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ORIGINAL ARTICLE

Altered Penetration of Polyethylene Glycols into Uninvolved Skin of Atopic Dermatitis Patients

Ivone Jakasa[1], Maarten M. Verberk[1], Maria Esposito[2], Jan D. Bos[3] and Sanja Kezic[1]

Involved regions of the skin in atopic dermatitis (AD) patients have an altered barrier function. Whether uninvolved skin also has a diminished barrier is controversial. To assess the barrier function of uninvolved skin in AD patients, the percutaneous penetration of polyethylene glycols (PEGs) of various molecular sizes was determined *in vivo* in AD patients and control subjects using tape stripping of the stratum corneum (SC). The diffusion and partition coefficients were determined using Fick's second law of diffusion. The SC thickness was similar in both groups; however, the *trans*-epidermal water loss was higher in atopic skin. The apparent diffusion coefficient of PEGs through atopic skin was twice as high as through normal skin, and decreased with increasing molecular weight (MW) in both groups. The partition coefficient in the skin of AD patients was half of that for normal skin but as for normal skin, there was no MW dependency. Although atopic skin exhibited altered barrier with respect to diffusion and partitioning, the permeability coefficients were nearly the same for atopic and normal skin. The results support the assumption of altered skin barrier of AD patients even in the skin that is visibly unaffected by disease.

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INTRODUCTION

The affected skin of patients with atopic dermatitis (AD) is known to have a defective barrier function. The alteration in the skin barrier *in vivo* is shown by the increase in *trans*-epidermal water loss (TEWL), in other words a poorer barrier to water transport (Seidenari and Giusti, 1995). On the other hand, the data on the permeability of uninvolved AD skin are contradictory. Some authors have reported higher TEWL *in vivo* in patients with AD history (Werner and Lindberg, 1985; Agner, 1990; Berardesca *et al.*, 1990; Watanabe *et al.*, 1991; Seidenari and Giusti, 1995; Di Nardo *et al.*, 1998; Tabata *et al.*, 1998), whereas others found no difference in comparison to normal skin (Seidenari, 1994; Tanaka *et al.*, 1997; Basketter *et al.*, 1998; Di Nardo *et al.*, 1998; Nicander and Ollmar, 2004). The well-known higher susceptibility to irritation in AD patients (Seidenari, 1994; Goffin and Pierard, 1996; Tabata *et al.*, 1998; Nicander and Ollmar, 2004) might be partly explained by higher skin permeability (de Jongh *et al.*, 2006). Increased penetration of theophylline in both involved and uninvolved AD skin *in vitro* was reported (Yoshiike *et al.*, 1993). In our previous *in vivo* study, the percutaneous penetration of sodium lauryl sulfate was altered in uninvolved skin of AD patients (Jakasa *et al.*, 2006).

Impaired skin barrier in AD has often been linked to the different lipid composition and structure of atopic stratum corneum (SC) in both involved as well as in uninvolved SC of AD patients (Imokawa *et al.*, 1991; Yamamoto *et al.*, 1991; Di Nardo *et al.*, 1998; Bleck *et al.*, 1999).

The *trans*-dermal permeability of many chemicals has been described using a model based on the octanol–water partition coefficient ($K_{o/w}$) and molecular weight (MW) of the chemical (Kasting *et al.*, 1987; Potts and Guy, 1992), showing exponential decrease of diffusion as a function of MW. Based on clinical experience, it was proposed that the absorption of molecules through normal human skin declines rapidly as MW increases over 500 Da (Bos and Meinardi, 2000). As regards to the role of MW in penetration through compromised skin *in vivo*, direct evidence is lacking.

This *in vivo* study investigated the skin penetration of polyethylene glycols (PEGs) of different MWs (150–590 Da) into uninvolved skin of AD patients compared to control subjects using tape stripping of the SC and the approach based on Fick's second law of diffusion for non-steady state condition (Pirot *et al.*, 1997). PEG is a hydrophilic polymer widely used in corneal and intestinal permeability research. The octanol/water partition coefficient ($\log K_{o/w} \sim -1.6$) does not change greatly with molecular size which makes PEGs, suitable model compounds that are not confounded by change in lipophilicity with molecular size (Hollander *et al.*, 1989).

