Dermal absorption of chemicals through normal and compromised skin

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Citation for published version (APA):
Jakasa, I. (2006). Dermal absorption of chemicals through normal and compromised skin

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Increased permeability for polyethylene glycols through skin compromised by sodium lauryl sulphate
(submitted to Exp Dermatol)
Abstract

In this in vivo human study we assessed the influence of skin damage by sodium lauryl sulphate (SLS) on percutaneous penetration of polyethylene glycols (PEGs) of different molecular weights (MW).

Percutaneous penetration of PEGs of various molecular sizes was determined using tape stripping of the stratum corneum (SC). The forearm skin of volunteers was pre-treated with 5% w/w SLS for 4 hours, and 24 hours later patches with PEGs were applied for 6 hours. After the end of application, the layers of the SC were removed consecutively using adhesive tape. The diffusion coefficient, stratum corneum/vehicle partition coefficient and the permeability coefficient were deduced by data regression to Fick's law written for unsteady-state diffusion across membrane from a constant source to a sink.

The trans-epidermal water loss (TEWL) was increased as a result of SLS treatment from $6.3 \pm 2.1$ g m$^{-2}$ h$^{-1}$ (mean ± SD) to $17.9 \pm 8.7$ g m$^{-2}$ h$^{-1}$. The diffusion coefficient for all PEGs was increased in the SLS damaged skin. The magnitude of the increase was smaller for higher MW. Also the partition coefficient of PEGs between SC and water was larger in the SLS compromised skin when compared to the normal skin and showed a tendency to increase with MW. The permeability coefficient decreased gradually with increasing MW of PEGs in both normal and SLS-compromised skin. SLS caused a three-fold increase of the permeability coefficient for all MWs ranging in normal skin from $0.34$ to $0.70 \times 10^{-5}$ cm h$^{-1}$ and in the SLS compromised skin from $1.20$ to $2.09 \times 10^{-5}$ cm h$^{-1}$ for MW of 590 to 282 Da.

Results of this study show the deleterious effect of SLS on the skin barrier for hydrophilic PEGs. A defective skin barrier will facilitate absorption of other chemicals that could lead to higher susceptibility and local skin effects.
Introduction

Most of the available skin permeability data are from normal intact human and animal skin. Data on permeability of chemicals in the compromised skin, in particular in humans in vivo, are scarce. Experimentally induced damage of the skin barrier has been used to study the influence of common disrupting chemicals (e.g. detergents) and also to model diseased skin characterized by defective skin barrier. As a result, various models based on chemical or mechanical barrier disruption have been developed. Tsai et al. investigated in an in vitro study using rat skin the influence of chemical barrier disruption on skin permeability [1-3]. In that study, it was shown that skin barrier disruption by acetone significantly enhanced permeability to both hydrophilic and amphiphilic compounds [1]. The influence of the pre-treatment of the skin with sodium lauryl sulphate (SLS) has shown to increase permeability of hydrophilic to moderately lipophilic compounds while influence of SLS was absent for highly lipophilic compounds [4-6].

Many surfactants, from pharmaceutical preparations to food products, are widely used as emulsifying, suspending, solubilizing and stabilizing agents. Anionic surfactants can penetrate and interact strongly with skin producing alterations in the barrier properties [7]. SLS in particular is able to cause variations in structural organization of lipids in the stratum corneum (SC) which are thought to be the most important pathway for chemical penetration and diffusion through SC [8-10].

In the present study we investigated the influence of SLS on the SC penetration of PEGs of different MWs (282 – 590 Da) in the human skin in vivo. PEG is a hydrophilic polymer widely used in corneal and intestinal permeability research. The octanol/water partition coefficient ($K_{ow}$) does not change considerably with molecular size, which makes PEGs suitable model compounds that are not confounded by changes in lipophilicity with molecular size [11]. In the study of Tsai et al. using in vitro rat skin, the penetration of polyethylene glycols (PEGs) of different sizes was shown to be enhanced by SLS [2-3]. Furthermore, in our parallel study we have found increased diffusion of PEGs in the skin of atopic dermatitis (AD) patients who are known for their defective barrier function [12]. Regarding the effect/role of molecular weight in penetration through skin compromised by SLS in vivo direct information/evidence is lacking.
Subjects and methods

Study population
Twenty healthy subjects, 11 males and 9 females, mean age 32 years (range 18-55 years), all Caucasians, participated in the study. Participants 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 the Atopy Score (the maximum is 34 points and a score ≥ 10 is considered as atopy) was derived [13]. The atopy score, expressed as mean ± SD, was 3.0 ± 2.4. Participants were not allowed to use soap, moisturizers or any other cosmetics or 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. The study was conducted according to the Declaration of Helsinki Principles.

