC from a non-exposed site was stripped off and served as a 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 1 h (TPM-2 shaker; Sarstedt, Nümbrecht, 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-well plate. After evaporation of the methanol, 200 μl Stains-All® (Sigma-Aldrich, St Louis, MO, U.S.A.) 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 strips. The methanol residue, containing tape strip with precipitated proteins on it after SLS analysis, was evaporated. One millilitre of 1 M NaOH was added to the strip and the vials were shaken for 2 h. The samples were left at room temperature overnight and the next day they were once more shaken for 2 h. One millilitre 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 the Bio-Rad DC protein assay method.
microassay\textsuperscript{27} using commercially available bovine serum albumin 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 \( \mu g \) SLS/\( \mu g \) protein. Assuming an SC density\textsuperscript{28} 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{29,30} (Figure 1), where \( C_{veh} \) is the SLS concentration applied (\( \mu g \) cm\(^{-3}\)), \( C(x) \) is the SLS concentration (\( \mu g \) cm\(^{-3}\)) at depth \( x \) (\( \mu m \)), \( K \) is the SC/water partition coefficient, \( L \) is the total thickness of the SC (\( \mu m \)), \( D \) is the diffusivity of SLS through the skin (cm\(^2\) h\(^{-1}\)) and \( t \) is the exposure duration (h).

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

**Figure 1.** Fick’s second law of diffusion. \( C_{veh} \) is the sodium lauryl sulphate (SLS) concentration applied (\( \mu g \) cm\(^{-3}\)), \( C(x) \) is the SLS concentration (\( \mu g \) cm\(^{-3}\)) at depth \( x \) (\( \mu m \)), \( K \) is the stratum corneum (SC)/water partition coefficient, \( L \) is the total thickness of the SC (\( \mu m \)), \( D \) is the diffusivity of SLS through the skin (cm\(^2\) h\(^{-1}\)) and \( t \) is the exposure duration (h).

The nonsteady-state diffusion equation (Figure 1) was fitted to the data where the rate constant for diffusion across the 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. Prism 4 software was used for curve fitting and statistical calculations (Graph Pad, San Diego, CA, U.S.A.). For statistical calculations Student’s t-test and one-way ANOVA with Bonferroni adjustment were used. P < 0.05 was considered significant.
Using the results of duplicate dermal exposures on two volar forearms we calculated the intrasubject variability as well as the intersubject variability in a restricted sense, i.e. after eliminating the intrasubject variability. For the latter we used the coefficient of variation (CV), where CV = \{\frac{\text{between subject variance} - \text{within subject variance}}{2}\}^{1/2}/\text{mean}. We assume that the intraindividual variation predominantly consists of the measurement variation and in a small part of the difference in permeability between both measured sites.

**Results**

A mean ± SD of 22 ± 9 strips for control subjects and 28 ± 9 for the patients with AD were needed to remove the SC completely. The mean ± SD amount of proteins removed from the exposure sites of the control subjects was 2206 ± 644 µg corresponding to an SC thickness of 8.7 ± 2.5 µm, while that of patients with AD was 2394 ± 491 µg, corresponding to an SC thickness of 9.4 ± 1.9 µm. Statistically acceptable \(r^2 \geq 0.95\) curve fitting was obtained for all control subjects and patients with AD. For two control subjects and two patients with AD fitting was obtained for only one of the duplicate measurements and for four control subjects curve fitting could only be performed using the pooled duplicate data.

Figure 2 shows the SLS concentration profile across the SC for one typical control subject and one AD patient as well as fitted curves obtained by nonlinear regression analysis (dashed lines).
Chapter 2.2

Figure 2. Sodium lauryl sulphate (SLS) concentration decay as a function of stratum corneum (SC) depth (x/L) in one control subject and one patient with atopic dermatitis (AD) after a 4-h exposure to 1% SLS. Nonlinear regression analysis was used to fit the equation given in Figure 1 to the data (dashed lines). D, diffusivity.

The penetration parameters are summarized in Table 1. We found no substantial difference between the two groups for SC thickness. TEWL was higher in patients with AD when compared with 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. In contrast, no significant correlation was found in patients with AD (r = -0.14, P = 0.55) (Figure 3).
The mean ± SD diffusivity was twice as high in patients with AD (12.7 ± 5.8 x 10^{-9} cm^2 h^{-1}) when compared with control subjects (6.2 ± 3.0 x 10^{-9} cm^2 h^{-1}, P < 0.001). We also compared, using one-way ANOVA, control subjects and patients with AD according to state of disease (active vs. inactive AD): the mean values of diffusivity in the three groups were significantly different (P < 0.001) (Figure 4). The mean value of diffusivity in patients with active AD was significantly higher compared with patients with inactive AD (P = 0.016) and control subjects (P < 0.001), but no significant difference was found between patients with inactive AD and control subjects (P > 0.05).
Chapter 2.2

Figure 3. Correlation between basal transepidermal water loss (TEWL) and stratum corneum (SC) thickness (μm) determined in control subjects and patients with atopic dermatitis (AD).