[1]Department: Coronel Institute of Occupational Health, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [2]Department of Dermatology, University of Rome ''Tor Vergata'', Rome, Italy and [3]Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Correspondence: Dr Ivone Jakasa, Department: Coronel Institute of Occupational Health, Academic Medical Center, Universiteit van Amsterdam, PO Box 22770, Amsterdam 1100 DE, The Netherlands. E-mail: i.jakasa@amc.uva.nl

Abbreviations: AD, atopic dermatitis; MW, molecular weight; PEG, polyethylene glycol; SC, stratum corneum; TEWL, trans-epidermal water loss

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RESULTS

To remove the SC completely, 28±5 strips were needed on average for control subjects and 28±7 for AD patients. Before exposure, TEWL was higher in the AD patients (8.4±4.3 g m^{-2} hour^{-1}) than the control subjects (6.3±2.1 g m^{-2} hour^{-1}, $P<0.05$). After the removal of exposure chamber containing PEG mixture, TEWL was 6.3±2.8 and 6.0±3.5 g m^{-2} hour^{-1} for AD patients and control subjects, respectively. We found no significant difference in the SC thickness between the two groups (8.7±2.5 and 9.4±1.9 μm for control subjects and AD patients, respectively).

Figure 1 shows a typical concentration profile for PEG282 and PEG590 across SC in a control subject and an AD patient together with the fitted curve which presents the solution to the Fick's second law of diffusion for non-steady state condition (dashed lines). The curve fitting could be performed for all control subjects and AD patients ($r^2>0.95$), except for PEG150. For PEG150, in four AD patients, a linear relationship was found between the concentration and SC depth, indicating steady-state absorption to which Fick's second law of diffusion cannot be used to derive diffusion coefficient. Further, in two control subjects, curve fitting could not be performed for PEG150 because of excessive variability of data points.

The results are summarized in Table 1. The apparent diffusion coefficient decreases with increasing MW of the penetrant. It was approximately twice as high for all PEGs in the AD patients as in the control subjects, except for PEG150, where it was only 60% higher in the AD patients ($P<0.0001$ for all PEGs). We also compared the control subjects and the AD patients according to state of disease: patients with active and inactive AD. As the result of one-way analysis of variance test for diffusion coefficient was significant ($P<0.05$), Bonferroni *post hoc* analyses were performed. The mean value of diffusion coefficient in patients with active AD was significantly higher than in the control subjects for all PEG molecules ($P<0.05$ for all PEGs). No significant difference was found between patients with inactive AD and the control