Penetration experiment
An application mixture of PEGs was made by dissolving 47.5 mg of monodispersed PEG150 (MW = 150.17 Da, Sigma, the Netherlands), 50.1 mg of monodispersed PEG282 (MW = 282.34 Da, Acros Organics, NY, USA), 102.9 mg of monodispersed PEG326 (MW = 326.4 Da, PolyPure, Norway), 199.1 mg of monodispersed PEG370 (MW = 370.4 Da, PolyPure, Norway), and 10 g of polydispersed PEG600 (average MW = 600 Da, Sigma, the Netherlands) in 2 ml of water. Subjects were exposed to the PEG application mixture (180 μL) using patch test chambers (Finn chambers®, 18 mm in diameter, Epitest Ltd., Finland) on volar arm for six hours. These prevented evaporation of water from the test site and this combined with excess PEG insured that the exposure concentration remained constant during the exposure. Another chamber containing the application mixture was applied onto the skin site pre-treated with SLS (≥ 99% purity; Fluka, Buchs, Switzerland). The treatment with SLS was performed 24 hrs before the application of PEG. This was done by applying 200 μL of a 5% (w/w) water solution of SLS for 4 h. Before application of the patch containing SLS or PEG and during tape striping the TEWL was measured using an Evaporimeter (VapoMeter SWL2g, Delfin Technologies, Ltd., Kuopio, Finland). Twenty minutes prior to application the subjects rested with their sleeves rolled-up in the examination room, where the temperature was 20-22 °C and relative humidity ranged between 50 and 60%. After the removal of the patch containing PEG, a piece of dry cell tissue was gently attached to the skin site to remove the residue of PEG mixture. Ten minutes after the end of exposure to PEGs, the SC layers were
sequentially removed with pre-cut Diamond tape pieces, 19 x 25 mm$^2$ (Diamond Ultra Clear tape, The Sellotape® Company, the Netherlands). Templates of Scanpor® tape were fixed on the skin around the application spot to limit the tape stripping of the exposed area (18 mm in diameter). To limit the tape stripping to the exposed area (18 mm in diameter) templates of Scanpor® tape were fixed on the skin around the application spot. 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. Total removal of the SC was evidenced by shiny appearance of the skin and by the TEWL > 100 g m$^{-2}$ h$^{-1}$. Each individual strip was placed into a glass vial and stored at -20 °C until analysis. The SC from a non-exposed site was stripped off and served as negative control.

**Analytical procedure**

The gas chromatographic method for determination of PEGs and the spectrophotometrical method for analysis of proteins in tape strips have been described extensively elsewhere [14, 15].

Concentration of PEGs on each strip was normalized for the amount of proteins and expressed as μg PEG/μg protein. Assuming an SC density of 1 g cm$^{-3}$ (Andersen and Cassidy, 1973) and uniform distributions of SC on the tapes and proteins within the SC (μg), the protein mass removed was converted to volume, enabling the depth of each strip in the SC (x). [16]. In our calculation of the SC solute concentration it was assumed that the protein concentration in the SC was (on average) 1g / mL SC.

**Data analysis**

The concentration of PEGs on each strip was plotted as a function of relative SC depth. To estimate the penetration parameters we used an approach based on Fick's second law of diffusion as described by Pirot et al. and a recent series of papers by Alberti et al. [17-21]. In this method, two parameters, K and D/L$^2$, are determined by best-fit regression of the concentration of PEGs as a function of relative SC depth (x/L) to the following equation

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

*Eq. 1*

where $C_{veh}$ is the applied PEGs concentration (μg cm$^{-3}$), C is the PEG concentration (μg cm$^{-3}$) at depth x (cm), K is the SC/water partition coefficient, L is the total thickness of the SC (cm), D is the effective diffusion coefficient of PEGs through the pseudo-homogeneous SC (cm$^2$ h$^{-1}$) and t is the exposure duration (h). The non-
steady state diffusion equation (Eq. 1) was fitted to the data and the rate constant for diffusion across SC (D/L², 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 permeability coefficient (Kp, cm h⁻¹) for each PEG oligomer was calculated from the relationship Kp = K*D/L. All concentration data were weighted equally in the regression analysis. The first strip was not included in the regression analysis, as it contained some residue of PEGs on the surface of the skin after end of exposure. For curve fitting and statistical calculations Prism 4 software (Graph Pad Software Inc., San Diego, CA, USA) was used. For statistical calculations Student’s paired two-tailed t-test was used and the p value < 0.05 was considered significant.