Figure 4. Mean ± SD diffusivity (D) and stratum corneum/water partition coefficient (K) of sodium lauryl sulphate in patients with active atopic dermatitis (AD\(_A\), n = 12), inactive atopic dermatitis (AD\(_I\), n = 8) and control subjects (n = 20).

The partition coefficient was somewhat lower in patients with AD (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).

To obtain insight into intraindividual and interindividual variation in diffusivity and partition coefficient we have used available duplicate data for 18 patients with AD and 14 control
subjects as shown in Table 1. The interindividual variation, expressed as the CV, was 48% and 55% in control subjects and patients with AD, respectively, for diffusivity, while the CV for partition coefficient was 46% in both groups. Overall, the interindividual variation was higher than the intraindividual variation in both parameters in both groups. The intraindividual variation in diffusivity was higher in patients with AD, while for the partition coefficient it was lower than in control subjects.

Discussion

In the present study we have assessed the penetration of SLS into the SC of patients with AD and control subjects using the noninvasive tape stripping technique. We have shown an increased diffusion in uninvolved AD skin when compared with 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 patients with AD, however, showed increased TEWL when compared with the skin of normal subjects (Table 1), indicating a less effective skin barrier for water. This is in agreement with the results of Laudanska et al., who also found higher TEWL in patients with AD in the state of remission of the skin lesions and of Seidenari and Giusti, who reported higher TEWL in involved and uninvolved skin of children affected by AD. The TEWL in control subjects was found to be inversely dependent on the SC thickness, which is in agreement with other studies. However, this relationship was not found in patients with AD, 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. As 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 probably also for a 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 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 towards movement of SLS, and the partition coefficient, are dependent on the composition and struc-
ture of the SC. We found the mean diffusivity across the SC to be two times higher in the skin of patients with AD when compared with control subjects. We have also looked into the differences in diffusivity of patients with AD according to state of disease (Figure 4). The diffusivity was higher in patients with active AD when compared with 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 not visibly affected by AD.

These findings are in the line with a study of de Jongh et al., where atopic persons (although with no history of AD) showed approximately 1.5 times higher diffusivity for SLS than nonatopics. In our accompanying study on the same subjects we found higher diffusivity also for polyethylene glycols of different molecular sizes in the skin of patients with AD compared with that of control subjects. Yosiike et al. reported increased penetration of theophylline not only in involved but also in uninvolved AD skin compared with control subjects. All these findings indicate that uninvolved atopic skin is more permeable for different compounds depending on their hydrophilicity and molecular size.

The skin of patients with AD showed a 30% lower solubility of SLS compared with normal skin, although the difference was not as high as that for diffusion. The partition coefficient was not significantly different when two groups of patients with AD 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 with diffusivity as the quality of the first data points largely influences the estimation outcome. To overcome this problem, a second prolonged experiment was recommended 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 the present study we chose a relatively short exposure duration as longer exposure to SLS would more probably lead to alteration of the skin barrier which might change the SC permeability.

In the present study we observed substantial inter- and intraindividual variation in both penetration parameters. The intraindividual variation in diffusivity was higher in patients with AD compared with 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 intraindividual variability in the partition coefficient was lower in patients with AD. As men-
tioned earlier, the determination of partition coefficient in a nonsteady state is largely influenced by the quality of the first data points. As the SLS concentration/SC depth curve (Figure 2) approaches linearity, as is the case with patients with AD, the intercept value from which partition coefficient is derived is less dependent on first data points and, therefore, the intraindividual variation decreases.

To summarize, the skin of patients with AD showed increased percutaneous penetration of SLS when compared with control subjects, supporting the hypothesis of impaired skin barrier even in the noninvolved skin. As a consequence, we expect that the defect skin barrier of patients with AD 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.

Acknowledgment

We are grateful to K.V. Sewnarain Sukul, C.E.T. Withagen and F. Calkoen-Kwa for technical assistance. The authors would like to thank the European Community for the financial support of this study, which was performed within the Fifth Framework Programme (project acronym: EDETOX).
References


33. Ya-Xian Z, Suetake T, Tagami H. Number of cell layers of the stratum corneum in normal skin - relationship to the anatomical location on the body, age, sex and physical parameters. *Arch Dermatol Res* 1999; **291**: 555-9.