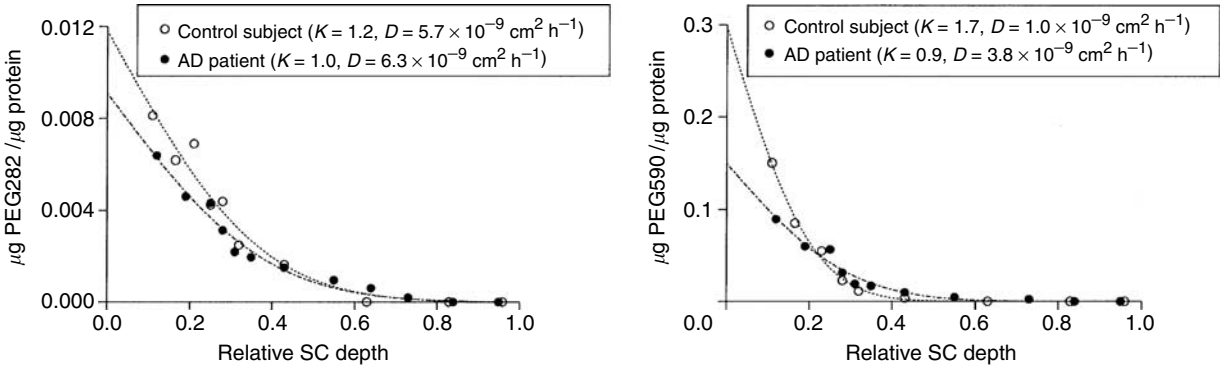

Figure 1. Concentration decay of PEG282 and PEG590 as a function of normalized position (x/L) in the SC in one control subject and one AD patient after 6 hours exposure to PEG mixture. Non-linear regression analysis was used to fit the equation (Equation 1) to the experimentally obtained data (dashed lines). The apparent diffusion coefficient is calculated from D/L^2 value determined from the slope of the curve, whereas the partition coefficient is determined from $K\times C_{veh}$ at the intercept at $x=0$.

Table 1. Penetration parameters of PEGs for AD patients and control subjects

	$D\times10^{-9}$ (cm^2 h^{-1})**		$D/L^2\times10^{-3}$ (h^{-1})*		K**		$K_p\times10^{-5}$ (cm h^{-1})#	
MW (Da)	AD	Ctrl	AD	Ctrl	AD	Ctrl	AD	Ctrl
150	13.3±6.7	8.4±5.2	15.9±7.4	10.8±6.6	1.02±0.29	1.66±0.70	1.34±0.42	1.25±0.39
282	9.2±5.1	4.5±2.9	11.5±8.4	5.6±3.0	0.93±0.38	1.76±1.00	0.84±0.55	0.68±0.24
326	8.2±4.2	4.5±2.8	9.9±5.2	5.5±2.9	0.86±0.35	1.72±0.99	0.69±0.30	0.66±0.25
370	7.6±3.8	4.1±2.7	9.2±4.7	4.9±2.7	0.86±0.37	1.74±0.96	0.65±0.40	0.60±0.22
414	6.7±3.6	3.5±2.2	7.7±3.4	4.2±2.0	0.85±0.37	1.78±0.94	0.54±0.19	0.55±0.21
458	6.2±3.2	3.2±1.9	7.1±3.2	3.9±1.8	0.82±0.38	1.78±0.92	0.48±0.22	0.51±0.19
502	6.1±3.0	2.9±1.7	7.1±3.3	3.6±1.6	0.81±0.38	1.78±0.85	0.49±0.27	0.48±0.19
546	5.0±3.0	2.4±1.4	5.8±3.5	3.0±1.4	0.80±0.43	1.87±0.78	0.37±0.23	0.44±0.19
590	3.8±2.8	1.9±1.1	4.5±3.5	2.4±1.2	0.96±0.53	1.85±0.82	0.34±0.27	0.35±0.19

AD, atopic dermatitis patients (mean±standard deviation, n=20); Ctrl, control subjects (mean±SD, n=20); D, apparent diffusion coefficient; D/L^2, diffusion rate constant; K, stratum corneum/water partition coefficient; K_p, permeability coefficient; MW, molecular weight; PEG, polyethylene glycol.
**$P<0.0001$, *P=0.0001–0.02, and #$P>0.1$ (for all PEG oligomers, one-tailed t-test).