Results

To remove the SC completely, on average 28 ± 5 and 23 ± 6 strips were needed for normal and SLS pre-treated sites, respectively. The SC thickness, expressed as mean ± SD was 8.7 ± 2.5 μm for the normal site, and 6.2 ± 3.0 μm, for the SLS pre-treated site. The amount of proteins in the SLS treated skin was in some subjects up to three times lower compared to the normal site. To enable comparison of the SC penetration between SLS treated and normal skin, only data obtained from the subjects with comparable (difference less then 15 %) protein amount were included in the calculations. In these subjects (n=10), the SC thickness, expressed as mean ± SD, was 9.1 ± 2.5 μm for the normal site and 8.4 ± 2.6 μm for the SLS treated site. Basal TEWL, expressed as mean ± SD (n=20), was 6.3 ± 2.1 g m² h⁻¹ and increased to 17.9 ± 8.7 g m² h⁻¹ 24 hours after SLS pretreatment (time point of the PEG application).

Figure 1 shows a typical concentration profile for PEG282 and PEG590 across SC of normal skin and skin compromised by SLS in one subject together with the fitted curve obtained by non-linear regression analysis (dashed lines). Statistically acceptable curve fitting (r² ≥ 0.95) was obtained for all subjects without SLS pre-treatment. However, after SLS pre-treatment curve fitting could not be performed for all MWs in two subjects due to scattered data points (Table 1). To enable paired analysis the calculations did take into consideration only the data obtained for both normal and SLS-compromised skin.

The results showed that the diffusion coefficient decreased as MW of the PEGs increased in both normal and SLS pretreated skin (Fig 2). The diffusion coefficient was approximately two times higher for PEG282 in SLS compromised skin and it
gradually decreased to be approximately 1.5 times higher for PEG590 when compared to normal skin (p < 0.05 for PEG282-370 while it was not significant for PEG414-590) (Fig 2).

**Fig 1:** Concentration decay of PEG282 and PEG590 as a function of normalized position (x/L) in the SC in normal and SLS compromised skin of one control subject after 6 hours exposure to PEG mixture. Non-linear regression analysis was used to fit the equation (Eq. 1) to the experimentally obtained data (dashed lines). The effective diffusion coefficient is calculated from $D/L^2$ value determined from the slope of the curve, while the partition coefficient is determined from $K*C_{veh}$ at the intercept at $x=0$.

Table 1: The ratios of penetration parameters for PEGs of different molecular weights before and after SLS treatment (geometric mean and 90% confidence interval)

<table>
<thead>
<tr>
<th>MW (Da)</th>
<th>$D \times 10^9$ (cm$^2$ h$^{-1}$)</th>
<th>90% CI ($D$)</th>
<th>K (unitless)</th>
<th>90% CI ($K$)</th>
<th>$K_p \times 10^5$ (cm h$^{-1}$)</th>
<th>90% CI ($K_p$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>282</td>
<td>2.08</td>
<td>1.03 - 4.18</td>
<td>1.20</td>
<td>0.82 - 1.77</td>
<td>2.94</td>
<td>1.87 - 4.62</td>
</tr>
<tr>
<td>326</td>
<td>1.79</td>
<td>0.88 - 3.62</td>
<td>1.28</td>
<td>0.89 - 1.86</td>
<td>2.68</td>
<td>1.67 - 4.31</td>
</tr>
<tr>
<td>370</td>
<td>1.79</td>
<td>0.95 - 3.37</td>
<td>1.36</td>
<td>0.94 - 1.98</td>
<td>2.87</td>
<td>1.88 - 4.37</td>
</tr>
<tr>
<td>414</td>
<td>1.76</td>
<td>1.02 - 3.03</td>
<td>1.51</td>
<td>1.09 - 2.09</td>
<td>3.06</td>
<td>2.10 - 4.48</td>
</tr>
<tr>
<td>458</td>
<td>1.66</td>
<td>0.99 - 2.77</td>
<td>1.60</td>
<td>1.15 - 2.23</td>
<td>3.08</td>
<td>2.10 - 4.52</td>
</tr>
<tr>
<td>502</td>
<td>1.45</td>
<td>0.87 - 2.41</td>
<td>1.85</td>
<td>1.32 - 2.58</td>
<td>3.09</td>
<td>2.07 - 4.61</td>
</tr>
<tr>
<td>546</td>
<td>1.44</td>
<td>0.89 - 2.33</td>
<td>1.78</td>
<td>1.22 - 2.58</td>
<td>2.96</td>
<td>1.86 - 4.72</td>
</tr>
<tr>
<td>590</td>
<td>1.48</td>
<td>0.97 - 2.25</td>
<td>2.04</td>
<td>1.45 - 2.89</td>
<td>3.48</td>
<td>2.35 - 5.16</td>
</tr>
</tbody>
</table>