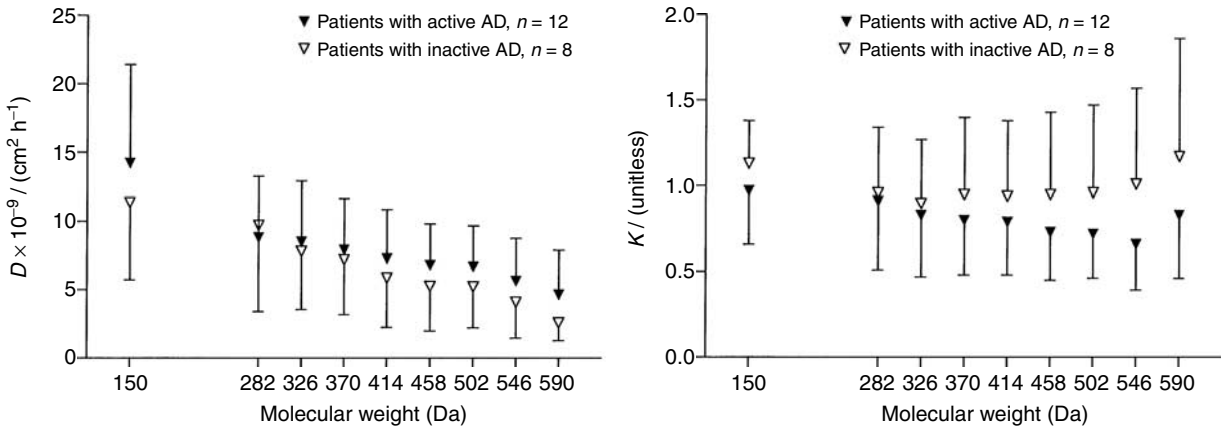

Figure 2. Average apparent diffusion coefficient (*D*) and partition coefficient (*K*) of PEG150–590 Da in the SC of patients with active and inactive AD after 6 hours dermal exposure to PEG mixture. The results are shown as mean ± SD.

subjects, or between AD patients with active and inactive AD (Figure 2). Consistent results were obtained for the diffusion rate constant (D/L^2) which was for PEGs 282–590 Da approximately twice as high in AD patients as in the control subjects, whereas for PEG150 it was only 65% higher in AD patients (Table 1).

The partition coefficient for AD patients was approximately half as large as in the control subjects for all PEG molecules, except for PEG150, where it was about 60% of that in the control subjects ($P<0.0001$ for all PEGs). We compared all three groups of subjects; again, the result of one-way analysis of variance test was significant ($P<0.01$) and Bonferroni *post hoc* analyses were performed. The partition coefficient was also significantly lower in patients with active or inactive AD than in the control subjects for all PEGs ($P<0.05$). There was no significant difference between patients with active and inactive AD (Figure 2).

The calculated permeability coefficients were nearly the same for both the AD patients and control subjects (Table 1).

The inter-individual differences in diffusion coefficient, partition coefficient, rate constant for diffusion, and permeability coefficient were considerable in both the AD patients and the control subjects. For different PEG oligomers, the observed range of the coefficient of variation in AD patients was 49–73% for the diffusion coefficient, 44–77% for the diffusion rate constant, 29–55% for the partition coefficient, and 32–80% for permeability coefficient; in control subjects, we found 58–66% for the diffusion coefficient, 45–62% for the diffusion rate constant, 42–58% for the partition coefficient, and 31–56% for permeability coefficient.

DISCUSSION

This *in vivo* study assessed the penetration of PEGs ranging in MW from 150 to 590 Da into the SC of AD patients and control subjects. The skin-stripping method used enabled us to estimate two penetration parameters, the effective diffusion coefficient in the SC and the partition coefficient between SC and vehicle from which the permeability coefficient could be calculated.

The diffusion coefficient across SC was about twice as high in AD patients as in the control subjects for all PEG oligomers. The consistent results were obtained for the diffusion rate constant, D/L^2. There was no clear tendency for an increase with state of disease (Figure 2). These findings are in line with our parallel study in the same subjects, where we found an increased diffusion coefficient and diffusion rate constant for sodium lauryl sulfate in uninvolved skin of AD patients compared to control subjects (Jakasa *et al.*, 2006). The higher diffusion of PEG we found in AD skin is consistent with the *in vitro* findings in hairless mice: the penetration of polydispersed PEG (PEG300, PEG600, and PEG1000) through normal skin and skin damaged by acetone, sodium lauryl sulfate, or tape stripping increased with the degree of barrier disruption as measured by TEWL (Tsai *et al.*, 2001, 2003).