$^*$MW = Molecular weight, $D$ = Diffusion coefficient, $K$ = Partition coefficient, $K_p$ = Permeability coefficient, CI = Confidence interval
Fig 2: Diffusion coefficient ($D$), partition coefficient ($K$) and permeability coefficient ($K_p$) of PEGs (282-590 Da) in normal and SLS compromised skin after 6 hours dermal exposure. The results are shown as mean ± SD, (* $p < 0.05$, ** $p < 0.01$).
The partition coefficient for normal skin was similar for all PEGs while in SLS compromised skin it had a tendency to increase with increased MW being more prominent for PEG 502-590 (p < 0.05) (Fig 2).

The permeability coefficient of PEGs of different MW was approximately three times higher in SLS compromised skin (p < 0.01 for all PEGs), and it gradually decreased with increasing MW in both normal and SLS-compromised skin. We found no correlation between basal TEWL or TEWL measured 24 hours after SLS pretreatment and any of the estimated penetration parameters.

Additionally, we calculated the ratios of all penetration parameters between normal skin and skin pre-treated with SLS. The geometric mean values of the ratios and corresponding 90% confidence interval are shown in Table 1.

The inter-individual differences in all measured penetration parameters were considerable for both normal and SLS compromised skin. The coefficient of variation for normal skin amounted from 64% to 82% for the diffusion coefficient, from 37% to 64 % for the partition coefficient, and from 43% to 64% for the permeability coefficient. The coefficient of variation for SLS compromised skin amounted from 44% to 75% for the diffusion coefficient, from 39% to 61% for the partition coefficient, and from 34% to 56% for the permeability coefficient.

Discussion

In the present study we assessed the penetration of PEGs ranging in the MW from 282 to 590 Da into the normal skin and skin compromised by SLS. From the amount of PEGs determined in the SC layers we estimated the effective diffusion coefficient and partition coefficient between SC and vehicle from which the permeability coefficient could be calculated. According to Fick’s law of diffusion, when the SC controls mass transfer through the skin, the skin flux of a penetrant is the product of the permeability coefficient and the concentration in the SC.

The SLS treatment caused moderate barrier impairment, the TEWL increased from 6.3 ± 2.1 g m⁻² h⁻¹ to 17.9 ± 8.7 g m⁻² h⁻¹. The average number of strips needed to remove the whole SC and the protein amount were significantly lower after SLS pretreatment, in some persons up to 3-fold. A possible explanation for the smaller amount of proteins could be partial loss of the SC after SLS pre-treatment. SLS is known to change the cohesiveness of the SC. The loss of proteins might have
occurred during the period between the removal of the SLS patch and the application of PEG leading to a thinner SC. Since SC is reported not to be homogenous across entire length, the comparison of the penetration between SLS treated and normal skin was not justified. Another reason for the lower protein amount in some subjects might be loss of SC during the removal of the PEG exposure chamber followed by gentle attaching of dry tissue paper. In this case, the upper layers of SC containing PEG will be lost. Of course, both possibilities might contribute to the loss of proteins making the outcomes unreliable. Therefore, for data analysis we have included only the paired data of subjects who had similar protein amount before and after SLS application ($n = 10$). The percutaneous penetration parameters and the TEWL values assessed before application of SLS did not statistically differ in these two groups of subjects (data not shown).

As expected, the diffusion coefficient was inversely dependent on MW in both normal and in SLS-compromised skin. The diffusion coefficient after pre-treatment with SLS increased for all PEG oligomers and this increase was more prominent for smaller oligomers. Enhanced diffusion coefficient in SLS pre-treated skin could be explained by the influence of SLS on the lipid bilayers of the SC. SLS has been shown to cause distinct abnormalities of the SC in its intercellular lipid domain leading to fluidization of the intercellular lipid domain [8, 9]. Recent work showed that SLS removed hydrophobic lipids (8-15%) while no removal of ceramides occurred [10, 22-23]. A similar effect of enhanced diffusion coefficient was found for PEGs, SLS and theophylline in patients with atopic dermatitis, whose skin is characterized by impaired intercellular lipid domain [12, 15, 24].