With respect to the diffusion coefficient in the SC, different mathematical functions have been proposed to describe the effect of solute size (Kasting *et al.*, 1987; Cleek and Bunge, 1993). In these functions, the diffusion coefficient decreases exponentially with increasing molecular size and predict a stronger effect of MW than the gradual decrease observed in our study, where in the range of MW from 280 to 590, this relation appeared to be linear (Figure 3). The experimentally determined dependence of permeability coefficients upon MW was less steep than predicted by Potts and Guy (1992) algorithm ($\log K_p = -2.72 + 0.71 \times \log K_{ow} - 0.0061 \times MW$) (Figure 4). For the prediction, we assumed the constant value of K_{ow} of −1.6 for all MW (Hollander *et al.*, 1989). This assumption is supported by the similar values of the partitioning coefficient we found for all PEG oligomers.

The results from our study are consistent with *in vitro* data obtained for hydrophilic compounds, showing weaker dependence of permeability coefficient on molecular size then predicted by Potts & Guy algorithm (Mitragotri, 2003). Recent *in vitro* study revealed that the impact of MW on the skin permeability depends on the penetrant hydrophilicity, showing that penetration of cyclosporin with MW > 1,000 Da could be substantially increased by introduction of polar side chains (Billich *et al.*, 2005).

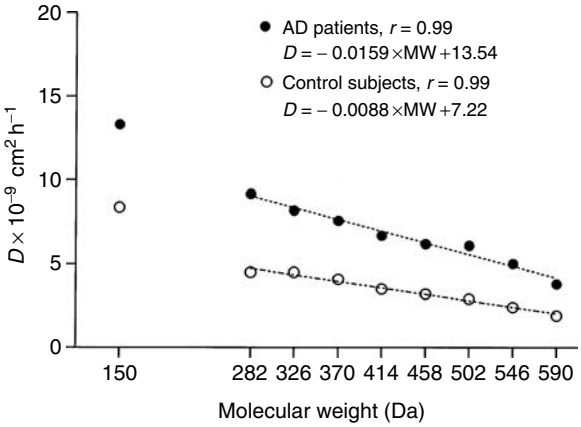

Figure 3. Apparent diffusion coefficients (*D*, mean values) versus the corresponding MWs of PEG150–590 Da in the SC of AD patients and control subjects after 6 hours dermal exposure to PEG mixture.

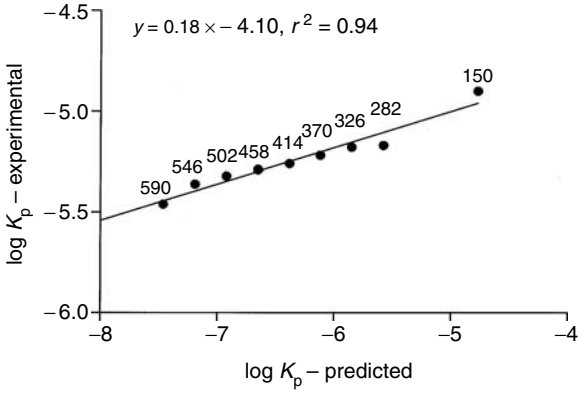

Figure 4. Comparison of the experimental values of the permeability coefficient (K_p) of PEG150–590 Da with those predicted by Potts and Guy algorithm $\log K_p = -2.72 + 0.71 \times \log K_{ow} - 0.0061 \times MW$, where value of $\log K_{ow}$ of −1.6 was taken for all MWs.

Not only the diffusion coefficient but also the partitioning of PEGs into AD skin was different from that in the control skin: the partition coefficient was twice as high in the control subjects as in the AD patients. There was also no clear tendency for a decrease with state of disease (Figure 2). Decreased partitioning of PEGs into diseased skin might be explained by the dryness of the AD skin: a smaller amount of water will decrease the partitioning of hydrophilic PEGs into the SC. In contrast to the diffusion coefficient, the partition coefficient was similar for all PEG oligomers. This was consistent with a similar octanol–water partition coefficient over a broad range of MWs (Hollander *et al.*, 1989).

The experimentally obtained permeability coefficients were similar in both AD patients and control subjects. Although AD patients showed increased diffusion coefficients, this increase was offset by a proportional decrease in the partition coefficients in AD patients. The steady-state flux will therefore be the same in both AD and normal skin; however, the AD patients will elicit a shorter lag time for transport of a chemical across the skin. For situations in which the exposure ends before steady state is reached during the exposure (the most common situation in real life), the flux into the body from the skin will be higher for the AD skin because D is larger. Although the total amount of PEG that eventually goes into the body from the skin will be larger for the control skin owing to higher partitioning, the rate that it goes into the body will be larger for the AD person. With respect to toxicity, the rate can be more important than the total amount. If the rate is slow enough, the body might be able to manage the chemical (i.e., metabolize it and eliminate it fast enough relative to the rate at which it enters the body).

Compounds which have such a low permeability like PEGs, will probably have a low relevance for pharmaceutical purposes. However, from a toxicological point of view, one has to keep in mind that also larger molecules are able to penetrate the skin, and as shown in this study this will be even more pronounced when the skin is impaired. As skin damaged mechanically, chemically or physiologically is not uncommon, when evaluating the health risk associated with skin exposure, penetration of higher MW compounds should be considered.

To summarize, the skin of AD patients showed increased diffusion coefficients for all PEGs when compared to control subjects. This supports the hypothesis that skin barrier function is altered even in skin that is visibly unaffected by AD. Possible implications of the findings are that AD patients might have a higher susceptibility for inflammation of the uninvolved skin, which in turn will increase permeability, resulting in a vicious circle. Additionally, in the design of new drugs, also compounds with larger MW could be suitable for therapeutic treatment.

MATERIALS AND METHODS

Study population

Twenty AD patients, 12 males and eight females, with a mean age of 29 years (range 18–54 years) and 20 healthy subjects, 11 males and nine females, with a mean age of 32 years (range 18–55 years), all Caucasians, participated in the study which was carried out in June and July 2004.

The AD patients were recruited from the outpatients clinic of the Academic Medical Center and diagnosed according to the Hanifin and Rajka (1980) criteria. We excluded patients who had received systemic therapy, such as corticosteroids and immunosuppressants, or phototherapy in the past 2 years. Subjects with concomitant ichthyosis vulgaris were also excluded. The test sites, both mid-volar arms had been free of dermatitis for at least 3 months before the experiment. The total eczema area and severity index (maximum 72 points) was assessed in the AD patients (Hanifin *et al.*, 2001). The severity of the disease was mild in all the patients and the median eczema area and severity index was 1.7 (ranging from 0.2 to 22.8). Twelve patients 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. The control subjects had no visible skin damage and no history of past or present AD or other dermatological diseases.

All the subjects completed the Erlangen questionnaire, from which an atopy score was calculated (maximum 34 points; a score $\geqslant 10$ is considered as atopy) (Diepgen, 1991). AD patients and control subjects had a score of 17.4 ± 6.6 and 3.0 ± 2.4 (mean $\pm$ SD), respectively.

The participants were not allowed to use soap, moisturizers, or any other cosmetics or creams on the lower mid-volar arms for 48 hours before and during the experiments. Written informed consent was obtained from all the subjects before the experiment. The Medical Ethics 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