The partition coefficient between SC and vehicle was similar for all PEG oligomers in normal skin, which is consistent with similar octanol-water partition coefficients of PEGs over a broad range of MWs [11]. The partitioning of PEGs in the SLS-compromised skin was higher than in normal skin. This better solubility of PEGs in the SC due to SLS pre-treatment might be explained by removal of hydrophobic lipids and induced hydration leading to more hydrophilic SC, as already suggested by others [18, 25-26]. In contrast to normal skin, partition coefficient was increasing with the MW of PEGs. The reason for this is not clear to us. In our parallel study with AD patients known for skin dryness, we found lower partition of PEGs in the SC compared to control subjects; however, this decrease was similar for all MWs [12].

The permeability coefficient, calculated from diffusion coefficient and partition coefficient values, was three-fold larger in the SLS compromised skin compared with the normal skin for all PEG oligomers, ranging 0.34 to $0.70 \times 10^{-5}$ cm h$^{-1}$ in normal
skin from and from 1.20 to 2.09 x 10^{-5} \text{ cm h}^{-1} in the SLS compromised skin (MW 590 to 282 Da). This was consistent with the values of 5 x 10^{-5} \text{ cm h}^{-1} to 8.3 x 10^{-5} \text{ cm h}^{-1} for PEG414 - PEG282 obtained in hairless mouse model [27]. The higher permeability coefficient of PEG found in the SLS compromised skin is consistent with the study of Tsai et al., who investigated the penetration of polydisperse PEGs (PEG300, PEG600 and PEG1000) in vitro in hairless mice after SLS pre-treatment [3]. In that study, the penetration of PEGs expressed as percentage of the applied dose increased with the degree of barrier disruption as measured by TEWL. Furthermore, they reported the shifting of the MW cut-off value for PEG in the damaged skin. In the normal skin a cut-off value of 414 Da was found; however, in the skin compromised by SLS the MW cut-off value was shifted to 766 Da. But, as discussed earlier, the reported cut-off value was probably more the result of the detection limit of the analytical method than the real molecular cut-off [3, Jakasa et al., submitted for publication]. In our study we see a gradual decrease of PEG penetration with increasing MW in both normal and SLS compromised skin. This is in accordance with Johnson et al., who reported that the diffusion coefficient of larger solutes (~350-500 Da) appear not to decrease at the same dramatic rate but instead remain relatively constant [28].

The effect of SLS on the percutaneous penetration has been shown to be dependent on the lipophilicity of the penetrant. Nielsen et al. investigated in vitro percutaneous penetration of a number of pesticides varying in lipophilicity through skin that was pre-treated with 0.1 and 0.3 % SLS for 3 hours [5]. He found that percutaneous penetration of more hydrophilic compounds was affected more. Penetration of lipophilic compounds (logK_{o/w} > 3) through the SLS treated skin increased little, whereas for less lipophilic compounds (logK_{o/w} of 0.7 and 1.7) the penetration increased two-fold. This is in agreement with the study of Borras-Blasco et al which investigated the effect of SLS on in vitro percutaneous absorption through rat skin of a number of compounds with a wide range of lipophilicity values (logK_{o/w} from -0.95 to 4.42) [4]. They showed that permeability increased only for compounds with logK_{o/w} <3. For a hydrophilic compound with a logK_{o/w} of -0.95 which was similar to that of PEGs (logK_{o/w} = -1.6) the permeability coefficient increased ten-fold. In our study the permeability coefficient was increased three- fold for all PEG oligomers. It has to be pointed out that the effect of SLS measured in various studies was influenced by the experimental design, in particular with reference to the time difference between SLS pre-treatment and penetrant application. In some studies, the penetrant was applied simultaneously with SLS, while in others immediately after removal of SLS or 24-48 hours after the SLS pre-treatment, when the irritation effect is highest. The nature and the magnitude of the effect will be therefore different.
In the present in vivo human study we have shown that SLS damages the skin leading to increased skin permeability of hydrophilic PEGs. The increase in permeability was due to enhancement in both diffusion and partitioning into the SC. In the skin that has been damaged by SLS exposure, the penetrated amount will be higher and larger molecules could penetrate in amounts that might be sufficient to exceed toxic levels. Therefore, when performing the risk assessment of dermal exposure, damaged skin, which occurs commonly, should be taken into consideration.

Acknowledgement
We are grateful to N. Mohammadi for the help during development of tape stripping method, and C. E. T. Withagen and F. Calkoen-Kwa for technical assistance. The authors would like to thank the European Community for providing financial support for part of this the study, which was carried out under the Fifth Framework Programme (project acronym: EDETOX).

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