The application mixture of PEGs was made by spiking 10 g of polydispersed PEG600 (average MW = 600 Da, Sigma, The Netherlands) with 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, New Jersey, NY), 102.9 mg of monodispersed PEG326 (MW = 326.4 Da, PolyPure, Norway), and 199.1 mg of monodispersed PEG370 (MW = 370.4 Da, PolyPure, Norway), to which 2 ml of water was added. Subjects were exposed for 6 hours to the PEG application mixture (180 μl) on the mid-volar arms using round patch test chambers (Finn Chambers®, 18 mm in diameter, Epitest Ltd, Finland). 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. The TEWL was measured on application sites, before patch application, after patch removal, and during the tape stripping procedure, using an evaporimeter (VapoMeter SWL2 g, Delfin Technologies Ltd, Kuopio, Finland). Twenty minutes prior the application, the subjects rested with their sleeves rolled up in the examination room, where the temperature was 20–22°C and the relative humidity ranged between 50 and 60%. After the removal of the patch containing PEG mixture, a piece of dry cell tissue was gently pressed to the skin site to remove the residue of PEG mixture. Templates of Scanpor® tape were fixed to the skin around the application spot to limit the tape stripping to exposed area (18 mm in diameter). Ten minutes after the end of exposure, the SC layers were sequentially removed with pre-cut pieces of Diamond tape, 19 × 25 mm (Diamond Ultra Clear tape, The Sellotape® Company, The Netherlands). The tape pieces were consecutively applied to the test site and uniformly pressed with a 1 kg stainless-steel roller, which was moved 10 times in two directions. The total removal of the SC was evidenced by the glistening, redness of the skin and additionally by checking visually that there was no SC present on the last obtained strips. We have also observed that the TEWL became very high ($>100\,g\,m^{-2}\,hour^{-1}$). Each individual strip was placed into a glass vial and stored at −20°C until analysis. The SC from a non-exposed site was harvested with the adhesive system described above and served as negative control.

Analytical procedure

The gas chromatographic method for the determination of PEGs in tape strips have been described in detail elsewhere (Jakasa *et al.*, 2004). Briefly, the method is based on extraction of PEGs from the tape strips with methanol (J.T. Baker, The Netherlands) and subsequent derivatization with pentafluoropropionic anhydride (Fluka, The Netherlands) at 70 ± 5°C for 30 minutes in dichloromethane (P.A., Merck, The Netherlands) and pyridine (Alltech, The Netherlands).

For the analysis of proteins, we used modified method of Dreher *et al.* (1998). Briefly, the remaining methanol solution containing tape strip was evaporated. One milliliter of 1 M sodium hydroxide (Merck, The Netherlands) was added to the strip and the vials were shaken for 2 hours. The samples were left at room temperature overnight and the next day they were shaken for 2 hours one more time. One milliliter of 1 M hydrochloric acid (Merck, The Netherlands) was added to neutralize basic solution. The protein assay was performed according to Bio-Rad DC protein microassay (Bio-Rad Laboratories, Germany) using commercially available BSA for standardization.

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

Data analysis

To estimate the penetration parameters, we used an approach based on Fick's second law of diffusion which applies to the non-steady state condition (Crank, 1975), as described extensively elsewhere (Pirot *et al.*, 1997). 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) (Figure 1) to the following equation:

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

where C_{veh} is the applied PEGs concentration ($\mu g\,cm^{-3}$), C is the PEG concentration ($\mu g\,cm^{-3}$) at depth × (cm), K is the SC/water partition coefficient, L is the total thickness of the SC (cm), D is the apparent diffusion coefficient of PEGs through the pseudo-homogeneous SC ($cm^2\,hour^{-1}$), D/L^2 ($hour^{-1}$) is the diffusion rate constant, and t is the exposure duration (hour). In deriving Equation 1, K and D/L^2 are assumed to be constant throughout the SC.

The permeability coefficient (K_p, $cm\,hour^{-1}$) for each PEG oligomer was calculated from the relationship $K_p = K \times D/L$. The first strip was not included in the regression analysis, as it contained some PEG residues on the surface of the skin after the end of exposure. All concentration data were weighted equally in the regression analysis. Prism 4 (Graph Pad Software Inc., San Diego, CA) and SPSS software were used (SPSS Inc., Chicago, IL) for curve fitting and statistical calculations. Student's t-test and one-way analysis of variance with Bonferroni post-test were used for statistical calculations and P-value <0.05 was considered significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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